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Entitled

Modeling of the diffusion effect of essential oil on the kinetics of germination and fungal growth.

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Dedication

Above all, we would like to express our gratitude to Allah, the Almighty, for granting us the health, courage, determination, and patience needed to accomplish this task.

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ملخص

تثير الإضافات الكيميائية مخاوف صحية وبيئية للمستهلكين، مما يدفع إلى التحول نحو بدائل أكثر صحة مثل الزيوت الأساسية. تهدف هذه الدراسة إلى التحقيق في تأثيرات انتشار الزيوت الأساسية من نباتين من النباتات الطبيعية ، وهما الثوم الجزائري والزعتر البري، على سرعة التنبت ونمو الفطريات لفطر Fusarium والعفن الأسود .A. niger طريقة استخلاص الزيوت المستخدمة كانت التقطير بالبخار باستخدام جهاز كليفنجر، وكانت طرق اختبار النشاط المضاد للفطريات هي الاتصال المباشر (مايكروديلوشن ، ماكروديلوشن) والتدخين. كانت سرعة نمو العفن الأسود أقل من 0.5 ملم / ساعة، مع أعلى معدلات تلاحظ للسيطرة وأقل تركيز للزيتين الأساسيين 1 و 2. بالنسبة للفطرياتFusarium ، كانت معدلات النمو (μ) دائماً أقل من 1 ملم / ساعة في جميع الظروف. كانت السيطرة تظهر أعلى معدل عند 0.709 ± 0.040 ملم / ساعة، تليها الزيت 1 بمعدل 0.780 ± 0.713 ملم / ساعة، والزيت 2 بمعدل 0.630 ± 0.029 ملم / ساعة لأقل تركيز من الزيت الأساسي. يظهر الزيت الأساسي 1 فعالية أكبر مقارنة بالزيت الأساسي 2 ضد الفطريات الاثنين المدروسة. أظهر الفطر Fusarium حساسية متزايدة بالنسبة للعفن .A. niger يوضح الزيت الأساسي 1 فعالية أكبر مقارنة بالزيت الأساسي 2 ضد الفطريات الاثنين المدروسة. أظهر الفطر Fusarium حساسية متزايدة بالنسبة للعفن .A. niger تخلص الدراسة إلى أن الخصائص الاستريوكيميائية لمركبات الزيوت الأساسية وتركيب البوليسكاريدات في جدران الخلايا الفطرية تلعب دوراً حاسماً في تحديد حساسيتها أو مقاومتها لمكونات الزيوت الأساسية النشطة. أظهر الزيت الأساسي 1 فعالية أعلى مقارنة بالزيت الأساسي 2 ضد كلتا الفطريات، مع الفطر Fusarium يظهر حساسية أكبر منA.niger .

الكلمات المفتاحية: حركية الإنبات, النمو الفطري, نمذجة تأثير انتشار الزيت العطري, التلامس المباشر, الزيت العطري, التعقيم بالدخان.

Abstract

Chemical additives pose health and environmental concerns for consumers, driving a shift towards healthier alternatives like essential oils. This study investigates the diffusion effects of essential oils from two plants, T. algeriensis and O. vulgare, on the kinetics of germination and fungal growth of Fusarium sp. and A. niger.
the used extraction method was Hydrodistillation using the Clevenger apparatus, the antifungal testing activity methodes were direct contact (Broth Macrodilution , Microdilution) and Fumigation . The growth rate of A. niger was less than 0.5 mm/h, with the highest rates observed for the control and the lowest concentration of both EO1 and EO 2.
For Fusarium sp., growth rates (µ) were consistently below 1 mm/h across all conditions. The control exhibited the highest rate at 0.709 ± 0.040 mm/h, followed by oil 1 at 0.780 ± 0.713 mm/h, and oil 2 at 0.630 ± 0.029 mm/h for the lowest concentration of essential oil.
EO 1 demonstrates greater efficacy compared to EO 2 against the two fungal species examined. Fusarium sp. displayed heightened sensitivity relative to A. niger. Essential oil 1 demonstrates greater efficacy compared to EO 2 against the two fungal species examined. Fusarium sp. displayed heightened sensitivity relative to A niger.
The study concludes that the stereochemical characteristics of EOs compounds and the composition of polysaccharides in fungal cell walls play a crucial role in determining their susceptibility or resistance to essential oil bioactive components. E0 1 exhibited superior effectiveness compared to EO 2 across both fungal species, with Fusarium sp. displaying greater sensitivity than A. niger.

Keywords: kinetics of germination, fungal growth, Modeling of the diffusion effect of essential oil, direct contact, essential oil, Fumigation

Résumé

Les additifs chimiques posent des préoccupations pour la santé et l'environnement des consommateurs, ce qui entraîne un changement vers des alternatives plus saines comme les huiles essentielles. Cette étude examine les effets de diffusion des huiles essentielles de deux plantes, T. algeriensis et O. vulgare, sur la cinétique de germination et de croissance fongique de Fusarium sp. et A. niger. La méthode d'extraction utilisée était l'hydrodistillation avec l'appareil de Clevenger, et les méthodes d'activité antifongique testées étaient le contact direct (macrodilution en bouillon, microdilution) et la fumigation. Le taux de croissance de A. niger était inférieur à 0,5 mm/h, avec les taux les plus élevés observés pour le témoin et la plus faible concentration des deux HE1 et HE2. Pour Fusarium sp., les taux de croissance (μ) étaient systématiquement inférieurs à 1 mm/h dans toutes les conditions. Le témoin présentait le taux le plus élevé à 0,709 ± 0,040 mm/h, suivi de l'huile 1 à 0,780 ± 0,713 mm/h, et l'huile 2 à 0,630 ± 0,029 mm/h pour la plus faible concentration d'huile essentielle. L'HE1 démontre une plus grande efficacité par rapport à l'HE2 contre les deux espèces fongiques examinées. Fusarium sp. a montré une sensibilité accrue par rapport à A. niger. L'huile essentielle 1 présente une plus grande efficacité par rapport à l'HE2 contre les deux espèces fongiques examinées. Fusarium sp. a montré une sensibilité accrue par rapport à A. niger. L'étude conclut que les caractéristiques stéréochimiques des composés des huiles essentielles et la composition des polysaccharides dans les parois cellulaires fongiques jouent un rôle crucial dans la détermination de leur sensibilité ou de leur résistance aux composants bioactifs des huiles essentielles. L'HE1 a présenté une efficacité supérieure par rapport à l'HE2 pour les deux espèces fongiques, avec Fusarium sp. montrant une sensibilité plus grande que A. niger

Mots Clés: **Cinétique de la germination, Croissance fongique, Modélisation de l'effet de diffusion des huiles essentielles, Contact direct, Huile essentielle, Fumigation**

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Introduction

Introduction

Modern consumer demands are a significant driving force within the contemporary food industry. Consumers prioritize healthy and safe food products that undergo minimal processing to retain their inherent natural properties. Nevertheless, some degree of processing remains inevitable to ensure the efficient transition of food from farm to table. However, ensuring food safety and quality remains a paramount concern throughout the processing chain. In this context, novel food processing and preservation technologies offer promising advancements. These technologies are lauded for their ability to deliver high-quality, safe food products while maintaining production efficiency. The integration of these novel technologies presents both exciting opportunities and intriguing challenges for the future of food processing. (Goyal, Veena, & Watharkar, 2023).

The contemporary industrial and economic landscape across nations, paramount attention is directed towards innovation, sustainability, and safety. This emphasis is congruent with the definition of sustainable development put forth by the United Nations World Commission on Environment and Development, which underscores the importance of satisfying current needs while safeguarding the capacity of future generations to fulfill their own. Within this framework, A design and development approach that prioritizes food system sustainability can resonate with consumers, offering a competitive advantage and fostering positive public relations opportunities.(French, 2004)

With the population steadily increasing and food demands escalating, there emerges a crucial need and an auspicious opportunity for the food industry to strike a balance between meeting market demands for food and addressing pressing environmental and social considerations.(Baldwin, 2015). The landscape of food preservation and processing has undergone significant evolution, presenting challenges that surpass those of previous eras. In response to contemporary needs, a range of innovative preservation methods is being devised to meet current demand, catering to economic efficiency and meeting consumer expectations in terms of nutritional value, taste, convenience, safety, absence of additives, cost-effectiveness, and environmental impact. Consequently, comprehending the intricate effects of each preservation approach on food has become paramount across various dimensions. (Rahman, 2012)

Polyphenols, a diverse class of secondary metabolites, are ubiquitous throughout the plant kingdom, found throughout various plant parts. Numerous studies have revealed that the unique chemical structure of plant-derived polyphenols confers a range of bioactivities, including antioxidant, antimicrobial, and preventative effects against chronic diseases The burgeoning field

of natural product development has positioned plant polyphenols as a research focus within food science due to their widespread presence in plants and diverse physiological functions. Notably, their exceptional properties present polyphenols as a viable alternative to synthetic additives currently used in the food industry for oils, seafood, meat, beverages, and food packaging materials.(Wu & Zhou, 2021).

There is an escalating demand within the food packaging sector for environmentally sustainable solutions, prompting heightened research and industry focus on sustainable, biodegradable, and edible materials. Various polymers, including starches, cellulose derivatives, chitosan/chitin, gums, proteins (both animal and plant-based), and lipids, are being integrated into edible films to enhance the longevity of food products. These materials offer advantages such as biocompatibility, effective moisture and gas barrier properties, non-toxicity, and environmental compatibility.(Mellinas et al., 2016)

Active, biodegradable, and edible packaging materials are crucial in the food industry due to increasing demand for sustainable alternatives that reduce disposal needs. However, achieving widespread commercial use requires advancements in scaling up production, addressing challenges like enhancing resistance to gases and liquids, assessing safety and biodegradability, and optimizing manufacturing techniques.(Jeevahan et al., 2020)

The scope of this study is the Modeling of the diffusion effect of essential oil on the kinetics of germination and fungal growth

Chapter I.
Bibliographic study

Chapitre I. Bibliographic study

I.1. Food preservation

I.1.1. Preservation motives

Foodborne diseases arise due to the consumption of food or beverages contaminated with deleterious agents, including physical, chemical, and biological contaminants. These illnesses encompass both foodborne intoxications and infections, which are occasionally misclassified as food poisoning. Among these agents, biological infectious agents hold particular significance, as they are responsible for the majority of foodborne diseases (OSHA, 2015).

Microbial food safety has risen to the forefront as a critical concern among consumers, food industries, and regulatory bodies globally. Intimately tied to the entirety of the food supply chain, encompassing production, processing, and distribution, microorganisms exert profound influence over the quality and safety of our dietary selections. While certain microbial species facilitate crucial food transformations, others present substantial hazards, underscoring the intricate balance between beneficial and detrimental microbial involvement in food systems (Pattanayaiying, H-Kittikun, & Cutter, 2015).

Preservation techniques in food science necessitate a comprehensive grasp of the complete food supply chain, spanning from cultivation through to distribution. This comprehensive strategy is fundamental for successful preservation and forms the foundation of food science and technology. Recognizing the particular characteristics needing preservation is vital, given their variability across different products (Gould, 1996).

For instance, in the process of food drying, aspects such as collapse and pore formation may have contrasting effects depending on the desired quality of the end product. Another significant consideration is the rationale behind the need for food preservation. The primary purposes of preserving food include addressing inadequacies in agricultural planning, creating value-added products, and offering dietary diversity (Desrosier & Desrosier, 1977).

I.1.2. Shelf life

Food spoilage occurs naturally, resulting in the gradual deterioration of color, texture, flavor, nutritional properties, and overall edibility. Consuming spoiled food can lead to illness and, in extreme cases, even death (Corradini, 2018). Taking into account their shelf life, food items can be categorized as (William H. Sperber, 2009):

a. *Perishable.* Typically spanning from several days to around three weeks, fall under the classification of perishable goods. This category encompasses items such as milk, dairy products, meats, poultry, eggs, and seafood. Without the implementation of appropriate preservation techniques, these commodities are susceptible to rapid deterioration.
b. *Semi-perishable.* Foods classified as semi-perishable have the capacity to be stored for an extended duration, typically around six months, when stored under ideal conditions. This subset of food items is categorized as semi-perishables. Examples encompass vegetables, fruits, cheeses, and potatoes.
c. *Non-perishable.* Non-perishable foods comprise a variety of natural and processed items characterized by an indefinite shelf life. These robust food items are capable of being stored safely for numerous years, or in some cases, even longer periods. Examples of non-perishable foods encompass dry beans, nuts, flour, sugar, canned fruits, mayonnaise, and peanut butter.

I.2. Food spoilage mechanisms

I.2.1. Physical spoilage

The initial type of spoilage that can affect food products arises from physical changes or instability. This includes damage such as bruising of fresh fruits and vegetables or the fracturing of dry, brittle items like potato chips and breakfast cereals. Bruising often occurs during transportation and distribution or as a result of dropping the products. Severe physical damage can render the product unacceptable to consumers (Moon et al., 2020). Moreover, other physical changes or instabilities typically involve moisture or mass transfer within the food. Changes in water content, whether through loss, gain, or migration, are common causes of food degradation. These changes can make the product unacceptable and often lead to microbial or chemical degradation. (Martinez & Whitaker, 1995)

I.2.2. Chemical spoilage

Food deterioration may also arise from chemical transformations involving its constituent elements such as proteins, lipids, and carbohydrates. The pace at which these chemical alterations occur is contingent upon various factors previously discussed, encompassing water activity, temperature, pH, and exposure to light or oxygen. Each reaction exhibits distinct optimal conditions; for instance, enzyme activity experiences a marked reduction at low water activity levels, particularly below the monolayer moisture threshold (Pyke & White, 2000).

I.2.3. Microbial spoilage

Bacteria, yeast, and mold are the main contributors to food spoilage. These microorganisms can modify the sensory characteristics of food, rendering it unsuitable or unappealing for consumption. They thrive in different food items, resulting in alterations in texture, flavor, aroma, or appearance.(Dilbaghi & Sharma, 2007).

a. Bacteria

Bacterial species that cause food spoilage are highly influenced by storage conditions, including temperature and packaging. In raw or fresh foods, spoilage in aerobic environments is typically caused by bacteria from the Pseudomonas genus, notably Pseudomonas fragi, Pseudomonas fluorescens, and Pseudomonas lundensis, which generate slime and unpleasant odors (Stanbridge & Davies, 1998). Lactic acid bacteria, including Lactobacillus and Leuconostoc species, along with Brochothrix thermosphacta, are significant spoilage organisms in vacuum-sealed or modified atmosphere packaging, leading to product souring. Spore-forming bacteria like Clostridium and Bacillus, particularly Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus pumilus, and Bacillus cereus, are linked to spoilage in bakery goods and other products.(Holzapfel, 1998).

b. Yeasts

Yeast spoilage was underestimated until the last century due to the relatively few foods and beverages in which yeasts surpass molds or bacteria in causing spoilage. The primary sources of food contamination by yeasts are the natural yeast populations present in raw ingredients and environmental contamination within manufacturing facilities. Once contamination occurs, yeasts significantly contribute to the spoilage of food and beverages, particularly through the alteration of fermented products. Yeasts employ various mechanisms in spoilage, including the production of lytic enzymes (such as lipases, proteases, and cellulases) and gas, the utilization of organic acids, and the induction of discoloration and off-flavors (Fleet, 2011).

c. Mold

Molds are filamentous fungi characterized by their lack of large fruiting bodies, unlike mushrooms. They play a crucial role in the decomposition and recycling of dead plant and animal matter in natural ecosystems. However, molds also pose a threat to a wide array of foods and materials valuable to humans. These fungi are particularly suited for growth within solid substrates, typically produce airborne spores, and depend on oxygen for their metabolic activities Chen (2022).

They can thrive across a broad spectrum of water activity (aw) values, pH levels, and temperatures by utilizing various substrates, including carbohydrates, organic acids, proteins, and lipids.(in't

Veld, 1996). As a result, molds demonstrate the capacity to proliferate in acidic substances like fruits or fruit juices(Lahlali, Serrhini, & Jijakli, 2005) , as well as in foods with moderate moisture levels such as bread and bakery items(Abellana et al., 1999). Additionally, molds exhibit growth potential in various food types including cereals, beverages, dairy products, and fermented goods, thereby contributing to the deterioration of a broad spectrum of food items.(Filtenborg, Frisvad, & Thrane, 1996)

The spoilage of food products by molds results in significant economic repercussions. Assessing these losses presents challenges as they vary based on factors such as geographical location, seasonal variations, and the specific type of product affected (Dantigny, Guilmart, & Bensoussan, 2005). Therefore, preventing mold spoilage is crucial for the food industry and can be achieved by minimizing contamination and mold growth within production facilities. Identifying and quantifying the factors contributing to mold contamination and growth is essential. Modeling tools, similar to those used for studying bacterial behavior, can be valuable for describing and understanding mold interactions with food matrices (Gibson & Hocking, 1997).

I.3. Factors determining microbial spoilage of foods

Food and beverage spoilage occurs due to the microbial action of various microorganisms. The microbial composition present in a specific food or beverage largely depends on the product's characteristics and its processing and storage methods. Factors influencing microbial growth in food can be classified into four categories: (Mossel et al., 1995)

a. *Intrinsic factors*: Intrinsic parameters are the physical, chemical, and structural characteristics inherent to the food itself. The most critical intrinsic factors include water activity, acidity, redox potential, available nutrients, and natural antimicrobial compounds.
b. *Extrinsic Factors:* Extrinsic parameters are environmental factors affecting the storage of food, particularly temperature, humidity, and atmospheric composition.

I.4. Bio-preservation

For many years, the food industry has employed a range of chemical agents to inhibit the proliferation of microorganisms responsible for food spoilage.(T. Mat thew Tay lor & Kanika Bhargava, 2019). The application of these chemicals, in conjunction with high-temperature processing, effectively extends the shelf life of foods by primarily disrupting the activities of pathogenic and spoilage microorganisms. However, this approach can adversely affect the organoleptic and sensory characteristics of certain foods (Mahmud & Khan, 2018).

As an alternative to traditional chemical and thermal preservation methods, there is a growing interest in defining novel preservation techniques like biopreservation. The aim is to ensure food safety without compromising quality standards, encompassing nutritional content as well as sensory attributes such as flavor, odor, color, and texture. This emphasis on meeting all these imperatives has spurred increased exploration into biopreservation methods (Anurova et al., 2019).

These naturally occurring preservatives represent bioactive compounds sourced from plants, animals, and beneficial microorganisms. They are increasingly recognized as credible substitutes for synthetic food additives. Many of these compounds originate from microorganisms,essential oils predominantly sourced from plants.(Smith-Palmer, Stewart, & Fyfe, 2001)

I.4.1. Spice and herbs

The terms "herbs" and "spices" have varied definitions in common languages, with the most widely accepted distinguishing herbs as originating from the green parts of a plant, such as stems and leaves. Unlike other plants used in food, herbs are typically employed in small quantities to impart flavor rather than substance to a dish. Spices, on the other hand, are distinct from herbs in that they are not derived from the green parts of plants but from other structures such as seeds, flowers, fruits, roots, or even bark.(Sánchez & Aznar, 2015)

Herbs and spices are widely distributed across the globe, with many originating from the Mediterranean region. Examples include rosemary, oregano, sage, thyme, peppermint, and garlic.(Christaki et al., 2012), Numerous extracts derived from herbs and spices exhibit antimicrobial activity against a broad spectrum of microorganisms, including bacteria, yeast, molds, and viruses. This antimicrobial efficacy is attributed to the presence of phytochemical constituents, notably phenolic compounds (Adeyinka & Richard, 2015).

I.4.2. Essential oils (EOs)

Essential oils, also referred to as volatile or ethereal oils, are aromatic oily liquids derived from various parts of plants, including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots, The composition of essential oils (EOs) from a specific plant species may vary across different harvesting seasons and geographical sources.(Bakkali et al., 2008).

There are notable differences between in vitro and in-food trials of plant-origin antimicrobials, primarily because only small percentages of essential oils (EOs) are acceptable in food products. Identifying the most effective essential oils depends on several factors, including the type of EO, its impact on organoleptic properties, composition, concentration, biological properties of the antimicrobial, the target microorganism, and the processing and storage conditions of the food

product in question (Gutierrez, Barry-Ryan, & Bourke, 2009). Despite some positive findings on the use of plant-origin natural antimicrobials in the food industry, two major challenges persist: the strong odors produced, particularly at high concentrations, and the increased cost of these materials (Proestos et al., 2008).

Studies have demonstrated that essential oils (EOs) can serve as an alternative preservative and pathogen-control method in various food products, including meat, fish, vegetables, rice, fruits, and dairy products. Research has shown that the antimicrobial activity of essential oils derived from *Rosmarinus officinalis against Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis, and Candida albicans* varies based on the location and seasonal variations of the plants.(Celiktas et al., 2007)

I.4.3. Phenolic components

Polyphenols are secondary compounds that are widely distributed throughout the plant kingdom. They are categorized into several classes based on the number of phenol rings they contain and the structural elements that bind these rings together: phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins), stilbenes, and lignans. These compounds are found in various plants and plant-derived foods.(Manach et al., 2004). The lipophilic nature of phenolics is pivotal for their antimicrobial activity, offering a distinct advantage. Lipophilic compounds have been observed to induce structural and functional damage to microorganisms by disrupting membrane permeability and upsetting the osmotic balance within the cell (Prakash et al., 2015) Polyphenols are increasingly of interest in the food industry due to their ability to retard the oxidative degradation of lipids, thereby enhancing the quality and nutritional value of food.(Kähkönen et al., 1999)

I.5. Aspergillus species

Aspergillus species are common in nature. They live as saprophytes in the soil but also on its surface and on plants. With the help of air currents, Aspergillus spores are transported and can settle on various substrates. Aspergillus can be isolated outside in the atmosphere, as well as inside homes, on clothing (ZITOUNI & BRIKI).

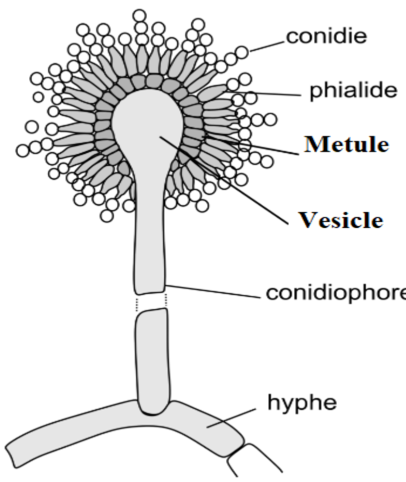


Figure 1. Diagram of the fungus of the genus "Aspergillus"(Siboukeur, Ould El Hadj, & Zargat, 2001)

Aspergillus niger is a filamentous fungus that is haploid and primarily utilized for waste management and biotransformations, along with its industrial applications such as citric acid production and the synthesis of extracellular enzymes. While it is commonly found in decaying vegetation, soil, or plants, it is not as hazardous as *Aspergillus fumigatus (*Nadumane, Venkatachalam, & Gajaraj, 2016*)*.

I.5.1. Cultural characteristics and macroscopic aspects

This fungus grows easily on Czapek medium, PDA, with a colony reaching 3 to 4 cm in 10 days. The extensive hyaline mycelium is largely submerged in the agar. Colonies initially appear white, then turn yellow, and finally develop black granules. Additionally, this fungus produces white aerial mycelium and numerous erect, powdery, dark brown-black sporulating structures, typically arranged in concentric circles. The reverse side is colorless to yellow. A pale yellow exudate may be produced in tiny droplets. This species exhibits rapid growth, with an optimal temperature range between 25 and 30°C, but it can grow up to 42°C. Its development is also inhibited by actidione (Krioui & Akroum, 2010).

Figure 2. *Aspergillus niger* colony on PDA “Source: Paul Cannon”

I.5.2. Microscopic morphology

The multiplication of this species is vegetative. There is no known sexual reproduction, nor presence of "Hülle cells". Instead, wide conidial heads are observed, initially spherical and later radiated, ranging from dark reddish-brown to black. They are carried by long conidiophores (1.5 to 3 mm long) with thick, smooth, colorless walls. The vesicle is globular, brown, and large (40 to 70 µm in diameter). The phialides, densely packed, are inserted into the vesicle via metulae arranged around the circumference of the vesicle. Metulae and phialides are slightly brownish. Conidia are produced in very long chains that tend to group into several compact columns over time. They are typically globular, brown, echinulate to very verrucose, and measure 3.5 to 5 µm in diameter. Pigmentation is not uniformly distributed across the entire surface of the conidium, but corresponds to ornamental granulations grouped in irregularly distributed ridges. The aspergillar head is therefore radially biseriate, and black at maturity (Siboukeur, Ould El Hadj, & Zargat, 2001).

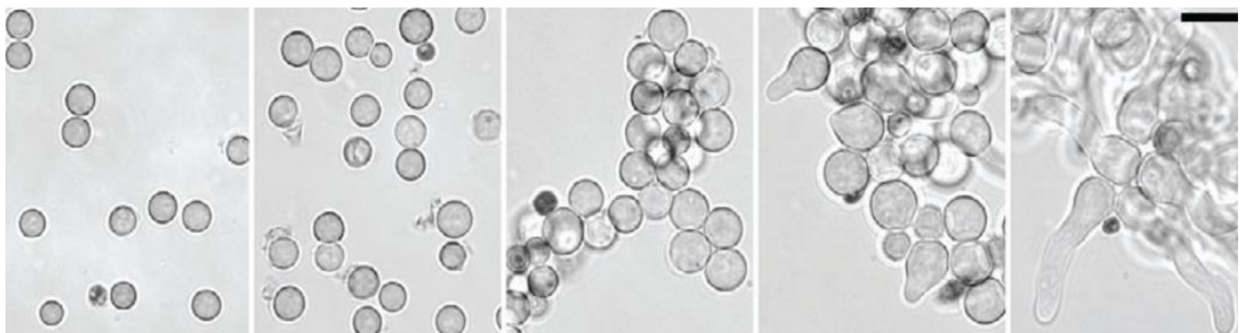


Figure 3. Germination of *A. niger* conidia as observed by bright-field

I.6. Fusarium

I.7. Fusarium graminearum

Fusarium graminearum is a filamentous fungus belonging to the genus Fusarium. It is a major plant pathogen, primarily responsible for causing Fusarium head blight (FHB) in cereal crops like wheat and barley. This pathogen produces several mycotoxins, including deoxynivalenol (DON), which present significant health risks to humans and animals upon ingestion. *F. graminearum* undergoes both sexual and asexual reproduction, with ascospores acting as the primary inoculum for infection. The fungus flourishes in warm and humid conditions, posing a continual threat to agricultural productivity and food safety (Bai & Shaner, 2004).

I.7.1. Cultural characteristics and macroscopic aspects

Fusarium graminearum demonstrates rapid growth on various standard mycological media, including Czapek yeast extract agar (CYA), malt extract agar (MEA), potato dextrose agar (PDA), and dichloran chloramphenicol peptide agar (DCPA). On CYA and MEA, colonies typically exhibit hues of grayish rose, grayish yellow, or lighter tones, with reverse sides ranging from orange-red to yellowish-brown. On PDA, colonies present a spectrum from yellowish-brown to reddish-brown, occasionally featuring a central aggregation of red-brown to orange areas bearing macroconidia, with the reverse side appearing dark red (Motarjemi, 2013). Fusarium graminearum grows at temperatures ranging from below 5°C to approximately 35°C, with optimal growth at 25°C. It can grow at a water activity as low as 0.90. Fusarium culmorum has a minimum growth temperature near 0°C, a maximum around 33°C, and an optimal temperature near 30°C. It can grow at a water activity as low as 0.87, which is lower than most other studied Fusarium species.(Pitt & Hocking, 2009)

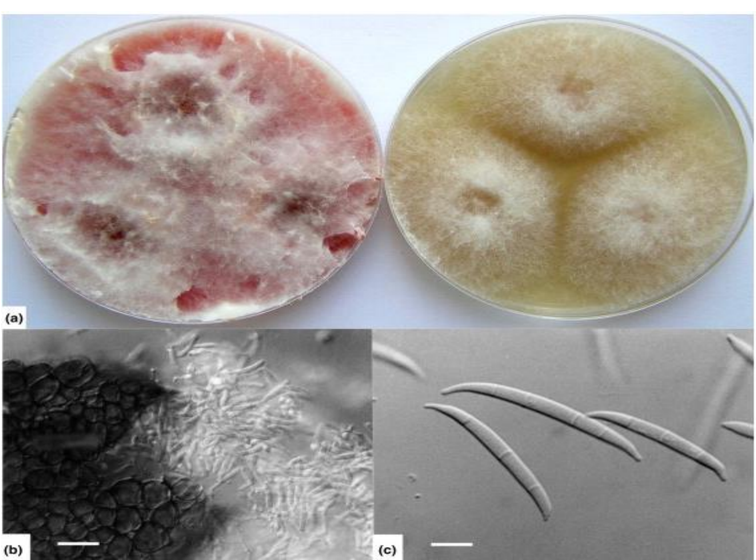


Figure 4. *Fusarium graminearum* (a) Colonies on PDA and dichloran chloramphenicol peptone agar (DCPA), 7 days, 25 °C; (b) Gibberella zeae perithecium and ascospores, bar=25 µm; (c) macroconidia, bar=10 µm (Motarjemi, 2013)

I.7.2. **Microscopic morphology**

The microscopic morphology of *Fusarium graminearum* is defined by several distinct features. The macroconidia are fusiform to sickle-shaped, typically measuring 20-60 µm in length and 3-5 µm in width, with 3-5 septa and smooth walls, produced abundantly in sporodochia. Microconidia are generally absent or infrequent. Chlamydospores are thick-walled resting spores formed within hyphae or macroconidia, functioning as survival structures. The hyphae are septate and hyaline, with branching conidiophores that produce macroconidia at their tips. Sporodochia are small, cushion-like clusters of hyphae that generate macroconidia and often appear reddish-brown to orange, contributing to the colony's coloration on certain media. These morphological characteristics are crucial for the identification and differentiation of *F. graminearum* from other Fusarium species and fungi (Guenther & Trail, 2005).

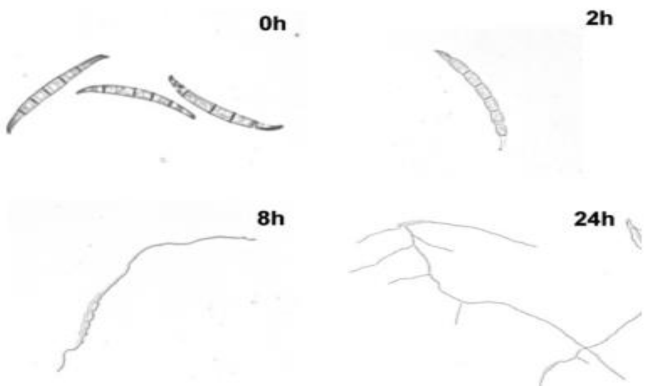


Figure 5. Light micrographs of F. *graminearum* macroconidia at the four time points used for microarray analysis (Seong et al., 2008).

I.8. *Thymus*

Thymus is a significant genus of plants that includes up to 400 species of aromatic and medicinal herbaceous perennials and shrubs. These species are predominantly found in the Mediterranean region and Asia. Traditionally, Thymus species are utilized as herbal teas, culinary spices, and condiments. Furthermore, their essential oils are among the world's top ten, renowned for their wide range of biological activities such as antioxidant, antibacterial, and age-delaying properties (Ziani et al., 2019). One of the most well-known Thymus species in North Africa is *T. algeriensis* (figure 6). *Thymus algeriensis* is a medicinal plant widely used by the north African population in traditional remedies for various inflammation-related conditions (Le Floc′h & Boulos, 2008).

Figure 6. Thymus algeriensis Boiss (A) and Aerial part (B) (Source: Biodiversidadvirtual.org/).

I.9. Organium

The genus Origanum includes approximately 38 species that are widely distributed across the Mediterranean, Euro-Siberian, and Irano-Siberian regions -Fig I.8-. As a significant member of the Lamiaceae family, this genus is highly valued for its essential oils and is known for its extensive morphological and chemical diversity (Agarwal, Singh, & Singh, 2017). *Origanum vulgare L.* (oregano) commonly known as oregano, is an aromatic perennial herb native to the Mediterranean region, low-growing perennial herb. It features a woody base and a spreading habit, forming a small, dense ground cover (figure 7).

Figure 7. *Origanum vulgare* L photo.

For centuries, *O. vulgare* has been traditionally used to flavor foods and treat various diseases due to its high essential oil content (Lukas et al., 2010). As early as the 7th century B.C., O. *vulgare* was used to season fish, meat, vegetables, and wine (Padulosi, 1997). Culinary subspecies of O. vulgare include ssp. gracile, ssp. glandulosum, and ssp. hirtum. In traditional medicine, *O. vulgare* has been used to treat respiratory disorders, stomachaches, painful menstruation, rheumatoid arthritis, nutritional disturbances, and urinary issues as a diuretic and antiurolithic (Bahmani, Khaksarian, & Rafieian-Kopaei, 2018). The aerial parts of the plant were most commonly used. In 2018, Bahmani et al. reviewed the therapeutic effects of *O. vulgare* based on Iran's ethnopharmacological records, noting its use in Iran for culinary purposes and in traditional medicine as a tonic, expectorant, carminative, stimulant, and antibacterial agent (Mozaffarian, 2013).

The forms of consumption for *O. vulgare* vary widely depending on the symptoms. These include tea or tincture, which are used to combat colds and digestive or respiratory disorders and to enhance overall health (Ličina et al., 2013). Decoctions or infusions of *O. vulgare* are known for their expectorant, antiseptic, digestive aid, and antispasmodic properties. Pieroni et al. reported the use of smoke inhalation from *O. vulgare* to relieve toothache (Pieroni, Quave, & Santoro, 2004).

I.10. Antifungal activity methods

To further explore the antifungal properties of these two oils, we intend to conduct the following microbial laboratory tests: Broth micro and macro dilution – Fumigation.

I.10.1. Dilution

Dilution methods are the most suitable for determining Minimum Inhibitory Concentration (MIC) values because they enable precise estimation of the concentration of the antimicrobial agent in either agar (agar dilution) or broth media (macrodilution or microdilution). These methods can quantitatively measure the in vitro antimicrobial activity against bacteria and fungi. Various guidelines exist for dilution antimicrobial susceptibility testing for both fastidious and non-

fastidious bacteria, yeast, and filamentous fungi, with the most recognized standards provided by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These guidelines offer a standardized procedure that can be practically implemented in most clinical microbiology laboratories. While the development of these methodological standards does not guarantee clinical relevance, it facilitates a uniform bioassay approach, aiding in the evaluation of the clinical significance of the results.(Pfaller, Sheehan, & Rex, 2004)

I.10.2. Broth dilution method

Broth micro- or macro-dilution is a fundamental method for antimicrobial susceptibility testing. The process involves creating two-fold dilutions of the antimicrobial agent (e.g., 1, 2, 4, 8, 16, and 32 µg/mL) in a liquid growth medium, which is then dispensed into tubes with a minimum volume of 2 mL (macrodilution) or into 96-well microtitration plates with smaller volumes (microdilution) . Each tube or well is inoculated with a microbial inoculum prepared in the same medium after diluting a standardized microbial suspension to the 0.5 McFarland scale.

After thorough mixing, the inoculated tubes or 96-well microtitration plates are incubated under appropriate conditions for the test microorganism, usually without agitation. The experimental methodology for accurately performing the microdilution is illustrated in Figure .(Balouiri, Sadiki, & Ibnsouda, 2016)

I.10.3. Broth microdilution

The Broth Dilution Method (BDM) is also appropriate for determining MIC values. In this approach, microorganisms are assessed for their capacity to generate visible growth on agar plates (agar dilution, AD) or in broth (broth dilution, BD) containing various dilutions of the antimicrobial agent. Each tube is inoculated with a known number of test microorganisms. In the classic tube or macrodilution method, 1 mL of the medium is diluted in test tubes. Only 0.1 mL of the medium is required for dilution, making it a microdilution method. After incubation overnight, the result (turbidity) can be assessed either visually or using a plate reader. This method is both cost-effective and efficient. However, when testing essential oils (EOs) with the Broth Dilution Method (BDM), a major challenge arises from their low solubility in water. To address this, various solvents such as dimethyl sulfoxide (DMSO) or ethanol, as well as detergents like Tween 20, are added to the tubes containing the EO being tested (Pauli & Schilcher, 2010).

I.10.4. Broth macrodilution volatilization

Broth macrodilution volatilization is a microbiological technique used to assess the effectiveness of antimicrobial agents, particularly those with volatile properties like essential oils. In this

method, different concentrations of the antimicrobial agent are mixed into liquid growth medium (broth) in test tubes. These tubes are sealed and placed in a controlled environment for incubation. During this process, the volatile components in the antimicrobial agent may evaporate from the broth, potentially leading to inconsistencies in concentration levels and affecting the accuracy of the results. This phenomenon is known as volatilization. To address this issue, precautions such as tightly sealing the test tubes and conducting the assay in a controlled environment are implemented (Houdkova et al., 2021).

I.10.5. Determination Minimal Fungicidal Concentration (MFC)

The Minimal Fungicidal Concentration (MFC) denotes the least concentration of an antifungal substance that induces the death or complete elimination of a fungal pathogen, as opposed to solely inhibiting its proliferation (Cantón et al., 2009).

I.10.6. Determination Minimal Inhibitory Concentration (MIC)

The MIC (Minimum Inhibitory Concentration) refers to the lowest concentration of an antimicrobial agent, measured in milligrams per milliliter (mg/mL), that inhibits the visible growth of a microorganism (Wiegand, Hilpert, & Hancock, 2008).

I.11. Predictive Microbiology

I.11.1. What is Predictive Microbiology?

It is a discipline that aims to develop mathematical models to predict the growth, survival, or decline capabilities of microbial populations in food based on influential environmental factors. To assess the microbiological stability of food or to evaluate their shelf life, conventional methods have been used for many years. However, the high cost of these experiments and their response time have sparked interest in computer simulation systems (Delhalle et al., 2012). Recommended by the AFSSA or by the European regulation on microbiological criteria in foodstuffs, the demand for mathematical modeling in microbiology was initiated in a regulatory context (European Food Safety Authority, 2010). Its applications can be summarized as follows (Delhalle et al., 2012):

- Determination of use-by dates (UBD): concept of minimum infectious dose (MID).
- Prediction of the growth of pathogenic or spoilage microorganisms in a specific food.
- Optimization of processes and their adaptation to ensure food safety: thermal treatments, storage conditions, etc.
- Quantitative Risk Assessment (QRA) (estimation of the evolution of the number of microorganisms in a production chain, evaluation of exposure to a pathogenic bacterium, consequences of this exposure).

- Assistance in the development of new products: formulation and process.
- Determination of critical limits of environmental factors. Example: determination of critical microbiological points (HACCP).

Predictive microbiology has experienced significant growth since the early 1980s. Many models have been developed to describe bacterial behavior based on the main physicochemical characteristics of food products (temperature, pH, water availability, preservative). The implemented modeling is divided into two stages corresponding to what is called primary modeling and secondary modeling. Primary models describe the evolution of bacterial density over time, and secondary models express the effect of environmental physicochemical factors on the parameters of the primary models (Couvert, 2006).

I.11.2. Growth Models

A primary growth model aims to describe the evolution over time of a well-defined microorganism in a given environment. This model results in a growth curve that is classically divided into 5 phases (Figure 8):

a. Lag phase (λ) where the initial population (N(0)) is constant and the growth rate (μ) is zero;
b. Acceleration phase where μ > 0;
c. Exponential phase where the growth rate reaches its maximum level (μmax);
d. Deceleration phase where the maximum growth rate decreases (μ < μmax);
e. Stationary phase where the population (N(t)) reaches its maximum concentration (Nmax).

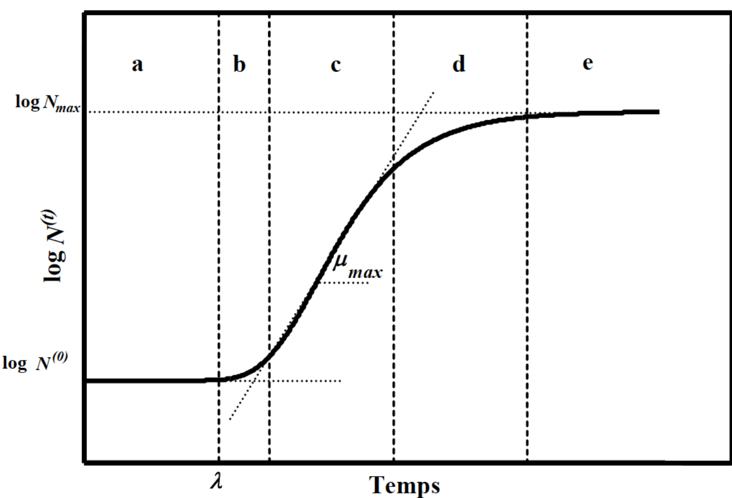


Figure 8. Main phases of a microbial growth curve and the model parameters that characterize it. (Belbahi, 2015)

Several primary growth models have been proposed to describe the growth kinetics of microbial populations in a given environment. The most well-known models are:

- Zwietering et al. (1990),
- Rosso (1995),
- Baranyi and Roberts (1994).

Zwietering et al. (1990) adapted the *Gompertz* model by incorporating the classic parameters of a growth curve with microbiological significance:

$$\log\left(N^{(t)}\right) = \log\left(N^{(0)}\right) + A.\exp\left\{-\exp\left[\frac{\mu_{max}e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

With $A = \log(N_{max}) - \log\left(N^{(0)}\right)$ et $e = \exp(1)$.

This model, which generates sigmoidal curves, has been commonly used due to its good fit with experimental growth data. However, some drawbacks have been reported. Rosso (1995) mentioned that the reparameterized Gompertz model leads to an overestimation of the growth rate µ_max compared to its classical definition (phase c of Figure 4). Therefore, Rosso (1995) developed a so-called logistic model with delay and break, which limits the problems mentioned above:

$$\log\left(N^{(t)}\right) = \begin{cases} \log\left(N^{(0)}\right) & , \quad t \leq \lambda \\ \log(N_{\max}) - \log\left[1 + \left(\frac{N_{\max}}{N^{(0)}} - 1\right)\exp\left(-\mu_{\max}(t-\lambda)\right)\right], & t > \lambda \end{cases} \quad (2)$$

The Baranyi and Roberts model (1994) is also one of the most widely used models for representing growth curves. It fits experimental growth data very well (good quality of fit) and has parameters with mechanistic meanings. However, the Baranyi model remains very complex and less used than that of Rosso (1995).

$$\frac{dN^{(t)}}{dt} = \mu_{max}\, N^{(t)}\, a(t)\, b(t) \quad (3)$$

$$a(t) = \frac{q_0}{q_0 + \exp(-\mu_{max}\, t)} \quad (4)$$

$$b(t) = 1 - \frac{N^{(t)}}{N_{max}} \quad (5)$$

Where $a(t)$is the increasing adjustment function depending on t, which converges towards 1 (varies between 0 and 1), and allows the transition from the lag phase to the exponential phase.

$b(t)$ is the decreasing braking function depending on $N^{(t)}$, which converges towards 0 (also varies between 0 and 1), and allows the transition from the exponential phase to the stationary phase. The parameter q_0 is given by h_0 which characterizes the physiological state of the inoculum $N^{(0)}$:

$$h_0 = \mu_{max}\,\lambda = \log\left(1 + \frac{1}{q_0}\right) \qquad (6)$$

The integrated form of the Baranyi and Roberts model (1994) is given by the following equation:"

$$N^{(t)} = N^{(0)} + \mu_{max}A^{(t)} - \log\left\{1 + \frac{\exp[\mu_{max}A^{(t)} - 1]}{\exp[N_{max} - N^{(0)}]}\right\} \qquad (7)$$

With :

$$A^{(t)} = t + \frac{1}{\mu_{max}}\log\left\{1 + \frac{\exp[-\mu_{max}\,t] + q_0}{1 + q_0}\right\} \qquad (8)$$

The Baranyi and Roberts model (1994) fits experimental growth data very well (good quality of fit) and has parameters with mechanistic meanings.

Chapter II.
Materials and Methods

Chapitre II. Materials and methods

II.1. Extraction procedures of essential oils

The essential oils derived from the two plants specimens (*Origanum* and *Thymus*) were obtained through hydrodistillation using the Clevenger apparatus. The process of essential oil extraction was conducted over a duration of three hours, with three repetitions performed for each sample accession. Following extraction, the volume of the obtained essential oil was quantified, and its weight percentage relative to the volume was computed. Subsequently, the essential oil was separated from the Clevenger apparatus and transferred into a glass vial for storage and stored in a refrigerator at a temperature of 4°C until they were ready for analysis.

Figure 9. Photo of extraction by hydrodistillation device, using the Clevenger apparatus

II.2. Preparation of the suspension spores

Cultures of *Aspergillus niger* and *Fusarium p.* were acquired, and replicates were generated. Physiological saline tubes containing a drop of Tween 80 were prepared and added to the cultures. The resulting mixtures were filtered to obtain solutions containing fungal spores. To determine the initial fungal spore count, a Malassez counting cell was utilized for each fungus.

To prepare the fungal culture medium, 5 mL of each fungal spore suspension were added to 45 mL of Sabouraud dextrose agar (SDA) containing 10% dimethyl sulfoxide (DMSO). This mixture ensures the even distribution of fungal spores within the agar medium, facilitating subsequent analysis.

II.3. Preparation of the essential oil broth

To prepare two tubes containing the essential oils from each plant mixed with broth, we added 120 µL of the respective essential oil to 5880 µL of broth. The first tube contained *Origanum* essential oil, while the second tube contained Thymus essential oil. A series of seven dilutions were prepared using 1.5 mL Eppendorf tubes, each containing 750 µL of broth. These dilutions were made from an Eppendorf tube that initially contained 1500 µL of essential oil broth. incubated at 24 ± 2°C for 24H,48H,72H. The results were observed using a microscope, and the fungal spores were counted using the Malassez counting cell.

II.3.1. Malassez counting cell method

A hemocytometer is a special gridded slide that allows the enumeration, within a precise and known volume, of all the visible elements under a 40x microscope objective. It must moisturize the side platforms with water, adhere the coverslip, and fill the counting chamber with the well-homogenized sample. After waiting for 10 minutes for the cells to sediment, count using a 40x objective lens the cells present in 4 rectangles or "counting units" according to the following rules.

Count all the cells within the rectangle as well as those overlapping the top and right edges. On a Malassez counting grid, one rectangle or counting unit contains 0.01 mm^3, which is equivalent to 0.01 μL of sample (recall 1 mm^3 = 1 µL = 1×10^{-3} mL = 1×10^{-6} L).

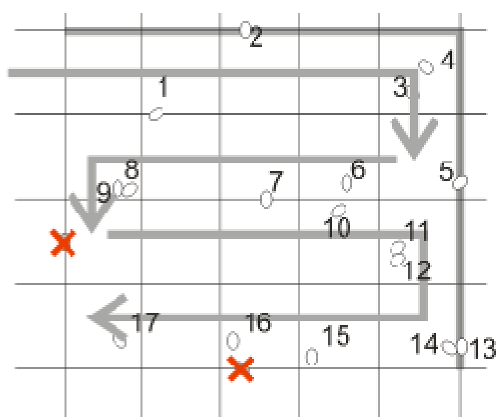


Figure 10. The enumeration of fungal spores using the Malassez counting cell

II.3.2. Broth microdilution for MIC and MFC

We conducted serial dilutions of the antimicrobial agent in broth within 96-well microtiter plates. Each well received a 50µL. Subsequently, we inoculated each well with a 50 µL, ensuring uniform concentration of microbial cells across all wells. The microtiter plate was then placed in an incubator set at 24±2 °C for a duration of 24 hours. Following the incubation period, each well was visually examined for microbial growth to ascertain the MIC.

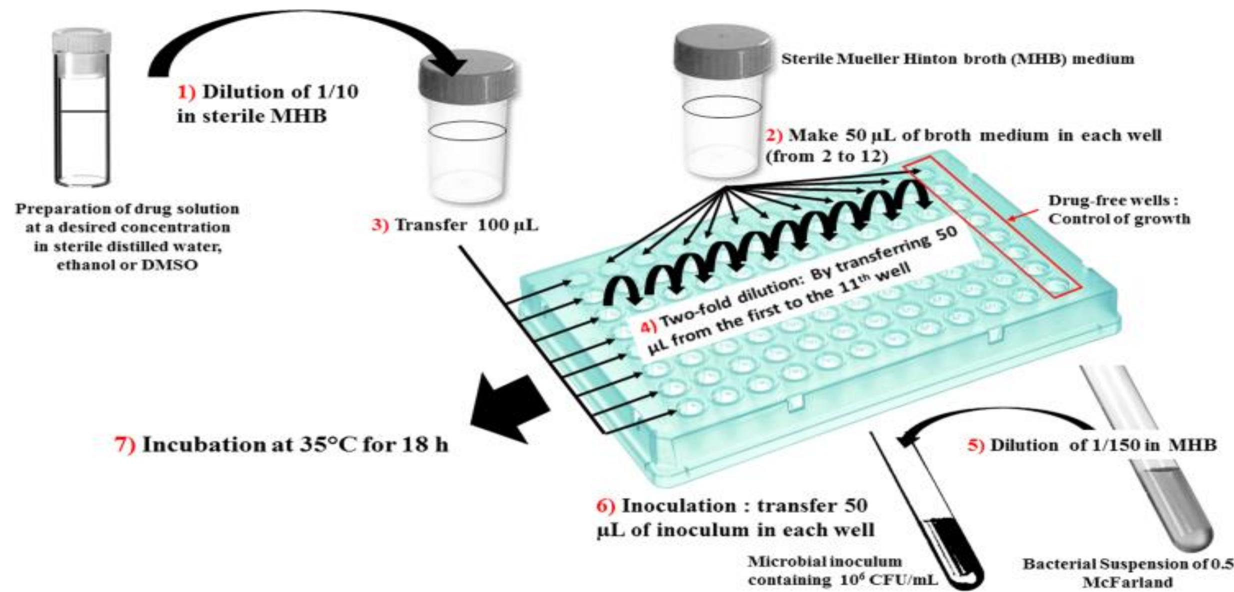


Figure 11. Broth microdilution for antifungal testing as recommended by CLSI protocol

II.4. Essential oils fumigation

The antifungal activity of essential oils against *Aspergillus Niger* and *Fusarium sp.* was investigated in laboratory conditions using fumigation method in SDA. Sterile filter paper discs (diameter 5 mm, Whatman No. 1) were placed in the inverted lid of the plates then different concentrations of essential oils were added. (35 µL of each different concentrations were added to the discs in a successive order).

Each treatment consisted of 01 replications in fumigation, 7 µL of the fungus mixture were separately added to the SDA. A series of 5 dilutions was initiated from an Eppendorf tube containing 100 µL of the essential oil mixed with 100 µL of DMSO. This process was repeated for the second essential oil. All the plates were incubated at 24 ± 2°C for 24h, the diameter of *Aspergillus Niger* and *Fusarium sp.* was measured in different plates continuously per 03 hours.

II.5. Growth assessment and kinetic modelling

The diameter of the developed mycelia *Aspergillus Niger* and *Fusarium sp.* was measured along two perpendicular axes. The mean values of the diameters at any time t was noted $d^{(t)}$, and it was plotted against time and fitted to a two-phase linear model for the estimation of the growth rate μ (mm day^{-1}) and the lag time λ (days) (Gougouli et al., 2011).

$$d^{(t)} = \begin{cases} d^0 & \text{for} \quad t \le \lambda \\ d^0 + \mu(t-\lambda) & \text{for} \quad t > \lambda \end{cases} \qquad (1)$$

Where d^0 is the diameter of the inoculated spore suspension (d^0= 4-5 mm).

II.6. Parameter estimation and statistical methods

The parameters of the two-phase linear model were estimated by nonlinear regression using a MATLAB 6.5 adjustment tool box (The MathWorks Inc., Natick, MA, USA). The root means squared error (*RMSE*) between all experimental and predicted data, adjusted coefficients of determination (adjusted R^2) and confidence intervals (calculated with 95% probability) were used as indicators of the quality of fit for the estimated parameters. Statistical significance ($p < .05$) of the effect of essential oils on the germination spores was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test.

Chapitre III. Results and discussion

III.1. Direct contact

III.1.1. Effect on the germination of *Aspergillus niger* spores

Throughout this section of the manuscript, the abbreviations C1, C2, C3, C4, C5, C6, and C7 will be used to represent the following concentrations: 15, 7.5, 3.75, 1.88, 0.94, 0.47, and 0.23 µg/ml, respectively. When O. vulgare EO was applied at varying concentrations from C1 to C7, the results, analyzed using the selected statistical model, indicated no germination in the first three dilutions (C1, C2, and C3). However, subsequent dilutions (C4, C5, C6, and C7) demonstrated germination in ascending order after 24 and 48 hours of treatment. Similarly, when T. algeriensis EO was applied at varying concentrations, the results indicated the presence of germination in ascending order starting from C2 after 24 and C1 after 48 hours of treatment with the EO, as shown in Table 1. (Manavathu, Alangaden, & Lerner, 1996)

Table 1. Effect of the concentration of two essential oils and incubation time on the germination of *Aspergillus niger* spores (10^6 spores/ml).

Concentration (µg/ml)	Essentiel oïl 1		Essentiel oïl 2	
	24 h	48 h	24 h	48 h
Control -	0	0	0	0
Control +	31.7 ± 11.9	MYC	31.7 ± 12	MYC
C1	0	0	0	9.0 ± 15.6
C2	0	0	4.0 ± 7.0 ^{a}	5.3 ± 8.4 ^{a}
C3	0	0	19.3 ± 10 ^{a}	18.0 ± 2.6 ^{a}
C4	7.3 ± 4.0 ^{a}	25.3 ± 1.2 ^{b}	18.7 ± 7.5 ^{a}	10.0 ± 4.4 ^{b}
C5	17.0 ± 7.0 ^{a}	21.7 ± 9.5 ^{a}	21.7 ± 3.5 ^{a}	21.7 ± 3.8 ^{a}
C6	24.3 ± 6.0 ^{a}	20.7 ± 6.4 ^{a}	26.7 ± 3.8 ^{a}	19.0 ± 2.0 ^{b}
C7	25.3 ± 5.0 ^{a}	32.7 ± 14.6 ^{a}	29.0 ± 6.9 ^{a}	13.7 ± 12.4 ^{b}

Values are means of three trials (n=3) ± standard deviation.
Same letters in lines refer to means not statistically different according to independent samples *t*-test.
MYC indicates that the field of observation is invaded by mycelium, making reading difficult.

We conclude that the essential oil of O. vulgare exhibits strong antifungal activity consistently across the two treatment durations. The minimum inhibitory concentration (MIC) was determined to be at the C3 dilution (1/8 MS), which remained constant after both 24 and 48 hours of treatment. However, starting from the C4 dilution (1/16 MS) to the C7 dilution (1/128 MS), the concentrations of the essential oil were not effective enough to inhibit the germination of A. niger spores.

We determine that the O.vulgare EO consistently demonstrates strong antifungal activity over both treatment periods. The minimum inhibitory concentration (MIC) was established at the C3 dilution (1/8 MS), maintaining its effectiveness after 24 and 48 hours of treatment. However, at dilutions from C4 (1/16 MS) to C7 (1/128 MS), the essential oil concentrations were insufficient to prevent the germination of A. niger spores.

Conversely, the EO of T. algeriensis displayed significantly weaker antifungal activity at various concentrations compared to that of O. vulgare. This indicates that A. niger may have a resistance mechanism against T. algeriensis EO. (Rao et al., 2010).

III.1.2. Effect on the germination of *Fusarium sp.* spores

Table 2. Effect of the concentration of two essential oils and incubation time on the germination of Fusarium sp. spores (10^6 spores /ml).

Concentration (µg/ml)	**Essentiel oïl 1**			**Essentiel oïl 2**		
	24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Control -	0	0	0	0	0	0
Control +	9.7 ± 3.5	MYC	MYC	9.7 ± 3.5	MYC	MYC
C1	0	0	0	0	0	0.0
C2	0	0	0	0	0	0.7
C3	0.3 ± 0.5^{a}	1.3 ± 1.2^{b}	2.7 ± 2.5^{c}	2.0 ± 1.7^{a}	6.3 ± 1.5^{b}	11.5 ± 7.8^{c}
C4	3.0 ± 2.6^{a}	4.3 ± 2.1^{ab}	5.0 ± 1.7^{b}	7.7 ± 4.0^{a}	8.7 ± 1.5^{a}	45.0 ± 15.0^{c}
C5	5.0 ± 2.0^{a}	6.0 ± 2.0^{a}	8.3 ± 10.4^{c}	10.7 ± 4.2^{a}	14.3 ± 6.1^{a}	MYC
C6	9.3 ± 0.6^{a}	19.0 ± 6.1^{b}	MYC	9.3 ± 7.5^{a}	12.7 ± 2.3^{a}	MYC
C7	$13.35.9^{a}$	36.0 ± 8.0^{b}	MYC	10.3 ± 1.5^{a}	17.3 ± 6.8^{b}	MYC

Values are means of three trials (n=3) ± standard deviation.
Same letters in lines refer to means not statistically different according to ANOVA and Tukey’s test.
MYC indicates that the field of observation is invaded by mycelium, making reading difficult.

When O. vulgare EO was applied at varying concentrations (C1, C2, C3, C4, C5, C6, C7), the results analyzed using the selected statistical model indicated no germination in the first two dilutions (C1, C2) during treatment after three durations (24h, 48h, 72h). However, subsequent dilutions (C3, C4, C5, C6, C7) demonstrated increasing germination during these periods. After 72 hours, the presence of mycelium in concentrations C6 and C7 complicated the results interpretation.

When T. algeriensis EO was applied at the same concentrations, no germination was observed in the first two dilutions (C1, C2) during the first two treatment times (24h, 48h), but weak germination was noted in C2 after 72h. Germination increased in subsequent dilutions (C3, C4, C5, C6, C7) over all three treatment times (24h, 48h, 72h), with significant mycelium presence

in concentrations C5 to C7, complicating result interpretation. This indicates that the effects of the essential oils from both plants are somewhat similar on Fusarium sp.These findings are summarized in Table 2 above.

III.1.3. Broth Microdilution For MIC And MFC

III.1.3.1. Minimal Inhibitory Concentration (MIC) For the O. vulgare and T. algeriensis essential oils:

Table 3: The Results Of Broth Microdilution Of Aspergillus Niger After 24h

Minimal Inhibitory Concentration (MIC)										
Essential Oil 01										
	SM	1	2	3	4	5	6	7	controle+	controle-
A	−	−	−	−	−	+	+	+		−
B	−	−	−	−	−	+	+	+	+	−
C	−	−	−	−	−	+	+	+	+	−
Essential Oil 02										
	SM	1	2	3	4	5	6	7	controle+	controle-
A	−	−	−	−	+	+	+	+	+	−
B	−	−	−	−	+	+	+	+	+	−
C	−	−	−	−	+	+	+	+	+	−

As shown in the table above, when O. vulgare EO was applied to A.niger spores, the broth became turbid starting from the 5th concentration and continued through the 7th concentration. Conversely, the broth remained clear from the MIC standard (MS) up to the 4th dilution. Similarly, when T. algeriensis EO was applied to A. niger spores, the broth became turbid starting from the 4th concentration and continued through the 7th concentration, while remaining clear from the MIC standard (MS) up to the 3rd dilution.

Table 4 :The Results Of Broth Microdilution Of Fusarium Sp After 24h

Minimal Inhibitory Concentration (MIC)										
Essential Oil 01										
	SM	1	2	3	4	5	6	7	controle+	controle-
A	−	−	−	+	+	+	+	+		−

B	−	−	−	+	+	+	+	+	+	−
C	−	−	−	+	+	+	+	+	+	−
Essential Oil 02										
	SM	1	2	3	4	5	6	7	controle+	controle-
A	−	−	−	+	+	+	+	+	+	−
B	−	−	−	+	+	+	+	+	+	−
C	−	−	−	+	+	+	+	+	+	−

As shown in the table above, when *O. vulgare* essential oil was applied to Fusarium sp spores, the broth became turbid starting from the 3rd concentration and continued through the 7th concentration. Conversely, the broth remained clear from the MIC standard (MS) up to the 2nd dilution.

when T. algeriensis essential oil was applied to Fusarium sp spores the broth became turbid starting from the 3rd concentration and continued through the 7th concentration. Conversely, the broth remained clear from the MIC up to the 2nd dilution.

The Minimum Inhibitory Concentrations (MICs) for the A. niger strains identified in this study were found to be 1/16 Mother Solution (MS) for O.. vulgare and 1/8 Mother Solution (MS) for T. algeriensis. These findings are consistent with those obtained using the broth macrodilution method.

The Minimum Inhibitory Concentrations (MICs) for the Fusarium sp strains identified in this study were found to be 1/4 Mother Solution (MS) for both *O. vulgare* and T. algeriensis. These findings aren't consistent with those obtained using the broth macrodilution method. which could be attributed to experimental errors, The contribution of individual components in both essential oils plays a significant role in their mechanisms of action. Therefore, isolating these components and studying their synergistic effects would provide further insights. (Lima et al., 2019).

III.1.3.2. Minimal Fungicidal Concentration (MFC) For The O. Vulgare And Thymus Algeriensis Essential Oils:

Table 5:The Results Of the MFC Test On Aspergillus Niger After 24h

Minimum fungicidal concentration (MFC)

Essential Oil 01						Essential Oil 02				
	SM	1	2	3	4	SM	1	2	3	4
A	−	−	−	+	+	+	+	+	−	+
B	−	−	−	+	−	+	+	+	−	+
C	−	−	−	+	−	+	+	+	+	+

As shown in the table above, when the broth containing *O. vulgare* essential oil was applied to A. niger spores and added to SDA, no growth was observed from the MIC standard (MS) up to the 2nd concentration. However, growth was observed at the 3rd and 4th concentrations.
As shown in the table above, when the broth containing *T. algeriensis* EO was applied to Aspergillus niger spores and added to SDA, growth was observed from the MIC standard (MS) up to the 4th concentrations.
The minimal fungicidal concentrations (MFCs) for the A. niger strains in this study were found to be 1/4 Mother Solution (MS) for the *O. vulgare* oil, while the *T. algeriensis* oil showed no fungicidal effect.

Table 6:The Results Of the MFC Test On Fusarium Sp After 24h

Minimum fungicidal concentration (MFC)										
Essential Oil 01						Essential Oil 02				
	SM	1	2	3	4	SM	1	2	3	4
A	−	−	+	+	+	+	+	+	+	+
B	−	+	+	+	−	+	+	+	+	+
C	−	−	+	+	+	+	+	+	+	+

The minimal fungicidal concentrations (MFCs) for the Fusarium sp. strains in this study were identified as 1/2 Mother Solution (MS) for the O. vulgare essential oil (EO), while the T. algeriensis EO exhibited no fungicidal effect. This indicates that O. vulgare EO has stronger antifungal activity and fungicidal properties, despite Thymol being the primary component of T. algeriensis (Ouakouak et al., 2021). In contrast, Origanum vulgare contains both Carvacrol and Thymol as its main components (Jafari Khorsand et al., 2022). These results suggest that the essential oil's composition plays a crucial role in its antimicrobial efficacy, however a comparative study is needed to confirm our hypothesis .(Zhang et al., 2019)

III.2. Effect of essential oil fumigation

III.2.1. Effect on the growth of *Aspergillus niger* spores

In this study, the growth kinetics of *Aspergillus niger* were investigated in a culture medium in a hermetically sealed Petri dish. The two-phase linear model proposed by Gougouli and Koutsoumanis (2010) was chosen as the primary growth model. This model is simple, robust, and perfectly describes the growth of molds. The kinetic parameters, the growth rate (μ) and the lag time (λ), were estimated for all concentrations of essential oil 1 (Table 8). The RMSE values (2.21 mm on average for all trials) proved the moderate quality of the fit of the two-phase linear model and indicated that the predictions describe the experimental data relatively satisfactorily.

The growth data of the *A. niger* strain is based on the diameter of the mycelia of this fungal strain. According to Figure 14, growth over time is linear after a lag phase. This lag phase is defined as the period during which the diameters of the mycelia remain equal to the initial diameter d = 4-5 mm. This diameter corresponds to the initial diameter of the spore suspension drop deposited on the agar. This method is based on a pre-established initial diameter that incorporates the spore germination process of the two species studied; this simplifies the acquisition of growth data and facilitates the estimation of the lag time λ.

The growth of molds in food or model food matrices (synthetic culture media) is inhibited by various environmental factors. In this study, the most important factors are temperature (set at 25°C), a_w (set at 0.993), and the concentration of essential oil in the pellets (diffused into the atmosphere of the dishes). The growth rate (μ) of *A. niger* decreases as the concentration of essential oil in the pellets increases (Table 8).

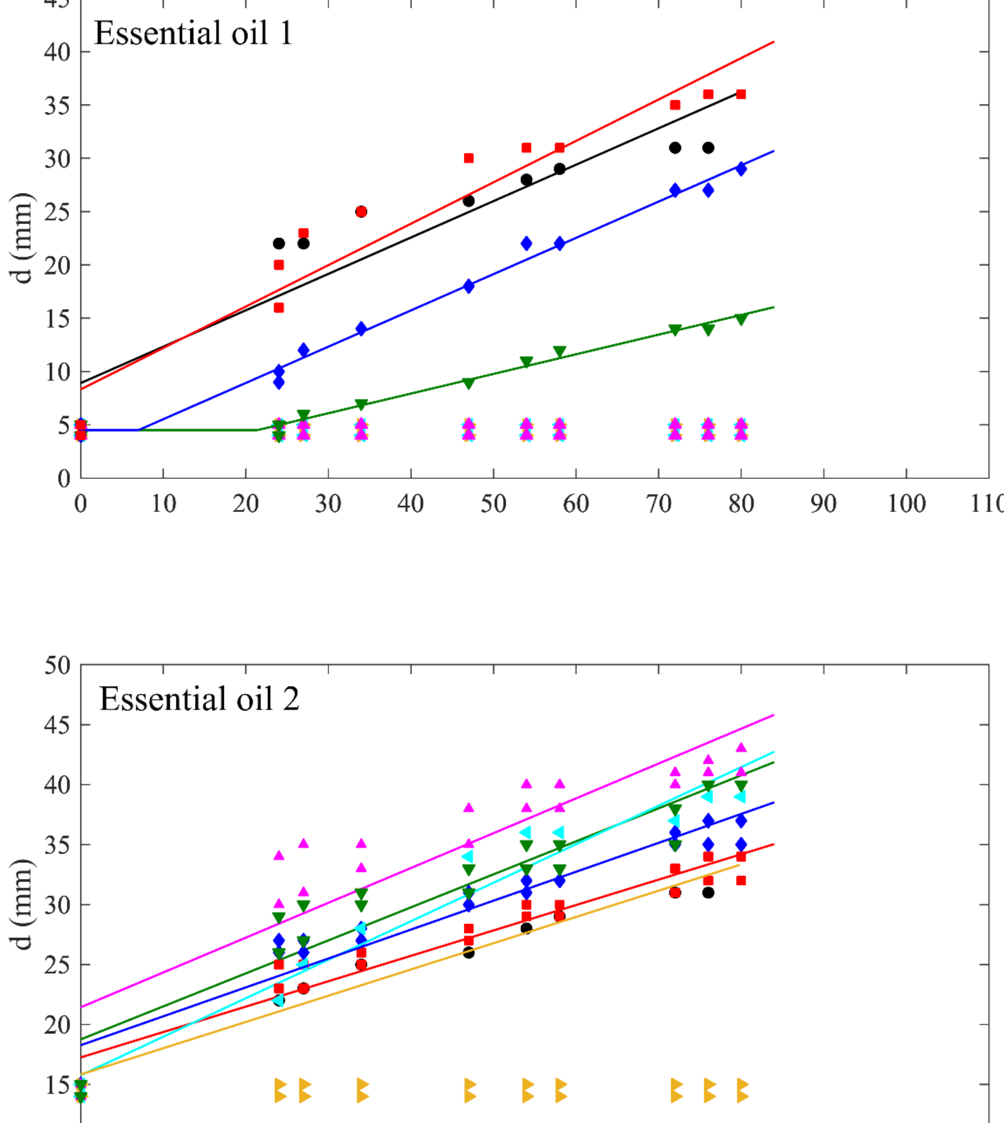


Figure 12. Effects of concertation of fumigated essential oil (● C1, ▶ C2, ◀ C3, ▲ C4, ◆ C5, and ■ C6) on the growth diameter *d* kinetic of *Aspergillus niger*. Solid lines represent the fit of the two-phase linear model to the d data, represented by symbols.

In bacterial growth, the lag time λ can be determined by the ratio between the amount of "work" a bacterium has to perform to adapt to its new environment and the rate at which it can perform it (Robinson et al., 1998). This adaptation work is called the physiological state of microbial cells; it is defined by the product μ x λ, and the latter is constant for identical pre-inoculation states of the cultures (Baranyi and Roberts, 1994). Unlike bacterial growth, the product μ x λ in molds has not been studied in detail in the literature (Gougouli and Koutsoumanis, 2010). In our study, the product μ x λ is relatively constant for the environmental factor studied (concentration of essential

oil in the pellets diffused into the atmosphere of the dishes). Gougouli and Koutsoumanis (2010) reported that the product μ x λ is relatively constant for the growth of other molds (*Penicillium expansum* and *Aspergillus niger*) at different incubation temperatures.

For all growth conditions of *A. niger*, the growth rate μ was less than 0.5 mm/h, with the highest rates observed for the control (0.342 ± 0.219 mm/h) and for the lowest concentration of the oil: 0.389 ± 0.082 and 0.212 ± 0.035 mm/h for oil 1 and oil 2, respectively. The increase in oil concentrations decreases the growth rate of the mycelia until the absence of growth at concentrations C3 and C1 for essential oils 1 and 2, respectively."

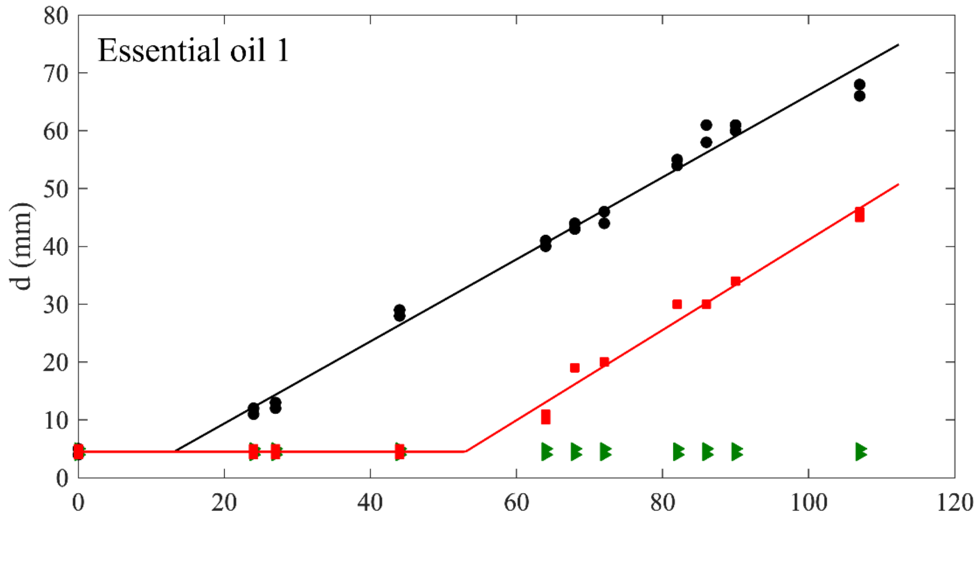
Table 7. Estimated growth kinetics parameters of *Aspergillus niger* at different concertation of fumigated essential oil. Mean values ± 95 % confidence interval.

	Control	C1	C2	C3	C4	C5	C6
Essential oil 1							
μ (mm/h)	0.342 ± 0.219	NG	NG	NG	0.184 ± 0.020	0.340 ± 0.029	0.389 ± 0.082
λ (h)	NC	-	-	-	21.4 ± 6.3	6.7± 5.6	NC
d^0 (mm)	NC	-	-	-	4.5 ± 0.9	4.5 ± 1.3	7.5 ± 1.6
RMSE	4.16	-	-	-	0.58	0.84	3.31
R^2	0.863	-	-	-	0.985	0.993	0.929
Essential oil 2							
μ (mm/h)	0.219 ± 0.040	NG	0.321 ± 0.057	0.290 ± 0.079	0.275 ± 0.052	0.241 ± 0.037	0.212 ± 0.035
λ (h)	NC	-	NC	NC	NC	NC	NC
d^0 (mm)	15.7 ± 12.0	-	NC	17.1 ± 10.2	NC	NC	16.7 ± 9.6
RMSE	1.326	-	2.23	3.72	2.420	1.93	1.58
R^2	0.963	-	0.954	0.805	0.902	0.917	0.927

RMSE: Root Mean Squared Error between experimental and estimated colony diameters.
NC: No Converged solution was obtained; NG: Not Growth.

III.2.2. Effect on the growth of *Fusarium sp.* spores

The growth kinetics of *Fusarium sp.* were investigated in a culture medium in a hermetically sealed Petri dish. The two-phase linear model proposed by Gougouli and Koutsoumanis (2010) was chosen as the primary growth model. The kinetic parameters (μ and λ) were estimated for all concentrations of essential oil 1 (Table 9). The *RMSE* values (1.77 mm on average for all trials) proved the moderate quality of the fit of the two-phase linear model and indicated that the predictions describe the experimental data relatively satisfactorily. The growth data of the *Fusarium sp* strain is based on the diameter of the mycelia of this fungal strain. According to Figure 15, growth over time is linear after a lag phase. The growth rate (*μ*) of *Fusarium sp* decreases as the concentration of essential oil in the pellets increases (Table 9).



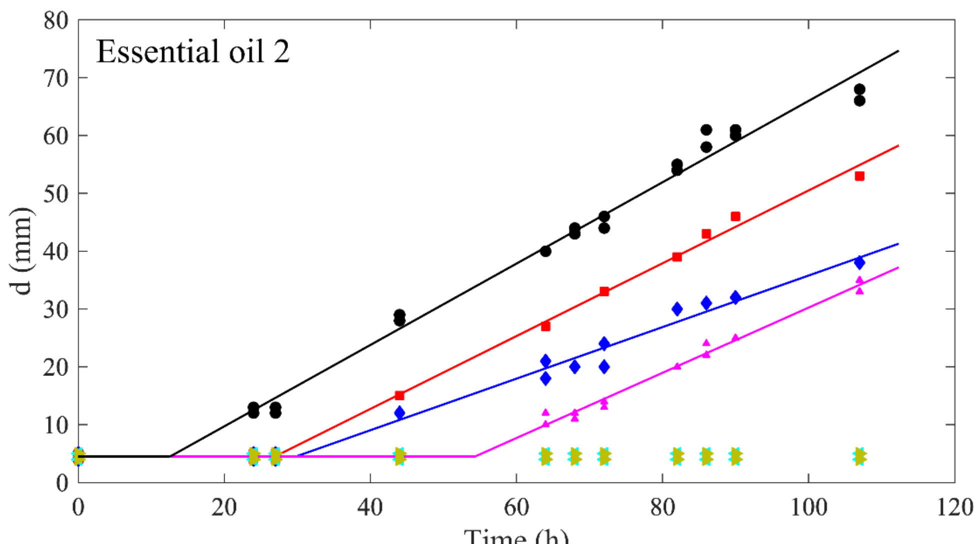


Figure 13. Effects of concertation of fumigated essential oil (● C1, ▶ C2, ◀ C3, ▲ C4, ◆ C5, and ■ C6) on the growth diameter *d* kinetic of *Fusarium sp.*. Solid lines represent the fit of the two-phase linear model to the d data, represented by symbols.

For all growth conditions of *Fusarium sp*, the growth rate μ was less than 1 mm/h, with the highest rates observed for the control (0.709 ± 0.040 mm/h) and for the lowest concentration of essential oil: 0.780 ± 0.713 and 0.630 ± 0.029 mm/h for oil 1 and oil 2, respectively. The increase in oil concentrations decreases the growth rate of the mycelia until the absence of growth at concentrations C5 and C2 for essential oils 1 and 2, respectively.

It is important to note that essential oil 1 is more effective than essential oil 2 for the two fungal species studied. Among these, *Fusarium sp*. exhibited a higher sensitivity than *Aspergillus niger*.

Table 8. Estimated growth kinetics parameters of *Fusarium sp.* at different concertation of fumigated essential oil. Mean values ± 95 % confidence interval.

	Control	C1	C2	C3	C4	C5	C6
Essential oil 1							
μ (mm/h)	0.709 ± 0.040	NG	NG	NG	NG	NG	0.780 ± 0.713
λ (h)	13.1 ± 5.7	-	-	-	-	-	53.0 ± 49.8
d^0 (mm)	4.5 ± 1.9	-	-	-	-	-	4.5 ± 3.3
RMSE	2.23	-	-	-	-	-	1.51
R^2	0.990	-	-	-	-	-	0.991
Essential oil 2							
μ (mm/h)	0.709 ± 0.040	NG	NG	0.564 ± 0.039	0.601 ± 0.109	0.446 ± 0.062	0.630 ± 0.029
λ (h)	13.1 ± 5.7	-	-	54.3 ± 2.4	41.1 ± 8.0	29.8 ± 7.4	26.9 ± 2.9
d^0 (mm)	4.5 ± 1.9	-	-	4.5 ± 0.7	4.5 ± 2.6	4.5 ± 1.3	4.5 ± 1.6
RMSE	2.23	-	-	0.94	2.94	1.51	1.04
R^2	0.99	-	-	0.992	0.961	0.986	0.998

RMSE: Root Mean Squared Error between experimental and estimated colony diameters.
NC: No Converged solution was obtained; NG: Not Growth.

Conclusion

The food industry employs diverse strategies to combat fungal growth and spoilage, yet reliance on chemical additives faces challenges due to consumer perception and regulatory constraints. Consequently, there is a burgeoning interest in exploring natural alternatives like essential oils for food protection. This study investigates the diffusion effects of essential oils from two plants, *Thymus algeriensis* and *Origanum vulgare*, on the germination kinetics and fungal growth of Fusarium sp. and A. niger.

Hydrodistillation using the Clevenger apparatus extracted the essential oils, which were then subjected to direct contact and fumigation antifungal tests.

Results revealed that *Origanum vulgare* essential oil exhibited significant antifungal activity against both fungal species compared to *Thymus algeriensis*. Notably, Origanum vulgare essential oil was more effective than *Thymus algeriensis*, with Fusarium sp. demonstrating greater sensitivity than A. niger. *Thymus algeriensis* oil showed no antifungal activity against A. niger due to α-glucan polysaccharides in the fungal cell membrane, providing significant resistance. MIC values for A. niger were EO1 (1/16 MS) and EO2 (1/8 MS), and for Fusarium sp., EO1 (1/4 MS) and EO2 (1/4 MS). MFC values for A. niger were EO1 (1/4 MS) and EO2 (1/2 MS), while EO2 displayed no fungicidal properties against Fusarium sp.

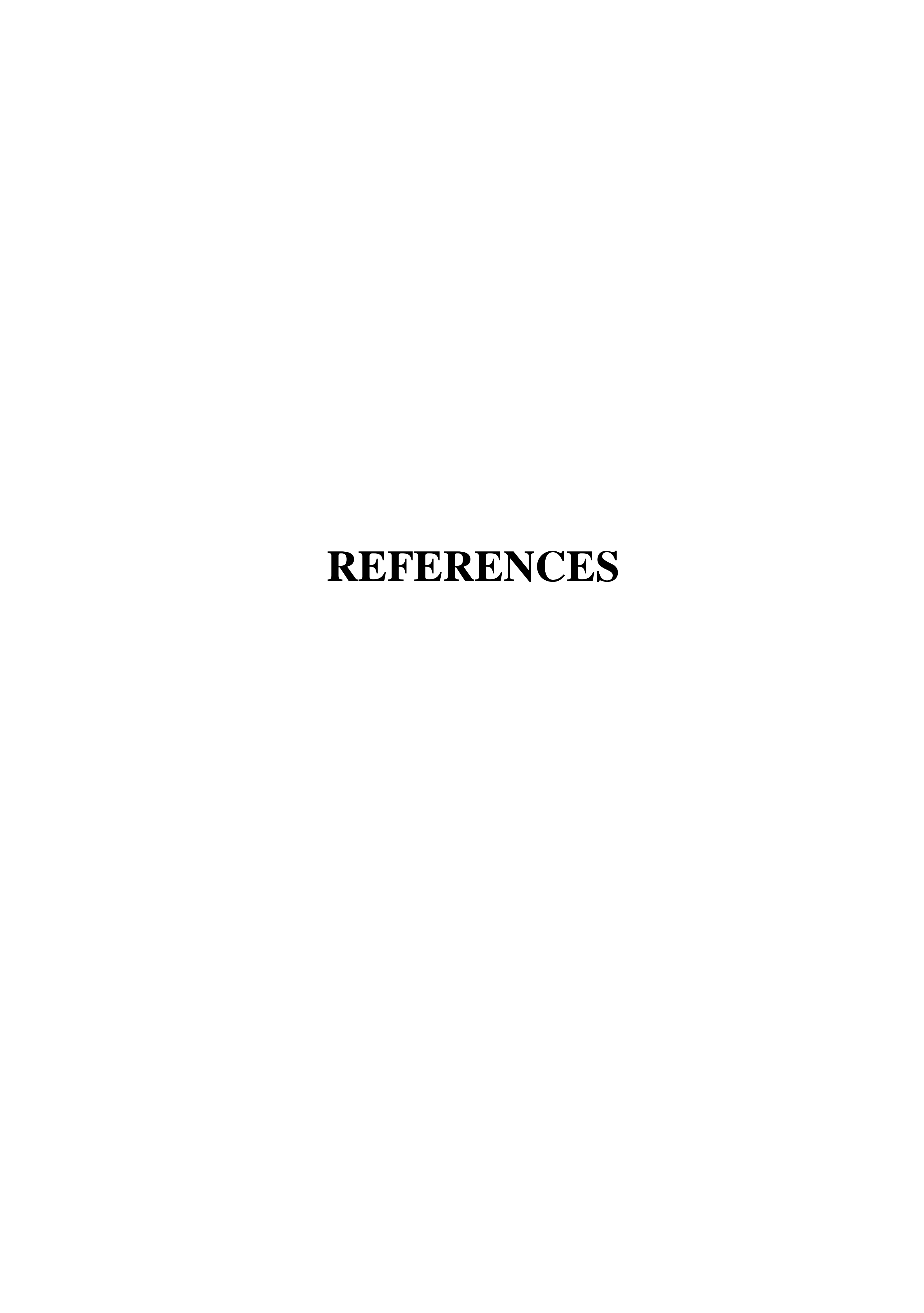
The growth rate of A. niger was less than 0.5 mm/h, with the highest rates observed for the control and the lowest concentration of both oil 1 and oil 2.

For Fusarium sp., growth rates (μ) were consistently below 1 mm/h across all conditions. The control exhibited the highest rate at 0.709 ± 0.040 mm/h, followed by oil 1 at 0.780 ± 0.713 mm/h, and oil 2 at 0.630 ± 0.029 mm/h for the lowest concentration of essential oil.

Fusarium sp. displayed heightened sensitivity relative to A. niger.

The study concludes that the stereochemical properties of essential oil compounds and polysaccharide structures in fungal cell walls significantly influence sensitivity or resistance to essential oil bioactive substances. Overall, essential oil 1 outperformed essential oil 2 for both fungal species, with Fusarium sp. showing higher sensitivity than A. niger.

Future research should aim to advance edible coating technologies and concentrate on refining the formulation of bio-based packaging materials and their application methods.



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