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Title

Antifungal activity assessment of some plant extracts against *Trichothecium* sp.

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(وَإِذْ تَأَذَّنَ رَبُّكُمْ لَئِن شَكَرْتُمْ لَأَزِيدَنَّكُمْ وَلَئِن كَفَرْتُمْ إِنَّ عَذَابِي لَشَدِيدٌ)

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All the thank to Mom for having been this institution where I learned the art of magnanimity and it is thanks to all that you have inculcated in me that I am here...

To the soul of my dear grandmother Nasria (رحمة الله عليها)

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To all those who have loved me for nothing, and have encouraged me all the time.

To those who want to see me succeed because they gave us the patience and courage to continue

And to all the people who know me...

Abdessamad

Dedication

In the name of Allah, the merciful, my Creator and my Master.

I'm dedicating this thesis to my beloved family who have meant and continue to mean so much to me. A special feeling of gratitude to my loving parents, my father El Hadj who has been a constant source of support and encouragement during this journey, and my mother M'hamed Habiba who has always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.

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Abstract

Trichothecium is a genus of plant pathogen fungi able to produce a wide variety of secondary metabolites including mycotoxins which can infect and spoil a variety of fruit crops, and thus, they have an economic impact on the farming industry in various countries worldwide.

This study aimed to assess the antifungal activity of aqueous and ethanolic extracts of *Juniperus oxycedrus*, *Peganum harmala*, *Nerium oleander* and *Rosmarinus officinalis* against the phytopathogen *Trichothecium* sp. using Agar well diffusion method.

The obtained results showed that the aqueous extracts of leaves of *R. officinalis* and *P. harmala* in addition to the ethanolic extracts of leaves and seeds of *P. harmala* and that of leaves of *J. oxycedrus* and *N. oleander* demonstrated an inhibitory action against the growth of *Trichothecium* sp.

This is the first study, to our knowledge, regarding the control of *Trichothecium* and could constitute a basis for the fight against such phytopathogens.

Keywords: *Trichothecium* sp., plant extracts, inhibitory concentration.

Résumé

Trichothecium est un genre de champignons phytopathogènes capables de produire une grande variété de métabolites secondaires, y compris des mycotoxines qui peuvent infecter et détériorer une variété de cultures fruitières, et ainsi, ils ont un impact économique sur l'industrie agricole dans divers pays du monde.

Cette étude a eu pour objectif l'évaluation de l'effet antifongique des extraits aqueux et éthanoliques de *Juniperus oxycedrus*, *Peganum harmala*, *Nerium oleander* et *Rosmarinus officinalis* contre le phytopathogène *Trichothecium* sp. en utilisant la méthode de diffusion sur gélose à partir des puits.

Les résultats obtenus ont montré que les extraits aqueux de feuilles de *R. officinalis* et *P. harmala* en plus des extraits éthanoliques de feuilles et graines de *P. harmala* et ceux des feuilles de *J. oxycedrus* et *N. oleander* ont un effet inhibiteur sur la croissance du mycélium de *Trichothecium* sp.

Cette étude constitue la première à notre connaissance concernant la lutte contre Trichothecium et peut constituer une base pour la lutte contre les agents phytopathogènes.

Mots clés:

Trichothecium sp., extraits de plantes, concentration minimale inhibitrice.

ملخص

شَعْرِيَّةُ القِراب هو جنس من الفطريات النباتية القادرة على إنتاج مجموعة واسعة من الأيضات الثانوية، بما في ذلك الميكوتوكسين، التي يمكن أن تصيب وتفسد مجموعة متنوعة من محاصيل الفاكهة، وبالتالي يكون لها تأثير اقتصادي على الصناعة الزراعية في مختلف البلدان حول العالم

وقد قيّمت هذه الدراسة النشاط المضاد للفطريات في المستخلصات المائية والإيثانولية لنباتات العرعر *Juniperus oxycedrus* ، الحرمل *Peganum harmala* ، الدفلى *Nerium oleander* وإكليل الجبل *Rosmarinus officinalis* ضد العامل الممرض النبات شعرية القراب (*Trichothecium* sp.)

أظهرت النتائج المتحصل عليها أن المستخلصات المائية لأوراق إكليل الجبل والحرمل وحتى المستخلصات الإيثانولية لأوراق و بذور الحرمل، بالإضافة إلى أوراق العرعر، إكليل الجبل والدفلى لديهم قدرة تثبيطية ضد نمو فطر شعرية القراب.

وهذه الدراسة هي الأولى، حسب علمنا، فيما يتعلق بالسيطرة على فطر شعرية القراب (*Trichothecium* sp.) ويمكن أن تكون أساسا للسيطرة على هذه العوامل المسببة للأمراض النباتية.

الكلمات المفتاحية: شَعْرِيَّةُ القِراب، مستخلصات نباتية، تركيز مثبط.

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INTRODUCTION

Introduction

Fungi have developed a plethora of strategies to colonize plants, and these interactions result in a broad spectrum of outcomes ranging from beneficial interactions to death of the host. With respect to plant pathogens, fungi represent probably the most diverse group of ecologically and economically relevant threats (Doehlemann et al. 2017). According to Bekkar (2014), plant pathogenic diseases reduce global agricultural production by 12-14 %, with 70 % of the damage being of fungal origin.

Trichothecium is a small heterogeneous genus of fungi present worldwide, contamination of food stuffs with Trichothecium spp. represents a real risk to the health of consumers because they are producers of mycotoxins. Tomato is very vulnerable to these fungi from the field through postharvest handling (Dal Bello 2008). Pink rot is a plant disease caused by *Trichothecium roseum* and can affect many fruits (apple, tomato, hami melon…etc.) as well as woody and herbaceous ornamentals (Sharma et al. 2014). This common and widespread disease can occur in commercial and residential plantings. As in other countries, diseases due to telluric fungi are frequently encountered in Algeria (Bekkar 2014).

The fresh fruit and vegetable industry faces the challenge of reducing its significant post-natal losses, largely due to disease and decay, without recourse to chemical treatments. To protect fruit and vegetables from pathogenic fungi during storage, the use of chemical fungicides has long been the most economically viable option. However, the increasing number of reported fungal-resistant pathogen strains and increased public awareness of the potential adverse effects of these chemicals on both human health and the environment have led to a review of their widespread use in agriculture (Charles et al. 2008).

With the increasing concerns of pesticide residues in agricultural products and environment as well as the incidence of resistance in plant pathogens against chemical pesticides, the use of non-chemical methods including natural metabolites have gained greater significance (Sarpeleh et al. 2009).

The use of plant extracts to protect stored products is one of the oldest practices in Africa due to the high rate of post-harvest losses on the continent. However, this practice was gradually abandoned with the emergence of synthetic pesticides during this century (Bouda et al. 2001).

In this perspective, our work consists in isolating and purifying the phytopathogen responsible of fruit rot *Trichothecium* sp. and evaluating the antifungal effect of aqueous and ethanolic extracts of some plants found in Algeria.

LITERATURE REVIEW

Literature review

1. *Trichothecium*

Trichothecium Link ex Fr. is a small and heterogeneous genus of fungi occurring throughout the world where 73 different species are recorded. *Trichothecium* Link was first reported in 1809. The main members of the genus are *Trichothecium polybrochum*, *Trichothecium cystosporium*, *Trichothecium pravicovi*, *Trichothecium luteum*, *Trichothecium parvum* and *Trichothecium roseum*. Conidiophores and conidia of the first three species are morphologically different from *T. roseum* (Persoon) Link ex S.F. Gray, the type species of the genus. Trichothecium species produce clusters of two-celled or single-septate conidia that are elliptical to pear-shaped (Bullerman 2003; Sharma et al. 2014). Species of Trichothecium are involved in food spoilage especially fruit rot during storage (Dal Bello 2008).

T. roseum, which is pink in color, is a common species that was placed in the form class Deuteromycetes or Fungi imperfecti with no sexual stages known in its life cycle (Sharma et al. 2014). It grows on various fruits and vegetables, as well as on cereal grains, such as barley, wheat, and corn, and cereal products such as flour but also on pecans, filberts, and meat. Some strains of Trichothecium produce the mycotoxins "trichothecenes" (Bullerman 2003).

1.1. Classification

The conidial stages of *Trichothecium* are very similar to the perfect fungi. Therefore, recent classification placed *Trichothecium* under the phylum Ascomycota. The current phylogenetic classification followed by the International Mycological Association, International Commission on the Taxonomy of Fungi, Systematic Mycology and Microbiology Laboratory (Fungal Databases, US Department of Agriculture Agricultural Research Service (USDA ARS), National Center for Biotechnology Information (NCBI), and Index Fungorum classified this fungus as:

- Kingdom: Fungi.
- Subkingdom: Dikarya.
- Phylum: Ascomycota.
- Subphylum: Pezizomycotina.
- Class: Sordariomycetes.
- Subclass: Hypocreomycetidae.
- Order: Hypocreales.
- Genus: Trichothecium.

1.2. Characteristic features

The morphological features of the *Trichothecium conidia* are shown in figure 1.

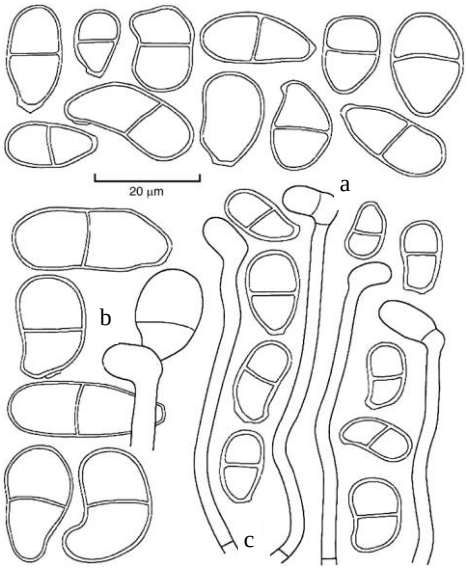

Figure 1. (a) *Trichothecium roseum* conidia; (b) *Trichothecium luteum* conidia and *conidiophores* with young conidia; (c) *Trichothecium parvum* conidia (Rifai and Cooke 1966).

a. Colony morphology

The colonies are flat, granular and powdery, white initially and then become pale pink to peach colored. Trichothecium grows rapidly at 25 °C on potato dextrose agar (PDA) and forms effused, velvety or powdery, whitish gray, yellowish, or pink colonies.

b. Mycelium

Trichothecium hyphae are septate, branched, smooth walled, hyaline or subhyaline.

c. Conidiophore

The conidiophores arise singly or in loose groups; erect, straight, or somewhat flexuous; mostly simple but occasionally branched, septate and scarcely swollen at the tip with meristematic apices capable of producing conidia in basipetal succession to form characteristic chains. The conidiophores are indistinguishable from the vegetative hyphae until the first conidium is produced. The conidiophore is shortened progressively with the formation of each conidium, that is, retrogressive conidial development. The characteristic basipetal catenulate conidial cluster and the asymmetric basal cell of the conidium are characters of great diagnostic value in assigning a species to the genus *Trichothecium* (Sharma et al. 2014).

2. Rot disease

Plant rot are diseases that can be caused by any of hundreds of species of soil-borne bacteria, fungi, and fungus like organisms (Oomycota). They are characterized by plant decomposition and putrefaction. The decay may be hard, dry, spongy, watery, mushy, or slimy and may affect any plant part (Encyclopaedia Britannica 2017).

2.1. Pink rot of apples

It is one of the principal postharvest diseases caused by *Trichothecium* that can affect Hami melons (Bi et al. 2003), apples and tomato (Sharma et al. 2014). The disease can be controlled by iprodione and azoxystrobin (Ma et al. 2004), however, they can be harmful to the environment and human health. In addition, they can induce fungicide pathogens resistance (Bi et al. 2005).

The disease is seen as a large lesion starting at the level of the stem scars or stylus, on the side of the fruits or from a wound such as a burst. The affected tissue initially turns dark green, browning slightly while a sour rot gradually develops. If the climatic conditions are wet, these lesions are covered with a rather characteristic pink mold. For instance, the symptoms on apples are observed as brown rot with pinkish spores on rotted tissue. (Hamid et al. 2014).

2.2. Control methods

Many crop plants are susceptible to various pathogens. Pesticide application is currently the primary means of control. This strategy can be effective but the repeated use of these substances generates pollution problems, as well as the rapid emergence and spread of strains of resistant pathogens (Bonzi 2007).

2.2.1. Pesticides

Pesticides used for managing fungi-caused fruit diseases are either fungicidal or fungi-static. Fungicides can be separated into two categories: protectants and systemic. Protectant fungicides protect the plant against infection at the site of application, and Systemic fungicides prevent disease from developing on parts of the plant away from the site of application. The application of fungicides in agriculture should be under strict regulation to ensure that commercialized foods are safe for consumption and pose negligible risks of acute toxicity due to carry-over (Veronica et al. 2019).

Besides, the use of pesticides generates many inconveniences. Indeed, the repeated use of active substances can generate the rapid emergence and spread of strains of resistant pathogens. In addition, pesticides pollute dangerously the environment and consequently affect human and

animal health. It is therefore imperative to find alternatives to these pesticides that are not harmful to the environment and human health (Charles et al. 2008). Furthermore, the excessive use of pesticides can also cause environmental problems; accumulation of pesticides in the environment disrupts the ecological balance and gives rise to pathogenic resistance to the pesticides (Veronica et al. 2019).

2.2.2. Using resistant plant varieties

Using resistant varieties, minimizing tree stress, and maintaining proper soil fertility reduces disease incidence since pathogens do not reproduce well on trees that are less susceptible to disease. As a result, the chance of resistance decreases. Avoid selecting sites with high disease pressure since this increases the chance of selecting resistant fungi.

2.2.3. Using plant extracts

The use of plant extracts has definite advantages. With the increase of chemicals prices and the scarcity of these products in local markets, products biodegradable from plants are a good alternative that allows producers to be able to protect their seeds at a relatively low cost. The reduction in the use of chemical pesticides due to the use of plant extracts contribute enormously to the reduction of environmental pollution and it also improves the public health.

In addition, because of the possible multiple resistances and side effects of the synthetic antimicrobial, increasing attention has been directed towards natural antimicrobial (Namiki 1990). Recently, the use of plant extract in the control of fungi is promising given their effectiveness and their environmental safety (Bonzi 2007).

a. *Peganum harmala* L.

P. harmala L. (Fig. 1), belonging to the family Zygophyllaceae, also known as Syrian rue and Harmal in north Africa (Esmaeil et al. 2011), is a herb that grows in Africa, the Middle East, India, South America, Mexico, southern USA and China (Kartal et al. 2003; Xiaojin et al. 2013). It is a medicinal plant with anti-microbial (Arshad et al. 2008), anti-inflammatory and analgesic properties (Monsef et al. 2004). Since ancient times, harmal was claimed to be an important medicinal plant. Its seeds were known to possess antimicrobial, antifungal and antiviral properties. Harmine, harmaline and their derivatives, harmal, harmalol, tetrahydroharmine and tetrahydroharmol isolated from the seeds of *P. harmala* have shown the anti-microbial and antifungal activity on human diseases (Hamid et al. 2009; Sarpeleh et al. 2009).

In Sarpeleh et al. (2009) study, the maximum antifungal activity was detected in seed extracts where harmine has been isolated in highest concentration suggesting that the antifungal activity observed in *P. harmala* is due to harmine.

Figure 2. *Peganum harmala* L. (Jinous and Fereshteh 2012).

Besides, to date there are few reports about studies on the bioactivity of *P. harmala* proteins. Luo et al. (2010) suggested that the crude protein extracts from Harmal could inhibit the proliferation of fungi. Xiaojin et al. (2013) isolated and characterized a novel antifungal protein (PHP) with a molecular weight of 16 kDa from *P. harmala* seeds for the first time. PHP exerted lipid binding, antifungal, antitumor, and anti-HIV-1 reverse transcriptase (RT) activities. Their results provide a scientific basis for the agricultural and medicinal use of this protein.

b. *Juniperus oxycedrus*

J. oxycedrus (Fig. 3) or cade juniper (Cupressaceae) is a shrub or small tree growing wild in stony places of the Mediterranean and Near East countries. It is one of the most appreciated plants for its richness in essential oil and its plethora of biologically active compounds extensively used in traditional medicine. *J. oxycedrus* was used for the treatment of various diseases, such as hyperglycemia, obesity, tuberculosis, bronchitis and pneumonia.

There are many reports on the chemical composition of the oils from Juniperus species, most of these reports indicate that α-pinene, manoyl oxide and Z-caryophyllene, δ-3-carene, geranyl acetone and caryophyllene oxide are the main constituents of these oils. Cedrol, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, monoterpene hydrocarbons and diterpenes were also reported (Ismail et al. 2012).

Figure 3. *Juniperus oxycedrus* (Lara et al. 2016).

Ismail et al. (2012) showed that there was a correlation between the antifungal activity and percentage of some components present in *J. oxycedrus* essential oils. Indeed, many authors have attributed the antifungal capacity of essential oils from different Juniperus, Calocedrus, Pistacia and Cupressus species to the presence of α-pinene, Z-caryophyllene and other sesquiterpenes.

c. *Rosmarinus officinalis* L.

R. officinalis or Rosemary (Lamiaceae) is a common dense, evergreen, aromatic shrub which belongs to mint family, grown in many parts of the world. The fresh and dried leaves are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods.

Historically, rosemary has been used as a medicinal agent to treat renal colic and dysmenorrhea. It has also been used to relieve symptoms caused by respiratory disorders and to stimulate the growth of hair. Extracts of rosemary are used in aromatherapy to treat anxiety-related conditions and to increase alertness.

There are 22 components, representing 97.41% of the total oil of *R. officinalis*. The major components are 1,8-cineole, α-Pinene, Camphor, Camphene and α -Pinene that have already been proven to possess antimicrobial activity (Yang et al. 2011).

Figure 4. *Rosmarinus officinalis* L.

d. *Nerium oleander*

N. oleander (Fig. 5), a member of the Apocynaceae family, is an ornamental plant that widely grows in the tropic and sub-tropic regions including Mediterranean area. Studies have shown that the extract of this plant contains cardiac glycosides, polysaccharides, some cardenolids and triterpenoids. Although the plant contains toxic agents, it is used against cancer, depression and cardiovascular purposes. In addition, the chemical extract of *N. oleander* reveals antimicrobial and antifungal activities, antidiabetic, antioxidant, antitumor and hepatoprotective activities (Yousra et al. 2021), antinociceptive, insecticidal and central nervous system depressant activity (Siddiqui et al. 2016). Singh et al. (2013) showed that the cardiac glycoside-enriched fraction of hot oleander extract, reduced infectivity of HIV (Giuseppina et al. 2019).

Elhoussine et al. (2010) analyses for flower part resulted in the identification of 34 compounds, representing 93.21% of the total oil. The major component was neriine (22.56 %), other predominant components were digitoxigénine (11.25 %), amorphane (8.11 %), 1.8-cineole (6.58 %), α-pinene (5.54 %), calarene (5.12 %), limonene (5.01 %), βphellandrene (4.84 %), terpinene-4-ol (3.98%), sabinene (3.22%), isoledene (2.94%), 3-carene (2.56%), humulene (2.29%), β-pinene (2.01 %) and cymen-8-ol (1.67 %).

Figure 5. *Nerium oleander*.

The extract of *N. oleander* has been shown to have antifungal activity against fungal strains *Candida albicans* and *Aspergillus flavus* and shows potency as that of standard fluconazol. (Nitave and Patil 2015). Siddiqui et al. (2016) demonstrated that all parts of *N. oleander*, leaves, stem and roots can reduce the growth of fungal colony. On commercial basis *N. oleander* is efficient in producing antifungal compounds alkaloids, flavonoids, carbohydrates, glycosides and tannins which can be exploited against many economically important pathogenic fungi (Siddiqui et al. 2016).

METHODOLOGY

Methodology

1. Aim of the study

This study aimed to evaluate the antifungal activity of aqueous and ethanolic extracts of *Rosmarinus officinalis*, *Peganum harmala* L., *Juniperus oxycedrus* and *Nerium oleander* against the phytopathogen *Trichothecium* sp.

2. Material and methods

2.1. Biological material

a. Plants

Plant extracts were prepared from four species; these are *Rosmarinus officinalis*, *Peganum harmala* L., *Juniperus oxycedrus* and *Nerium oleander*, that were collected during March 2021 from Tiaret, Algeria.

b. *Trichothecium* sp.

Trichothecium sp. was isolated from rotted leaves of tomato.

2.2. Methods

2.2.1. Plant extracts preparation

Aqueous and ethanolic extracts (10 % w/v) were prepared according to (Abubakar and Haque 2020). Leaves of all the tested plants were used in addition to *P. harmala* seeds, they were shade dried and then grinded to powder. 10 g of the powder was added to 100 ml of distilled water for the aqueous extract and to 100 ml of 10 % ethanolic solution for alcoholic extract. They were left to macerate 72h during which mild agitation was performed. After the maceration period the extracts were filtered with Whatman filter paper (3 mm). The filtrate was then used for antifungal activity testing.

2.2.2. *Trichothecium* sp. isolation

Rotted leaves of tomato were placed on slices of disinfected potato and were incubated for 5 days at room temperature (≈ 25°C). After this period of time, a fragment from formed mycelia was transferred on potato dextrose agar (PDA) supplemented with gentamycin and incubated at room temperature until purification of the isolate. Macroscopic and Microscopic observations were performed on the isolate to ensure its purity.

2.2.3. Antifungal activity testing

Agar well diffusion method was used to test the antifungal action of the four plant extracts against the isolated *Trichothecium* sp. (Ncube et al. 2008). Briefly, Petri dishes containing 20 ml of PDA were used. Wells (6 mm in diameter) were made using Pasteur pipette (Fig. 6). A disk of agar containing the fungus was placed on the centre of Petri dishes containing the sterile MH and nutrient agar and then the wells were filled with 10 µl of each plant extract to be tested. The dishes were incubated at room temperature during 5 days. After incubation, diameter of fungus growth was measured.

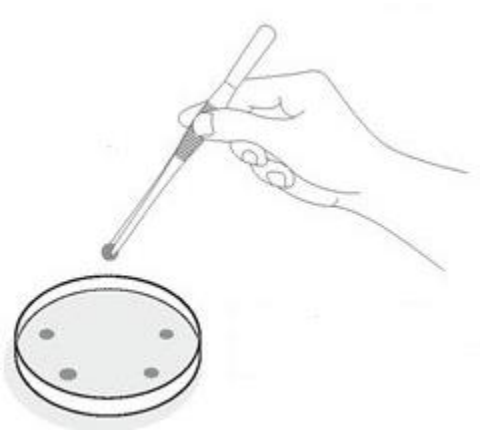

Figure 6. Agar well diffusion method used to test the antifungal activity.

RESULTS

Results

1. Isolated *Trichothecium* sp.

After several subculturing of the colonies formed on the agar, the obtained pure culture was observed at the microscope.

The macroscopic and microscopic observations revealed characteristic features of Trichothecium species; that is white powdery appearance of the colony (Fig. 7a) and cluster of two-celled septate pear-shaped conidia (Fig. 7b).

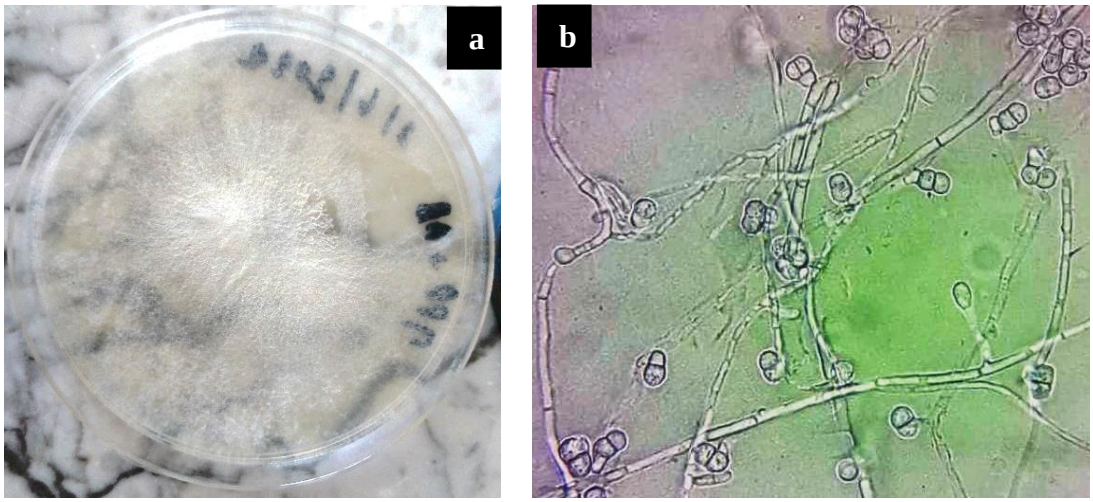

Figure 7. Macroscopic (a) and microscopic (b) observations of *Trichothecium* sp.

2. Antifungal activity

Obtained results of the antifungal activity testing after 5 days incubation of *Trichothecium* sp. with the different plant extracts are shown in figures 8 to 10.

We note on figure 8 that the mycelial growth of the colony of *Trichothecium* sp. does not go far from the center of Petri dish and is limited by the ethanolic extracts of *P. harmala* leaves (Ps) and *J. oxycedrus* leaves (J). This result demonstrates the antifungal action of the plant ethanolic extracts.

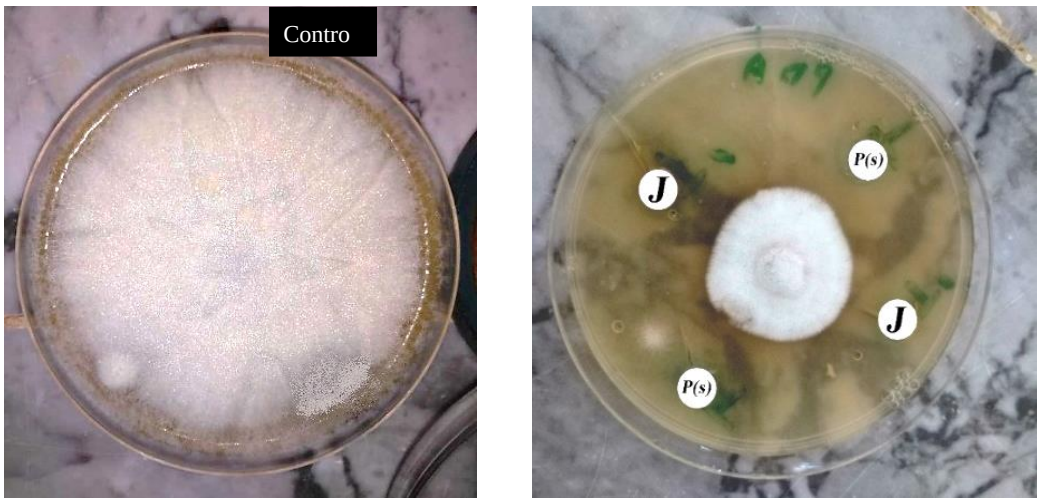

Figure 8. Mycelial growth of *Trichothecium* sp. in the presence of ethanolic extracts of *P. harmala* seeds (Ps) and *J. oxycedrus* leaves (J).

Besides, the aqueous and ethanolic extracts of *P. harmala* leaves also showed a limitation of the growth of the fungus demonstrating their antifungal activity (Fig. 9).

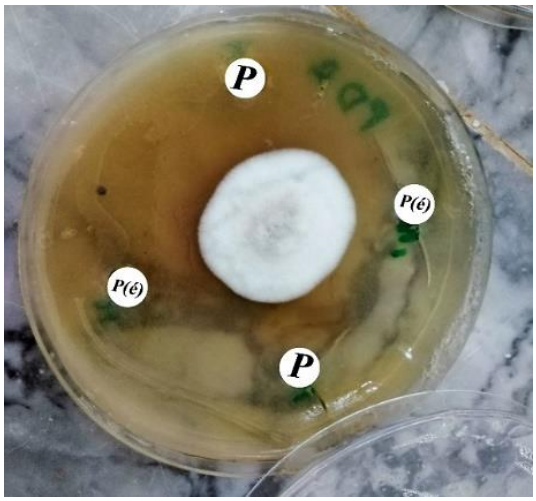

Figure 9. Mycelial growth of *Trichothecium* sp. in the presence of aqueous (P) and ethanolic (Pé) extracts of *P. harmala* leaves.

Regarding *R. officinalis* leaves' aqueous (R) and ethanolic (Ré) extracts, we noted a mycelial growth near the well containing the ethanolic extract while the growth is limited in the vicinity of the aqueous extract (Fig. 10). However, we noted the growth of bacterial colonies in all the wells containing the extracts. The result suggests that the ethanolic extract (10 %) of *R. officinalis* does not have an inhibitory effect against *Trichothecium* sp. While the inhibition seen in the aqueous extract may either mean that the extract has an inhibition action or that there was an antagonistic interaction between the fungus and the developed bacteria.

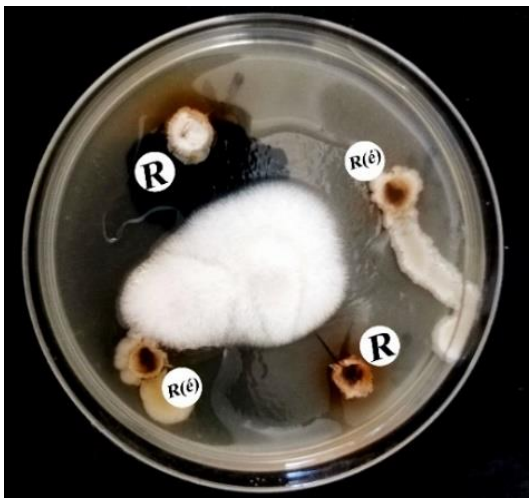

Figure 10. Mycelial growth of *Trichothecium* sp. in the presence of *R. officinalis* leaves aqueous (R) and ethanolic (Ré) extracts.

Furthermore, the ethanolic extract of *N. oleander* (Ne) inhibited the mycelial growth of *Trichothecium* sp. while we noticed a growth of the fungus near the well containing the aqueous extract (N) (Fig. 11).

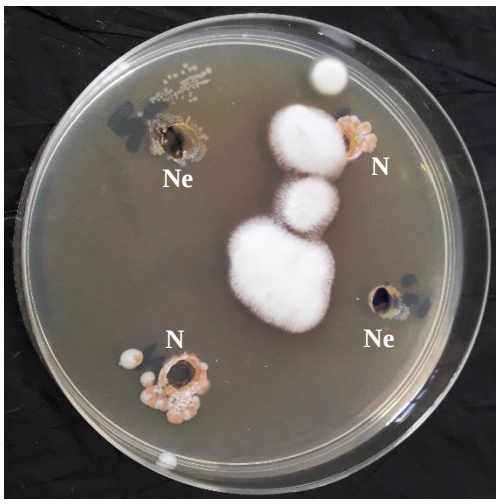

Figure 11. Mycelial growth of *Trichothecium* sp. in the presence of *N. oleander* leaves aqueous (N) and ethanolic (Ne) extracts.

*** The aqueous extracts of *P. harmala* seed and *J. oxycedrus* leaves were not assayed because the extracts have developed molds on their surfaces.**

DISCUSSION

Discussion

This study aimed to evaluate the antifungal effect of aqueous and ethanolic extracts of four plant species (*P. harmala*, *J. oxycedrus*, *R. officinalis* and *N. oleander*) against *Trichothecium* sp. This study is the first to our knowledge carried out on *Trichothecium sp.*

Except the aqueous extracts of *P. harmala* leaves and *J. oxycedrus* leaves and the ethanolic extract of *R. officinalis* leaves; all the extracts have demonstrated an inhibitory activity on the growth of the isolated phytopathogen *Trichothecium* sp.

The biological activity of plant extracts is mainly due to secondary metabolites found in all parts of the plant (Pagare et al. 2015). The choice of the solvent used during extraction process is important and depends on the type of plant, part of plant to be extracted and nature of the bioactive compounds. Water and ethanol are polar solvents and are often used in the extraction of polar compounds. Water dissolves a wide range of substances; it is cheap, nontoxic, nonflammable, and highly polar. However, it promotes bacterial and mold growth; it may cause hydrolysis, and a large amount of heat is required to concentrate the extract (Abubakar and Haque 2020). This can explain why some of our extracts were damaged by molds. As well, alcoholic solvents are miscible in water, they are nontoxic at low concentration, and are self-preservative at a concentration above 20 % (Abubakar and Haque 2020). In our study, we used ethanol as a solvent at a concentration of 10 %; the low concentration of ethanol can explain why molds have developed near the well containing the ethanolic extract of *R. officinalis* leaves.

In this study, the aqueous extract and ethanolic extracts of *P. harmala* seeds in addition to the ethanolic extract of its leaves have shown an inhibitory action against the tested fungus. Several studies have shown the antimicrobial activity of *P. harmala* extracts especially that of its seeds. This activity is mainly attributed to its bioactive compounds mainly alkaloids (harmaline, harmine and harmane) that are found in higher amounts in the seeds in addition to polyphenols, tannins and saponins (Ait Abderrahim 2018). Studies have shown that the antifungal activity of *P. harmala* is mainly due to harmine that was demonstrated to be highly soluble in water (Sarpeleh et al. 2009). Another study showed that *P. harmala* inhibited mycelia growth of *Alternaria alternate*, *Penicillium degitatum*, *Rhizopus stuolonifer* and *Magnaporthe grisea* (Xiaojin et al. 2013). Alcoholic extract of *P. harmala* seeds also showed an inhibitory effect on *Candida glabrata and Candida albicans* (Jinous and Fereshteh 2012).

Besides, studies showed that *J. oxycedrus* ethanolic extract has antifungal action mainly due to the presence of α-pinene, manoyl oxide, Z-caryophyllene and geranyl acetone (Ismail et al. 2012). Terfaya et al. (2017) showed that essential oil of *J. oxycedrus* have a significative inhibitory activity against six fungal strains of *Fusarium oxysporum f. sp Albedinis* in addition to dermatophyte strains (Cavaleiro et al. 2006).

Studies demonstrated the presence of polyphenols, particularly flavonoids, phenolic acids and phenolic terpenes in *R. officinalis* (Ibañez, et al. 2003). Several studies have shown the antimicrobial activity of *R. officinalis* essential oil, they attributed its inhibitory effects to the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol (Ait Abderrahim et al. 2017). Studies of Tirumalasetty, et al. (2014) reported that the antimicrobial activity of rosemary is highly attributed to carnosol and carnosic acid (phenol diterpenes). However, Moreno, et al. (2006) and Ivanovic, et al. (2012) demonstrated that the effectiveness of rosemary is related to a possible synergy between the rosmarinic phenolic acid and the carnosic acid diterpene.

Sepehri et al. (2016) demonstrated the antifungal activity of ethanolic extract of *R. officinalis* against *Candida albicans*. α-pinene, 1,8-cineole, and camphor are the major components of rosemary chemotypes and have been shown to possess antifungal activity (Matsuzaki et al. 2013). The essential oil of rosemary showed antimicrobial activity against *Candida albicans and Aspergillus niger* (Yang et al. 2011). Inhibitory activity was also observed against *Cryptococcus neoformans* for the majority of *R. officinalis* chemotypes tested (Satyal et al. 2017).

Regarding *N. oleander*, studies have shown that this plant contains numerous toxic compounds that are present in all parts of the plant; mainly oleandrin and nerine that are cardiac glycosides as well as cardenolides compounds that inhibit cellular membranes. A large amount of polyphenols is also present in the leaves of *N. oleander*. Cinnamic acid is the main component, the other components are epicatechin, catechin and chlorogenic acid in addition to the presence of tannins, alkaloids, saponins and terpenes which are known for their antioxidant and antimicrobial activity (Ait Abderrahim et al. 2017).

Studies have shown that *N. Oleander* is efficient in producing antifungal compounds that is; alkaloids, flavonoids, carbohydrates, glycosides and tannins that can be exploited against many economically important fungal pathogens (Siddiqui et al. 2016). *N. oleander* extract has been shown to reduce the growth of *Fusarium solani* and *Fusarium oxysporum* (Hadizadeh et al. 2009). It also has anti-mycotic activity against *Alternaria alternate* and *Rizoctonia solani* using agar dilution bioassay (Gupta et al. 2010).

CONCLUSION

Conclusion

Trichothecium spp. are plant pathogens able to produce a wide variety of secondary metabolites including mycotoxins which can infect and spoil a variety of food and fruit crops, thus, they have an economic impact on the farming industry in various countries worldwide.

Our study constitutes the first to our knowledge that assesses the antifungal action of some local plant extracts against *Trichothecium* sp.

Throughout this study we were able to demonstrated that the aqueous extracts of leaves of *R. officinalis* and *P. harmala* in addition to the ethanolic extracts of leaves and seeds of *P. harmala* and that of leaves of *J. oxycedrus* and *N. oleander*; have an inhibitory action against the mycelial growth of *Trichothecium* sp.

Although our results are preliminary, they constitute a basis for the use of plants as antifungal agents for the control of the rotting agents of fruit and vegetables specifically, and for the control of food spoilage agents in general

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