EFFECT OF ESSENTIAL OILS ON FUNGAL GROWTH AND FUNGAL INFECTIONS

BY

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A thesis submitted for the degree of
Master of Science
in the Faculty of Science at the University of Glasgow

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October 1993

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A Jeanne,
En souvenirs de nos
cueillettes fructueuses

ACKNOWLEDGEMENTS

I wish to acknowledge Dale Walters for his patience, time, support and guidance throughout the course of the experiments and the preparation of this thesis.

Thanks to EEC and Région Picardie for their contribution to the funding of my research.

Many thanks are due to Katja Svoboda for her support and to Neil MacRoberts for his help and advice on statistical analysis.

I also wish to thank Andy Jackson for his practical assistance and helpful discussions, Neil Havis and Janice Hampson for their practical help, and Anne, Rhona, and Katie for their patience in improving my English and for their complicity.

I thank all the staff and students of the Plant Science Department for all the smiles that helped me to keep mine.

Je tiens également à remercier toutes les personnes , proches et enseignants, qui ont participé à mon éducation, et plus particulièrement mes parents qui m'ont toujours fait confiance.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABBREVIATIONS

BP : ß-pinene
car : carvacrol
DF : degree of freedom
DMAPP : dimethyl allyl pyrophosphate
FPP : farnesyl pyrophosphate
GLC : gas-liquid-chromatography
GPP : geranyl pyrophosphate
g-ter : γ -terpinene
HC : high concentration
IPP : isopentenyl pyrophosphate
iso : isopinocampheol
l-BA : l-bornyl acetate
LC : low concentration
LSD : least significant difference
MS : mean square
NC : normal concentration
p-cym : para-cymene
pino : pinocamphone
SS : sum of squares
thy : thymol

SUMMARY

Investigations were made on the effect of hyssop and oregano essential oils on plant diseases. This involved an examination of the effect of the oil on infection of barley and apple with powdery mildew and broad bean with rust, and on mycelial growth of *Pyrenophora avenae* and *Pyricularia oryzae*. Germination of *Botrytis fabae* conidia and *Uromyces viciae-fabae* uredospores, in the presence of the oils, was also studied. The effects of the main components of the essential oils were also tested in several *in vitro* and *in vivo* experiments.

The *in vivo* experiments yielded various results which were not significantly different from controls. Thus, pre-inoculation treatment of broad bean and apple with oregano oil increased infection with rust and powdery mildew, while post-inoculation treatment of barley and pre-inoculation treatment of apple with hyssop oil, and a post-inoculation treatment of apple with oregano oil, all reduced powdery mildew infection. In addition, both ß-pinene and isopinocampheol controlled barley powdery mildew infection. Suspensions of 0.4% and 0.04% oregano oil completely inhibited mycelial growth of *P. avenae* and *P. oryzae*. This inhibition was shown to be due to vapours of the oils. Combinations of components of hyssop oil containing isopinocampheol slowed down growth of *P. oryzae* and inhibited

growth of *P. avenae*. Thymol, and especially carvacrol, both components of oregano oil, reduced mycelial growth of *P. avenae* and *P. oryzae* considerably.
Suspensions of hyssop oil reduced germination of conidia of *B. fabae* and uredospores of *U. viciae-fabae*, while oregano oil reduced germination of *B. fabae* conidia.

Thus, although the two essential oils examined here were powerful inhibitors of fungal growth *in vitro*, the oils possessed very little activity on the plant.

INTRODUCTION

Since the 1950's agricultural practices have been deeply modified. The role of farmers in the EEC after the second world war was to provide food and to reach self-sufficiency. Pesticides and selected varieties were the tools they were given to achieve this aim. The goal of self-sufficiency has been reached and overtaken within the EEC, and other western countries. Indeed, these countries now have to face over-production. However, 40 years of intensive farming practice has also created several problems.

The intensive use of the same pesticides led to resistance to those pesticides developing in target organisms and to their accumulation in the environment. Some pesticides can still be detected at very high level years after their use was forbidden by law.

The notion of the accumulation of pesticides in the food chain had not been integrated into the early toxicological tests. Eventually unplanned consequences of continual pesticide use became apparent. The question of healthy food appeared.

These problems are no longer the concern judge of ecologists but now also concern the man in the street, the authorities and all levels of agribusiness (agrofurniture, farmers, transformers).

To deal with these concerns, new agricultural practices and

systems are studied and the agriculturalists are asked to find solutions, like novel forms of disease control. The idea was that these would replace existing methods that no longer work effectively. It was also hoped that many different novel forms of control would be developed, thus reducing the risks (the risk of inefficiency by abuse and the secondary effects of the practice itself on the environment and human health). Clearly, the development of effective, novel means of disease control should be based on a thorough knowledge of plant: pathogen systems.

The plant itself provides researchers with a wide range of compounds which might be useful in this fight against diseases. Thus, a new generation of fungicides based on chemicals derived from compounds found in plants is a possibility. This is especially true of essential oils, many of which are known to possess antimicrobial properties.

Chapter 1

LITERATURE REVIEW

1 NOVEL FORMS OF DISEASE CONTROL

As knowledge of plants, pathogens and the complex interaction between plants and pathogens increases new forms of disease control that use this new knowledge appear. These new ways of controlling disease are numerous and many are based on completely novel modes of action. Only three novel forms of disease control will be described in the following paragraphs.

1.1. Biological control

Biological control is based upon the exploitation of the relationship that exists in the field between the host, the pathogen, parasites of the latter, and other non-pathogenic organisms. Biological control uses agents or antagonists which become active and effective after their introduction, or after modification of the cropping conditions or of the environment. The direct introduction of antagonists, which is less common, can take the form of, for example, the introduction of an attenuated strains of viruses to control virulent strains. Biological control agents are essentially responsible for antibiosis, competition or hyperparasitism (Waage & Greated, 1988 ; Strange, 1993).

1.1.1. Exploiting antibiosis

In antibiosis, control of pathogens occurs through the production of an antibiotic compound.

Antibiotic production is thought by some workers to be an important characteristic of successful biocontrol agent. Indeed, antibiotic producing strains of bacteria have been used successfully against *Nectria galligena* on apple and fireblight caused by *Erwinia amylovora* (Cook & Baker, 1989). However, since antibiosis implies antibiotic synthesis, there is a risk associated with the use of antibiotic-producing organisms for the protection of produce for human consumption. This approach could select microorganisms not sensitive to antibiotics produced by the biocontrol agent as well as antibiotics commonly used to control human infection.

1.1.2. Exploiting competition

Competition for substrates is particularly vigorous in soil. In the rhizosphere there is a balance between pathogenic and saprophytic microorganisms. However, the latter can out-compete the former and colonise the rhizosphere. For example, the incorporation of barley straw in the soil encourages the population of saprophytes that use nitrogen to assimilate it. However nitrogen is essential for germination and penetration of *Fusarium solani* f.sp.*phaseoli*, the cause of bean root rot. So *Fusarium solani* f.sp. *phaseoli* is controlled because it

is deprived of nitrogen when barley straw is incorporated into the soil. Also competition can be exploited for the control of pathogens on citrus fruits during storage. Thus, a washed fruit rots quicker than a non washed fruit (Chalutz & Wilson, 1990).

1.1.3. Exploiting hyperparasitism

Plant pathogens can be parasitised or lysed by other organisms. Here, control can be achieved by introducing the parasite of the pathogen or by making the environment required for growth of the biocontrol agent more favourable. For example, a system of regular weekly plantings of radish seeds allows a population of mycoparasitic *Trichoderma* spp. to increase in the soil. This increase is followed by a decrease in *Rhizoctonia solani*, the cause of the damping-off in radish seedlings (Henis & Ghaffar & Baker, 1978)
In this approach, the biological agent must be compatible with other micro-organisms in rhizosphere or phyllosphere. It has to be able to survive under field conditions and to colonise the part of the plant it is supposed to protect (Baker & Scher, 1987).

1.1.4. Limits to biological control

The poor efficiency of fungicides in controlling soil-borne

pathogens, and the development of fungicide resistance were important in the development of biological control. However, despite the advantages, there are problems with biological control. One of the limits to the development of biological control is the fact that the environment has more influence on the activity of the biocontrol agent than on pesticides used for chemical control (Baker & Scher, 1987). The interactions in the biological system and also the efficiency of such systems are difficult to predict. Moreover, biological control is slower to take effect than fungicidal control. Further problems appear with the production and storage of antagonists, as living organisms, in sufficient quantities for large scale field-use.
Certainly, for biological control to be successful, a long-term view is required.

1.2. Induced resistance

Plants have a range of resistance mechanisms, which can be split roughly into two groups : passive and active mechanisms. In the latter case, the resistance mechanisms only come into operation after attempted pathogen infection. It has been known for some time that prior inoculation of a plant with a pathogen can make that plant more resistant to subsequent infection (Taylor, 1987). This 'induced resistance' can be localised and systemic, and although they appear to be related, the processes which are activated in

response to infection are different in the two cases.

1.2.1. Localised induced resistance

In the phenomenon of localised induced resistance, the reduction in disease severity occurs in tissue that has already been inoculated. In many cases phytoalexin production is induced at the site of infection and these compounds accumulate in the host tissue very close to the site of inoculation. In some cases however, phytoalexins are not associated with the induction of local resistance. Indeed in some experiments, resistance may have actually resulted from increased bacterial antagonism on leaf surfaces, and had nothing to do with resistance induced within the plant (Taylor, 1987).

1.2.2. Induced systemic resistance

In the phenomenon of induced systemic resistance, resistance occurs later on another part of the plant, following prior inoculation on a different part of this plant. That is to say that the initial infection occurs on one part of the plant (a lower leaf, for example) and if infection was attempted another part of the plant later (*ie* an upper leaf), the latter becomes intensely resistant. There is one limit to the development of this ability : there is a time lag before

systemic protection is detected.

The protection mechanisms that act at the site of the second infection are various : papilla formation, lignification, induction of glucanase and chitinase activities, and the activation of peroxidase involved, perhaps, in lignification (Dean & Kuc, 1985). The activation of these mechanisms requires the reception of a signal originating from the part of the plant that was previously inoculated. Recent work suggests that in some systems, salicylic acid seems to be the signal or part of the signalling mechanism involved in systemic induced resistance (Raskin, 1992). So it appears that infection triggers off the synthesis of salicylic acid in the infected leaf, which moves in the phloem to the upper leaves. The time necessary for the synthesis and transport of salicylic acid would explain the time lag between the initial infection and the appearance of the induced resistance.

The possible exploitation of systemic induced resistance has created much excitement. Two means by which systemic induced resistance might be used practically include application of salicylic acid analogues (Walters *et al.*, 1993) and increasing the amount of mRNA coding for the synthesis of enzymes producing salicylic acid (Day, 1993).

1.3. Film-forming polymers

The majority of foliar-infecting fungal pathogens need to penetrate the cuticle and cell wall of leaf tissues quickly,

before food reserves in the spores runs out. Surely then, if the leaf surface was coated, making it more difficult for spores to penetrate the leaf, fungal infection might be reduced. One approach to achieve this might be to use film-forming polymers.

Film-forming polymers can take various forms : oils, waxes, polyterpenes and alcohols. They are used at present as antitranspirants and to delay desiccation of agricultural and horticultural crops. Another major use is in conjunction with fungicides, as stickers. But for several years they have been tested as a means of controlling foliar infections by reducing leaf penetration by fungal pathogens. Thus, Ziv and Frederiksen (1987) have shown control of wheat powdery mildew and wheat rust using film-forming polymers. They noticed that the control was more effective on the upper side of the treated leaves and increased with leaf age. A fungistatic effect of film-forming polymers was shown by Elad *et al.* (1990). Film-forming polymers reduce germination of *Botrytis cinerea* conidia, elongation of the germ tube and linear growth of mycelium.

Applied pre- or post-inoculation, film-forming polymers significantly reduced powdery mildew infection on barley seedlings. The best control followed pre-inoculation sprays with the polymers (Walters, 1991). In the same experiment, however, not all the polymers were equally effective in controlling mildew.

In several trials addition of fungicide to film-forming polymers produced no extra benefit. Thus, Elad *et al.* (1990)

noticed no additive statistical effect of chlorothalonil plus a polymer for the control of grey mould on tomato, and although Walters (1991) noticed a greater reduction in infection when Emerald and Vapour Gard were applied with difluoromethylornithine (a polyamine biosynthesis inhibitor), than when difluoromethylornithine was used alone, the reduction was not significantly different.

Ziv and Frederiksen (1987) suggested that polymers may act first because coated surfaces are hydrophobic, thus creating a low water potential at infection sites and secondly, the coated surfaces may be impenetrable because of the thickness, or resistance to enzymatic attack of the coating material. Since film-forming polymers are non phytotoxic, permeable to gases, have excellent weathering properties and are biodegradable, they may pose little environmental threat. In addition, they might alleviate some of the problems of fungicide resistance.

2 ESSENTIAL OILS

2.1. General

All true essential oils are secondary metabolites of plant products and in some instances the oil produced in one part of a plant is different from that produced in another. In some instances the oils are stored in specialised oil cells,

Plate 1 : Glandular secretory cells on the surface of a hyssop leaf (X 40 with incident illumination)

Plate 2 : Peltate glandular trichome of hyssop leaf (X 400 with interference contrast)

Plate 3 : Peltate glandular trichome of oregano leaf (bright field, stained ; X 400)

Plate 1

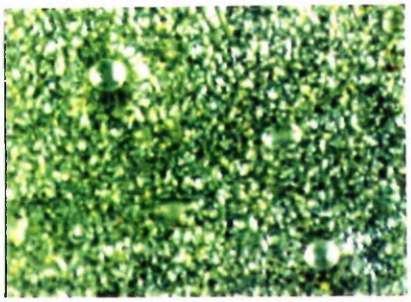


Plate 2

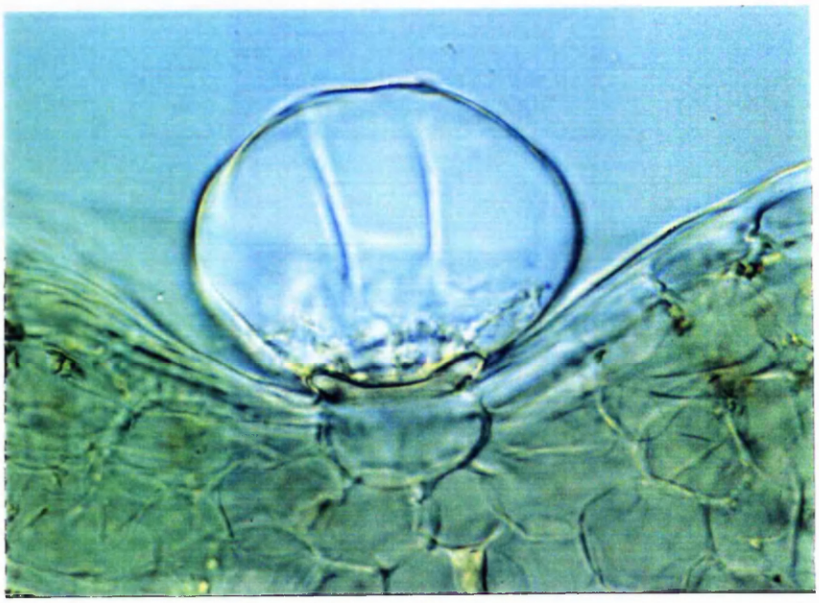


Plate 3

whilst in others they are not specifically located (Anon, 1984).

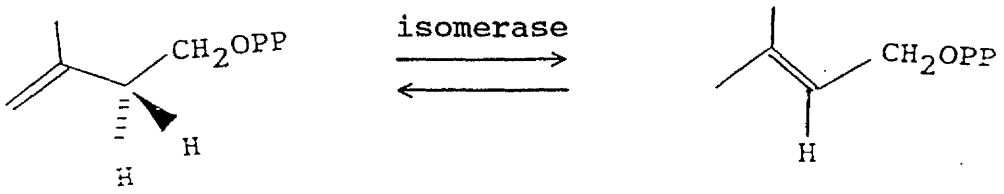
2.1.1. Secretion and storage of essential oil in the plant

The essential oil is secreted and stored by glandular trichomes which originate from epidermal cells and develop on the surface of various organs (Plate 1 and Plate 2).
For example, the leaves of oregano bear on both sides numerous glandular hairs (60-80 μm in diameter). The glandular hairs can be divided into the peltate hairs (one basal cell, one stalk cell, a secretory head and peripheral cells) (Plate 3), and into the capitate hairs of type I and II (one basal cell, one to three stalk cells, and one head cell which shape varies with the type of capitate hair). There are differences in the mode of secretion between the different kinds of glandular hair. In the case of the capitate hairs the material is secreted to the outside whilst it is accumulated above all the head cells in a space formed by detachment and elevation of their cuticle in the peltate hair.

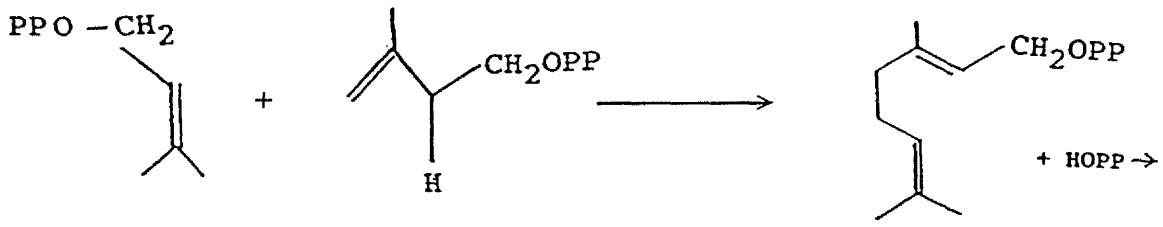
2.1.2. Chemical composition and principle of extraction

With the exception of oils derived from glycosides, volatile

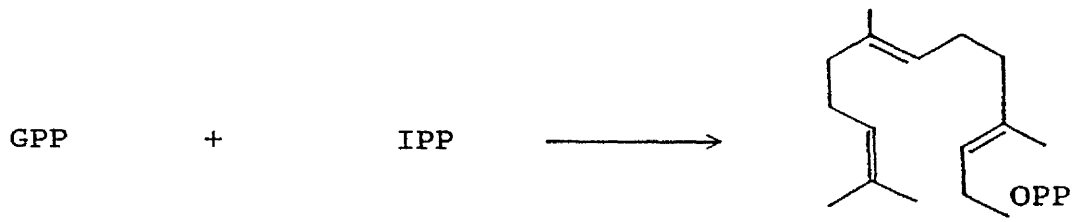
Figure 1 : Biosynthetic pathway : from the isopentenyl pyrophosphate unit (first precursor) to farnesyl pyrophosphate (first step in the synthesis of sesquiterpenes).



IPP DMAPP



DMAPP IPP GPP



FPP

oils are generally mixtures of hydrocarbons and oxygenated compound derived from these hydrocarbons. The odour and taste of volatile oils is mainly determined by these oxygenated constituents, which are usually appreciably soluble in water, but more soluble in alcohol. Many oils are terpenoids in origin (Trease & Evans, 1983).

Essential oils are normally extracted by water or steam distillation processes from specified parts of plants, or by cold expression from the pericarp of citrus fruits, where the essential oil will contain proportions of non volatile products.

2.1.3. Biosynthetic pathway

Most of the main components of essential oils are terpenoids. Their chemical form is $(C_5H_8)_n$ and they all originate from the isoprene unit. Their biosynthesis begins with the formation of isopentenyl pyrophosphate (IPP), the first precursor, from mevalonic acid. IPP can be transformed by an isomerase into dimethyl allyl pyrophosphate (DMAPP). The association of a unit of DMAPP with a unit of IPP leads to the formation of geranyl pyrophosphate (GPP), the starting point for monoterpene biosynthesis.
Subsequently, the association of a unit of GPP with a unit of IPP leads to the formation of farnesyl pyrophosphate (FPP), the first step in the synthesis of sesquiterpenes (Figure 1).

2.1.4. Variation in the composition of essential oils

The composition of the essential oils from one species of plant can vary enormously. This can arise from variation in the environment. The composition of essential oils is related to plant metabolism. So differences in soil and climate (water, temperature, light) will generate differences in quality of essential oils (Hornok, 1992).

2.1.5. Biological properties of essential oils

The biological properties of essential oils are numerous and varied, and their effects can be very potent.
Maruzzella & Robbins (1961) reported different properties of essential oils, including :

-inhibition of seed germination
-antimicrobial properties (see below)
-inhibition protozoa growth
-inhibition fibroblasts growth
-inhibition carcinomas growth
-inhibition of the growth of animal cells and tissues

2.1.6. Antimicrobial properties

Maruzzella & Balter (1959) tested 119 essential oils for

activity against 12 phytopathogenic fungi. They found that 100 of them possessed antifungal activity against at least 2 of the fungi tested. Oregano oil was one of the most efficient, inhibiting growth of *Botrytis allii* and *Diplodia maydis*. In later work, Maruzzella (1963,a) showed that various perfume oils could inhibit or prevent *in vitro* growth of 5 dermatophytes. The dilutions used were very high and were still effective at dilutions between 1:500 to 1:13000. In fact, phytopathogenic fungi were less susceptible to the oils than dermatophytes. Thus, Maruzzella (1963,b) reported that of 17 perfume oils that inhibited growth of 5 phytopathogenic fungi, most were effective at dilutions of 1:500 to 1:2000. Interestingly, Maruzzella *et al* (1963) found that only the vapours of aromatic chemicals were able to inhibit growth of phytopathogenic bacteria. Of 254 vapours tested, 180 (*ie* 70%) were found to produce zones of inhibition against at least one of the five test organisms. In each of his papers on phytopathogenic micro-organisms, Maruzzella suggested, as future work, studies using oils as a component of a preparation to control diseases. To date, it appears that no work has been performed in this area.

Plate 4 : Hyssop (*Hyssopus officinalis*)

Plate 5 : Oregano (*Origanum vulgare*)

Plate 4

Plate 5

2.2. Oregano and hyssop oils

Hyssopus officinalis (Plate 4) and *Origanum vulgare* (Plate 5) both belong to the *Labiatae*. This family contains many species of medicinal and aromatic plants. A large number of *Labiatae* are also cultivated either as ornamentals or as kitchen herbs. Upwards of 60 genera are grown in temperate regions alone. Many species are cultivated commercially, mostly as aromatic herbs. Also, in different parts of the world, native species of *Labiatae* are much used by the local people (Heywood , 1978).

2.2.1. Chemical composition

The chemical composition of oregano and hyssop oil varies in function of various factors listed in the 2.1.4.
The table 1 shows the difference of composition of several hyssop oils from different origins analysed by different methods (at different periods) and different workers. Nearly all of them contain ∝-pinene, ß-pinene, pinocamphone in various proportions. Eventhough a range of methods of analysis were used, differences between the various sources of oil still exited.
It is necessary to precise that the term 'oregano' covers at least 39 species in 16 genera all over the world (Fleischer & Sneer, 1982). It gathers plants which give oils with high level of carvacrol and thymol. The table 2 shows the

Table 1 : Composition of different hyssop oils analysed by various workers

	H	S	G	K	J	L
∝-pinene	*	7.3	*		1.41	0.74
ß-pinene	*	5.3	14		22.87	8.8
3 carine	*					
para-cymene	*				0.4	0.07
cis-ocimene					*	0.11
trans-ocimene						0.31
∝-terpinene		9.4				
∝-terpineol				*	*	1
∝-terpinyl acetate				*		
terpine-4-ol						0.33
camphene	*		*		traces	0.14
pinocamphone	*	46.7	50		12.18	42.66
pinocampheol		2.1	*			
isopinocamphone					32.57	30.88
hydroxy-2-isopinocamphone					0.65	0.33
1,8-cineole				*		0.64
linalool				*		
bornyl acetate				*		
myrcene					1.84	0.72
limonene					*	0.68
myrtenate de methyl					*	
acetate de myrtenyl					traces	
myrtenol					*	0.37
myrtenol methyl ester					2.72	
myrtenyl methyl ether						3.97
thujone					traces	0.08
isothujone					traces	
pinocarvone					traces	
estragole					*	
caryophyllene					*	0.42
∝-humulene					*	
allo-aromadendrene					0.54	0.82
germacrene D					*	0.40
bicyclo-germacrene					traces	
δ -cadinene					*	0.15
ɣ -cadinene					traces	
ß -cadinene					traces	
T-cadinol					traces	
calemenene					traces	traces
methyleugenol					0.54	0.09
nerolidol					0.37	0.09
elemol					1.67	0.36
carvacrol					traces	traces
spatulenol					2.19	0.68
sabinene						1.72
methyl chavicol						0.09
caryophyllene oxyde						0.23
1-octene-3-ol						0.11

H = HILAL et al. (1978) - hyssop from Egypt
S = SHARMA et al. (1963) - hyssop from India
G = GUENTHER (review of 1949)
K = KHODZHIMATOV et al. (1975) - hyssop from Tashkent
J = JOULAIN (1976) - hyssop from Hungary
L = LAWRENCE (1984) - hyssop from North America

* = present but concentration unknown
composition expressed in percentage of oil

Table 2 : Composition of different oregano oil analysed by various workers

	1	2	3
thymol	*	50	1.1-7.4
carvacrol	*		62.4-82.6
sabinene	13.5		trace
trans-sabinene hydrate			trace-0.1
cis-ß-ocimene	13.5		
trans-ß-ccimene	3.8		
caryophyllene	9.2		
+ cis-dihydrocarvone			0.4-1.0
caryophyllene oxide II			trace-0.1
trans, trans ∝-farnesene	2.4		
p-cymene	3.1	17.5	2.7-8.8
p-cymen-8-ol			trace-0.2
linalool + ß-bourbonene + 4,5-epoxy-p-menth-1-ene + cis-sabinene hydrate + cis-p-menth-2-en-1-ol	7.3		0.1-0.4
trans-2-hexenal	6.6		
∝-terpinene			0.3-0.7
ɣ-terpinene	1.9		1.3-2.3
∝-terpineol			0.3-0.7
terpinen-4-ol			0.4-0.7
terpinolene			trace-0.1
terpinolene epoxyde			trace-0.2
3-octanol	1.5		
d-pentene		10.5	
∝-pinene			0.4-0.7
ß-pinene			trace-0.2
myrcene			0.4-0.8
camphene			trace-0.3
limonene			0.1-0.2
1-octen-3-ol			0.1-0.4
3-octanol			trace-0.1
∝-murolene + carvone + ß-bisabolene			0.2-0.6
methyl carvacrol + aromadendrene			0.4-0.8
∝-maaliene +terpinen 4-yl acetate + transdihydrocarvone + methyl benzoate			0.8-1.5
∝-humulene			0.1-0.4
humulene epoxyde II			trace-0.1
bornyl acetate + ß-ylangene + trans ∝-bergamotene			trace-0.2
δ-cadiene			trace-0.1
∝-cadinene			trace-0.1
ɣ-cadinene			trace-0.2
cadin-1(16),4-diene + viridiflorene + ɣ-murolene			0.1-0.2
∝-thujene			trace-0.2
1,8-cineole			trace-0.1
∝-p dimethylstyrene			trace-0.1
copaene			trace-0.1
calamenene			trace-0.1
methyl chavicol			trace-0.1

1 = PRAKASH V. (1990) - analysis by Maarse and Van Os
Origanum vulgare

2 = PRAKASH V. (1990)
Origanum vulgare L. var Viride

3 = LAWRENCE B.M. (1984) - analysis by Ietswaart
Origanum vulgare L. var Viride

difference of composition of several oregano oils. In *Origanum vulgare*, carvacrol is the main constituent (Prakash, 1990).

2.2.2. Medicinal uses and properties of hyssop

The essential oil has been known since 1754 and has replaced the dried plant gradually since that time.
Used internally, the oil eases and modifies expectoration and it possesses carminative, diuretic, vermifuge and emmenagogue properties (Lautié & Passebecq, 1979 ; Valnet, 1982 ; Simon, 1984) (see the glossary of medical terms in Table 1 in Appendix 1)
The main interests in hyssop for external use are the cicatrizing and resolvent properties. Hyssop is also reported to have antiviral properties (against herpes simplex virus) (Mabey, 1988).

2.2.3. Culinary uses of hyssop

The flowering tops and leaves of hyssop are used as flavouring agents in such beverages as teas, tonics, and bitters, and to a limited extent in vegetable dishes, soups, salad and candied products. In the past hyssop was one of the

main constituents of the *absinthe* and now the essential oil is used in liqueurs (Joulain, 1976).

2.2.4. Medicinal uses and properties of oregano

Origanum vulgare is officially accepted in some countries for its medicinal value and it is used extensively in homoeopathy.

The oil used internally has an antispasmodic, stomachic, carminative, expectorant, antiseptic effect properties (Lautié & Passebecq, 1979 ; Valnet,1982 ; Simon *et al.*, 1984) (see the glossary of medical terms in Table 1 in Appendix 1). It is used as a treatment against parasites and as an analgesic. It is also used for gargling and in baths and also in healing lotions for wounds (usually in conjunction with other herbs). It is reported to have a stimulating effect on growth of hair (Valnet, 1982 ; Prakash, 1990), and to remove lice (Leung, 1980). Oregano oil is a powerful disinfectant, and two of its constituents, carvacrol and thymol, are considered to be anthelminthic and antifungal agents (Guenther, 1949 ; Windholz, 1976).

2.2.5. Culinary uses of oregano

The fresh and dried plant or the essential oil are used

extensively for food flavouring both in industrial products and in the home. The main use of oregano is on pizza. This is the main reason for its development during the last three decades. It is also used to flavour tomato or bean soup, egg dishes, meats (sausages, pork, lamb, poultry and game), vegetables and seafood salad. It is also an essential ingredient of chilli powder and it is used in chilli con carne and many mexican dishes. In addition, it is eaten as a vegetable in India (Prakash, 1990). Oregano has been and is still used in several drinks, especially alcoholic liqueurs. Prior to the introduction of hops, oregano was used to flavour ale and beer (Muenscher & Rice, 1955).

3 PATHOGENS

3.1. Pathogens studied *in vivo*

3.1.1. Barley powdery mildew

Since 1903, when the first severe epidemic of barley mildew occurred, barley mildew has remained a continual problem in Europe (Wolfe & Schwarzbach, 1978). It is even considered to be the most serious disease of barley in Great Britain (Gair & Jenkins & Lester, 1972).

3.1.1.1. Effects on the plant and symptoms of infection

Erysiphe graminis is an obligate parasite. Mildew infection has a profound effect on the growth and physiology of the plant, affecting photosynthesis, respiration, assimilate partitioning and mineral relation of the plant (Walters, 1985). The loss of crop yield can reach 40% or even more, but is usually around 10-20%.
The first symptoms of mildew are small white fluffy pustules on the leaf surface.

3.1.1.2. Life cycle of the pathogen

Mildew overwinters as mycelium on green living material (volunteers and autumn sown crops). Cleistothecia are also a source of inoculum, when they release the sexually produced ascospores in humid weather during autumn and spring. In the spring, when the temperature rises, dormant mycelium starts to grow and conidia are produced. Severe attacks may be expected when the host is growing rapidly, when the weather is warm (15-22°c) and when there is an abundance of conidia. The optimal weather conditions for the germination of conidia are 15°c and 95% RH.

3.1.1.3. Control

Chemical control is widely practised. The first major systemic fungicides to be used to control barley mildew were:

-ethirimol (2amino pyrimidine ; direct action) used largely as a seed treatment

-tridemorph (morpholine ; protectant and eradicant effect) used solely as a foliar spray

These were introduced in the mid-sixties and have been used widely because of their efficiency. Insensitivity of the pathogen to these fungicides increased over the years and the fungicides have become non effective (Wolfe, 1984)

Today, because of the problem of fungicide resistance, mixtures are used commonly. Thus, mixture of triazoles and morpholines are used to control mildew in most spring barley crops (Carlile, 1988).

Disease resistance is very important in the control of barley powdery mildew. It is the most economic method of control, when the degree of resistance amounts to near immunity. But when the area of cultivation of a variety with single gene resistance increases, the frequency of the matching pathogenicity gene in the pathogen increases as well. This leads to the development of resistant strains of the mildew and the eventual loss of resistance in the variety (Wolfe & Schwarzbach, 1978). Also, the continued extensive use of one type of fungicide (*e.g.* triazoles) leads to a pathogen adaptation and the consequent risk of large scale and more severe loss of effectiveness of the compounds in controlling

mildew and other pathogens.

The simplest strategy to control barley powdery mildew would be to diversify among varieties and fungicides. Wolfe suggested using variety mixtures in 1984. Although it has been consistently demonstrated that the use of crop mixture reduces mildew infection and leads to yield increases of 6-10% (Carlile, 1988 ; Villich-Meller, 1992). This approach has not been adopted in a large scale in the United Kingdom.

3.1.2. Apple powdery mildew

3.1.2.1. Effects on the plant and symptoms of infection

Podosphaera leucotricha Ell. & Ev. Salm. is responsible for apple powdery mildew. It is the most important disease of apple in the United Kingdom (Blake, Hunter, Souter;1982).

If the young leaves that appear in the spring are infected by powdery mildew, they can become narrow, curled and covered with a white mealy powder. The flowers may also seem small and paler than normal. Severely infected shoots may be seen later, partly or wholly defoliated. Then, assimilates that should be used for fruit production are diverted into vegetative growth. The disease also leads to russetting on young fruit which reduces the market value (Burchill, 1978).

3.1.2.2. Life cycle of the pathogen

The fungus overwinters on the host as dormant mycelium in blossom buds. When it develops at the beginning of the spring, it starts the primary infection. Conidia are then produced and spread by the wind, leading to secondary infection. Mildew is normally seen on the under surface of the leaves (Burchill, 1978). The hyphae grow over the surface and sometimes produce perithecia that contain asci. These will release ascospores during the spring (Wormald, 1955).

3.1.2.3. Control

The first fungicide to be used against apple powdery mildew was sulfur. This was mainly protectant in action and treatment needed to be repeated every 10-15 days.

In the 1950's and 1960's the dinitrophenols (surface fungicide with curative and protectant action) displaced sulfur. For example, dinocap became the predominant treatment against powdery mildew in dessert apples in the United Kingdom (Bent, 1978).

Today, the systemic fungicides used against apple powdery mildew are triazoles (like triadimefon, eradicant and protectant), fenarimol (pyrimidine) and triforine (protectant, curative and translaminar effects).

Curative fungicides of the second generation, *e.g.* pyrifenox, now provide, effective control of apple powdery mildew

(Zeller, 1989).

A biological control of apple powdery mildew based on root extracts of *Rumex obtusifolius* has been successfully tried. Those extracts significantly reduced infection under greenhouse conditions, but were less effective in fields experiments. Nevertheless, biological plant protection measures reduce diseases rather than completely control them, like conventional fungicides.(Bosshard & Schuepp & Siegfried, 1987)

Northover and Schneider have recently reported (1993) a prophylactic and a therapeutic activity of canola oil that is comparable to the effectiveness of dinocap in controlling apple powdery mildew.

Resistant varieties are available. However, varieties resistant to apple powdery mildew are more susceptible to apple scab and varieties resistant to apple scab are more susceptible to apple powdery mildew (Zeller, 1992).

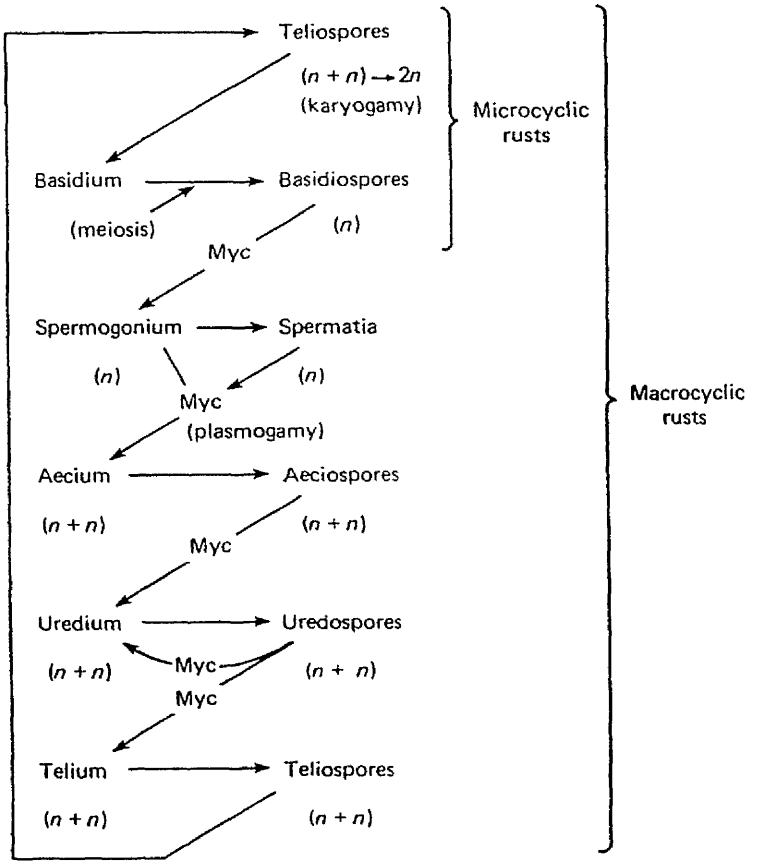
3.1.3. Broad bean rust

3.1.3.1. Effects on the plant and symptoms in infection

Uromyces viciae-fabae (Pers) Schroet. is responsible for broad bean rust.

Small red-brown rust pustules, surrounded by chlorotic haloes

Figure 2 : The kinds and sequence of spores and spore-producing structures in the rust fungi along with the nuclear condition of each. Myc = mycelium



are often seen over leaf surfaces late in the growing season. An early infection can lead to defoliation of plants (Parry, 1990).

Infection mainly affects leaf activity during the pod filling stage and the reduction in leaf area caused by the pathogen is responsible for a shortage of assimilates during the latter stage of growth (Williams, 1978). However, the reduction in yield is slight.

3.1.3.2. Cycle of the pathogen

Uromyces viciae-fabae is a macrocyclic rust, producing spermagonia, aecia, uredia ,telia (Figure 2).

The fungus overwinters as uredospores or resting mycelium on living plant material (volunteer beans, old green bean debris and autumn sown crops).

New infection is due predominantly to urediospores, although if the climate is not too severe, aeciospores can also act as a source of inoculum.

3.1.3.3. Control

Broad bean rust can be controlled using fenpropimorph (contact and systemic morpholine fungicide). There is no useful resistance available to broad bean rust.

3.2. Pathogens studied *in vitro*

3.2.1. *Pyricularia oryzae*

Pyricularia oryzae Cav. causes blast disease of rice. It is one of the most serious diseases of rice.
Conidia are the main source of inoculum.
The disease is favoured by high R.H. (90-92%), high light intensity, long photoperiods, windy weather, unbalanced nitrogen nutrition and a night temperature around 20°c alternating with a day temperature of 30-35°c.

In the past Blasticidin S (antibiotic) and phenylmercury acetate have been widely used for control of blast. Because of their high toxicity to mammals and because Blasticidin S damages rice at concentrations not greatly above those needed to control rice blast disease (Hassal, 1990 ; Marsh, 1977), they are no longer used. Kasugamycin has also been used for its eradicant effect. Now research on the control of rice blast is oriented towards resistance in the rice plant (Kato *et al.*, 1983 ; Neto *et al.*, 1991 ; Cartwright *et al.*, 1977). Interestingly, some compounds which accumulate during resistance reaction have been tested for antifungal activity (Yoshida *et al.*, 1990 ; Volp'in, 1991). Thus, Neto has isolated (-)-jasmonic acid from wild rice (*Oryzae officinalis*), resistant to *P. oryzae*, and found that it inhibits spore germination completely at 250 ppm.

3.2.2. *Pyrenophora avenae*

This fungus causes leaf stripe and seedling blotch of oats. The perithecial stage, *Pyrenophora avenae*, is rare in the field. The conidial stage of reproduction is *Helminthosporium avenae*.

The disease is seed-borne and primary inoculation occurs by conidia and mycelium. Secondary infection is due to conidia. High humidity favours development of the secondary phase (CMI description no 389)

Conidia are produced sparingly on nutrient media, but abundantly in tap-water agar.

The optimum temperature for growth is 30°c.

Because control using seed dressings (thiram and dithiocarbamates) is successful, it is not a disease of major importance (Jones & Clifford, 1978).

3.2.3. *Botrytis fabae*

Botrytis fabae is responsible, together with *Botrytis cinerea* for chocolate spot on broad bean. Two phases of chocolate spot can be seen : a non-aggressive one (small discrete red brown lesions) and an aggressive one (lesions much larger and darker in colour) (Parry, 1990). The later phase is much more often due to *B. fabae* than to infection by *B. cinerea* (Harrison, 1988). It occurs when there is a long period of high humidity or when the plant is under stress.

B. fabae is a saprophytic fungus.

The asexual spores of *B. fabae* are macroconidia (=conidia) and microconidia. The sexual stage of *B. fabae* is unknown.

Conidia of *B. fabae* do not germinate if the relative humidity is not high. Thus, they will not infect bean leaves if the relative humidity is less than 84-85%.

Microconidia are produced by *B. fabae* on old colonies or as a response to prolonged conditions unfavourable to fungal growth (Urbash, 1984).

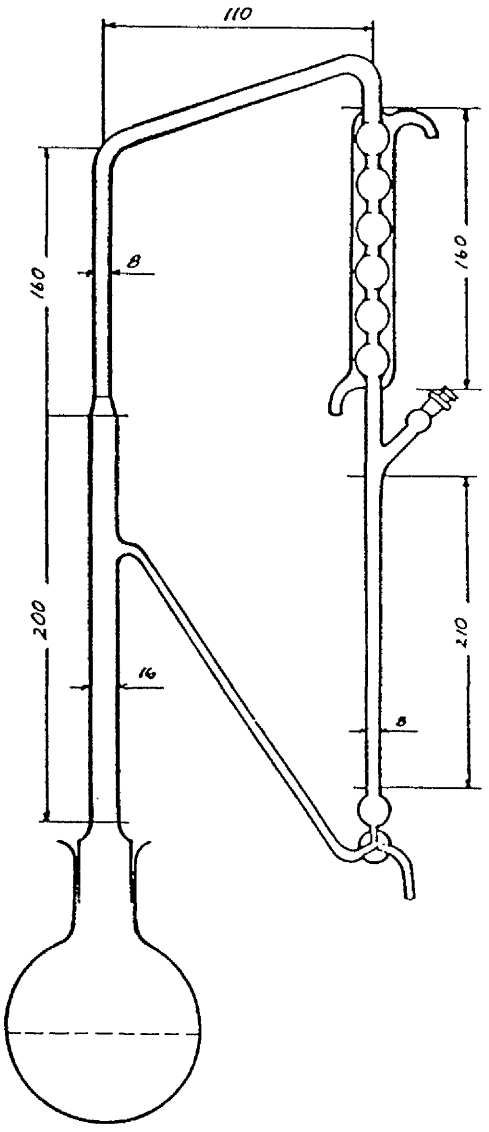
Sclerotia can germinate in three ways. Firstly, they may produce mycelium which commonly occurs on agar media. Secondly, sclerotia may produce apothecia. Thirdly, they can germinate by producing conidiophores directly from sclerotia. To obtain conidia from hyphae on agar the mean optimum temperature must be around 17.3°c and the wavelength of irradiation between 375 and 400 nm, or 675 nm (which is less effective).

Aggressive chocolate spot occurring relatively early in the season can reduce yield by 50%. For this reason chocolate spot must be controlled. Cultural control of the disease is aimed at reducing development of the aggressive phase. Chemical control is widely practised (Parry, 1990). However, because of problems with fungicides resistance and the lack of effective resistance in the crop, new means of control chocolate spot are sought.

Chapter 2

MATERIALS AND METHODS

Figure 3 : British *Pharmacopoeia* distillation apparatus



1 DISTILLATION

Distillation, like solvent extraction and pressing, is one procedure for extracting essential oils from plants. With this method, the volatile compounds and compounds insoluble in water can be extracted. The essential oil components with boiling points up to 300°c can also be extracted from plants at ambient pressure and at a temperature below 100°c (Hornok, 1992).

The apparatus used to distil the dried material of hyssop and oregano was identical to that specified in the *European Pharmacopoeia* (Figure 3)(Lou & Zhi-cen, 1980).

Plant material was distilled for 2 h. An average of 1.35 ml of oil was obtained from the distillation of 80 g of dry hyssop flower tops and 1.5 l of distilled water. This yielded an average of 0.6 ml of oil . Higher yield of oil in the flowering parts of hyssop have been reported by Werker *et al.* (1985).

After distillation, the oil was stored in vials in a refrigerator at 2-6°c.

2 GAS-LIQUID-CHROMATOGRAPHY

Different oil samples were analysed by Gas-Liquid-Chromatography (GLC). These included hyssop flowering tops cultivated under cover and outside, hyssop seedlings, hyssop leaves and oregano. The analysis was performed using a United

Technologies Packard 439 Gas Chromatograph connected to a Hewlett Packard Integrator 3390 A.

The operating conditions were as follows :

Injector temperature	250 °c
Detector temperature	250 °c
Starting temperature	50 °c
Finishing temperature	200 °c
Programming rate	5 °c/min
Initial time held for	10 min
Final time held for	12 min
N flow rate	30 ml/min
H flow rate	50 ml/min
Air flow rate	300 ml/min
Sample size	0.2 ul
Split injection	1:100
Chart speed	0.5 cm/min

Between each injection the syringe was rinsed using ether. To determine the major components of the oils, the retention time for compounds which constitute greater than 3% of the oil composition were compared with the retention time of known standards.

3 EXPERIMENTS *IN VIVO*

3.1. Growth of plant material

3.1.1. Barley

Barley (*Hordeum vulgare* L. cv. Golden promise) was grown either in a heated ventilated glasshouse or in a growth room.

In the glasshouse the temperature was 18-22°c during the day and 10°c at night. In addition to natural daylight plants were grown under 400 W mercury vapour lamps to provide a 16 h photoperiod during the winter. In the growth room, the temperature was 22-24°c during the day and 15-16°c at night. A light intensity of 250 μmol m^{-2} s^{-1} was provided by fluorescent strip lighting for 16 hours per day. The plants were watered regularly without addition of nutrients. Thirteen seeds were sown per tray of Fisons Levington M3 compost. The extra plants were thinned out to leave 10 replicate plants per treatment.

3.1.2. Broad bean

Broad beans (*Vicia faba* L. cv. Three Fold White) were grown either in a glasshouse (described above) or in a growth cabinet, where the temperature was set at 13°c at night and 16°c during the 16 h photoperiod. The plants were watered regularly without addition of nutrients. Five seeds were sown per 15 cm diameter pot, in Fisons Levington M3 compost. The experiment was run with four replicates per treatment.

3.1.3. Apples

Seeds of apple (*Malus bitenfelder*) were stratified by placing them in cold storage (-10°c) for 14 weeks in trays containing

a mixture of 3 volumes of Fison's Livington compost to one volume of sand. After this time the trays were removed from cold storage and placed in a heated, ventilated glasshouse for the germination of the seeds. When the seedlings had 2 leaves, the seedlings were potted individually into one per 8 cm diameter pots containing Fisons Livington M3 compost. The apple plants were watered regularly without addition of nutrients.

3.2. Inoculation with the pathogen and maintenance of pathogen on host

3.2.1. Barley powdery mildew

The stock plants were grown as described above. Stock plants that were heavily infected with *Erysiphe graminis f.sp. hordei Marchal* were gently shaken above the plant that had to be inoculated. In an experiment, stock plants that had been inoculated at the same time were used for inoculation of experimental plant, thus ensure that conidia of a similar age were used. As far as possible, attempts were made to apply a similar inoculum load onto each experimental plant. Stock plants , inoculated experimental plants and controls were kept in different compartments of the glasshouse.

3.2.2. Broad bean rust

The stock plants were grown as described above. Uredospores of rust (*Uromyces viciae-fabae* (Pers) Schroet.) were collected in a petri dish using a fine-hair brush. They were added to distilled water to obtain a concentration of 40 000 spores/ml. This spore concentration was checked using the Improved Neubauer haemocytometer. The spore suspension was agitated with a magnetic stirrer for 5 minutes. Then 4 drops of Tween 20 were added to 100 ml of the spore suspension. All leaves were inoculated on stock plants while only the second pair of leaves was inoculated on experimental plants. Plants were inoculated by spraying leaves to run-off with the spore suspension using a disposable aerosol sprayer. The plants were then covered with a polyethylene bag for 48 h to increase the relative humidity required for infection.

3.2.3. Apple powdery mildew

The stock plants were grown as described above. The upper surface of stock plant leaves were brushed to remove mildew conidia. Experimental plants were inoculated by gently brushing the conidia onto the under surfaces of the leaves since new infections are known to occur on the under surfaces of apple leaves (Burchill, 1978)

3.3. Application of essential oils to plants

The preparation of the oil suspensions and their application to plants were similar for barley, broad bean and apple.
Each suspension was prepared by adding the required quantity of oil to distilled water to obtain the desired concentration. Tween 20 was used as a surfactant : 8 drops/100ml were added to each treatment and to the control.
Suspensions of crystallised components of hyssop or oregano oil were prepared by dissolving 1 g of an individual component in 1 ml ether. Desired concentrations were obtained by adding the appropriate volume of water containing Tween 20. Controls containing different amounts of ether concentration were used in the experiment.
The oil suspensions were applied using a disposable aerosol sprayer onto all leaves on barley and apple seedlings and to the upper surfaces of the second pair of leaves for broad bean. The suspension was carefully mixed, before and during the application and was sprayed to run-off.

3.4. Disease assessment

3.4.1. Barley powdery mildew

The percentage area of the second leaf that was covered with powdery mildew was assessed using a visual key (MAFF, 1976) on the 6th day after inoculation.

3.4.2. Broad bean rust

On the 17th day after the inoculation, infection intensity was assessed and expressed as number of rust pustules/cm^2 of leaf. The area of each of the second pair of leaves was determined using a leaf area meter. All pustules on the leaf were counted.

3.4.3. Apple mildew

The percentage of the area of the third leaf that was affected by powdery mildew was assessed using a visual key, on the 13th, 15th, and 17th days after inoculation.

4 EXPERIMENTS *IN VITRO*

4.1. Mycelial growth

Pyrenophora avenae (Ito and Kuribayashi apud Ito, culture no 296817) and *Pyricularia oryzae* were grown on potato dextrose agar (PDA) in 8cm diameter Petri dishes.
For inoculation, mycelium was taken from the peripheral edge of stock cultures. Plugs of mycelium were removed with a 10

mm cork borer and then inverted and placed in the centre of each Petri dish, the mycelium facing the medium.

Essential oils are considered to be sterile (Zaika, 1988) so they were added to the 150 ml flask of PDA just before using it (temperature between 45°c and 50°c) to reach the concentration of 0.01%, 0.05%, 0.1%, 0.4% for hyssop oil and 0.005%, 0.01%, 0.02% for oregano oil.

Each plate was filled with 20 ml of medium containing the essential oil that had been thoroughly mixed. Three replicates were used for each treatment.

For the components of the oils, the concentration used was calculated by using the efficient concentration of the oil they come from and their rate in this oil (that has been determined by GLC).

Of the individual components of the essential oil used in this study, ß-pinene and isopinocampheol are liquid, while pinocamphone, l-bornyl acetate, carvacrol, thymol are crystals. A precaution was taken when using the crystallised compounds : they were kept in the refrigerator until the very last moment and then weighed and immediately added to the medium. This method was necessary with compounds that become very sticky when the temperature rises slightly. All the compounds were dissolved by adding them to the warm medium that was kept stirred with a magnetic stirrer for several minutes. The media were stirred until the added compounds has dissolved but because of the volatile nature of most of the compounds, the stirring was not prolonged.

The effects of volatile components of oils were tested using

three compartment Petri dishes. Two compartments containing PDA : one compartment was inoculated with *P.avenae* and the second one with *P.oryzae*. The third compartment was filled with 7 ml of PDA containing various concentrations of essential oil. In this way, the fungi were not in direct contact with the oil (as in the previously described method) and only the vapours could affect the growth of the mycelium. In order to test whether this vapour of the essential oil exerted fungistatic or fungicidal effect on mycelial growth, the following experiment was performed. PDA was poured into 2 of the 3 compartments of a 3-compartment Petri dish and these were inoculated with the 2 fungi (*P.avenae* and *P.oryzae*). On the second day, PDA containing varying concentrations of oil was added to the third compartment. On the fourth day, a plug was taken from the peripheral edge of the colony, if there had been no growth, and this was inoculated onto a PDA plate. If the mycelium grew, the effect of the oil was fungistatic. On the other hand, if the mycelium did not grow the effect of the oil was fungicidal.

In all the experiments, fungal growth was assessed on the second, fourth and sixth day for *P.avenae* and on the third, sixth and ninth day for *P.oryzae*, which grew more slowly. The colony radius was measured in mm, excluding the plug. An average was taken of the three measurements made on each Petri dish.

4.2. Germination test

4.2.1. Maintenance of the fungi

Botrytis fabae can be grown easily *in vitro*. The medium used to induce *B. fabae* spore production was as described by Leach & Moore (1966). The concentration of inorganic salts used in this medium was high. About 200g of unmacerated bean leaf material was autoclaved for 20 min in 1 l of distilled water, after which 20 g agar, 20 g sodium nitrate and 160 g sucrose were added. The stock culture of spores was prepared in aseptic conditions : a suspension of spores was made by scraping the surface of the culture in the presence of sterile distilled water. This suspension was used to inoculate the Leach & Moore medium in petri dishes. The inoculated Petri dish were placed in an incubator (with a 16 h photoperiod) at 20°c with near UV. radiation. In the following fortnight, spores appeared and could be used to set up the experiment.

For *Uromyces viciae-fabae*, which could not be grown *in vitro*, the spores where collected from stock plants as described in a previous section.

4.2.2. Studies of germination on glass slides

A spore suspension of *B. fabae*, prepared as described above,

was passed through four layers of muslin to remove mycelial debris (Doherty & Preece, 1978). Distilled water was added to the suspension to adjust the concentration to 10^5 spores/ml, and was checked with an Improved Neubauer Haemocytometer. The slides were set up using De Cal's method (1988), but this method was subsequently improved to respond to the exigencies of the essential oils. The oil suspension was prepared by adding oil to distilled water to obtain concentrations of 0%, 0.1%, and 0.4% for hyssop oil and 0%, 0.01% and 0.04% for oregano oil. Then a drop of Tween 20 was added to the solution which was shaken on a rotamixer. A glass slide was laid on a glass rod on moist filter paper in a petri dish. Onto this slide, a 15 µl droplet of spore suspension was mixed with a 30 µl droplet of oil suspension. Five replicates were set up for each treatment. All petri dishes were left for 20 h, on a tray in a polyethylene bag at 20°c. Thus, the slides were kept moist. After 20 h, each slide was stained with lactophenol cotton blue to stop further germ tube formation and growth (Bell & Daly, 1962). In the second set of experiments and thereafter, Tween 20 was added to the lactophenol cotton blue to facilitate the action of lactophenol, since the oil had formed a barrier around the spores. The slides were then examined under a microscope at *400 magnification. The percentage germination was assessed by counting the number of germinated spores out of 100 spores.

Spores of *Uromyces viciae fabae* were collected as indicated above. Because these spores produce self inhibitors when they

are clumped, they need special treatment to release the self-inhibitors. Around 50 mg of rust spores were floated on 100 ml sterile distilled water as a monosporic layer at 4°c for 16 h. They were then washed with distilled water and suspended in distilled water at a concentration of 50000 spores/ml, checked using an Improved neubauer Haemocytometer. The following stages were the same as described above for *B. fabae*.

The slides were examined under a microscope at *100 magnification. The percentage germination was assessed by counting the number of germinated spores from 100 spores.

5 STATISTICAL ANALYSIS

Data were analysed using the statistical software Package Minitab, Release 7.1 Standard Version (Minitab, Inc. 1989). Means and standard error of the mean were calculated using the 'describe' command. The regression equation was obtained by using the 'regression' command. Significance was determined using Student's t-test and one-way analysis of variance for the *in vitro* experiments and two-way analysis of variance for the *in vivo* experiments. Data from the unbalanced experiment examining the effect of the components of essential oils on barley powdery mildew, were analysed using Genstat.

In the expression of the results, significant differences are shown as P=0.05 *, P=0.01 **, P=0.001 ***.

Chapter 3

RESULTS

1 DISTILLATION OF ESSENTIAL OILS AND ANALYSIS OF COMPONENTS BY GAS LIQUID CHROMATOGRAPHY

1.1 Composition of hyssop oil

Several samples of hyssop oil were analysed by GLC . These samples included flowers of hyssop grown under plastic and outside, and from hyssop seedlings and hyssop leaves collected at the second cut.

The yield of oil extracted from the dried plant depended upon the origin of the plant material : the flowers of hyssop grown under plastic yielded 1.71% oil, hyssop grown outside yielded 1.63%, seedlings yielded 1.0% and leaves 0.84%. Some of the components of the oil could not be determined precisely. Nevertheless, the oil from the different samples contained :

- ß-pinene
- isopinocampheol or isopinocamphene or pinocamphone (these components could not be separated satisfactorily by GLC)
- pinocamphene

Some of the components seemed to be specific to certain types of sample *e.g.* bornyl acetate was detected in flowers of hyssop grown outside and hyssop seedlings from the second cut, borneol was detected in hyssop leaves from the second cut (Table 3 ; Figure 4).

Figure 4 : Part of the monoterpenoid synthetic pathway : chemical formulae of components of hyssop and oregano oil

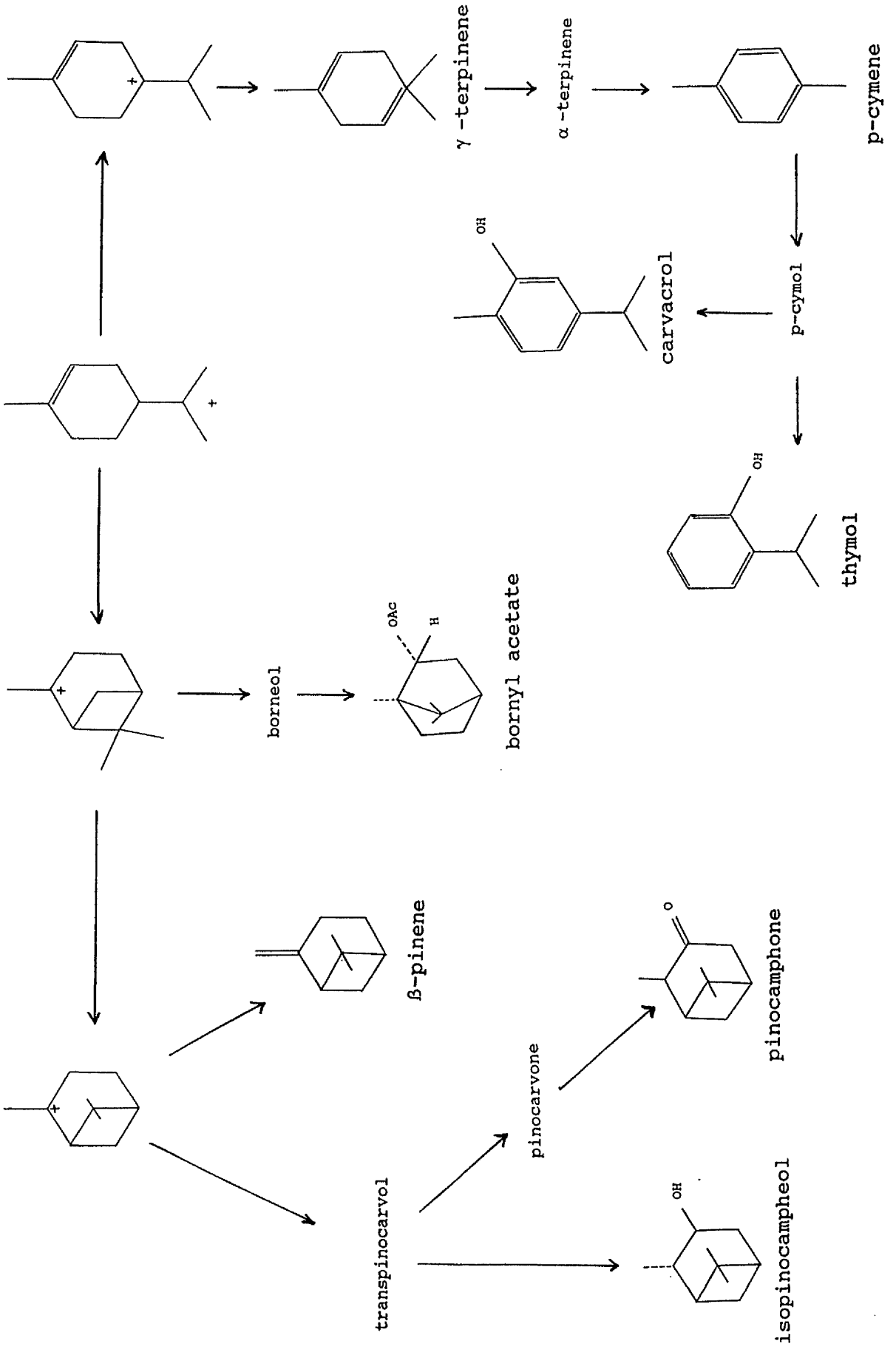


Table 3 : Composition of hyssop oil analysed by gas liquid chromatography

components	percentage of oil			
	hyssop flowers		hyssop seedling	hyssop leaves
	plastic	outside	2nd cut	2nd cut
ß-pinene	7.96	8.98	7.18	4.01
isopinocampheol or isopinocamphene or pinocamphone	55.82	47.23	57.67	53.29
pinocamphene	22.14	18.05	20.49	11.09
bornyl acetate		4.30	3.18	
borneol				4.19

Only the components which represented more than 3% of the oil were taken into account

Table 4: Composition of oregano oil analysed by gas liquid chromatography

components	percentage of the oil
γ -terpinene	6.20
p -cymene	2.88
thymol or carvacrol	71.70

one component (5.26%) has not been determined

Only the components which represented more than 3% of the oil were taken in account

1.2. Composition of oregano oil

A sample of oregano oil was analysed by GLC. Distillation of oregano plants yielded about 1% oil when grown under plastic and 0.6% when grown outside. This sample of oil contained γ-terpinene, p-cymene (two precursors of thymol and carvacrol). A third peak on the GLC trace could not been identified precisely : it was thymol or carvacrol, or a mixture of both of them (Table 4 ; Figure 4).

2 EXPERIMENTS *IN VIVO*

Experiments were planned differently for barley and broad beans and for the apple seedlings. In barley and broad bean experiments, oregano and hyssop oil were sprayed in one trial before inoculation and in an other trial after inoculation. In apple experiments, hyssop oil was sprayed before and after inoculation in the same experiments. This experiment was repeated with oregano oil.

2.1 Effect of essential oils on infection of barley with powdery mildew

2.1.1. Pre-inoculation treatment with hyssop oil

In an initial experiment, suspensions between 0.1% and 3.2%

Plate 6 : Phytotoxicity of oregano oil on barley

Plate 7 : Phytotoxicity of hyssop oil (0.16%) on broad bean leaves

Plate 8 : Phytotoxicity of hyssop oil on a broad bean leaf

Plate 6

Plate 7

Plate 8

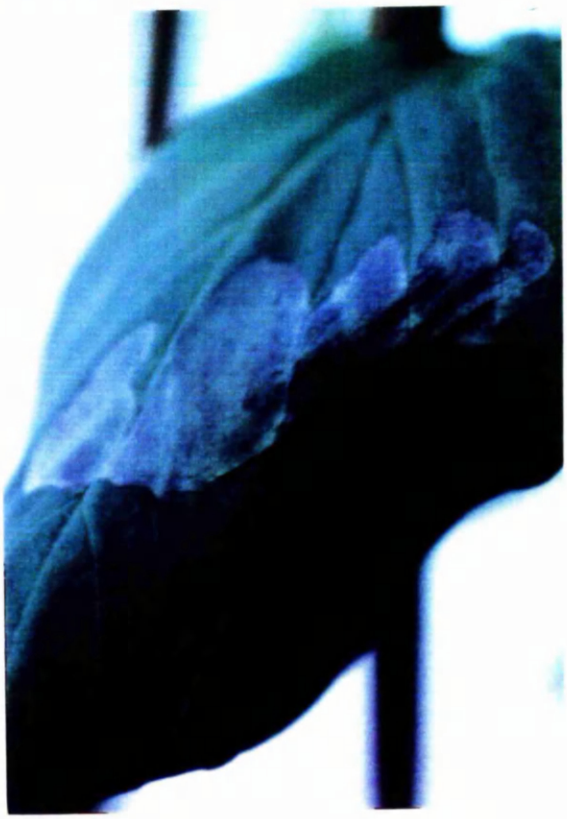


Figure 5 : Percentage leaf area infected with barley powdery mildew, following treatment with various concentrations of hyssop oil, 1, 2 or 3 days before inoculation. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Significant differences from the control are shown as *. Standard error and statistics data are shown in Table 2 in Appendix 2.

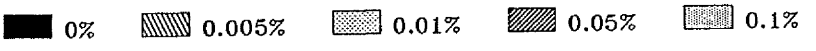


Figure 6 : Percentage leaf area infected with barley powdery mildew, following treatment with various concentrations of oregano oil, 1, 2 or 3 days before inoculation. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Standard error and statistics data are shown in Table 3 in Appendix 2.

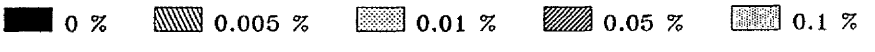


Figure 5

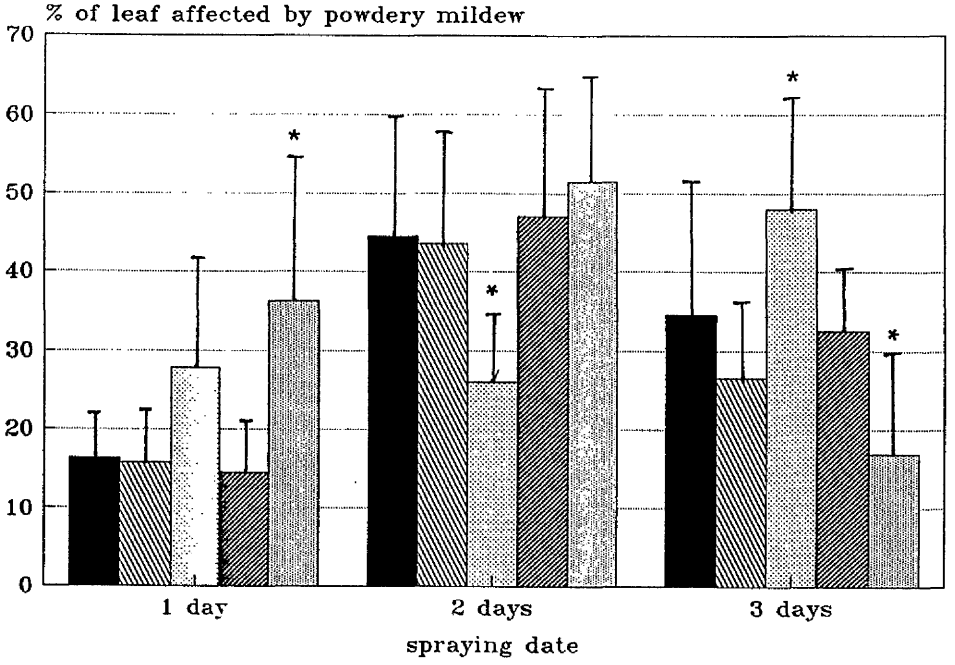
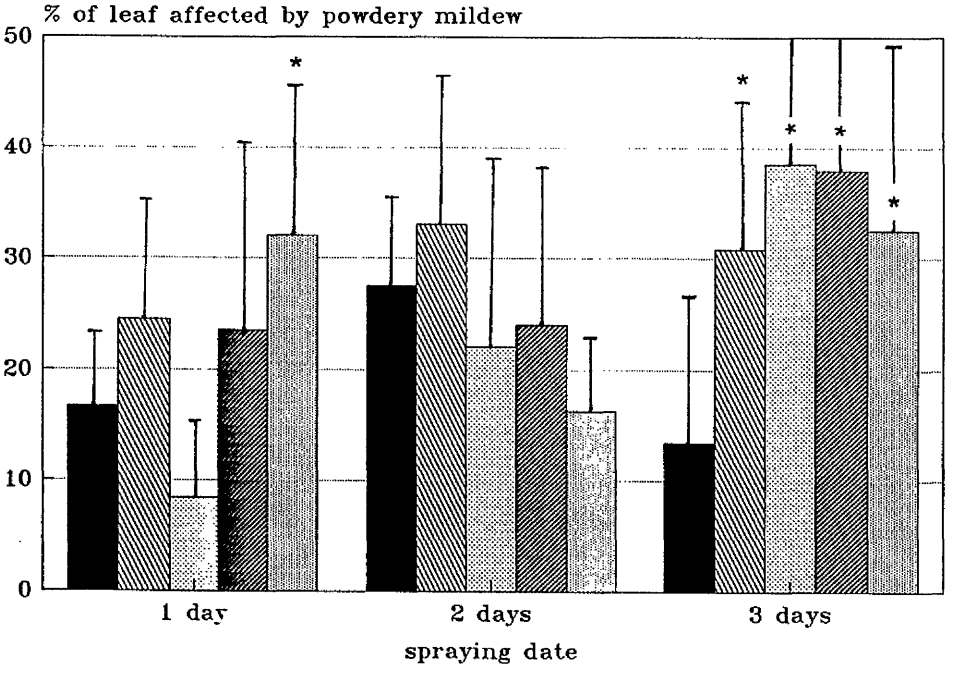


Figure 6



of hyssop oil had been used to treat barley plants. Strong phytotoxicity had been noticed in the 0.2% treatment, the day following application (Plate 6). For this reason, a range of lower concentrations of oil was used.

There was considerable variation in mildew infection between treatments *e.g.* the control for the different spraying times (Figure 5 ; Statistics shown in Table 2 in Appendix 2). When the oil was applied one day before inoculation, infection was increased by 0.1% and 0.01% concentration. In contrast, when applied 2 days before inoculation, the 0.1% oil concentration had a smaller effect on infection, while treatment with 0.01% oil actually reduced mildew infection. When those treatments were applied three days before inoculation, all treatments except 0.01% reduced infection.

2.1.2. Pre-inoculation treatment with oregano oil

In an initial experiment, suspensions between 0.1% and 3.2% of oregano oil had been used to treat barley plants. Strong phytotoxicity had been observed at the lowest concentration (0.1%), the day following treatment. For this reason, a range of lower concentrations was used.

Some variation was observed in the level of mildew infection in the different controls (Figure 6 ; Statistics shown in Table 3 in Appendix 2). When the oil was sprayed one day before inoculation, 0.01% oregano oil reduced infection while all the other concentrations, and especially 0.1% oregano

Figure 7 : Percentage leaf area infected with barley powdery mildew, following treatment with various concentrations of hyssop oil, 1, 2 or 3 days after inoculation. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Significant differences from the control are shown as *. Standard error and statistics data are shown in Table 4 in Appendix 2.

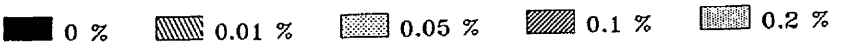


Figure 8 : Percentage leaf area infected with barley powdery mildew, following treatment with various concentrations of oregano oil, 1, 2 or 3 days after inoculation. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Standard error and statistics data are shown in Table 5 in Appendix 2.

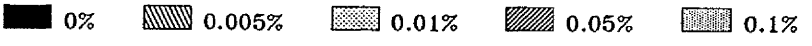


Figure 7

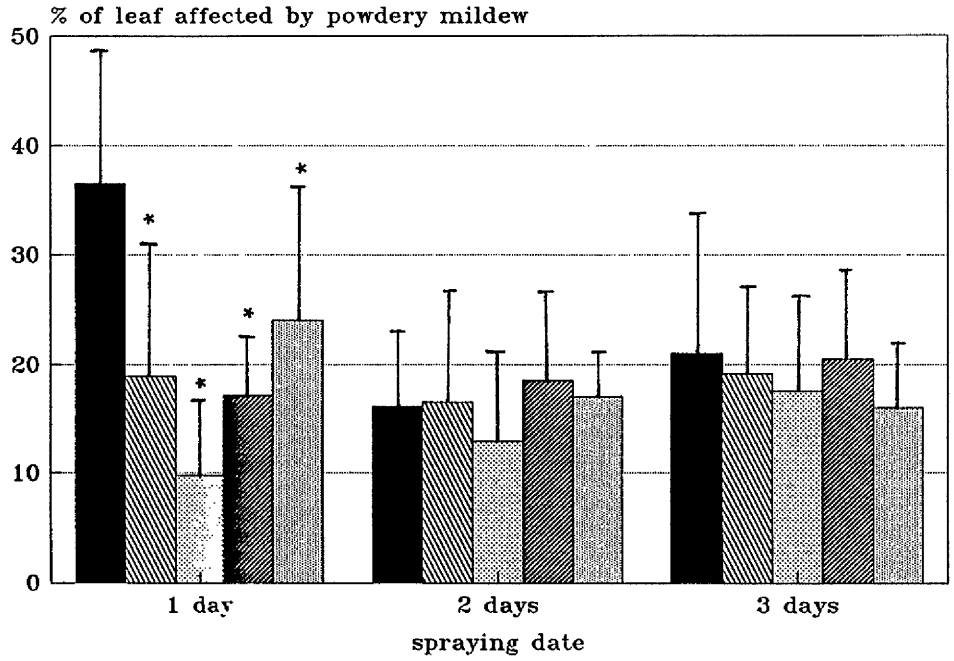
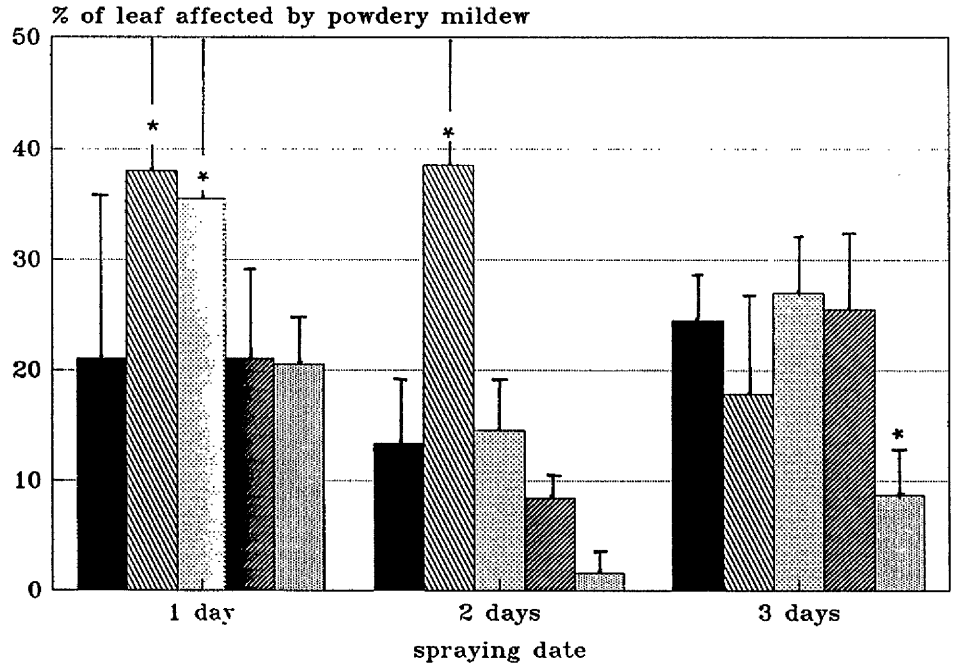


Figure 8



oil, increased mildew infection. When the oil was sprayed 2 days before inoculation, the concentration of 0.005% oregano oil increased infection, while all the other concentrations reduced it, especially treatment with 0.1% oregano oil. When the oil was sprayed 3 days before inoculation, all the treatments increased powdery mildew infection substantially.

2.1.3. Post-inoculation treatment with hyssop oil

Some variation was observed in mildew infection in the different controls (Figure 7 ; Statistics shown in Table 4 in Appendix 2). Thus, although infection ranged from 16-21% for the 2 and 3 days after inoculation controls, mildew infection in the 1 day post-inoculation control was 36%. When the oil was sprayed 1 day after inoculation, the level of infection in each treatment was much lower than the control, especially the concentration of 0.05% hyssop oil. When the oil was sprayed 2 days after inoculation, there was little effect on mildew infection, compared to the control. When the oil was sprayed 3 days after inoculation all treatments reduced infection, albeit to a small extent.

2.1.4. Post-inoculation treatment with oregano oil

When the oil was sprayed 1 day after inoculation, the concentrations of 0.005% and 0.01% oregano oil increased

infection substantially (Figure 8 ; Statistics shown in Table 5 in Appendix 2). When the oil was sprayed 2 days after inoculation, there was a large reduction in infection following treatment with 0.1% oil and a large increase using 0.01% oil. When the oil was sprayed 3 days after inoculation, the concentrations of 0.05% and notably 0.1% oregano oil reduced mildew infection.

2.2. Effects of essential oils on infection of broad bean with rust

2.2.1. Pre-inoculation treatment with hyssop oil

In an initial experiment, suspensions of hyssop oil at various concentrations between 0.1% and 3.2% had been used. No phytotoxicity had been noticed on the day following treatment, but on the 15th day after inoculation phytotoxicity was visible on some plants (Plates 7 & 8).
When the oil was sprayed one day before inoculation, 0.05% hyssop oil reduced infection while 0.2% oil increased infection considerably (Figure 9 ; Statistics shown in Table 6 in Appendix 2). When the oil was applied 2 days before inoculation, all treatments reduced infection, especially treatment with 0.01% oil. When the oil was sprayed 3 days before inoculation, 0.05% and 0.2% oregano oil reduced mildew infection while 0.1% oil increased infection.

Figure 9 : Number of rust pustules per cm^2 of broad bean leaves, following treatment with various concentrations of hyssop oil, 1, 2 or 3 days before inoculation.
Values are the means of 4 replicates. Significant differences from the control are shown as *. Standard error and statistics data are shown in Table 6 in Appendix 2.

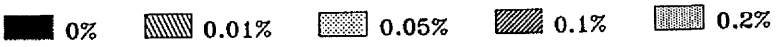


Figure 10 : Number of rust pustules per cm^2 of broad bean leaf, following treatment with various concentrations of oregano oil, 1, 2 or 3 days before inoculation.
Values are the means of 4 replicates. Significant differences from the control are shown as *. Standard error and statistics data are shown in Table 7 in Appendix 2.

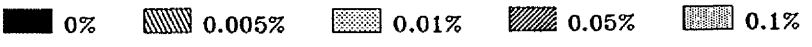


Figure 9

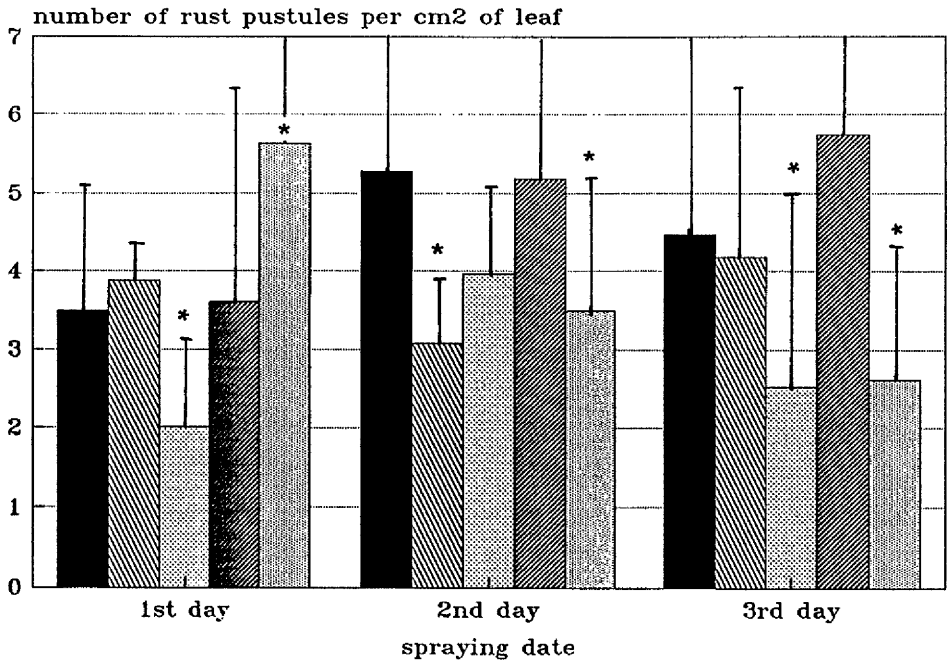
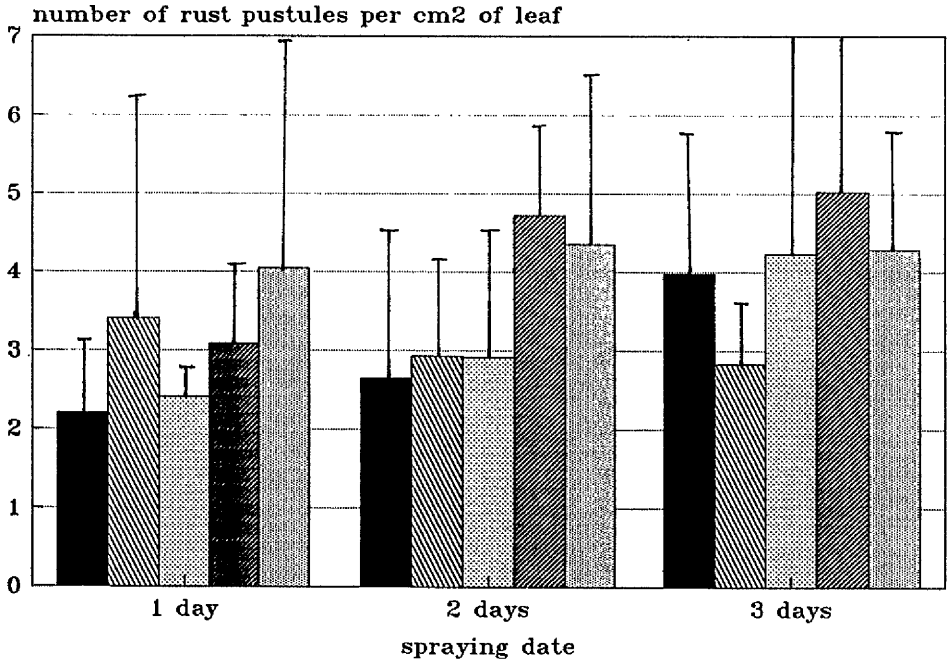


Figure 10



2.2.2. Pre-inoculation treatment with oregano oil

In an initial experiment, suspensions of hyssop oil at various concentrations between 0.1% and 3.2% had been used. Phytotoxicity was noticed following treatment with 0.2% oil on the second day after spraying.

When the oil was sprayed 1 day before inoculation, rust infection was higher in all treatments than the control (Figure 10 ; Statistics shown in Table 7 in Appendix 2). The same was true when the oil was sprayed two days before inoculation. Here, infection was greatly increased following applications of 0.05% and 0.1% oil. When the oil was sprayed 3 days before inoculation, 0.01% and 0.1% oregano oil reduced infection, while 0.05% oil increased infection.

2.2.3. Post-inoculation treatment with hyssop oil

There was considerable variation in rust infection in the different controls in this experiment (Figure 11 ; Statistics shown in Table 8 in Appendix 2). When the oil was applied one day post-inoculation, rust infection was reduced, especially following treatments with 0.1% oil. Application of hyssop oil 2 days after inoculation reduced rust infection, although here, the reduction caused by application of 0.1% oil was not as great as observed 1 day post-inoculation. When the oil was sprayed 3 days after inoculation, there was little difference between treatment and the control.

Figure 11 : Number of rust pustules per cm^2 of broad bean leaves, following treatment with various concentrations of hyssop oil, 1, 2 or 3 days after inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 8 in Appendix 2.

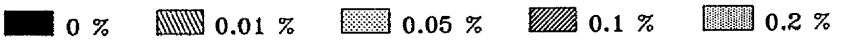


Figure 12 : Number of rust pustules per cm^2 of broad bean leaves, following treatment with various concentrations of oregano oil, 1, 2 or 3 days after inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 9 in Appendix 2.

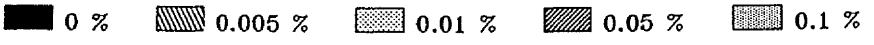


Figure 11

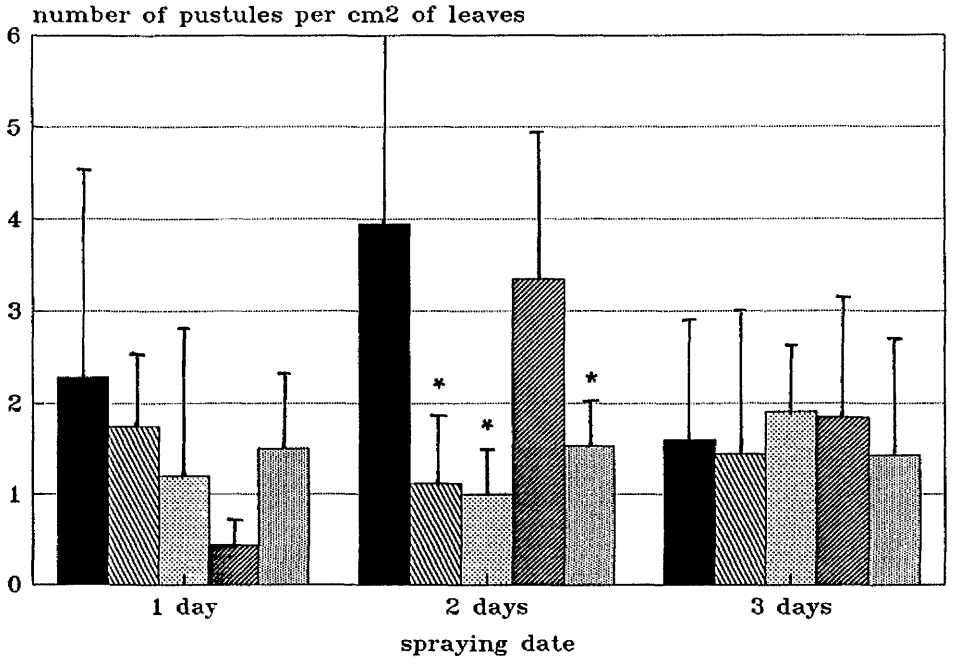
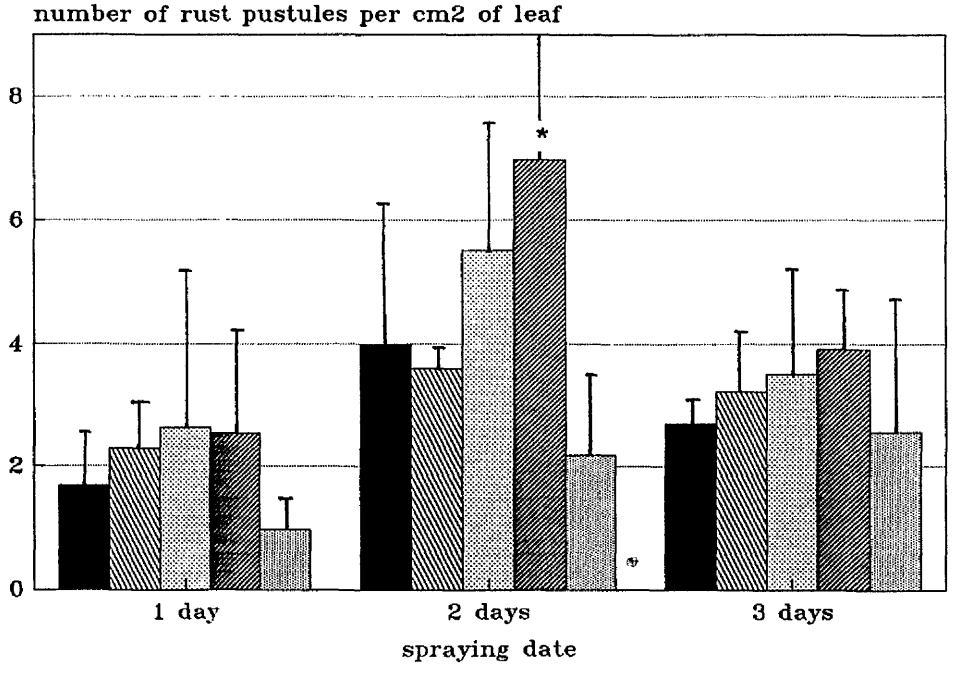


Figure 12



2.2.4. Post-inoculation treatment with oregano oil

Some variation was observed in rust infection on the different controls (Figure 12 ; Statistics shown in Table 9 in Appendix 2). When the oil was sprayed 1 day after inoculation, it increased the level of disease except at the concentration of 0.1%, which reduced mildew. When the oil was sprayed 2 days after inoculation, the effect varied greatly : concentrations of 0.01% and 0.05% increased infection, while 0.005% and especially 0.1% oil reduced infection. When the oil was applied 3 days after inoculation, it increased rust infection, except at the concentration of 0.1% oregano oil.

2.3. Effect of oils on infection of apple seedlings with powdery mildew

2.3.1. Pre-inoculation treatment with hyssop oil

Phytotoxicity was observed on plant treated with 0.32% hyssop oil. No symptoms or phytotoxicity were observed on plant treated with lower concentration of the oil. The effect of hyssop oil on mildew infection were variable (Figure 13 ; Statistics shown in Table 10 in Appendix 2). Thus, although by 13 days after inoculation most oil concentration had mildew infection on apple seedlings, by the 15^{th} and 17^{th} days after inoculation, some of these reduction had disappeared. In any case, none of the changes in infection

Figure 13 : Percentage leaf area infected with apple powdery mildew, following treatment with various concentrations of hyssop oil, 2 days before inoculation. Infection was assessed 13, 15 and 17 days after the inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 10 in Appendix 2.

Figure 14 : Percentage leaf area infected with apple powdery mildew, following treatment with various concentrations of hyssop oil, 2 days after inoculation. Infection was assessed 13, 15 and 17 days after the inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 10 in Appendix 2.

Figure 13

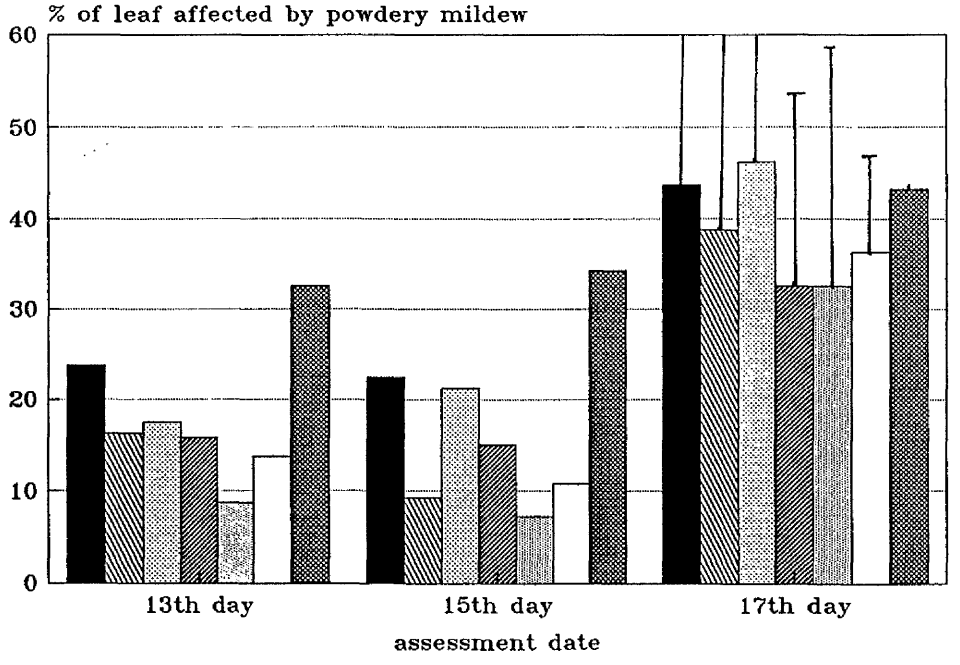
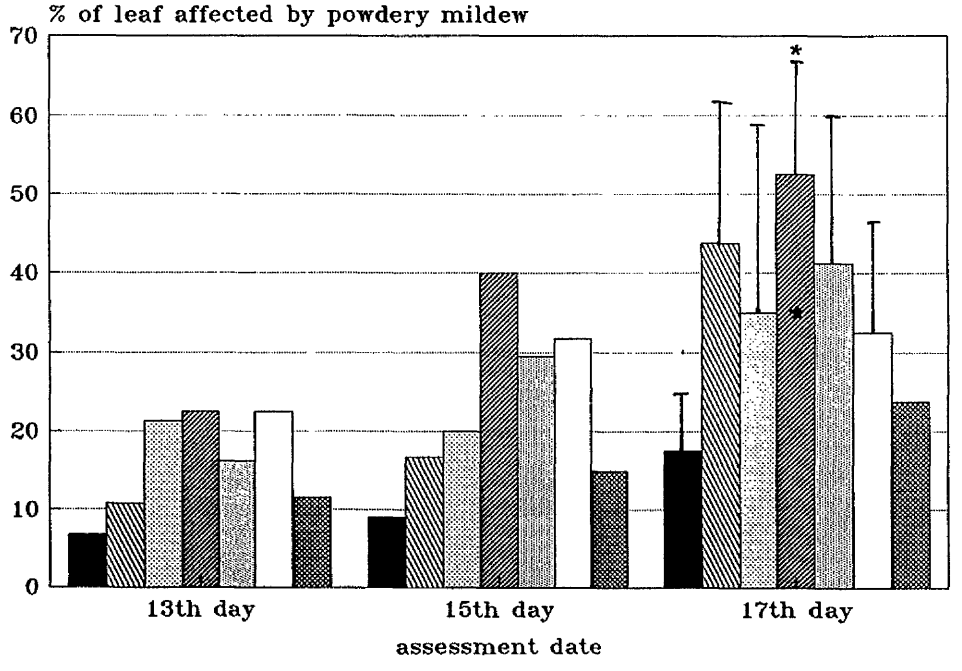


Figure 14



observed were significantly different from the control.

2.3.2 Post-inoculation treatment with hyssop oil

Phytotoxicity was observed on plants treated with the suspension of 0.32% hyssop oil. Plants in all treatments were more infected than controls. Maximum infection was observed using the 0.04% concentration of hyssop oil (Figure 14 ; Statistics shown in Table 10 in Appendix 2). However, only the 0.01%, 0.04% and 0.08% treatment were significantly different from the control.

2.3.3. Summary of the effects of hyssop oil

When plants were sprayed with hyssop oil 2 days before inoculation, they were less infected than the control. In contrast, when the plants were sprayed 2 days after inoculation, they were more mildewed than the control.

2.3.4. Pre-inoculation treatment with oregano oil

Phytotoxicity was observed on 2 of the 4 replicates treated with a suspension of 0.2% oregano oil. 13 days after inoculation, most of the treatments showed the same level of infection as the control, except for treatment with 0.05% oil where the level of infection was double than the control. By

Figure 15 : Percentage leaf area infected with apple powdery mildew, following treatment with various concentrations of oregano oil, 2 days before inoculation. Infection was assessed 13, 15 and 17 days after the inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 11 in Appendix 2.

Figure 16 : Percentage leaf area infected with apple powdery mildew, following treatment with various concentrations of oregano oil, 2 days after inoculation. Infection was assessed 13, 15 and 17 days after the inoculation.
Values are the means of 4 replicates. Standard error and statistics data are shown in Table 10 in Appendix 2.

Figure 15

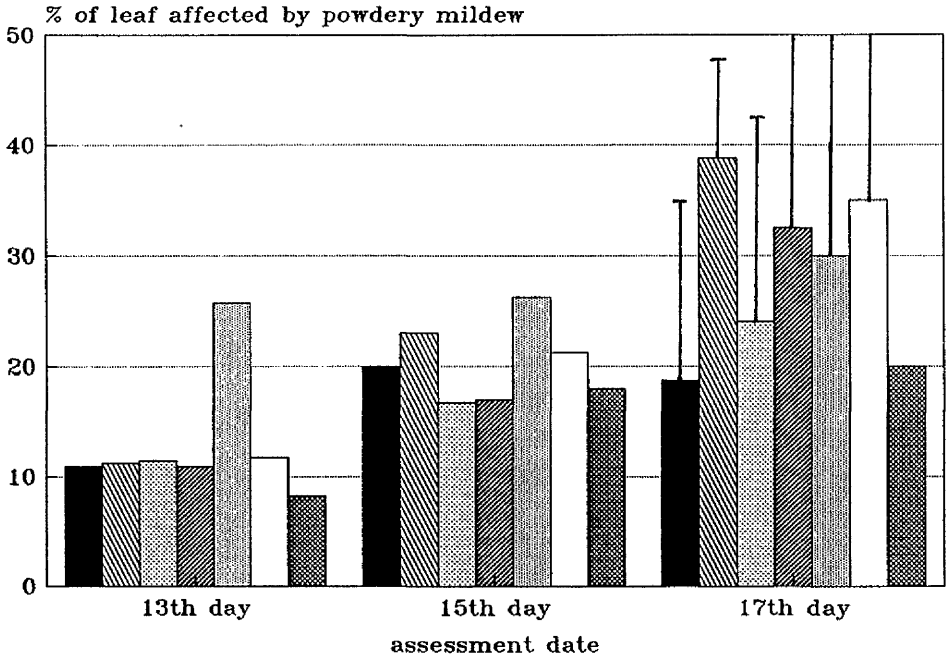
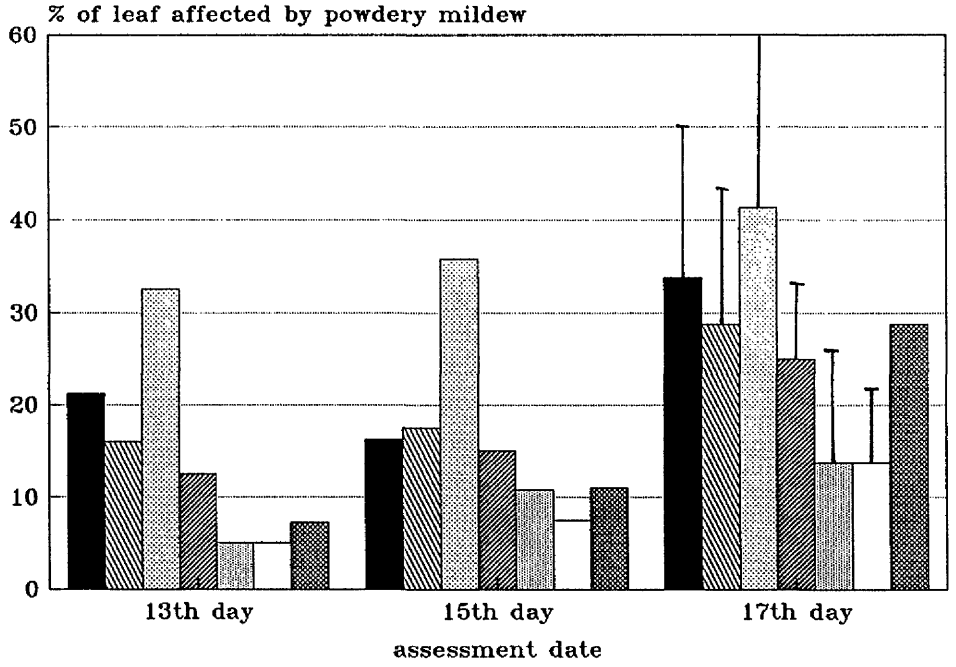


Figure 16



15 days after inoculation, the treatments with 0.005%, 0.05% and 0.1% oregano oil were higher than the control. Treatment with the other concentrations reduced mildew infection compared to the control. By 17 days after inoculation, mildew infection in all treatments was much higher than the control (Figure 15 ; Statistics shown in Table 11 in Appendix 2). None of the treatments were significantly different from the control.

2.3.5. Post-inoculation treatment with oregano oil

Phytotoxicity was noticed on 2 of the 4 replicates treated with the suspension of 0.2% oregano oil. Mildew infection following treatment with 0.01% concentration of oil was always greater than the control. However, all of the other concentrations reduced powdery mildew infection, especially the 0.05% and 0.1% concentration of oregano oil (Figure 16 ; Statistics shown in Table 11 in Appendix 2). Although these reduction in mildew infection were observed on the 15^{th} and 17^{th} days after inoculation, on the 15^{th} day after inoculation, mildew infection of plants which received 0.005% and 0.02% oil was near control values. Only the reduction in mildew infection resulting from treatment with 0.05% and 0.1% oil were significantly different from the control.

Figure 17 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of hyssop oil 1 day before or 1 day after inoculation. Treatments with isopinocampheol (0.01%, 0.05% and 0.2%), l-bornyl acetate (0.002%), -pinene(0.008%) were compared to a control not containing ether. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Statistics data are shown in Table 12 in Appendix 3.

- control
- iso LC : isopinocampheol at a low concentration (0.01%)
- iso NC : isopinocampheol at a natural concentration (0.05%)
- iso HC : isopinocampheol at a high concentration (0.2%)
- lBA LC : l-bornyl acetate at a low concentration (0.002%)
- BP : ß-pinene

Figure 18 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of hyssop oil 1 day before or 1 day after inoculation. Treatments with l-bornyl acetate (0.004% and 0.02%), pinocamphone (0.01%), isopinocampheol (0.05%) + l-bornyl acetate (0.04%) were compared to a control containing 5% ether. Infection was assessed 6 days after inoculation. Values are the means of 10 replicates. Statistics data are shown in Table 12 in Appendix 3.

- control 5% ether
- iso + lBA : isopinocampheol at a natural concentration (0.05%) + l-bornyl acetate at a natural concentration (0.04%)
- lBA NC : l-bornyl acetate at a natural concentration (0.04%)
- lBA HC : l-bornyl acetate at a high concentration (0.02%)
- pino LC : pinocamphone at a low concentration (0.01%)

Figure 19 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of hyssop oil 1 day before or 1 day after inoculation. Treatments with isopinocampheol (0.05%) + pinocamphone (0.05%), pinocamphone (0.05% and 0.2%) were compared to a control containing 25% ether. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Statistics data are shown in Table 12 in Appendix 3.

- control 25% ether
- iso + pino : isopinocampheol and pinocamphone at a natural concentration (0.05%)
- pino NC : pinocamphone at a natural concentration (0.05%)
- pino HC : pinocamphone at a high concentration (0.2%)

Figure 17

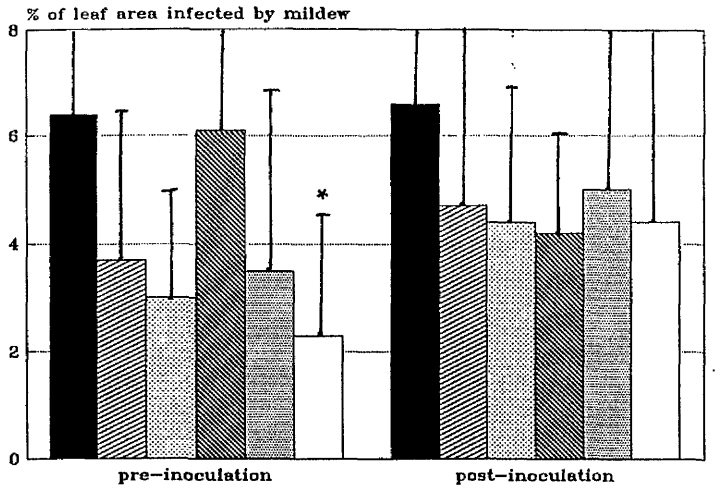


Figure 18

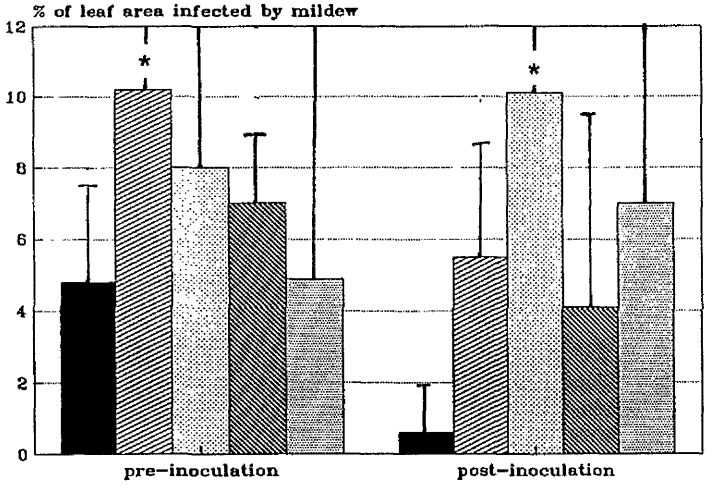
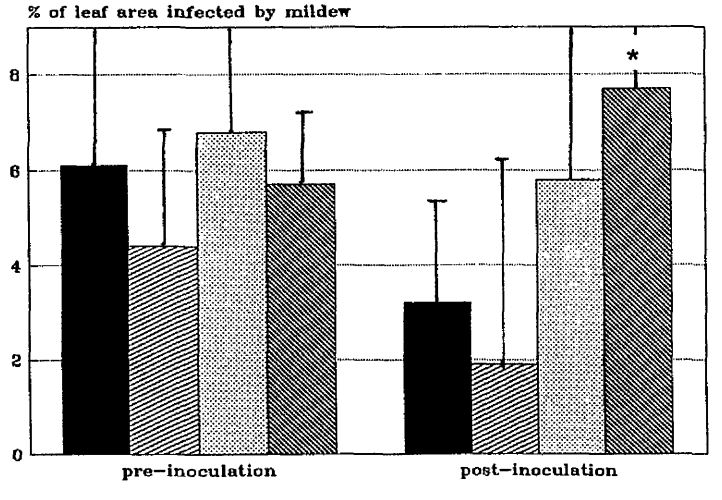


Figure 19



2.3.6. Summary of the effects of oregano oil

When plants were sprayed with oregano oil 2 days before inoculation, mildew infection was increased compared to control. On the other hand, when the plants were sprayed 2 days after inoculation, the treated plants were less infected, in most cases, than the control.

2.4. Effect of the components of hyssop oil on barley powdery mildew

All the treatments, when compared to the control (without ether), were less covered in mildew, although only the treatment with ß-pinene reduced infection significantly. In contrast, all the treatments, when compared to the control containing 5% ether, were significantly more covered in mildew. When compared to the control containing 25% ether, treatment with pinocamphone at a high and a low concentration were more heavily infected by powdery mildew, especially following post-inoculation treatment, while treatment with isopinocampheol + pinocamphone reduced powdery mildew infection. Interestingly, addition of effect to the control reduced mildew infection, especially in post-inoculation treatment. (Figure 17, 18, 19 ; Statistics shown in Table 12 in Appendix 3)

Figure 20 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of oregano oil 1 day before or 1 day after inoculation. Treatments with carvacrol (0.36%), thymol (0.36%), p-cymene (0.002%) were compared to a control containing 0.36% ether. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Statistics data are shown in Table 13 in Appendix 3.

- control 0.36% ether
- thymol LC : thymol at a low concentration (0.36%)
- carvacrol LC : carvacrol at a low concentration (0.36%)
- p-cymene

Figure 21 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of oregano oil 1 day before or 1 day after inoculation. Treatments with carvacrol (0.72%), thymol (0.72%), thymol (0.36%) + carvacrol (0.36%) were compared to a control containing 0.72% ether. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Statistics data are shown in Table 13 in Appendix 3.

- control 0.72% ether
- thymol NC : thymol at a natural concentration (0.72%)
- carvacrol NC : carvacrol at a natural concentration (0.72%)
- thy + car : thymol and carvacrol

Figure 22 : Percentage leaf area infected with barley powdery mildew, following treatment with various components of oregano oil 1 day before or 1 day after inoculation. Treatments with carvacrol (1.5%), thymol (1.5%) were compared to a control containing 1.5% ether. Infection was assessed 6 days after inoculation.
Values are the means of 10 replicates. Statistics data are shown in Table 13 in Appendix 3.

- control 1.5% ether
- thymol HC : thymol at a high concentration (1.5%)
- carvacrol HC : carvacrol at a high concentration (1.5%)

Figure 20

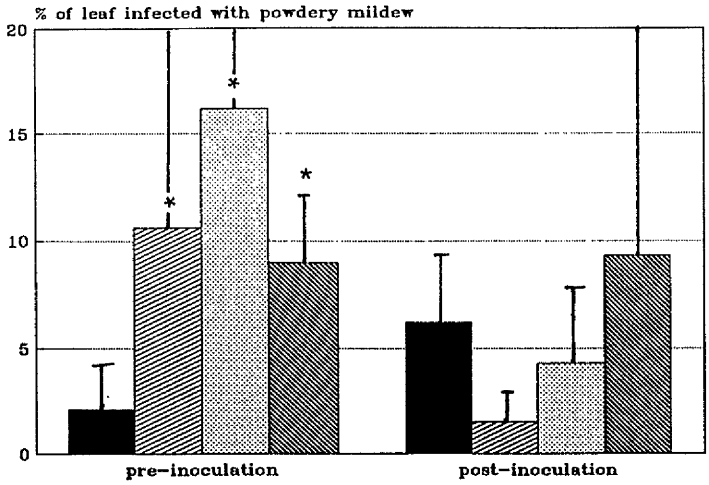


Figure 21

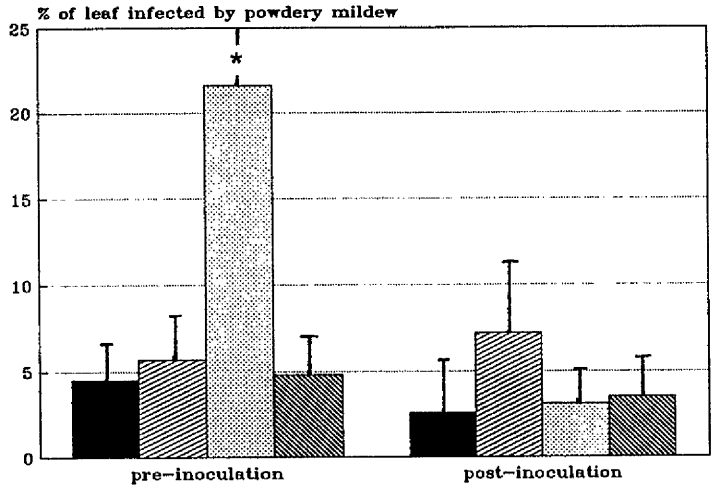
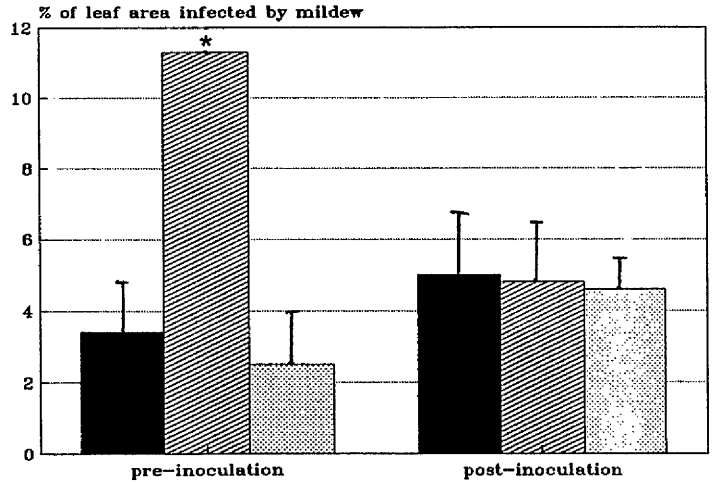


Figure 22



2.5. Effect of the components of oregano oil on barley powdery mildew

Controls containing ether were less infected with mildew than controls without ether. These reductions in infection were significant. In contrast, none of the treatments has a significant effect on mildew infection when compared to the appropriate control, although some treatments *e.g.* carvacrol at a low and a natural endogenous concentration increased infection, when others *e.g.* thymol at a low concentration reduced mildew infection (Figure 20, 21, 22 ; Statistics shown in Table 13 Appendix 3)

3 EXPERIMENTS *IN VITRO*

3.1. Effect of essential oils on germination of spores of *Botrytis fabae* and *Uromyces viciae-fabae*

3.1.1 Effect of hyssop oil on germination of spores of *Botrytis fabae*

There was a small reduction in the *in vitro* germination of *B. fabae* spores using hyssop oil as 0.1% and 0.4% (Figure 23). Thus using 0.4% hyssop oil, the percentage germination was

Figure 23 : Effect of hyssop oil on *Botrytis fabae* germination.
Values are the means of 5 replicates. Statistics data are shown in Table 14 in Appendix 4.

Figure 24: Effect of hyssop oil on *Uromyces viciae-fabae* germination.
Values are the means of 5 replicates. Statistics data are shown in Table 14 in Appendix 4.

Figure 23

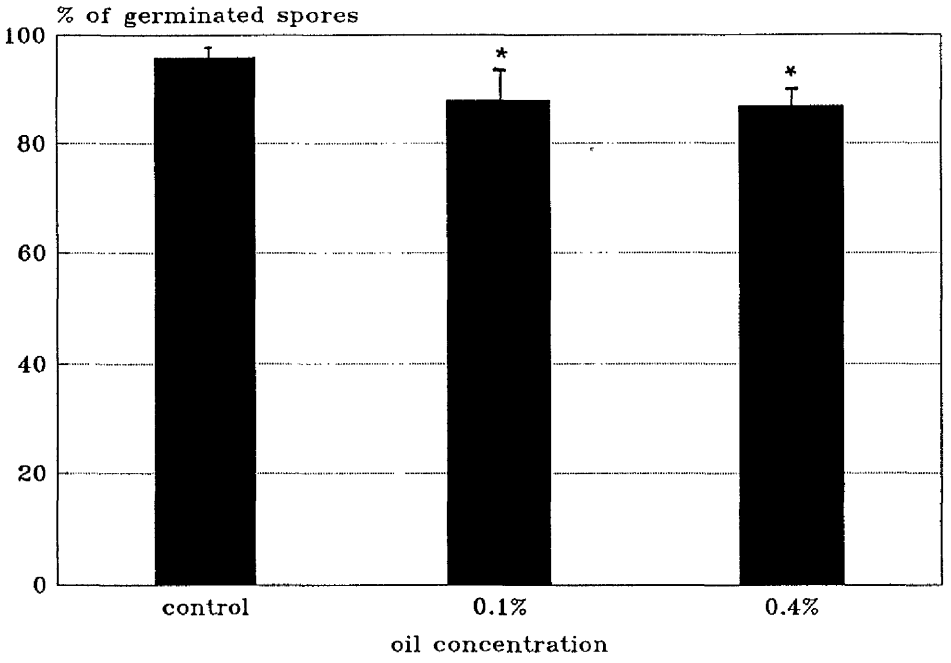
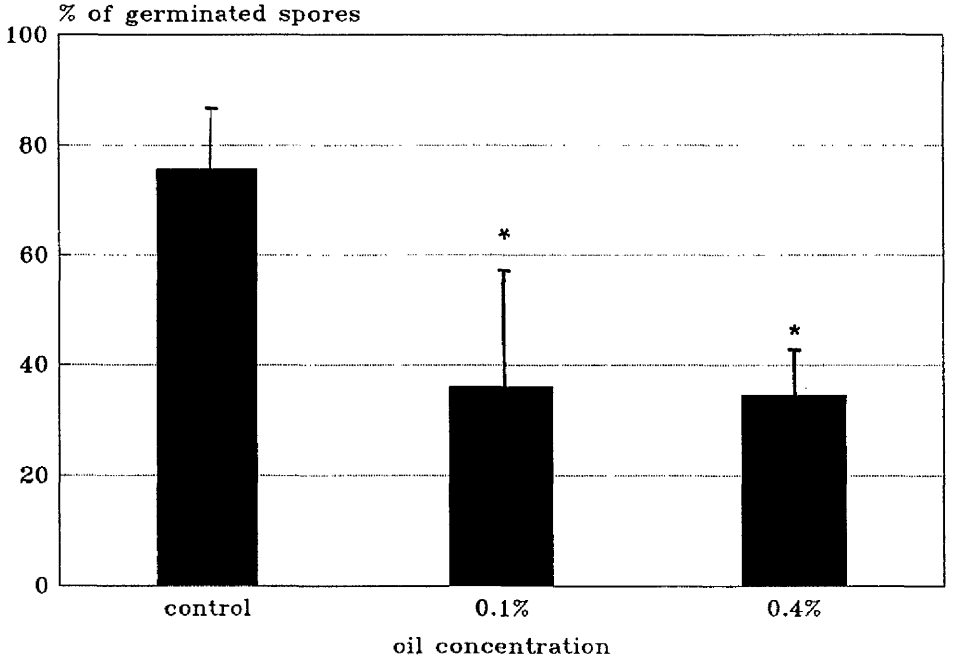


Figure 24



86.8%, while it was 95.8% for the control. A one-way analysis of variance showed that these effects of hyssop oil on *Botrytis fabae* germination were significant. (Statistics shown in Table 14 in Appendix 4)

3.1.2. Effects of hyssop oil on germination of uredospores of *Uromyces fabae*

There were substantial reductions in the germination of uredospores of *U. viciae-fabae* using hyssop oil as 0.1% and 0.4% (Figure 24). Thus, using 0.1% hyssop oil, the percentage germination was less than half that of the control (75.6% for the control and 36% with 0.1% of oil). A one-way analysis of variance showed that these reductions were highly significant (Statistics shown in Table 14 Appendix 4).

3.1.3 Effect of oregano oil on germination of spores of *Botrytis fabae*

Germination of *B. fabae* spores was reduced by 0.04% and 0.16% oregano oil (Figure 25). There was a small difference between the control (95.2% germination) and treatment with 0.04% hyssop oil (90% germination) but the reduction was much greater between the control (95.2%) and treatment with 0.16% oil (72.75% of germination). A one-way analysis of variance showed that these reduction were significant (Statistics

Figure 25 : Effect of oregano oil on *Botrytis fabae* germination.
Values are the means of 5 replicates. Statistics data are shown in Table 15 in Appendix 4.

Figure 26 : Effect of oregano oil on *Uromyces viciae-fabae* germination.
Values are the means of 5 replicates. Statistics data are shown in Table 15 in Appendix 4.

Figure 25

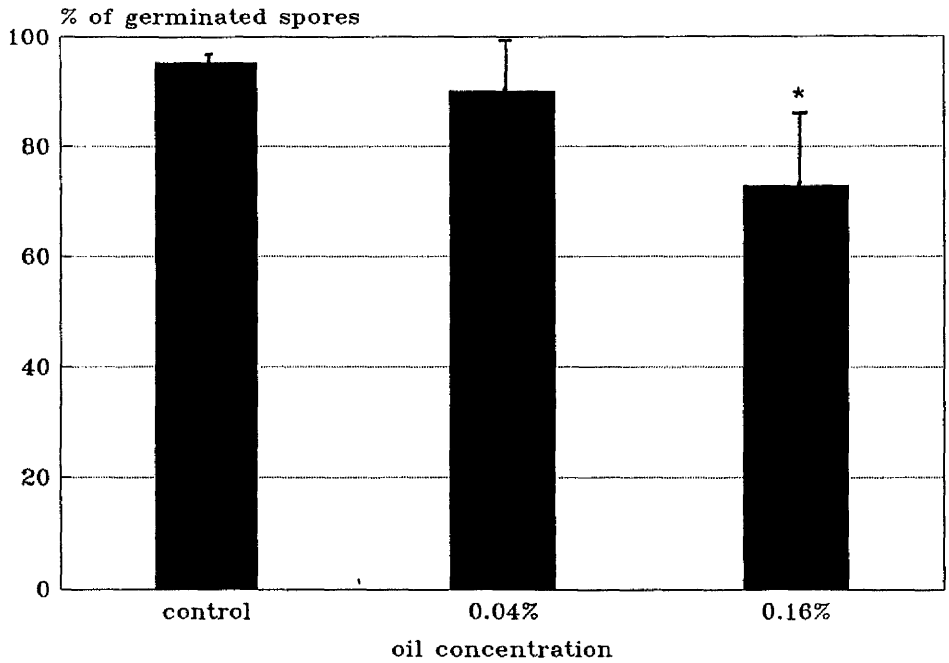
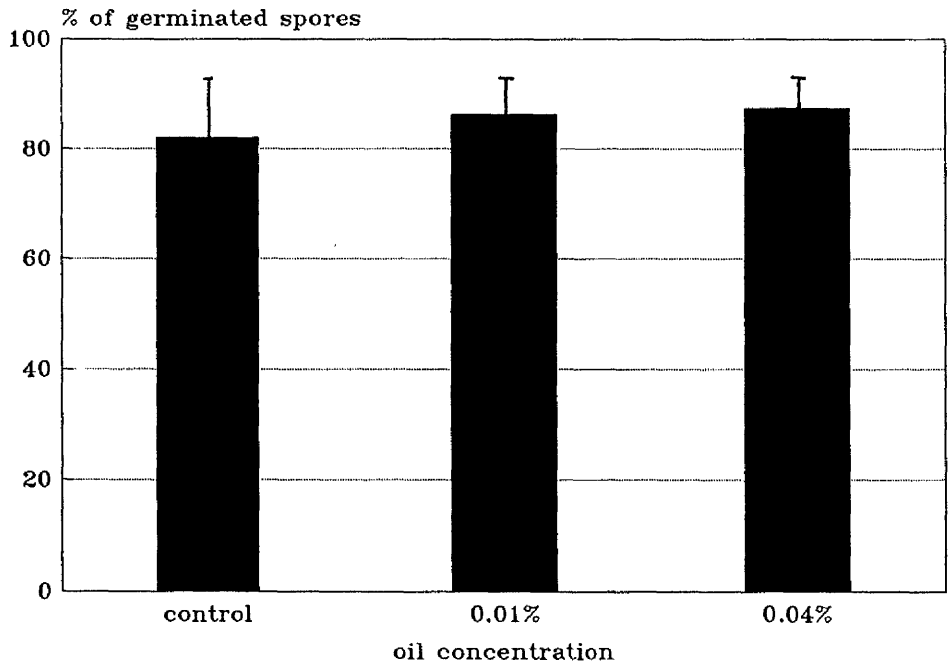


Figure 26



shown in Table 15 in Appendix 4).

3.1.4. Effect of oregano oil on germination of uredospores of *Uromyces viciae-fabae*

Germination of uredospores of *U. viciae-fabae* was actually increased by oregano oil (Figure 26). The percentage germination rose from 82% for the control to 87.4% for treatment with 0.04% oregano oil. A one-way analysis of variance showed that these changes were not significant (Statistics shown in Table 15 in Appendix 4).

3.2 Effect of essential oils on mycelial growth of *P.avenae* and *P.oryzae*

3.2.1. Effect of hyssop oil on mycelial growth of *P.avenae*

Although 0.01% hyssop oil has no effect on mycelial growth of *P. avenae*, fungal growth was substantially reduced by exposure to 0.05% and 0.1% hyssop oil. Indeed, there was no fungal growth on medium containing 0.1% hyssop oil by 4 days after inoculation of plates, while 2 days later, fungal growth was just 8% that of the control (Figure 27, 28 ;

Figure 27 : Effect of various concentrations of hyssop oil on the mycelial growth of *P.avenae*. Growth was completely inhibited by 0.4% hyssop oil.
Values are the means of 3 replicates. Statistics for these data are presented in Table 16 in Appendix 5.

Figure 28 : Regression equations for *P.avenae* growth on media containing various concentrations of hyssop oil.

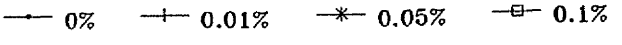
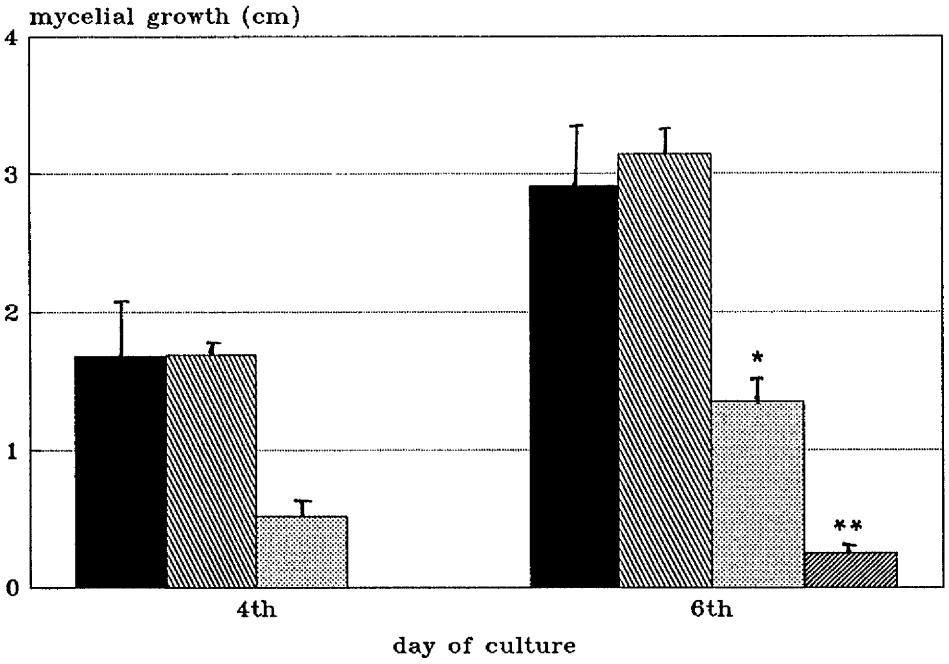
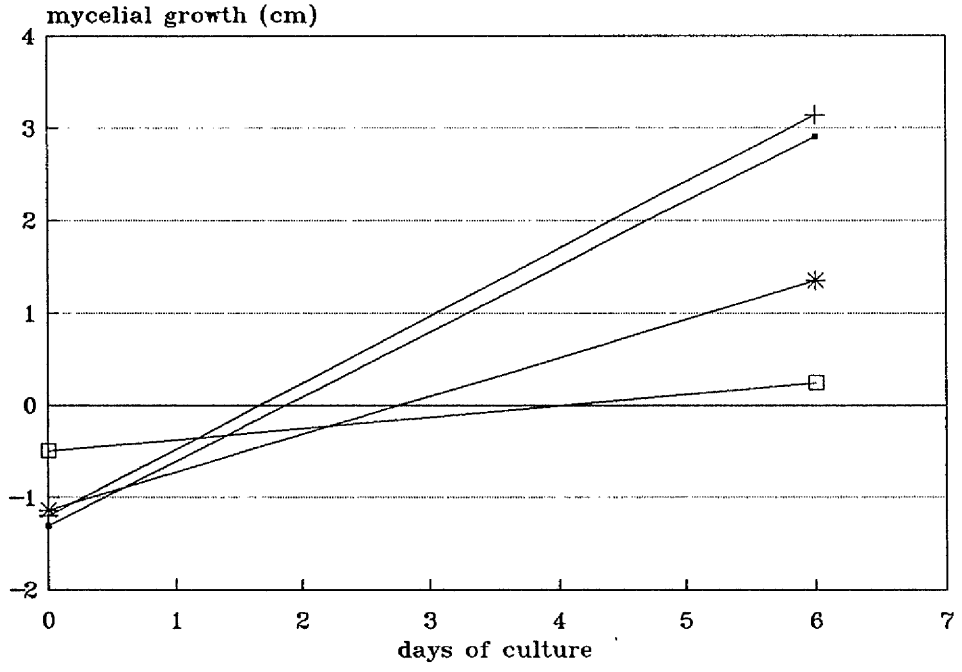


Figure 27



1st set

Figure 28



1st set

Figure 29 : Effect of various concentrations of hyssop oil on the mycelial growth of *P.oryzae*. Growth was completely inhibited by 0.4% hyssop oil.
Values are the means of 3 replicates. Statistics for these data are shown in Table 17 in Appendix 5.

Figure 30 : Regression equations for *P.oryzae* growth on media containing various concentrations of hyssop oil.

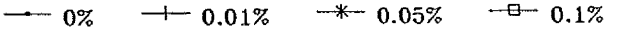
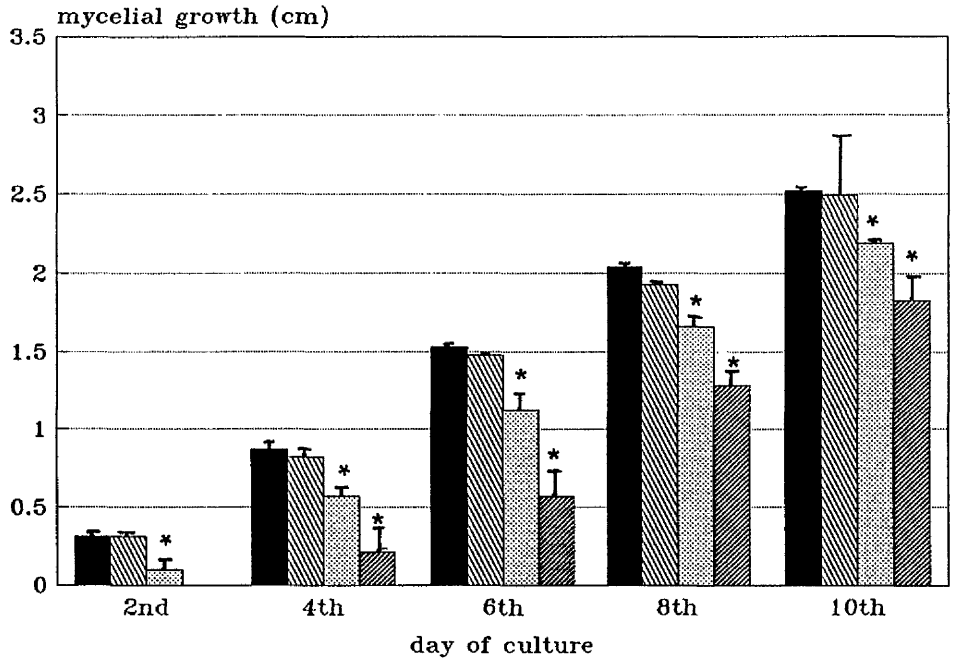


Figure 29



1st set

Figure 30

1st set

Figure 31 : Effect of various concentrations of oregano oil on the mycelial growth of *P.avenae*. Growth was completely inhibited by 0.01% oregano oil.
Values are the means of 3 replicates. Statistics for these data are shown in Table 18 in Appendix 5.

Figure 32 : Regression equations for *P.avenae* growth on media containing various concentrations of oregano oil.

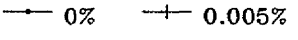
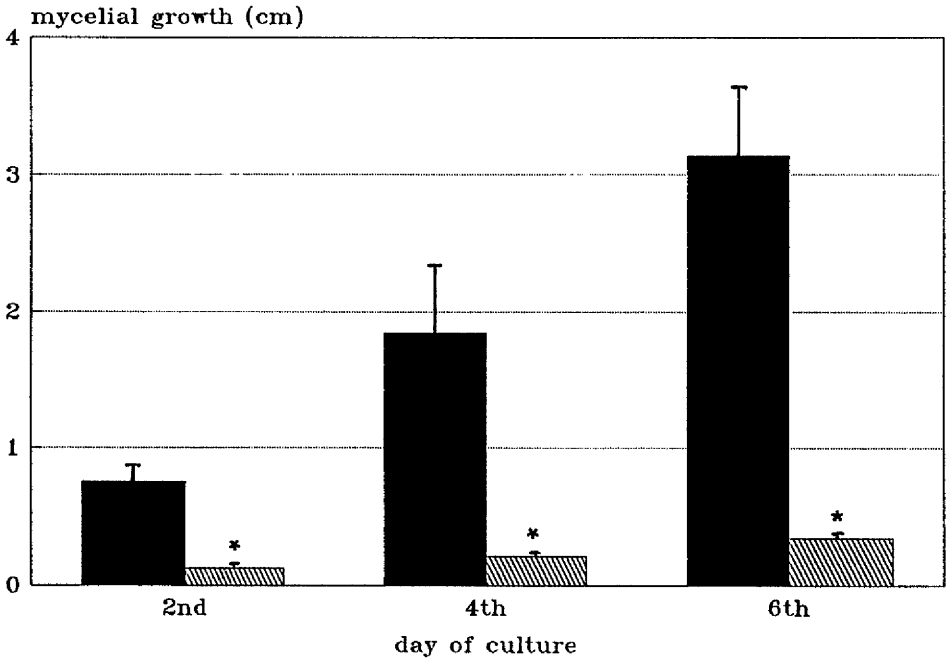
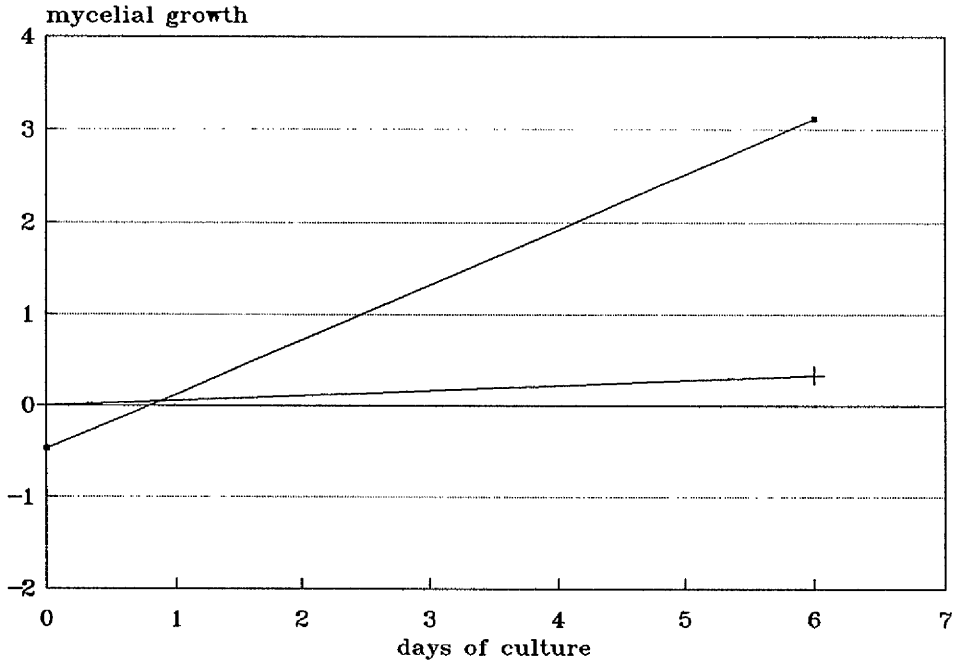


Figure 31



3rd set

Figure 32



3rd set

Statistics shown in Table 16 in Appendix 5).

3.2.2. Effect of hyssop oil on mycelial growth of *P.oryzae*

P.oryzae did not grow on medium containing 0.4% hyssop oil. In contrast, although fungal growth was reduced by 0.05% and 0.1% oil, these growth reduction were small. Hyssop oil at 0.01% had little effect on growth of *P.oryzae* (Figure 29, 30; Statistics shown in Table 17 in Appendix 5).

3.2.3. Effect of oregano oil on mycelial growth of *P.avenae*

Mycelial growth of *P. avenae* was very substantially reduced on exposure to 0.005% oregano oil.
After 6 days of culture in 0.01% oregano oil, some growth of *P.avenae* was visible on the plug, but growth was completely inhibited by the concentration of 0.02% (Figure 31, 32 ; Statistics shown in Table 18 in Appendix 5).

3.2.4. Effect of oregano oil on mycelial growth of *P.oryzae*

Oregano oil at 0.005% reduced growth of *P. oryzae*

Plate 9 : Effect of oregano oil on mycelial growth of *P.oryzae*

Plate 9

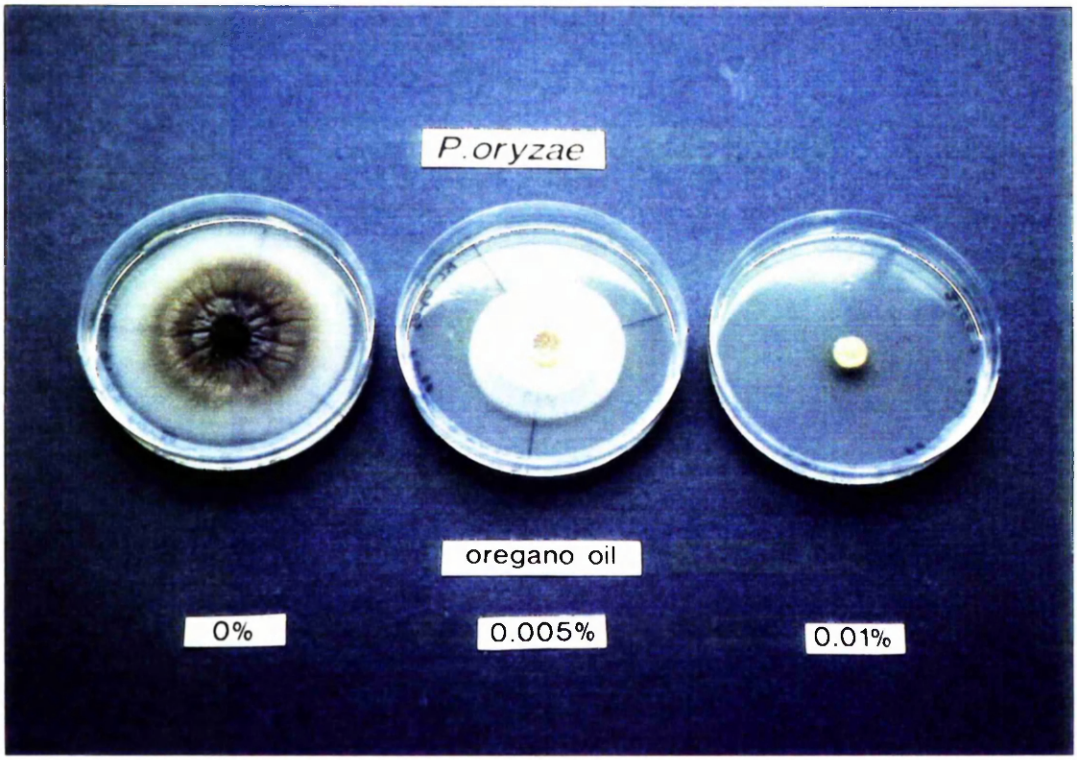


Figure 33 : Effect of various concentrations of oregano oil on the mycelial growth of *P.oryzae*. Growth was completely inhibited by 0.01% oregano oil.
Values are the means of 3 replicates. Statistics for these data are shown in Table 19 in Appendix 5.

Figure 34 : Regression equations for *P.oryzae* growth on media containing various concentrations of oregano oil.

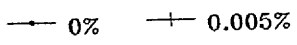
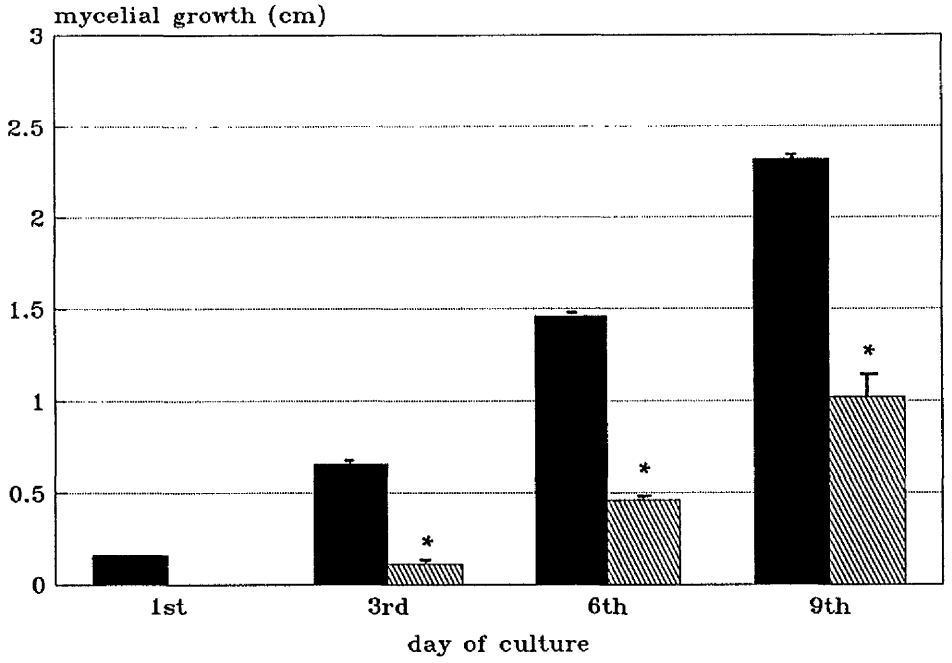
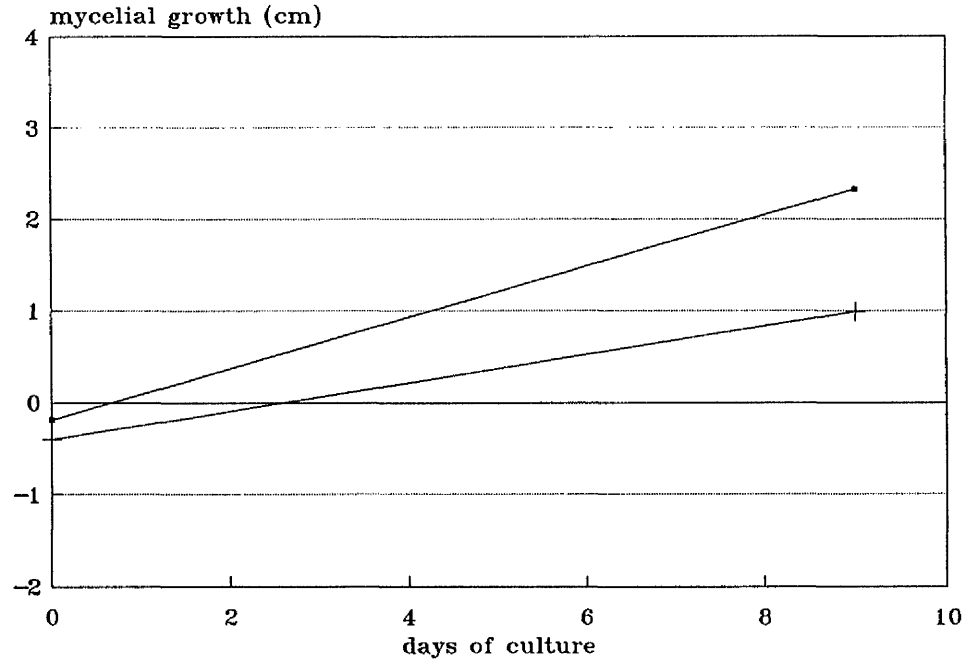


Figure 33



4th set

Figure 34

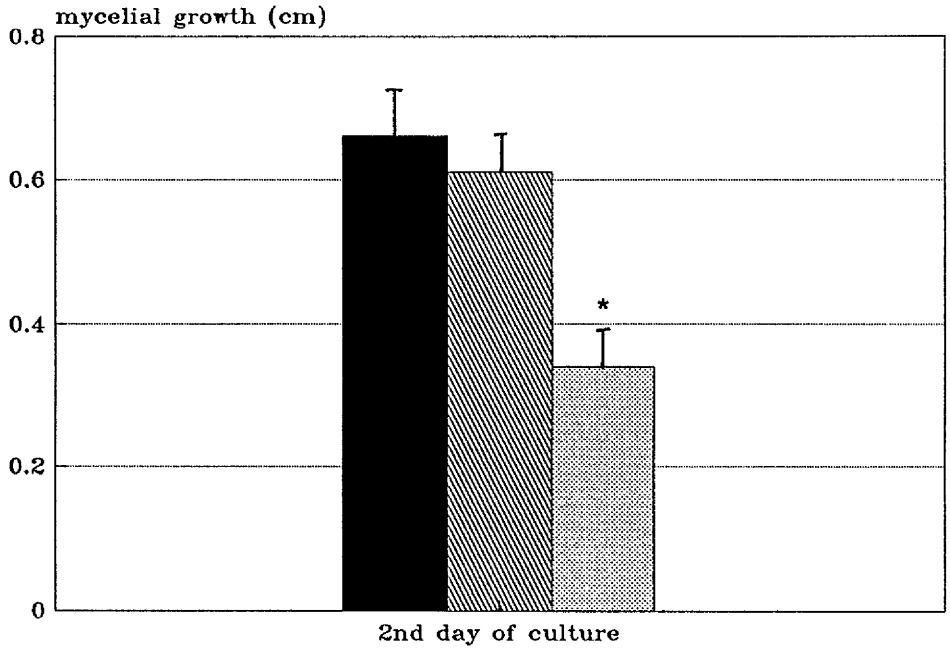


4th set

Figure 35 : Effect of volatile components of hyssop oil at various concentrations on the mycelial growth of *P.avenae*. Growth was completely inhibited by 0.4% hyssop oil.
Values are the means of 3 replicates. Statistics for these data are shown in table 20 in Appendix 6.

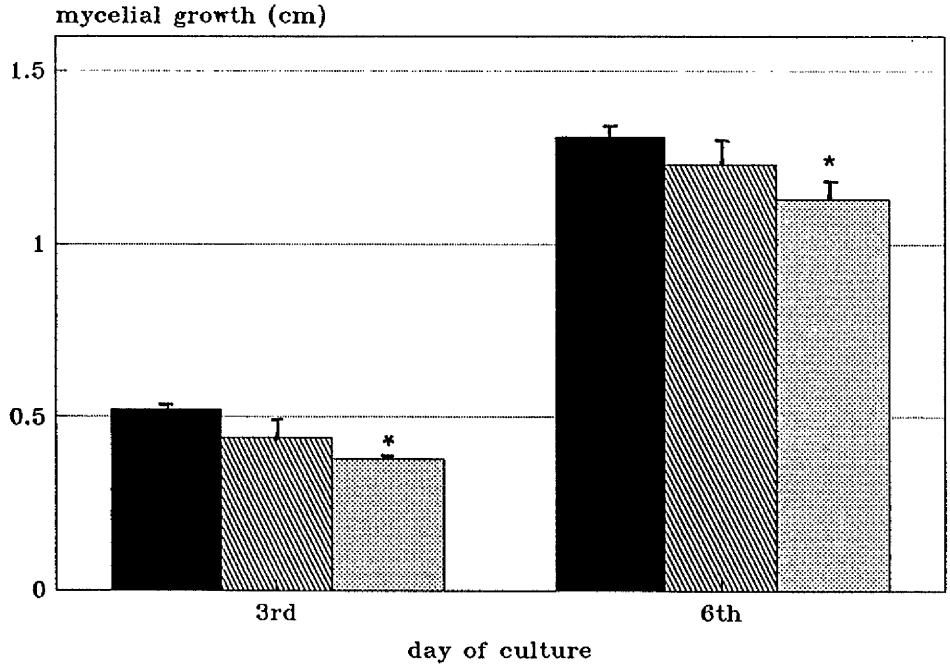
Figure 36 : Effect of the volatile components of hyssop oil at various concentrations on the mycelial growth of *P.oryzae*. Growth was completely inhibited by 0.4% hyssop oil.
Values are the means of 3 replicates. Statistics for these are shown in Table 21 in Appendix 6.

Figure 35



10th set

Figure 36



10th set

Figure 37 : Effect of the volatile components of oregano oil at various concentrations on the mycelial growth of *P.avenae*. Growth was completely inhibited by 0.04% oregano oil.
Values are the means of 3 replicates. Statistics for these data are shown in Table 22 in Appendix 6.

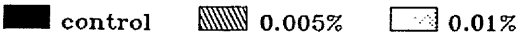


Figure 38 : Effect of the volatile components of oregano oil at various concentrations on the mycelial growth of *P.oryzae*. Growth was completely inhibited by 0.04% oregano oil.
Values are the means of 3 replicates. Statistics for these data are shown in Table 23 in Appendix 6.

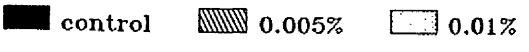
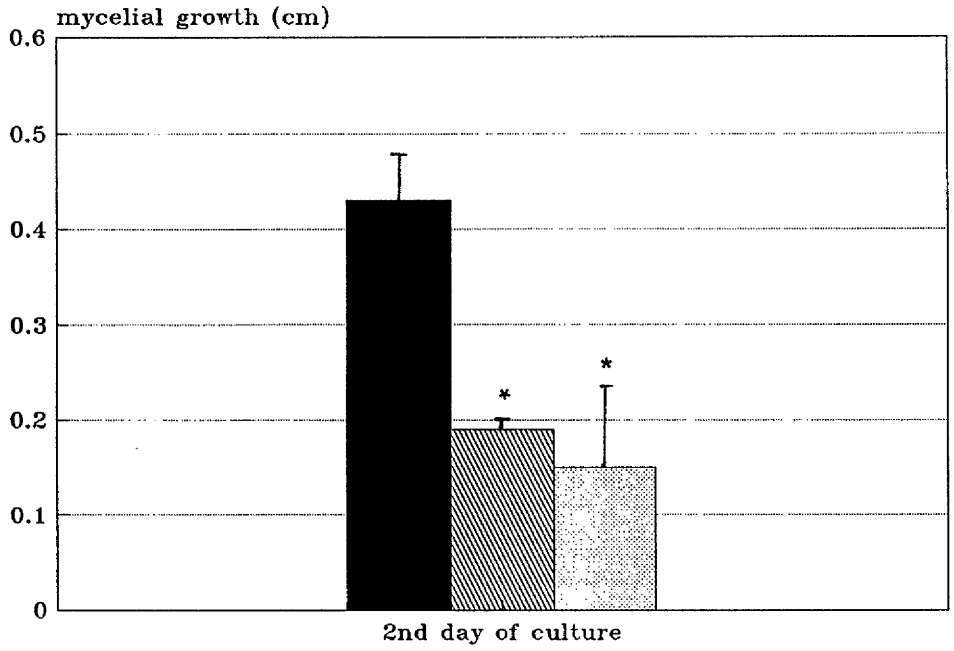
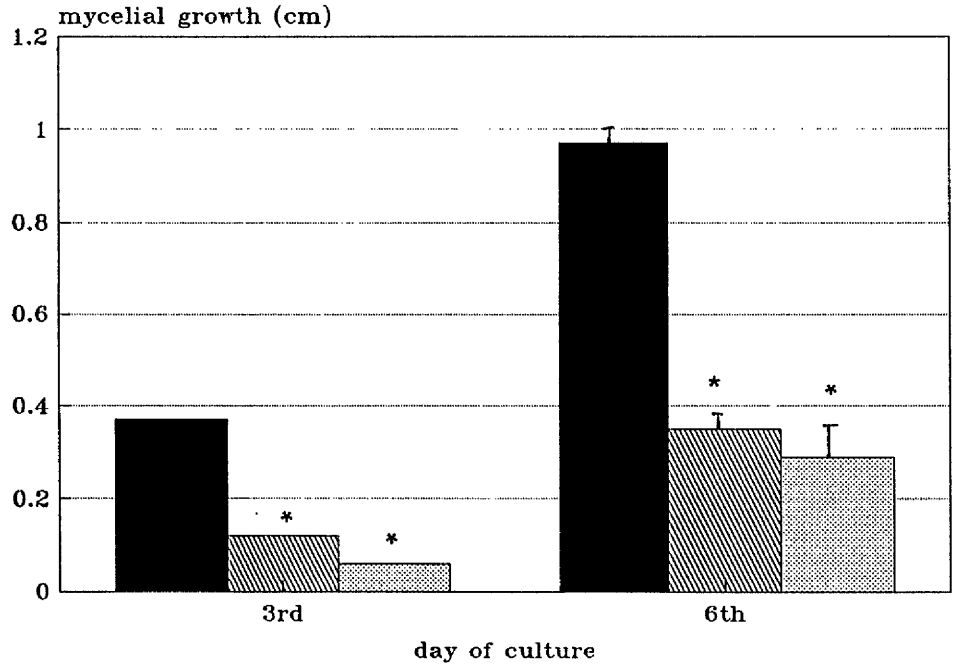


Figure 37



11bth set

Figure 38



11bth set

substantially. Growth of *P.oryzae* was completely inhibited by 0.01% oregano oil (Plate 9) (Figure 33, 34 ; Statistics for these data are shown in Table 19 in Appendix 5).

3.3. Effect of the volatile components of oils on mycelial growth

3.3.1. Effect of the volatile components of hyssop oil on mycelial growth

Four days after exposure, the vapours from the medium containing 0.4% hyssop oil completely inhibited mycelial growth of *P.avenae* and *P.oryzae* (Plate 10). Although the vapours from 0.1% hyssop oil reduced growth of *P. avenae* by approximately 50% (Figure 35 ; Statistics shown in Table 20 in Appendix 6), the vapours from the same concentration of oil has considerably less effect on growth of *P. oryzae* (Figure 36 ; Statistics shown in Table 21 in Appendix 6).

3.3.2. Effect of the volatile components of oregano oil on mycelial growth

The volatile components of oregano oil at 0.005% and 0.01% inhibited mycelial growth of both *P. avenae* and *P. oryzae* substantially (Figure 37, 38 ; Statistics shown in Table 22, 23 in Appendix 6).

Plate 10 : Effect of hyssop oil vapours on mycelial growth of *P. avenae* and *P. oryzae*
The vapours from the medium containing 0.4% hyssop oil completely inhibited the growth of *P. avenae* and *P. oryzae*

Plate 11 : Effect of oregano oil vapours on mycelial growth of *P. avenae* and *P.oryzae*
The vapours from the medium containing 0.04% oregano oil completely inhibited the growth of *P. avenae* and *P. oryzae*

Plate 10

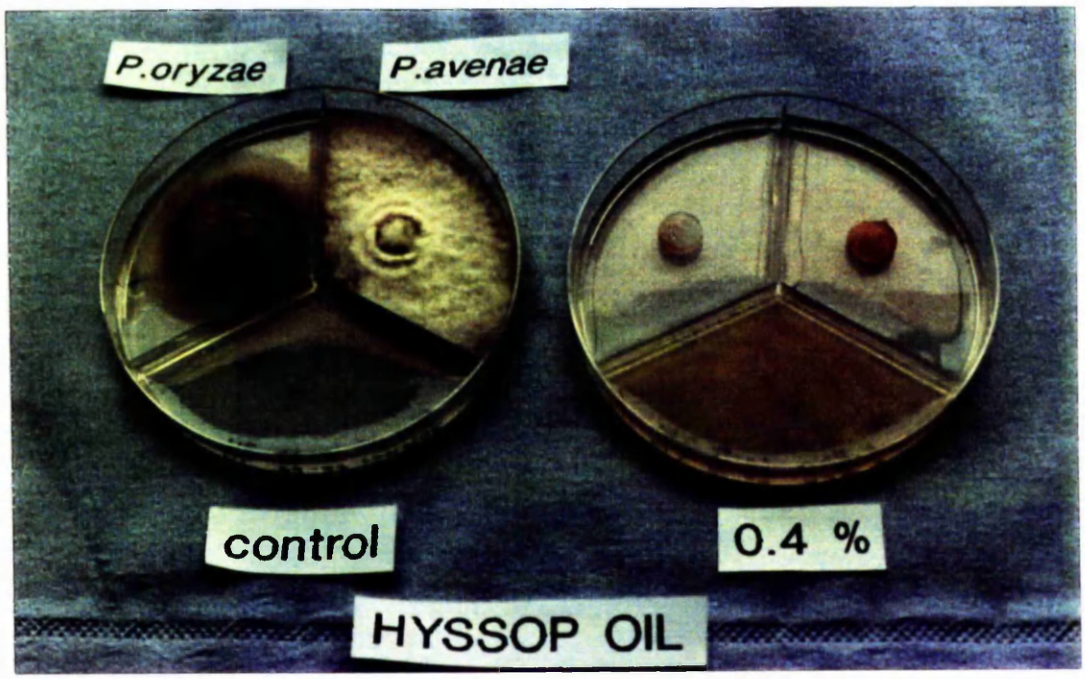
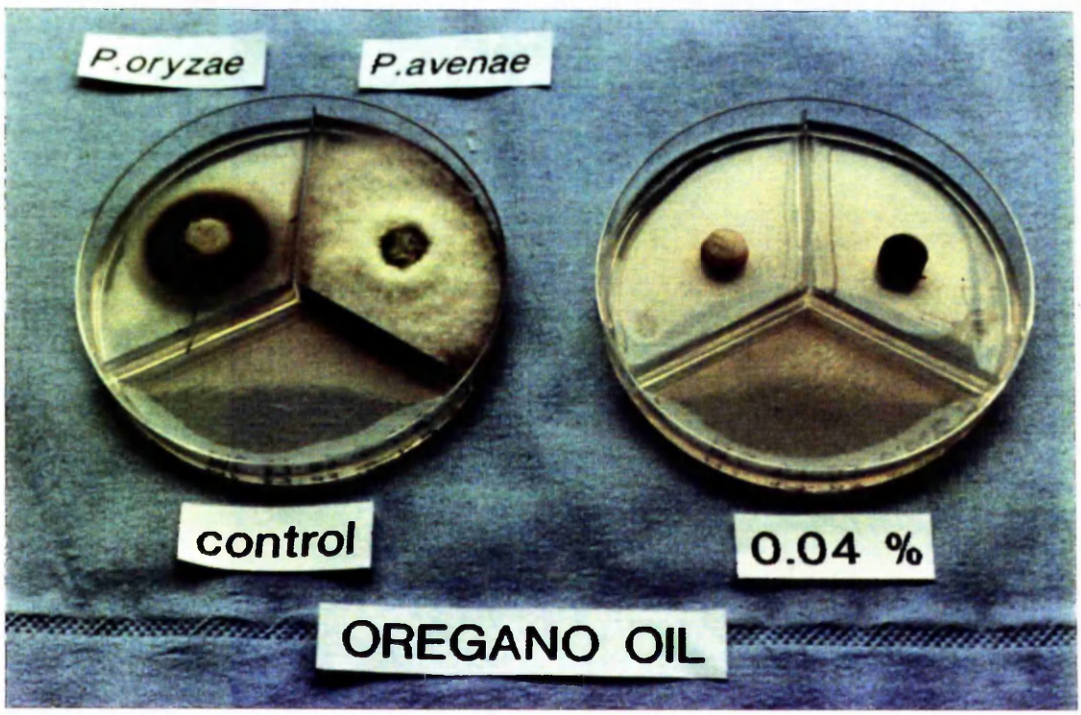


Plate 11



Four days after exposure, vapours from the medium containing 0.04% oregano oil completely inhibited the growth of *P.avenae* and *P.oryzae* (Plate 11)

3.3.3. Determination of the type of effect of hyssop oil on mycelial growth

The inhibition of the growth was not as powerful as the inhibition observed previously. In the most concentrated treatment the colonies grew slowly except on the side close to the compartment of the Petri dish that contained the oil and where the growth was completely stopped. Plugs have been taken from the side that showed the inhibition and have been used to inoculate an agar Petri dish. Mycelium grew from this plug.

3.3.4. Determination of the type of effect of oregano oil on mycelial growth

The inhibition of the growth was also not as powerful as the inhibition previously. The most concentrated treatment did not the stop of the growth. A lack of time did not allow to do again this experiment with a more concentrated medium.

Plate 12 : Variation in fungal growth between replicates

Plate 13 : Effect of l-bornyl acetate on mycelial growth of *P.avenae*
High concentration of l-bornyl acetate affected the shape and appearance of the fungal colony of *P. avenae*

Plate 12

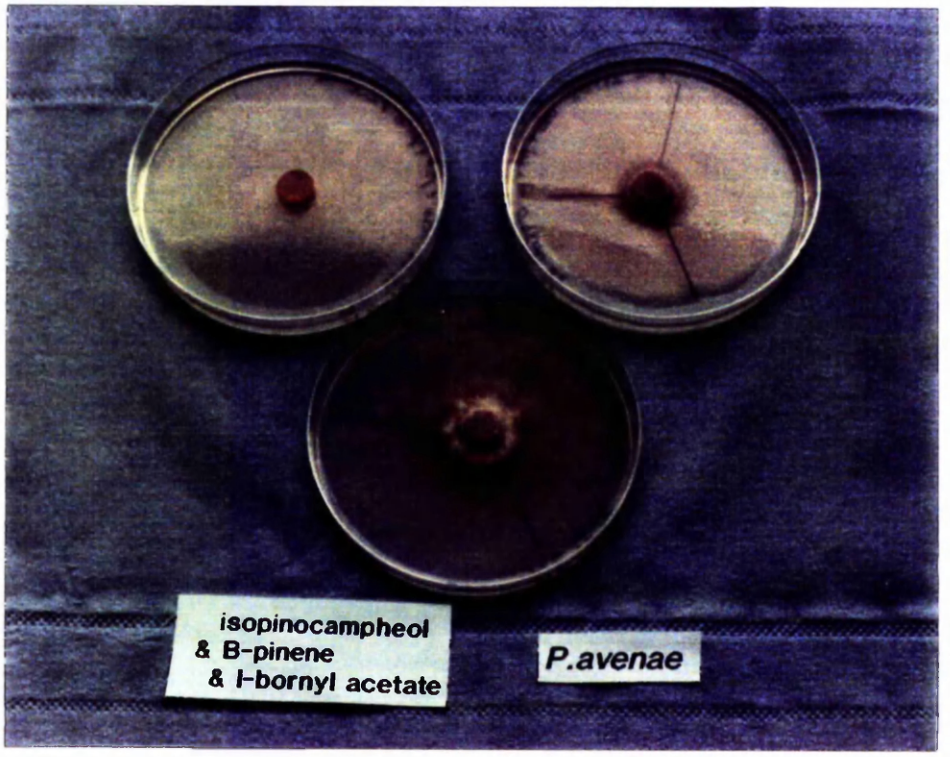


Plate 13

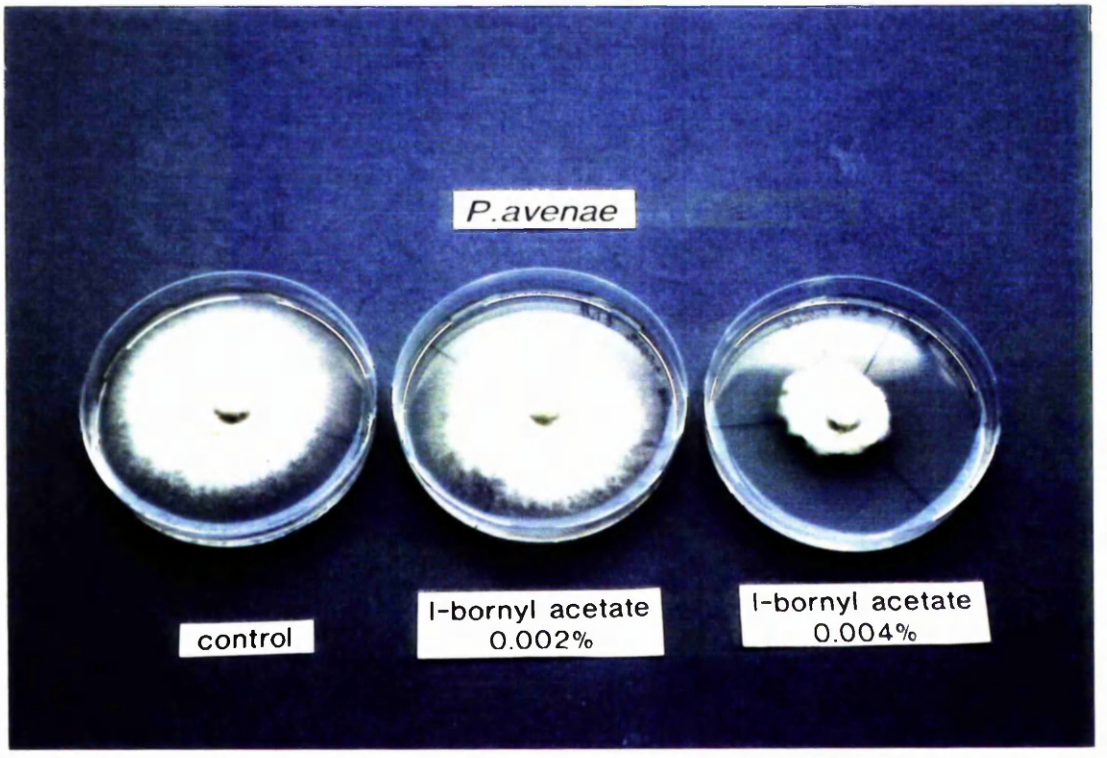


Table 5 : Comparison of the sensitivity of *P.avenae* and *P.oryzae* to the components of hyssop oil

Oil components	*P.avenae*		*P.oryzae*	
iso 0.05%	VS		S	
iso HC	VS	#	VS	#
pino HC	VS		VS	
lBA 0.08%	VS		VS	
pino+iso	VS			
iso+lBA	VS	#	VS	
iso+BP	VS	#	VS	
pino+iso+lBA	VS	#	VS	
pino+BP+lBA	VS			
iso+BP+lBA	VS	#	VS	
iso+BP+pino	VS			
iso+BP+pino+lBA	VS	#	VS	#

VS : very significantly different from the control (P = 0.01)
S : significantly different from the control (P = 0.05)
: no growth at all

Table 6 : Comparison of the sensitivity of *P.avenae* and *P.oryzae* to the components of oregano oil

Oil components	*P.avenae*		*P.oryzae*	
p-cym	VS			
car	VS	#	VS	#
thy	VS		VS	
thy+car	VS	#	VS	#
g-ter+p-cym +thy+car	VS	#	VS	#

VS : very significantly different from the control (P = 0.01)
S : significantly different from the control (P = 0.05)
: no growth at all

3.4. Effect of components of essential oils on mycelial growth

The results of experiments examining the effects of components of the oil on mycelial growth are presented on histograms (Figure 39 to Figure 48). A summary of these results and a comparison between the response of *P.avenae* and *P.oryzae* are presented in Table 5 and Table 6. The effect of the different oil components were analysed using Dunnett's test (LSD = least significant difference).

3.4.1. Effect of components of hyssop oil on mycelial growth of P. avenae

Two concentrations of ß-pinene and 1-bornyl acetate (the normal endogenous concentration and a higher one), and combination of components (each of them at the endogenous concentration), were used. Although great care was taken in the mixing of the essential oil components, complete homogenisation was not always achieved. This may have been responsible, in part, for the variation in fungal growth observed between replicates (Plate 12).

1-bornyl acetate and ß-pinene used on their own at the natural endogenous concentration, tended to increase the growth of *P.avenae*, although these increases were not significant (Figure 39 ; Statistics shown in Table 24 in Appendix 7).

Plate 14 : Medium containing isopinocampheol : needle-shaped crystals appeared on the surface of the medium containing a high concentration of isopinocampheol

Plate 14

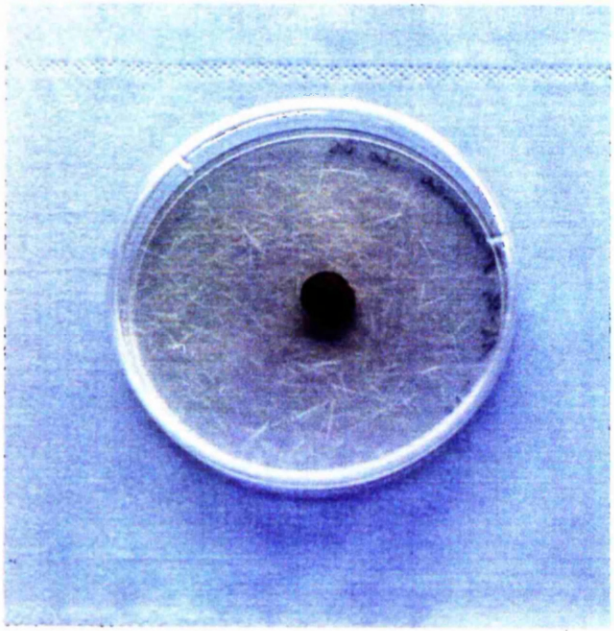


Figure 39 : Effect on the growth of *P.avenae*, of ß-pinene and 1-bornyl acetate at a natural endogenous concentration and at a higher concentration.
Values are the means of 3 replicates. Significant differences are shown as *. Statistics for these data are presented in Table 24 in Appendix 7.

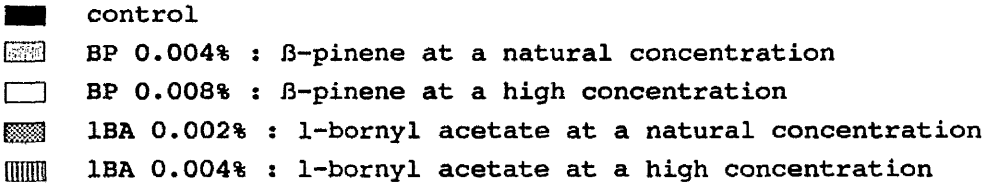


Figure 40 : Effect on the growth of *P.avenae*, of 1-bornyl acetate at various concentrations.
Values are the means of 3 replicates. Statistics for these data are presented in Table 25 in Appendix 7.

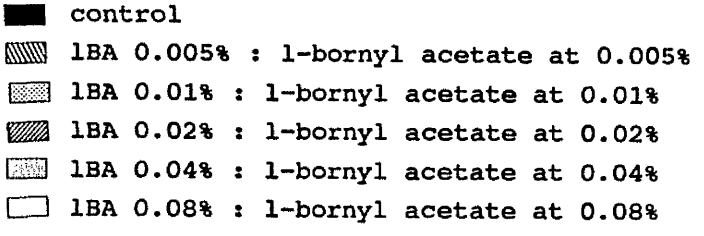
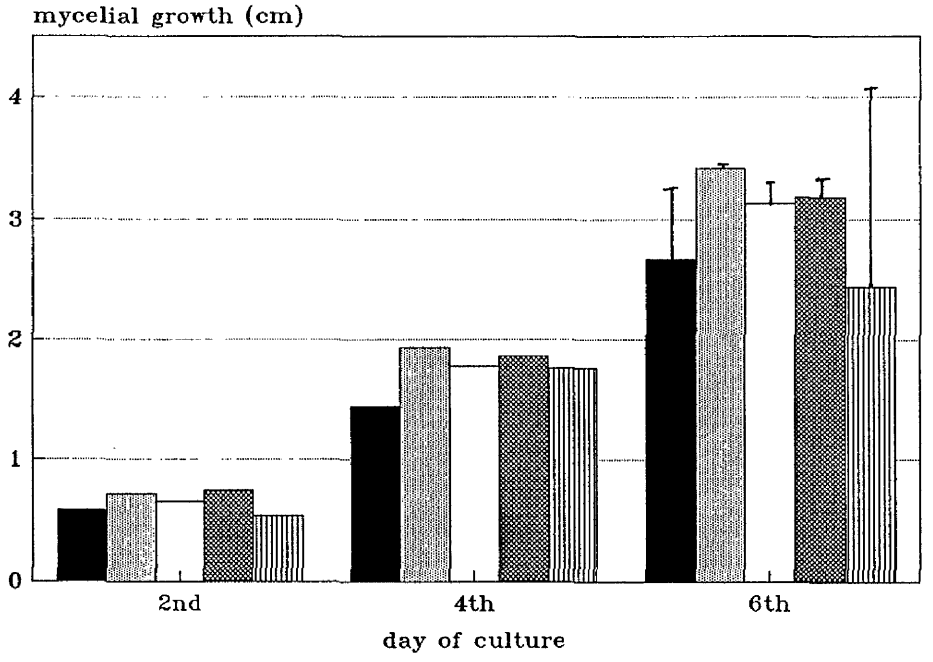
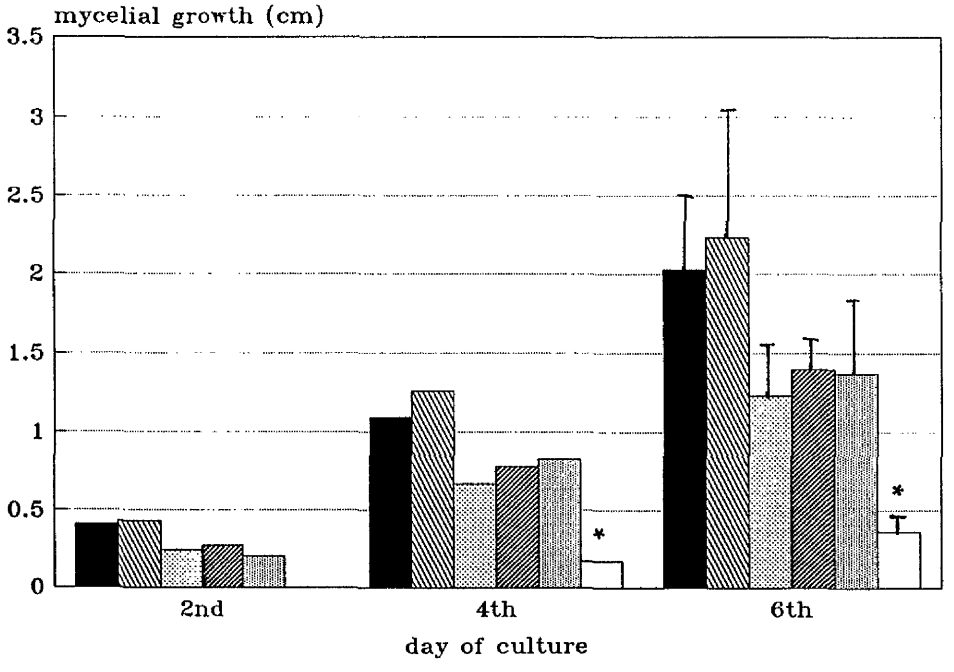


Figure 39



5th set

Figure 40



7th set

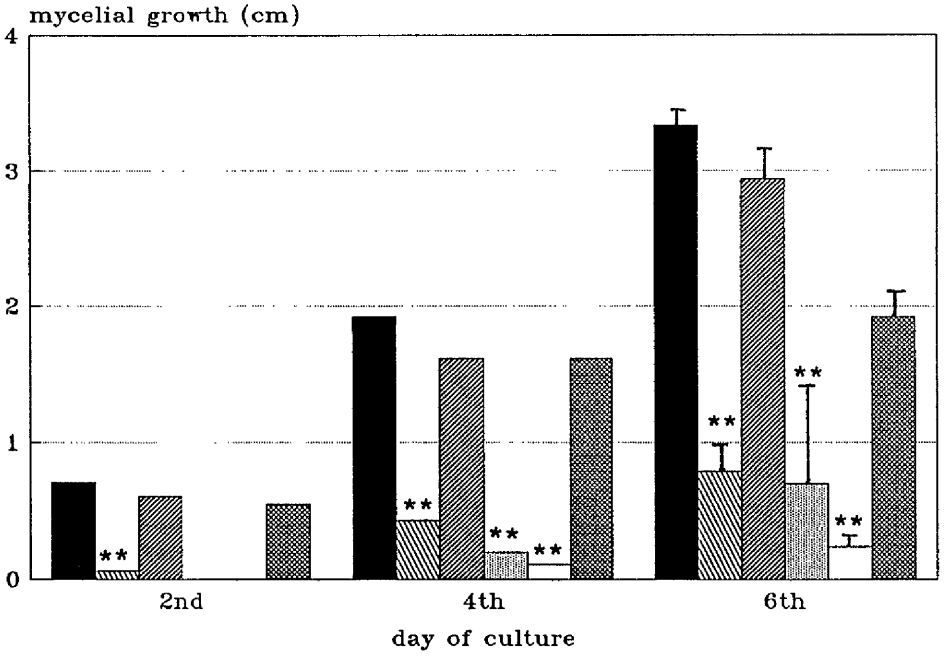
Figure 41 : Effect on the growth of *P.avenae*, of isopinocampheol (natural and higher concentration : 0.05%, 0.3%), pinocamphone (natural and higher concentration : 0.02%, 0.13%), pinocamphone (0.02%) + isopinocampheol (0.004%), pinocamphone (0.02%) + ß-pinene (0.008%), isopinocampheol (0.05%) + 1-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%).
The growth of *P.avenae* was completely inhibited when it was treated with isopinocampheol at a high concentration (0.3%), with isopinocampheol (0.05%) + 1-bornyl acetate, with isopinocampheol (0.05%) + ß-pinene (0.008%).
Values are the means of 3 replicates. Statistics for these data are presented in Table 26 in Appendix 7.

- control
- iso 0.05% : isopinocampheol at a natural concentration
- pino 0.02% : pinocamphone at a natural concentration
- pino 0.1% : pinocamphone at a high concentration
- pino + iso : isopinocampheol and pinocamphone
- pino + BP : pinocamphone and ß-pinene

Figure 42 : Effect on the growth of *P.avenae* of pinocamphone (0.02%) + 1-bornyl acetate (0.004%), pinocamphone (0.02%) + ß-pinene (0.008%) + 1-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%) + pinocamphone (0.02%), pinocamphone (0.02%) + isopinocampheol (0.05%) + 1-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%) + 1-bornyl acetate (0.004%), pinocamphone (0.02%) + isopinocampheol (0.05%) + ß-pinene (0.008%) + 1-bornyl acetate (0.004%). The three last treatments inhibited the growth of *P.avenae* completely. Values are the means of 3 replicates. Statistics for these data are presented in Table 27 in Appendix 7.

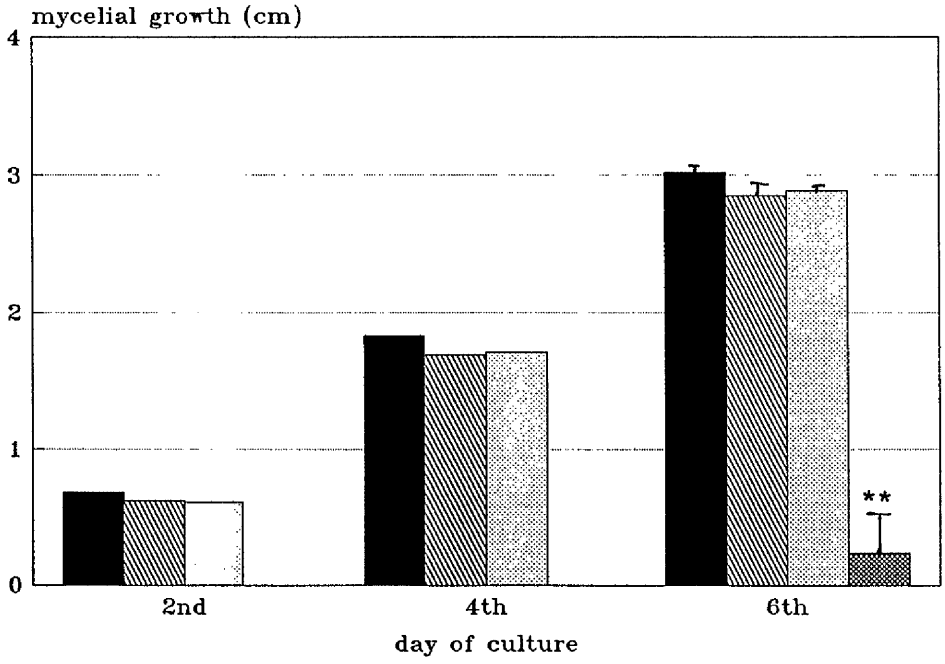
- control
- pino + 1BA : pinocamphone and 1-bornyl acetate
- pino + BP + 1BA : pinocamphone and ß-pinene + 1-bornyl acetate
- iso + BP + pino : isopinocampheol and ß-pinene and pinocamphone

Figure 41



8th set

Figure 42



9th set

A subsequent experiment was performed using l-bornyl acetate, since the natural endogenous concentration did not show any depressing effect on growth of *P.avenae* and the higher concentration affected the shape and the appearance of the fungal colony (Plate 13). Indeed, concentration of l-bornyl acetate between 0.01% and 0.08% (especially the latter concentration) reduced fungal growth substantially (Figure 40; Statistics shown in Table 25 in Appendix 7). Isopinocampheol used at the natural concentration had a very significant effect on the growth of *P.avenae* reducing it substantially. Also, the appearance of the medium containing isopinocampheol was interesting, with needle-shaped crystals appearing on the surface of the medium (Plate 14). The effect of pinocamphone was not significant. However, all combinations containing isopinocampheol had a strong depressing effect on the growth of *P.avenae* and most of them inhibited fungal growth completely (Figure 41, 42 ; Statistics shown in Table 26, 27 in Appendix 7). The synergistic effect of the components of the oil is highlighted by plate 15. Three of the components (isopinocampheol, ß-pinene, l-bornyl acetate) in combination reduced growth of *P.avenae* considerably, while these three components together with pinocamphone completely inhibited fungal growth.

Plate 15 : Effect of some components of hyssop oil on mycelial growth of *P.avenae*

Plate 16 : Effect of some components of hyssop oil on mycelial growth of *P.oryzae*

Plate 15

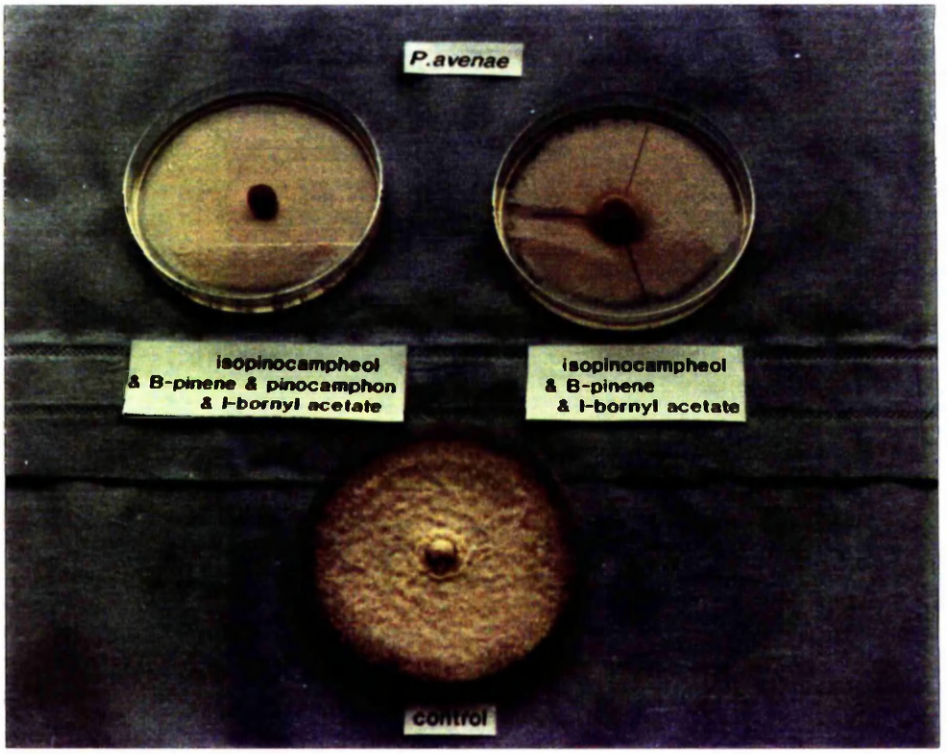


Plate 16

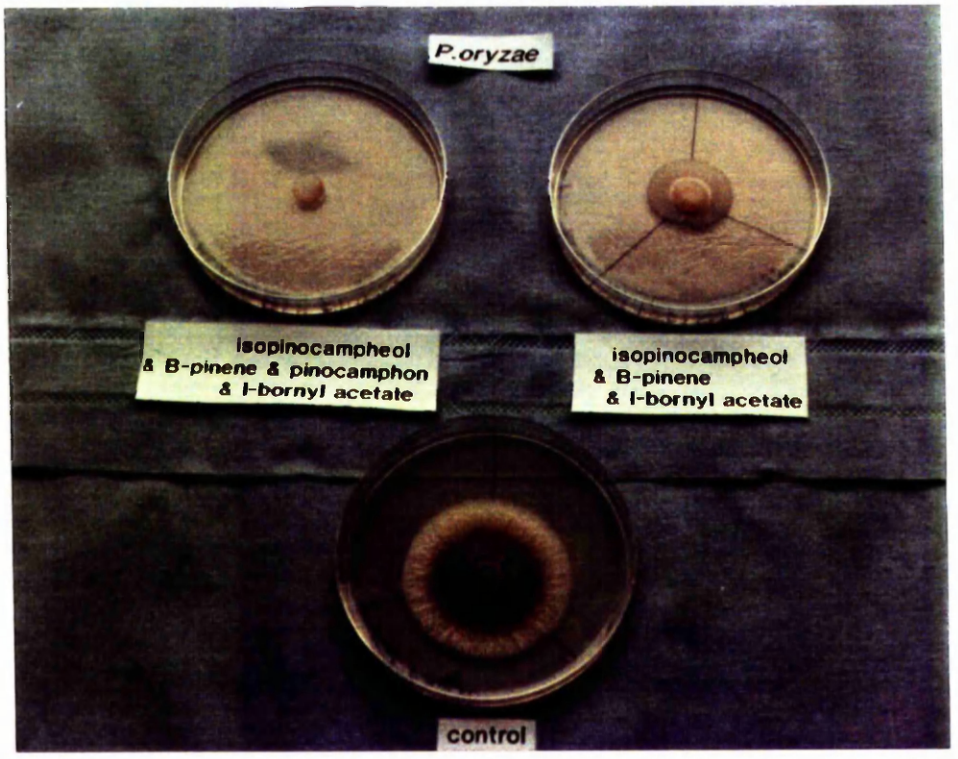


Figure 43 : Effect on the growth of *P.oryzae*, of ß-pinene and 1-bornyl acetate at a natural concentration and at a higher concentration.
Values are the means of 3 replicates. Significant differences are shown as *. Statistics for these data are presented in Table 28 in Appendix 7.

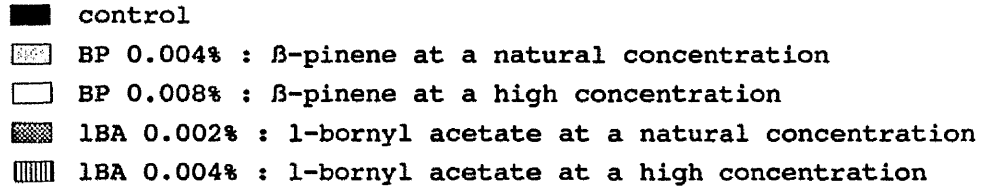


Figure 44 : Effect on the growth of *P.oryzae* of 1-bornyl acetate at various concentrations.
Values are the means of 3 replicates. Statistics for these data are presented in Table 29 in Appendix 7.

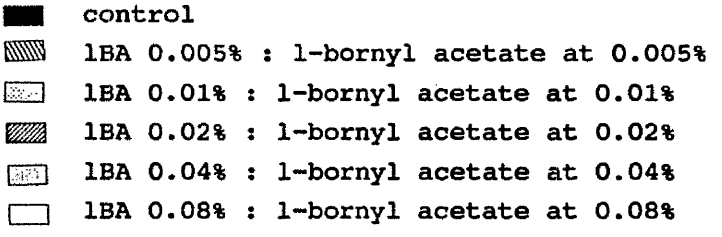
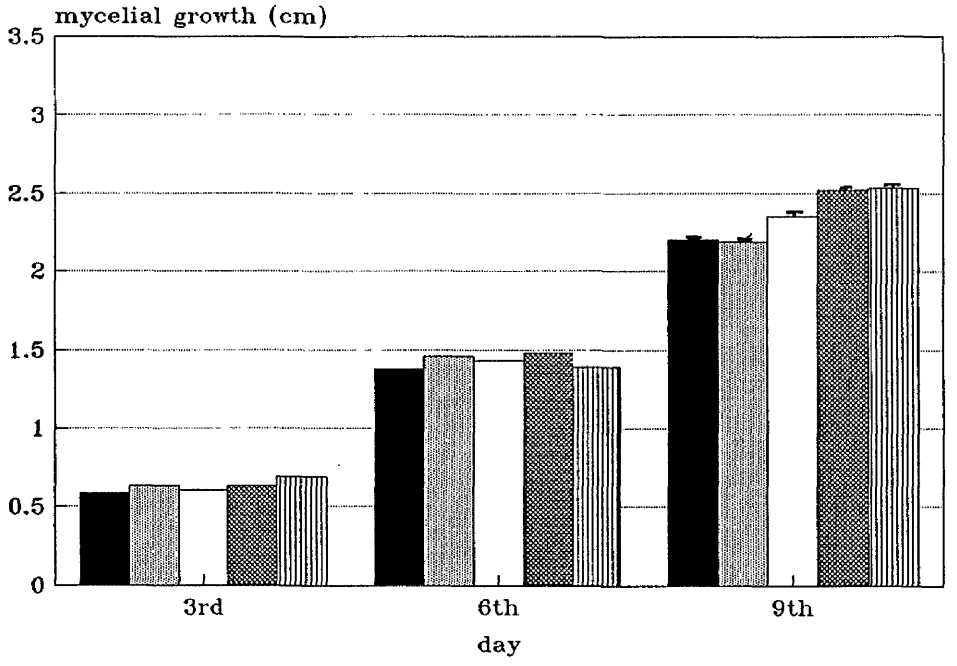
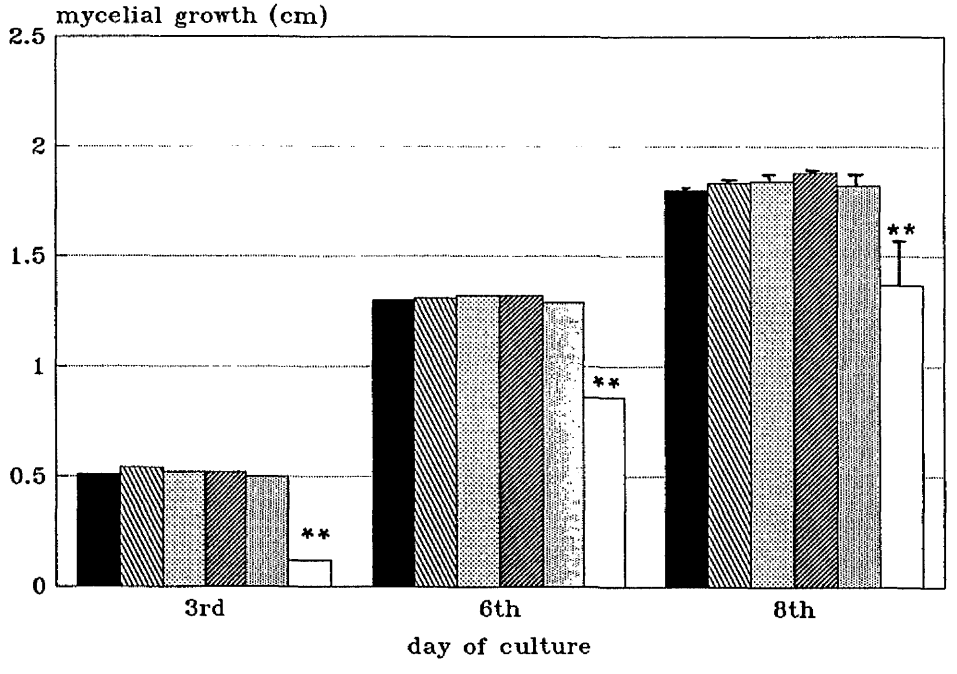


Figure 43



5th set

Figure 44



7th set

Figure 45 : Effect on the growth of *P.oryzae*, of isopinocampheol (natural and higher concentration : 0.05%, 3%), pinocamphone (natural and higher concentration : 0.02%, 0.13%), pinocamphone (0.02%) + isopinocampheol (0.004%), pinocamphone (0.02%) + ß-pinene (0.008%), isopinocampheol (0.05%) + l-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%).
The growth of *P.oryzae* was completely inhibited when it was treated with isopinocampheol at a high concentration (0.3%). Values are the means of 3 replicates. Statistics for these data are presented in Table 30 in Appendix ?.

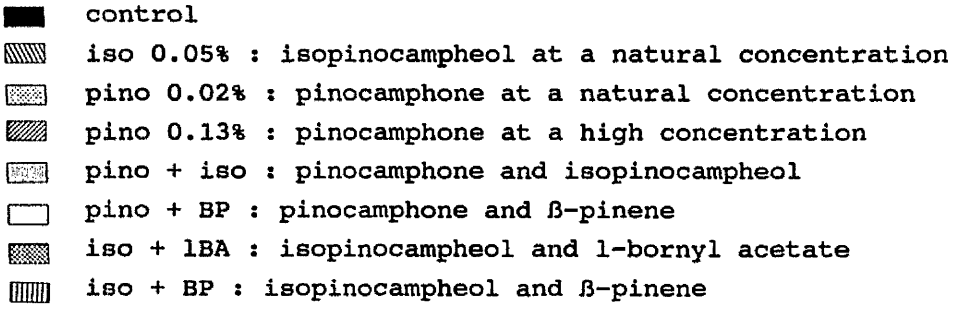


Figure 46 : Effect on the growth of *P.oryzae* of pinocamphone (0.02%) + l-bornyl acetate (0.004%), pinocamphone (0.02%) + ß-pinene (0.008%) + l-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%) + pinocamphone (0.02%), pinocamphone (0.02%) + isopinocampheol (0.05%) + l-bornyl acetate (0.004%), isopinocampheol (0.05%) + ß-pinene (0.008%) + l-bornyl acetate (0.004%), pinocamphone (0.02%) + isopinocampheol (0.05%) + ß-pinene (0.008%) + l-bornyl acetate (0.004%). The last treatment inhibited the growth of *P.oryzae* completely.
Values are the means of 3 replicates. Statistics for these data are presented in Table 31 in Appendix 7.

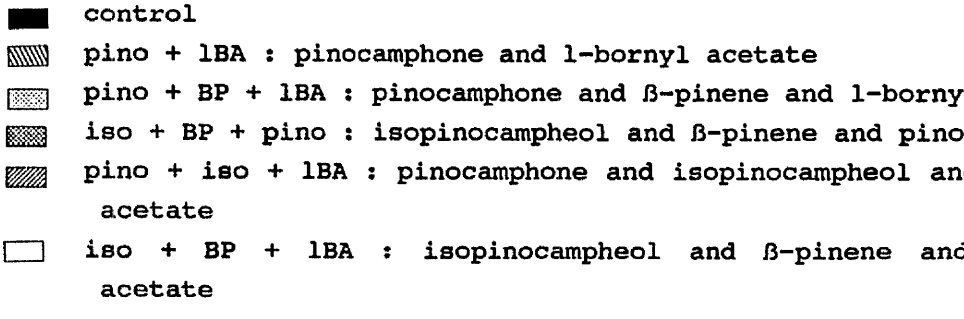
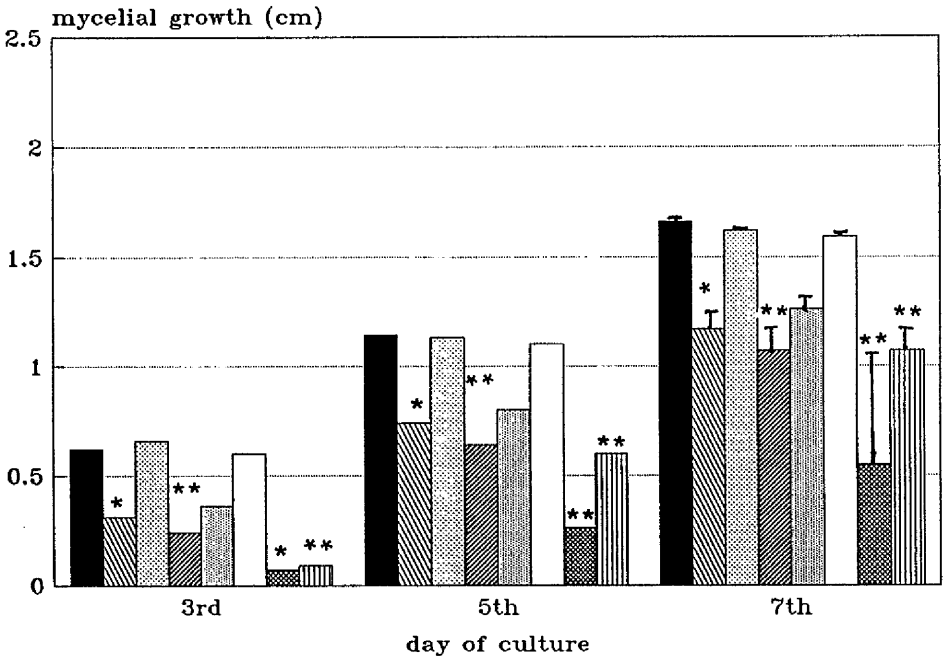
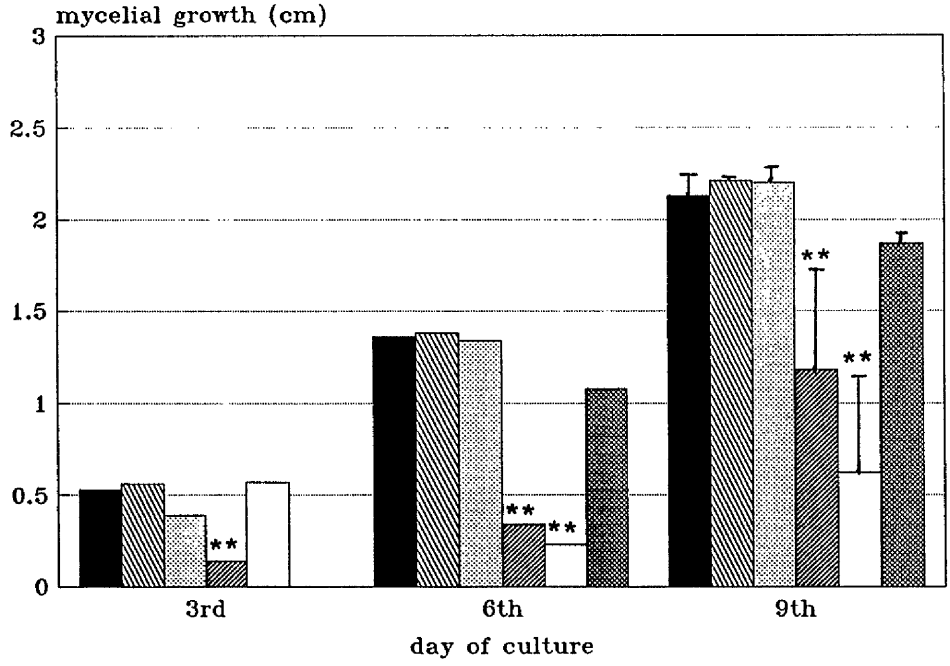


Figure 45



8th set

Figure 46



9th set

3.4.2. Effects of components of hyssop oil on mycelial growth of *P.oryzae*

The growth of *P.oryzae* was not affected, or was slightly increased, by ß-pinene, l-bornyl acetate (Figure 43 ; Statistics shown in Table 28 in Appendix 7). In contrast, l-bornyl acetate at 0.08% reduced the growth of *P.oryzae* very significantly (Figure 44 ; Statistics shown in Table 29 in Appendix 7). Isopinocampheol used at a natural endogenous concentration, had a significant depressing effect on growth of *P.oryzae* while pinocamphone at a normal concentration had no effect. The combination pinocamphone + isopinocampheol also reduced growth of *P.oryzae*. Moreover, the growth of *P.oryzae* decreased very significantly when it was treated with the following combination of components : isopinocampheol + l-bornyl acetate, isopinocampheol + ß-pinene, pinocamphone + isopinocampheol + l-bornyl acetate, isopinocampheol + ß-pinene + l-bornyl acetate, isopinocampheol + l-bornyl acetate + ß-pinene + pinocamphone. The last combination was the only treatment, based on the natural endogenous concentration, that was able to inhibit *P.oryzae* growth completely (Figure 45, 46 ; Statistics shown in Table 30, 31 in Appendix 7).
The same synergistic effect observed previously with *P.avenae* was also noticed with *P.oryzae* (Plate 16).

Plate 17 : Effect of some components of oregano oil on mycelial growth of *P. avenae*

Plate 18 : Effect of some components of oregano oil on mycelial growth of *P. oryzae*

Plate 17

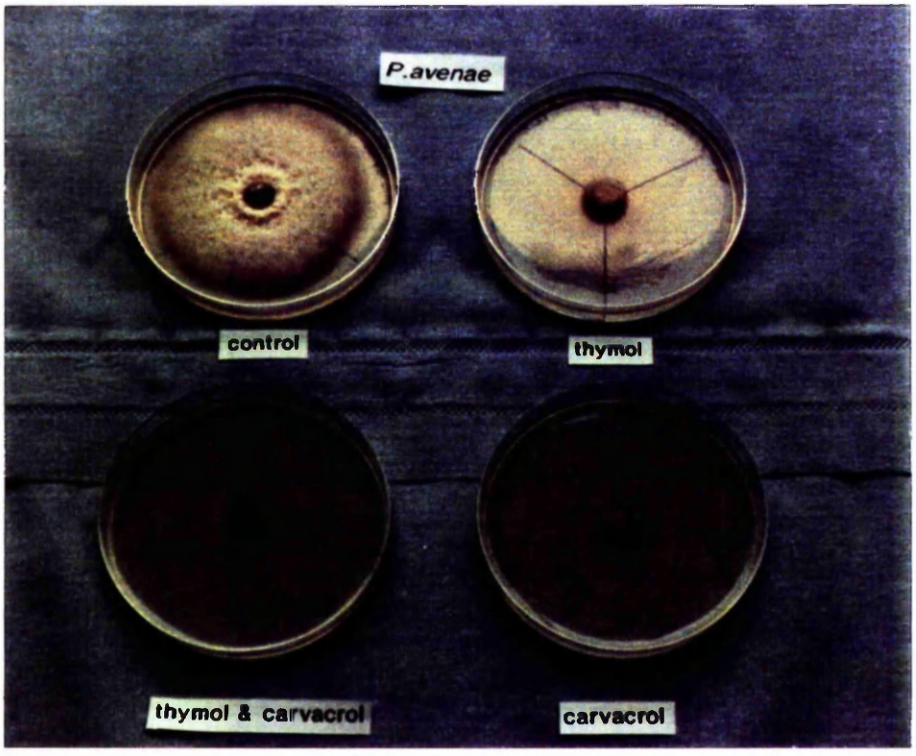


Plate 18

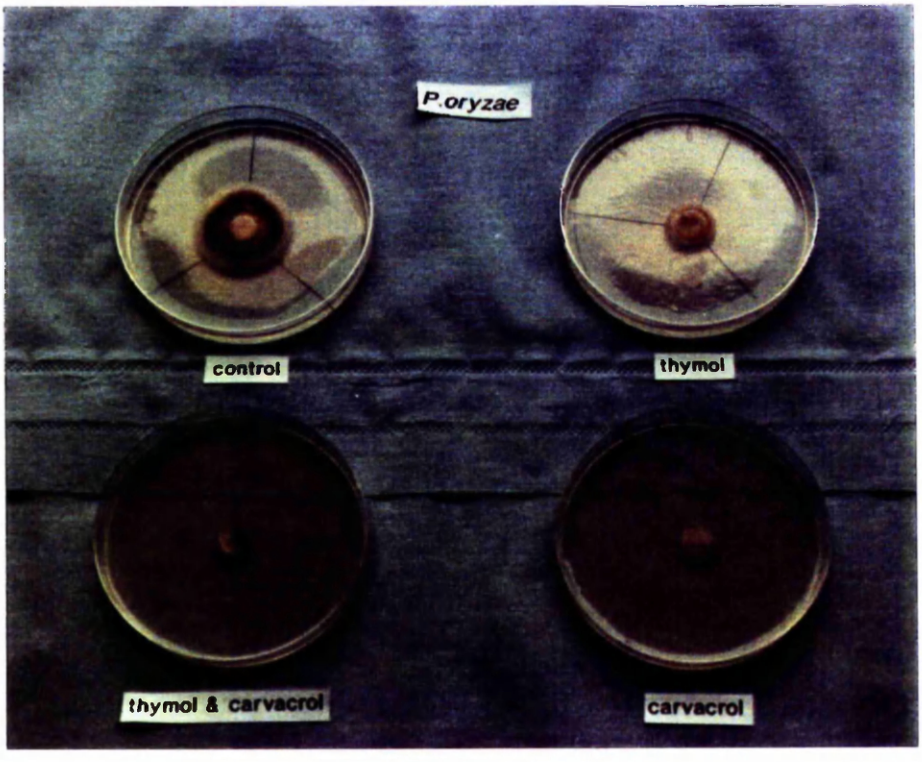


Figure 47 : Effect on the growth of *P.avenae*, of γ -terpinene (0.002%), p-cymene (0.002%), thymol (0.7%), carvacrol (0.7%) and thymol (0.35%) + carvacrol (0.35%).
The growth of *P.avenae* was completely inhibited by carvacrol, carvacrol + thymol, γ -terpinene + p-cymene + thymol + carvacrol.
Values are the means of 3 replicates. Significant differences are shown as *. Statistics for these data are presented in Table 32 in Appendix 7.

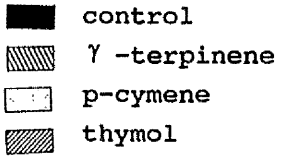


Figure 48 : Effect on the growth of *P.oryzae* of γ -terpinene (0.002%), p-cymene (0.002%), thymol (0.7%), carvacrol (0.7%) and thymol (0.35%) + carvacrol (0.35%).
The growth of *P.oryzae* was completely inhibited by carvacrol, carvacrol + thymol, γ -terpinene + p-cymene + thymol + carvacrol.
Values are the means of 3 replicates. Significant differences are shown as *. Statistics for these data are presented in Table 33 in Appendix 7.

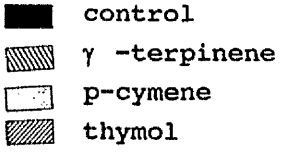


Figure 47

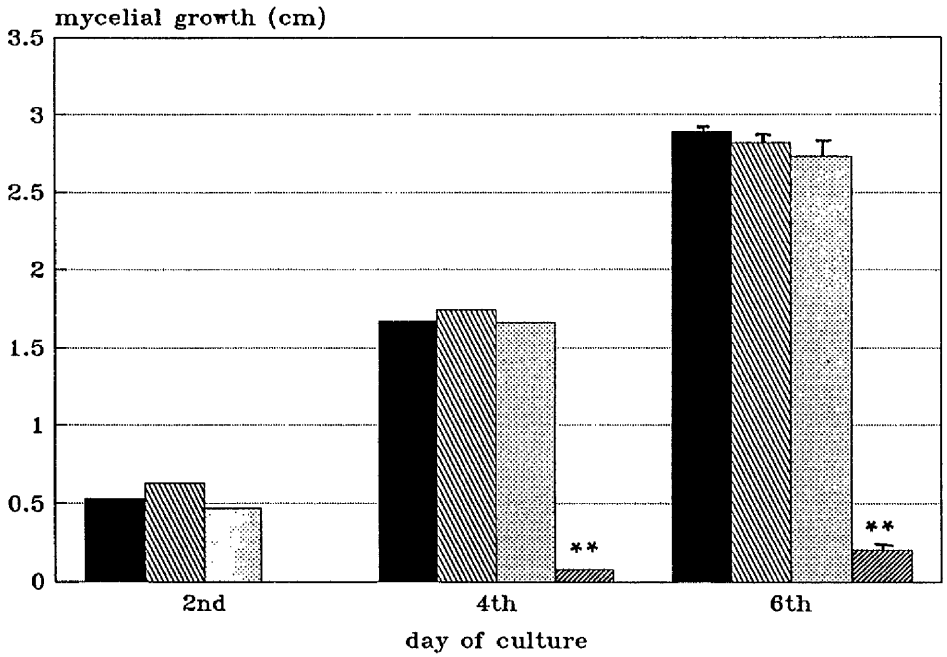


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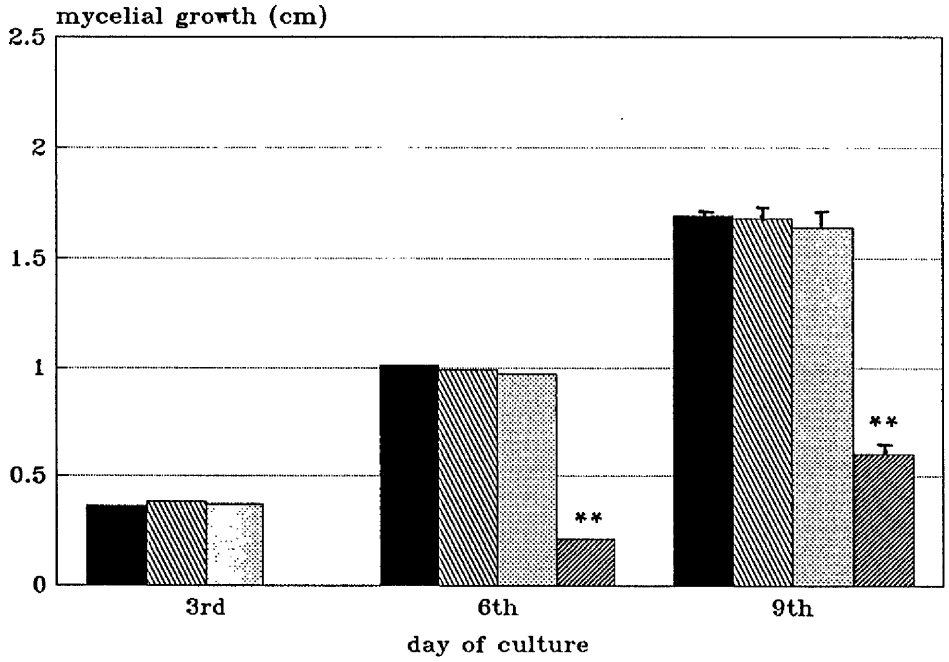
Figure 48



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3.4.3. Effect of components of oregano oil on fungal growth of *P.avenae*

The growth of *P.avenae* was not significantly affected by γ-terpinene. The reduction in growth following exposure to p-cymene was small but significant. The substantial reduction following growth in thymol and the complete inhibition of growth due to carvacrol were also highly significant (Figure 47 ; Statistics shown in Table 32 in Appendix 7). The plate 17 shows the slight growth of *P.avenae* on thymol, the growth of *P.avenae* on the plug, but not on the medium containing carvacrol, and the total inhibition (no growth either on the medium or on the plug) on medium containing thymol + carvacrol.

3.4.4. Effect of components of oregano oil on fungal growth of *P.oryzae*

The growth of *P.oryzae* was not significantly affected by γ-terpinene and p-cymene. The large reduction in growth on medium attended with thymol and the complete inhibition on carvacrol, were highly significant (Figure 48 ; Statistics shown in Table 33 in Appendix 33).

The plate 18 shows the slight growth of *P.oryzae* on thymol and the complete inhibition on carvacrol and carvacrol + thymol.

Chapter 4

DISCUSSION

In the present study, quite different results were observed in the *in vitro* and *in vivo* experiments. The *in vitro* studies showed clearly the efficacy of both oils against fungal pathogens, while the results of the *in vivo* studies were less clear cut. This difference between *in vitro* and *in vivo* experiments may be the reason why papers dealing with *in vitro* effect of essential oils on mycelial growth are far more abundant than papers dealing with disease control by essential oils. In fact, despite exhaustive literature searches, no papers dealing with the *in vivo* effects of essential oils on plant diseases could be found. However, *in vitro* tests of essential oils against microorganisms have been much more oriented towards human pathogens and spoilage microorganisms than towards plant pathogens.

In 3 germination tests out of 4, the oil reduced spore germination. Thus, hyssop oil reduced germination of *B. fabae* conidia and *U. viciae-fabae* uredospores, and oregano oil reduced *B. fabae* conidial germination. The experiment dealing with the effect of oregano oil on germination of *U. viciae-fabae* uredospores should be repeated, since in the other experiments the oils reduced spore germination, while oregano oil actually increased germination of rust uredospores.

A suspension of 0.4% hyssop oil completely inhibited *in vitro* mycelial growth of both *P. avenae* and *P. oryzae*. In contrast, a suspension of just 0.01% oregano oil inhibited growth of the same fungi. Indeed, oregano oil was 40 times stronger than hyssop oil in inhibiting *P. avenae* and *P.*

oryzae mycelial growth.

This antifungal action was probably due mainly to the volatile components of the oils. The vapours emanating from the medium containing 0.4% hyssop oil or 0.04% oregano oil inhibited mycelial growth of *P. avenae* and *P. oryzae*. The vapours of oregano oil were 10 times stronger than the vapours of hyssop oil. Thus, Moleyar and Narasimhan (1986) underlined the fact that the volatile compounds could accumulate over the agar medium when they were incorporated into it. So the inhibition of fungal growth by volatile compounds in the agar medium probably reflected the combined activity of the vapours and the compounds incorporated in the medium. Because of this, these authors could explain why they obtained different inhibition in liquid medium. According to Zaika (1988), the degree of observed microbial inhibition depends on the methods employed to test for antimicrobial activity.

If the volatile nature of an oil can improve its antifungal activity , then Zaika (1988) stressed that this could also be a disadvantage, since the loss of the inhibiting substance via evaporation, following prolonged incubation, could occur and growth could take place. Indeed, Zaika (19988) concluded that essential oils in contact with microorganisms in the test medium possessed greater germicidal powers than they did in their vaporous state.

In the experiment performed to determine whether the effect of the oils was fungicidal or fungistatic, the concentrations of oils used were not high enough. Indeed, the

oregano oil did not completely inhibit fungal growth. Thus, the conclusion that hyssop oil has a fungistatic rather than a fungicidal effect, has to be made cautiously. This method, where the mycelium has already grown before the oil is added, seems to require the use of higher oil concentrations before any conclusion about the type of effect of the oil can be made. This could be related to the volatile nature of the oils, as discussed above.

The effect of the oil on fungal infection of plants is not so clear. Thus, the pre-inoculation treatment of broad bean and apple with oregano oil increased infection, while post-inoculation treatment of barley and a pre-inoculation treatment of apple with hyssop oil, and a post-inoculation treatment of apple with oregano oil, all decreased infections. In general, pre-inoculation treatment had either an increasing (oregano oil) or a decreasing (hyssop oil) effect, whereas post-inoculation treatments with either oregano or hyssop oils tended to decrease infection. Only very low concentrations could be applied because of the phytotoxic effect of the oils.

Maruzzella & Balter (1959) had performed only *in vitro* experiments and had not performed work *in vivo* when he suggested that essential oils might be added to fungicides to enhance their efficacy. Indeed, he was attempting apply to phytopathogenic microorganisms, a property discovered on human pathogens. Indeed, some essential oils enhance the antibacterial activity of certain antibiotics (Halbeisen,

1956). Given that the essential oils from both oregano and hyssop had little effect on fungal infection on plants, it was decided to examine whether the components of the oils might exert antifungal effects.

P. avenae was sensitive to a greater number of components of hyssop oil than was *P. oryzae*. Thus, *P. avenae* was sensitive to 12 components, or associations of components, compared to 9 for *P. oryzae*. Indeed, *P. oryzae* was only completely inhibited by 2 components or associations of components, while *P. avenae* was completely inhibited by 6 of them. All the associations which inhibited growth of *P. avenae* contained isopinocampheol in combination with other components or isopinocampheol alone, at a high concentration. As for *P. oryzae*, its growth was inhibited only by isopinocampheol alone at a high concentration and by the mixture of isopinocampheol, pinocamphone, ß-pinene and l-bornyl acetate. Isopinocampheol, the compound that is very likely to be the main component of hyssop oil, seems to have an important role in the antifungal effects of hyssop oil. But the addition of the other components to isopinocampheol is not less important : the strongest antifungal effect was achieved when the mixture of components was closer to the composition of hyssop oil.

There was not a great difference between the sensitivity of *P. avenae* and *P. oryzae* to oregano oil : *P. avenae* was sensitive to 5 components or associations of components of

oregano oil, while *P. oryzae* was sensitive to 4 of them. However, the same components completely inhibited both fungi : carvacrol and mixtures containing carvacrol. Thymol was also a very potent inhibitor of fungal growth.

Interestingly, Deans and Svoboda (1990) claimed that essential oils from plants containing phenolic monoterpenes were most effective at inhibiting microbial growth. Indeed, carvacrol and thymol have already been reported to be powerful inhibitors of fungal and bacterial growth (Hitokoto *et al.*, 1980). Their effect against phytopathogenic fungi like *Fusarium monoliforme* and *Rhizoctonia solani* has been reported by Yegen *et al*. Zaika (1988) even refers to Martindale 's work (1910) which showed that oregano, thymol and carvacrol were much more active than phenol in destroying microorganisms.

Kurita *et al* (1981) attributed this efficiency to the alkyl group added to the benzene ring of phenol. They noticed that the activity of the phenolic compounds appeared to be dependent upon the size of the added alkyl group : the bigger the alkyl group, the greater the antifungal activity. They also found that hydrocarbons, including ß-pinene and p-cymene, were almost ineffective in inhibiting the growth of any of the fungi they tested, even when they were used at a concentration of 2 mM (=about 0.3%). The present study gave similar results, except that p-cymene could slow down growth of *P. avenae*. In terms of antibacterial activity, Maruzzella *et al* (1963) suggested that oxides > ketones > acids > aldehydes > alcohols > esters > phenols > acetals >

hydrocarbons > lactones. Here phenol appeared to be more active than hydrocarbons, although they were not the most antibacterial compounds. Moreover, Maruzzella *et al* (1963) noticed that this ranking of the antimicrobial activity of aromatic chemical vapours against phytopathogenic bacteria was close to the activity observed on human pathogenic bacteria and fungi.

For both hyssop and oregano oil, the components which showed the strongest inhibitory effects were the major components of the oil : isopinocampheol from hyssop oil, and carvacrol and thymol from oregano oil. Indeed, Hitokoto (1980) also found that the inhibitory effects of 3 powdered spices were largely accounted for by the main component of the essential oils in each sample (eugenol from cloves, anethol from star anise seeds, thymol from thyme). Zaika (1988) agreed that the antiseptic power of spices and herbs could be attributed, in some cases, to the major components of the oil. In contrast, Carlton *et al*. (1992) suggested that separate components of the oil have differing modes of action which complement one another in the whole oil. They suggest that a complex oil would likely present a great barrier to pathogen adaptation, than would a relatively simple mixture of terpenes. Unfortunately, the additive and the synergistic effects of the oils studied in this thesis cannot be discussed further, since the single major component of oregano and hyssop oil, and their combination, were not examined in the same experiment.

The experiment examining the components of hyssop and oregano

oil had been carried on barley powdery mildew, only because of the lack of time. Nevertheless, some results were obtained, even if they were not in complete agreement with the results of the *in vitro* experiments. The *in vivo* screening gave ß-pinene as the only component with significant antifungal activity, while the *in vitro* screens showed ß-pinene to be much less effective. On the other hand, isopinocampheol did reduce infection by barley powdery mildew, even if the reduction was not significant. As a matter of fact, the reduction in barley powdery mildew infection by isopinocampheol occurred at each concentration and was constant, whether isopinocampheol was applied pre- or post-inoculation. So the *in vivo* effect of isopinocampheol tended to agree with the results of the *in vitro* experiments. Results obtained using with the components of hyssop and oregano oils were inconclusive, partly because of the level of infection on control plants.

From the work discussed above, we know that hyssop and oregano oils affected germination of fungal spores and mycelial growth. More precisely, the volatile components were probably responsible for the fungistatic effects observed. Moreover, among these components, isopinocampheol from hyssop oil, and carvacrol and thymol from oregano oil, were strongly antifungal. Indeed, the fact that the components which were active against mycelial growth are volatile is very important. In the *in vitro* experiments, the environment is closed (*i*.e. in a Petri dish), while no such containment

existed in the *in vivo* experiment. So the vapours were confined in the *in vitro* experiments, while they were able to diffuse away from the plant in the *in vivo* experiments. So the concentration of vapour at the leaf surface *in vivo* would have actually been lower than the concentration of vapour present in the Petri dish in *in vitro* experiments, even though the same concentration was used in each case. The application of higher oil concentration *in vivo* experiments was impossible due to phytotoxicity. Indeed, the phytotoxicity could explain the increase in infection observed in some treatments : the oil could have injured the cuticle of the leaf and facilitated penetration by the fungus.

The effects of the volatile components and the main components of the oils have not been tested on spore germination. It is possible that the components which might inhibit spore germination might not necessarily the same as the components which inhibited mycelial growth in the work described above. Moreover, the efficacy of a volatile component would be greater if applied just before or just after inoculation, when the concentration of the vapour would still be high at the inoculation site. In this way it might be possible to explain the effect of ß-pinene on infection, where ß-pinene was effective when it was sprayed 1 day before or 1 day after inoculation.

After noticing the inhibitory effect of hyssop and oregano oils on mycelial growth of *P.avenae* and *P.oryzae*, and on germination of *B.fabae* and *U.viciae-fabae*, it would be interesting and useful to observe the effects of the components of hyssop and oregano oil on these processes. Thus, the components which were active against barley powdery mildew and the components which inhibited mycelial growth could be compared to the components which inhibited germination. Further work could test the additive or even the synergistic effect of the addition of essential oils or essential oil components to common fungicides.

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LIST OF APPENDICES

Table 1 : GLOSSARY OF MEDICAL TERMS

Analgesic : relieves or diminishes pain

Antiseptic : destroys germs and microorganisms that produce disease

Antispasmodic : relieves or prevents involuntary muscular spasms

Carminative : expels gas from the intestines

Cicatrizing : accelerates the formation of scar tissue

Diuretic : promotes the flow of urine

Emmenagogue : restores the menstrual flow

Expectorant : clears out phlegm from the chest by coughing

Resolvent : a preparation to soothe inflammatory conditions

Stomachic : aids the stomach action

Vermifuge : a medicament to expel intestinal parasites

Table 2 : Effect of hyssop oil applied pre-inoculation, on infection of barley seedlings with powdery mildew

Oil concentration	% of leaf area infected		
	1 day before	2 days before	3 days before
0%	16.3 ± 2.19	44.5 ± 5.24	34.5 ± 5.75
0.01%	15.8 ± 2.15	43.5 ± 4.78	26.5 ± 3.25
0.05%	27.8 ± 4.93	25.5 ± 2.93	48.0 ± 4.90
0.1%	14.5 ± 2.17	47.0 ± 5.54	32.5 ± 2.61
0.2%	36.3 ± 5.93	51.5 ± 4.41	16.9 ± 4.30

SED(df=135) = ± 6.05

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	10377	5188	
oil concentration	4	724	181	
interaction	8	11915	1489	8.13 *
error	135	24756	183	
total	149	47772		

Table 3 : Effect of oregano oil applied pre-inoculation, on infection of barley seedlings with powdery mildew

Oil concentration	% of leaf area infected		
	1 day before	2 days before	3 days before
0%	16.7 ± 2.15	27.5 ± 2.50	13.9 ± 4.32
0.005%	24.5 ± 3.69	33.0 ± 4.23	30.8 ± 4.19
0.01%	8.4 ± 2.10	22.5 ± 5.44	38.5 ± 6.24
0.05%	23.5 ± 5.28	24.0 ± 4.40	37.9 ± 6.13
0.1%	32.0 ± 4.10	16.3 ± 2.31	32.5 ± 4.90

SED(df=135) = ± 6.13

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	2401	1201	
oil concentration	4	2086	522	
interaction	8	6589	824	4.38 ***
error	135	25409	188	
total	149	36485		

Table 4 : Effect of hyssop oil applied post-inoculation, on infection of barley seedlings with powdery mildew

Oil concentration	% of leaf area infected		
	1 day after	2 days after	3 days after
0%	36.5 ± 3.88	16.1 ± 2.28	21.0 ± 4.20
0.01%	18.9 ± 3.60	16.5 ± 3.34	19.1 ± 2.50
0.05%	9.8 ± 2.05	12.9 ± 2.86	17.5 ± 2.80
0.1%	17.1 ± 1.79	18.5 ± 2.89	20.5 ± 2.73
0.2%	24.0 ± 4.00	17.0 ± 1.11	16.0 ± 1.80

SED(df=135) = ± 4.13

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	640.4	320.2	
oil concentration	4	1874.2	468.6	
interaction	8	2408.2	301.0	3.52 ***
error	135	11526.6	85.4	
total	149	16449.4		

Table 5 : Effect of oregano oil applied post-inoculation, on infection of barley seedlings with barley powdery mildew

Oil concentration	% of leaf area infected		
	1 day after	2 days after	3 days after
0%	21.0 ± 4.82	10.2 ± 1.77	24.5 ± 1.38
0.005%	38.0 ± 5.07	38.5 ± 7.49	17.8 ± 2.97
0.01%	35.5 ± 4.80	14.5 ± 1.70	27.2 ± 1.69
0.05%	21.0 ± 20.00	8.4 ± 0.70	25.5 ± 2.29
0.1%	20.5 ± 20.00	1.6 ± 0.67	8.7 ± 1.30

SED(df=135) = ± 4.60

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	3573	1787	
oil concentration	4	7652	1913	
interaction	8	5498	687	6.48 ***
error	135	14368	106	
total	149	31092		

Table 6 : Effect of hyssop oil applied pre-inoculation, on infection of broad bean with rust

Oil concentration	number of rust pustules per cm2 of leaf		
	1 day before	2 days before	3 days before
0%	3.48 ± 0.898	5.27 ± 1.550	4.46 ± 1.370
0.01%	3.87 ± 0.265	3.07 ± 0.437	4.18 ± 1.150
0.05%	2.00 ± 0.628	3.96 ± 0.627	2.53 ± 0.821
0.1%	3.6 ± 1.46	5.18 ± 1.460	5.74 ± 2.980
0.2%	5.6 ± 1.15	3.48 ± 0.935	2.60 ± 0.567
SED(df=45) = ± 1.78			

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	2.32	1.16	0.118
oil concentration	4	27.68	6.92	1.093
interaction	8	44.00	5.50	0.869
error	45	285.00	6.33	
total	59	359.00		

Table 7 : Effect of oregano oil applied pre-inoculation, on infection of broad bean with rust

Oil concentration	number of rust pustules per cm2 of leaf		
	1 day before	2 days before	3 days before
0%	2.20 ± 0.495	2.64 ± 1.000	3.98 ± 0.971
0.005%	3.40 ± 1.540	2.92 ± 0.654	2.83 ± 0.421
0.01%	2.40 ± 0.139	2.90 ± 0.888	4.23 ± 1.610
0.05%	3.08 ± 0.553	4.71 ± 0.602	5.02 ± 1.340
0.1%	4.05 ± 1.580	4.35 ± 1.140	4.29 ± 0.822

SED(df=45) = ± 1.43

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	10.89	5.44	0.118
oil concentration	4	20.86	5.22	1.093
interaction	8	12.76	1.59	0.869
error	45	185.92	4.13	
total	59	230.43		

Table 8 : Effect of hyssop oil applied post-inoculation, on infection of broad bean with rust

Oil concentration	number of rust pustules per cm2 of leaf		
	1 day before	2 days before	3 days before
0%	2.28 ± 1.170	3.95 ± 1.060	1.60 ± 0.671
0.01%	1.74 ± 0.440	1.12 ± 0.389	1.44 ± 0.835
0.05%	1.20 ± 0.831	0.99 ± 0.272	1.90 ± 0.444
0.1%	0.43 ± 0.152	3.34 ± 0.876	1.84 ± 0.669
0.2%	1.50 ± 0.427	1.53 ± 0.264	1.43 ± 0.681

SED(df=45) = ± 0.96

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	6.03	3.01	1.635
oil concentration	4	12.79	3.20	1.739
interaction	8	25.16	3.14	1.707
error	45	82.81	1.84	
total	59	126.78		

Table 9 : Effect of oregano oil applied post-inoculation, on infection of broad bean with rust

Oil concentration	number of rust pustules per cm2 of leaf		
	1 day before	2 days before	3 days before
0%	1.68 ± 0.449	3.99 ± 1.190	2.69 ± 0.227
0.005%	2.28 ± 0.374	3.59 ± 0.208	3.21 ± 0.500
0.01%	2.62 ± 1.360	5.50 ± 1.120	3.50 ± 0.885
0.05%	2.52 ± 0.847	6.97 ± 2.590	3.91 ± 0.546
0.1%	0.96 ± 0.270	2.16 ± 0.738	2.55 ± 1.140
SED(df=45) = ± 1.44			

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
spraying date	2	59.02	29.51	7.094 **
oil concentration	4	47.56	11.89	2.858 *
interaction	8	19.52	2.44	0.586
error	45	187.34	4.16	
total	59	313.44		

Table 10 : Effect of hyssop oil on infection of apple seedling with powdery mildew

Oil concentration	% of leaf area infected	
	Pre-inoculation	Post-inoculation
0%	43.8 ± 14.30	17.50 ± 4.33
0.01%	38.7 ± 13.30	43.75 ± 9.44
0.02%	46.2 ± 12.10	35.0 ± 12.6
0.04%	32.5 ± 11.10	52.50 ± 7.5
0.08%	32.5 ± 13.60	41.3 ± 10.1
0.16%	36.25 ± 5.54	32.50 ± 7.77
SED(df=36) = ± 15.02		

Two-way analysis of variance based on the assessment on the 17th day after the inoculation

SOURCE OF VARIATION	DF	SS	MS	F
day of spraying	1	19	19	0.04
concentration	5	848	170	0.37
interaction	5	2644	529	0.11
error	36	16237	451	
total	47	19748		

Table 11 : Effect of oregano oil on infection of apple seedling with powdery mildew

Oil concentration	% of leaf area infected			
	Pre-inoculation		Post-inoculation	
0%	18.7	± 8.26	33.7	± 6.88
0.005%	38.7	± 4.27	32.5	± 5.95
0.01%	24.0	± 9.27	41.3	±12.60
0.02%	32.5	±11.10	25.0	± 3.54
0.04%	30.0	±13.40	13.7	± 5.15
0.08%	35.0	±16.70	13.7	± 3.75
0.16%	20.0	± 4.08	28.7	± 8.98
SED(df=36) = ± 13.23				

Two-way analysis of variance based on the assessment on the 17th day after the inoculation

SOURCE OF VARIATION	DF	SS	MS	F
day of spraying	1	120	120	0.091
concentration	5	1067	214	0.59
interaction	5	2547	509	1.141
error	36	12595	350	
total	47	16329		

Table 12 : Effect of the components of hyssop oil on infection of barley with powdery mildew

Oil concentration	% leaf area infected		
	Pre-inoculation	Post-inoculation	Mean
control	6.40	6.60	6.50
1BA LC	3.00	4.40	3.70
iso LC	3.50	5.00	4.25
iso NC	3.70	4.70	4.20
iso HC	6.10	4.20	5.15
BP	2.30	4.40	3.35
iso+1BA	8.00	10.10	9.05
5% ether	4.80	0.60	2.70
1BA NC	7.00	4.10	5.55
1BA HC	10.20	5.50	7.85
pino LC	4.90	7.00	5.95
25% ether	6.10	3.20	4.65
pino NC	6.80	5.80	6.30
pino HC	5.70	7.70	6.70
iso+pino	4.40	1.90	3.15
Mean	5.53	5.01	5.27

SED components = 1.410
SED sprayingdate = 0.515
SED interaction = 1.994

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	VR	F. PR
rep. stratum	9	108.56	12.06		
components	14	918.78	65.63	3.30	< 0.001
spraying date	1	19.76	19.76	0.99	0.320
interaction	14	429.69	30.69	1.54	0.096
error	261	5190.34	19.89		
total	299	6667.13			

Table 13 : Effect of the components of oregano oil on infection of barley with powdery mildew

Oil concentration	% leaf area infected	
	Pre-inoculation	Post-inoculation
control	8.90	3.50
0.36% ether	2.10	6.20
thymol LC	10.60	1.50
carvacrol LC	16.20	4.30
p-cymene	9.00	9.30
0.72% ether	4.50	2.60
thymol NC	5.70	7.20
carvacrol NC	21.60	3.10
thymol + carcacrol	4.80	3.50
1.5% ether	3.40	5.00
thymol HC	11.30	4.80
carvacrol HC	2.50	4.60

SED components = 1.792
SED sprayingdate = 0.732
SED interaction = 2.534

Two-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	VR	F. PR
rep. stratum	9	387.73	43.08		
components	11	1834.88	166.81	5.20	< 0.001
spraying date	1	843.75	843.75	26.28	< 0.001
interactiopn	11	2503.75	227.61	7.09	< 0.001
error	207	6645.87	32.11		
total	239	12215.98			

Table 14 : Effect of hyssop oil on germination of spores of *Botrytis fabae* and *Uromyces viciae-fabae*

Oil concentration	% spore germination	
	Botrytis fabae	*Uromyces viciae-fabae*
control	0.9580 ± 0.0102	0.756 ± 0.0506
0.1%	0.8780 ± 0.0218	0.360 ± 0.103
0.4%	0.8680 ± 0.0139	0.3440 ± 0.0403
SED(df=12)	± 0.023	± 0.099

Botrytis fabae : One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F	P
concentration	2	0.0243	0.0122	9.46	0.003
error	12	0.0154	0.0013		
total	14	0.0398			

Uromyces viciae-fabae : One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F	P
concentration	2	0.545	0.272	11.11	0.002
error	12	0.294	0.024		
total	14	0.839			

Table 15 : Effect of oregano oil on germination of spores of *Botrytis fabae* and *Uromyces viciae-fabae*

Oil concentration	% spore germination	
	Botrytis fabae	*Uromyces viciae-fabae*
control	0.952 ± 0.008	0.820 ± 0.050
0.01%		0.862 ± 0.030
0.04%	0.900 ± 0.042	0.874 ± 0.028
0.16%	0.727 ± 0.064	
SED(df=12)	± 0.056	± 0.053

Botrytis fabae : One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F	P
concentration	2	0.1193	0.0597	7.65	0.008
error	12	0.0858	0.0079		
total	14	0.2051			

Uromyces viciae-fabae : One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F	P
concentration	2	0.0080	0.0040	0.58	0.575
error	12	0.0832	0.0069		
total	14	0.0912			

Table 16 : Parameters of the regression equations of *P.avenae* growth (on media containing various concentrations of hyssop oil) with the corresponding standard deviation and t-ratio.

Oil concentration	const	stdev	t-rat	grad	stdev	t-rat
0%	-1.310	0.856	-1.53	0.703	0.167	4.19
0.01%	-1.196	0.240	-4.98	0.723	0.047	15.34
0.05%	-1.136	0.294	-3.86	0.415	0.057	7.19
0.1%	-0.500	0.073	-6.79	0.125	0.014	8.66

const : constant / stdev : standard deviation / t-rat : t-ratio / grad : gradient

One-way analysis of variance based on the final assessment

SOURCE OF VARIATION	DF	SS	MS	F
factor	4	25.532	6.3883	131.1
error	10	0.487	0.048	
total	14	26.040		

Table 17 : Parameters of the regression equations of *P.oryzae* growth (on media containing various concentrations of hyssop oil) with the corresponding standard deviation and t-ratio.

Oil concentration	const	stdev	t-rat	grad	stdev	t-rat
0%	-0.219	0.035	-6.21	0.279	0.005	52.43
0.01%	-0.140	0.111	-1.26	0.250	0.016	14.91
0.05%	-0.453	0.040	-11.0	0.263	0.006	42.70
0.1%	-0.600	0.091	-6.58	0.237	0.013	17.21

const : constant / stdev : standard deviation / t-rat : t-ratio / grad : gradient

One-way analysis of variance based on the final assessment

SOURCE OF VARIATION	DF	SS	MS	F
factor	4	12.338	3.084	81.83
error	10	0.376	0.037	
total	14	12.714		

Table 18 : Parameters of the regression equations of *P.avenae* growth (on media containing various concentrations of oregano oil) with the corresponding standard deviation and t-ratio.

Oil concentration	const	stdev	t-rat	grad	stdev	t-rat
0%	-0.473	0.343	-1.38	0.596	0.079	7.51
0.005%	-0.001	0.018	0.06	0.055	0.004	12.78

const : constant / stdev : standard deviation / t-rat : t-ratio / grad : gradient

One-way analysis of variance based on the final assessment

SOURCE OF VARIATION	DF	SS	MS	F
factor	2	17.798	8.899	105.8
error	6	0.505	0.084	
total	8	18.304		

Table 19 : Parameters of the regression equations of *P.oryzae* growth (on media containing various concentrations of oregano oil) with the corresponding standard deviation and t-ratio.

Oil concentration	const	stdev	t-rat	grad	stdev	t-rat
0%	-0.184	0.039	-4.70	0.277	0.006	45.83
0.005%	-0.398	0.075	-5.36	0.153	0.011	13.27

const : constant / stdev : standard deviation / t-rat : t-ratio / grad : gradient

One-way analysis of variance based on the final assessment

SOURCE OF VARIATION	DF	SS	MS	F
factor	2	8.1369	4.0684	888.7
error	6	0.027	0.004	
total	8	8.1644		

Table 20 : Effect of the volatile components of hyssop oil on the growth of *P.avenae* : one-way analysis of variance based on the final assessment.

SOURCE OF VARIATION	DF	SS	MS	F
factor	3	0.8390	0.279	117.7
error	8	0.019	0.002	
total	11	0.8580		

Table 21 : Effect of the volatile components of hyssop oil on the growth of *P.oryzae* : one-way analysis of variance based on the final assessment.

SOURCE OF VARIATION	DF	SS	MS	F
factor	3	3.4139	1.137	571.3
error	8	0.0159	0.002	
total	11	3.4298		

Table 22 : Effect of the volatile components of oregano oil on the growth of *P.avenae* : one-way analysis of variance based on the final assessment.

SOURCE OF VARIATION	DF	SS	MS	F
factor	3	0.2937	0.097	29.52
error	8	0.0265	0.003	
total	11	0.3202		

Table 23 : Effect of the volatile components of oregano oil on the growth of *P.oryzae* : one-way analysis of variance based on the final assessment.

SOURCE OF VARIATION	DF	SS	MS	F
factor	3	1.4860	0.495	245.6
error	8	0.0161	0.002	
total	11	1.5022		

Table 24 : Effect of ß-pinene and l-bornyl acetate on the growth of *P.avenae*

Components	Mycelial growth
A : control	2.67 ± 0.330
B : ß-pinene 0.004%	3.42 ± 0.029
C : ß-pinene 0.008%	3.13 ± 0.120
D : l-bornyl acetate 0.002%	3.18 ± 0.087
E : l-bornyl acetate 0.004%	2.44 ± 0.920

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	4	1.944	0.486	0.83
error	10	5.852	0.585	
total	14	7.797		

Dunnett's test

LSD = 1.854 (P =0.05)

B-A = 0.75

C-A = 0.46

D-A = 0.51

E-A = -0.23

Table 25 : Effect of 1-bornyl acetate at various concentrations on the growth of *P.avenae*

Components	Mycelial growth
A : control	1.99 ± 0.290
B : 1-bornyl acetate 0.005%	2.23 ± 0.500
C : 1-bornyl acetate 0.01%	1.23 ± 0.180
D : 1-bornyl acetate 0.02%	1.40 ± 0.130
E : 1-bornyl acetate 0.04%	1.37 ± 0.330
F : 1-bornyl acetate 0.08%	0.35 ± 0.070

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	5	6.468	1.294	5.21
error	12	2.981	0.248	
total	17	9.449		

Dunnett's test

LSD = 1.220 (P = 0.05) (*)
1.581 (P = 0.01) (**)

B-A = 0.24
C-A = -0.76
D-A = -0.59
E-A = -0.62
F-A = -1.64 **

Table 26 : Effect of various components and mixtures of components of hyssop oil on the growth of *P.avenae*

Components	Mycelial growth	
A : control	3.34	± 0.072
B : iso 0.05%	0.79	± 0.107
C : iso HC	0.00	± 0.000
D : pino 0.02%	2.94	± 0.129
E : pino HC	0.70	± 0.404
F : pino + iso	0.24	± 0.046
G : pino + ßP	2.92	± 0.107
H : iso + lBA	0.00	± 0.000
I : iso + ßP	0.00	± 0.000

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	8	48.7279	6.0910	86.96
error	18	1.2608	0.07	
total	26	49.9887		

Dunnett's test

LSD = 0.659 (P = 0.05) (*)
0.821 (P = 0.01) (**)

B-A = -2.55 **
C-A = -3.34 **
D-A = -0.4
E-A = -2.64 **
F-A = -3.1 **
G-A = -0.42
H-A = -3.34 **
I-A = -3.34 **

Table 27 : Effect of various combinations of components of hyssop oil on the growth of *P.avenae*

Components	Mycelial growth	
A : control	3.02	± 0.029
B : pino + lBA	2.83	± 0.049
C : pino + ßP + lBA	2.89	± 0.020
D : iso + ßP + pino	0.23	± 0.186
E : pino + iso + lBA	0.00	± 0.000
F : iso + ßP + lBA	0.00	± 0.000
G : iso + ßP + pino + lBA	0.00	± 0.000

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	6	42.164	7.027	430.3
error	14	0.228	0.016	
total	20	42.393		

Dunnett's test

LSD = 0.315 (P = 0.05) (*)
0.400 (P = 0.01) (**)

B-A = -0.19		F-A = -3.02	**
C-A = -0.13		G-A = -3.02	**
D-A = -2.79	**		
E-A = -3.02	**		

Table 28 : Effect of ß-pinene and l-bornyl acetate on the growth of *P.oryzae*

Components	Mycelial growth
A : control	2.20 ± 0.000
B : ß-pinene 0.004%	2.19 ± 0.010
C : ß-pinene 0.008%	2.35 ± 0.023
D : l-bornyl acetate 0.002%	2.22 ± 0.010
E : l-bornyl acetate 0.004%	2.23 ± 0.020

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	4	0.047	0.0119	17.2
error	10	0.007	0.0007	
total	14	0.054		

Dunnett's test

LSD = 0.202 (P = 0.05) (*)
0.268 (P = 0.01) (**)

B-A = -0.01
C-A = 0.15
D-A = 0.02
E-A = 0.03

Table 29 : Effect of 1-bornyl acetate at various concentrations on the growth of *P.oryzae*

Components	Mycelial growth
A : control	1.80 ± 0.000
B : 1-bornyl acetate 0.005%	1.83 ± 0.020
C : 1-bornyl acetate 0.01%	1.84 ± 0.013
D : 1-bornyl acetate 0.02%	1.88 ± 0.010
E : 1-bornyl acetate 0.04%	1.82 ± 0.029
F : 1-bornyl acetate 0.08%	1.37 ± 0.130

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	5	0.537	0.1075	11.68
error	12	0.110	0.0092	
total	17	0.648		

Dunnett's test

LSD = 0.235 (P = 0.05) (*)
0.305 (P = 0.01) (**)

B-A = 0.03
C-A = 0.04
D-A = 0.08
E-A = 0.02
F-A = -0.43 **

Table 30 : Effect of various components and mixtures of components of hyssop oil on the growth of *P.oryzae*

Components	Mycelial growth
A : control	1.65 ± 0.013
B : iso 0.05%	1.16 ± 0.049
C : iso HC	0.00 ± 0.000
D : pino 0.02%	1.62 ± 0.010
E : pino HC	1.07 ± 0.062
F : pino + iso	1.25 ± 0.029
G : pino + ßP	1.59 ± 0.010
H : iso + lBA	0.55 ± 0.294
I : iso + ßP	1.07 ± 0.057

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	8	7.065	0.883	27.2
error	18	0.584	0.032	
total	26	7.649		

Dunnett's test

LSD = 0.449 (P = 0.05) (*)
0.560 (P = 0.01) (**)

B-A = -0.49	*	F-A = -0.40	
C-A = -1.65	**	G-A = -0.06	
D-A = -0.03		H-A = -1.10	**
E-A = -0.58	**	I-A = -0.58	**

Table 31 : Effect of various combinations of components of hyssop oil on the growth of *P.oryzae*

Components	Mycelial growth
A : control	2.13 ± 0.066
B : pino + 1BA	2.21 ± 0.010
C : pino + ßP + 1BA	2.20 ± 0.040
D : iso + ßP + pino	1.86 ± 0.033
E : pino + iso + 1BA	1.18 ± 0.312
F : iso + ßP + 1BA	0.62 ± 0.302
G : iso + ßP + pino + 1BA	0.00 ± 0.000

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	6	13.933	2.322	27.62
error	14	1.176	0.084	
total	20	15.110		

Dunnett's test

LSD = 0.715 (P = 0.05) (*)
0.909 (P = 0.01) (**)

B-A = 0.08
C-A = 0.07
D-A = -0.27
E-A = -0.95 **
F-A = -1.51 **
G-A = -2.13 **

Table 32 : Effect of various components of oregano oil on the growth of *P.avenae*

Components	Mycelial growth
A : control	2.89 ± 0.020
B : γ-terpinene	2.81 ± 0.029
C : p-cymene	2.73 ± 0.052
D : thymol	0.20 ± 0.017
E : carvacrol	0.00 ± 0.000
F : thymol + carvacrol	0.00 ± 0.000
G : γ-terpinene + p-cymene + thymol + carvacrol	0.00 ± 0.000

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	6	39.366	6.561	3541.95
error	14	0.025	0.001	
total	20	39.391		

Dunnett's test

LSD = 0.106 (P = 0.05) (*)
0.135 (P = 0.01) (**)

B-A = -0.09		E-A = -2.89	**
C-A = -0.16	**	F-A = -2.89	**
D-A = -2.69	**	G-A = -2.89	**

Table 33 : Effect of various components of oregano oil on the growth of *P.oryzae*

Components	Mycelial growth
A : control	1.68 ± 0.013
B : γ-terpinene	1.67 ± 0.023
C : p-cymene	1.64 ± 0.046
D : thymol	0.60 ± 0.017
E : carvacrol	0.00 ± 0.000
F : thymol + carvacrol	0.00 ± 0.000
G : γ-terpinene + p-cymene + thymol + carvacrol	0.00 ± 0.000

One-way analysis of variance

SOURCE OF VARIATION	DF	SS	MS	F
factor	6	12.677	2.112	1540.70
error	14	0.019	0.001	
total	20	12.696		

Dunnett's test

LSD = 0.091 (P = 0.05) (*)
0.116 (P = 0.01) (**)

B-A = -0.01		E-A = -1.68	**
C-A = -0.04		F-A = -1.68	**
D-A = -1.08	**	G-A = -1.68	**