

IV. SUMMARY AND CONCLUSION

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages during pre-harvest and post-harvest for crops, vegetables and fruits. The microbiology of cereals and fruits are highly dominated by fungi, being *Aspergillus spp.* and *Fusarium spp.*, the most commonly isolated genera. Losses of plants quantity and quality due to plant infected with fungal pathogens and/or their toxins of great concern to public health it can only be reduced if protected the plants or crops by controlling fungal pathogens with a proper fungicides to protect the pre-harvest plants and reduce food borne outbreaks and assure consumers a continuing safe, wholesome, and nutritious food supply. Also food borne illness caused by consumption of contaminated foods with pathogenic fungi has been.

Chemical fungicides may be available to effectively control plants fungal diseases; Excessive application of these synthetic fungicides may not always be desirable. The improper use of these fungicides harmful effects on the health of human, animals and environment and the no longer make them attractive to use.

So that, the present work aimed to replace the undesirable and unsafe organic fungicides with natural and safe sources for potent biocontrol to destructive plant fungal diseases. The main objective of the present study was to investigate antifungal properties of wild and medicinal plants extracts and essential oils against some phytopathogenic fungi, causal agents of destructive diseases for economical crops, vegetables and fruits. The under investigation Fungi were: *Aspergillus flavus*, *A. oryzae*, *A. fumigatus*, *Penicillium chryogenum*, *p. commune*, *Fusarium oxysporum*, *F. brachygibbosum*, *F. solani*, and *Rhizoctonia solani*,

All the fungal species were isolated and studied morphologically using Traditional practices for studies of fungi include their macroscopic features as well as microscopic characteristics.

In order to confirm Traditional morphological characterizations of the fungal isolates by molecular identification by preparation of genomic DNA for different nine

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isolates and amplified ITS region by convention PCR using universal primers (ITS1, ITS4) and purifies the obtained PCR product. The purified PCR product was subject to DNA sequence and analyzed by the basic local alignment search tool (BLAST) at NCBI database to identify and differentiate between the isolated the fungal isolates. on the basis of AGS ct to DNA sequencing. The obtained sequences of ITS region rDNA ITS region with PCR technique were purified and the culture techniques Our results of DNA preparation and ITS region sequence and phylogenetic analysis were providing a sufficient genetic scaffolding to detect and precisely classify and reliably differentiate studied nine fungal species. Precise identification and diagnosis of plant pathogens during early stages of infection can help a lot in better management of the diseases. Soil borne pathogen being most difficult to identify during initial stages of infection can be successfully detected by molecular methods.

The overall evaluation of this study concludes that the investigated of medicinal plant extracts and essential oils have good antifungal activities revealing high inhibition properties The antifungal activities results *in vitro*: showed that the plant extracts from *Zingiber officinale*, *Anethum graveolens*, *Alium sativum*, *Foeniculum vulgare*, *Cassia Senna*, *mentha varidis*, *Syzygium aromaticum*, *Thymus vulgaries*, *Alium cepa*, *Olea europaea*, *Lavandula angustifolia*, *Nigella Sativa* and *ziziphus spina-csisti* were more effective for inhibiting the growth of nine tested fungi causing plant diseases than the rest of plant extracts under investigation .The best of these plant extracts to inhibit the growth of *F. oxysporum*, *F. solani* *F. brachygibbosum*, *R. solani*, *A. Fumigatus*, *A. flavus*, *F.oryzae*, *P. chryogenum* and *P. commune* were *Zingiber officinale*, *Anethum graveolens*, *Alium sativum*, *Foeniculum vulgare*, *Cassia Senna*, *mentha varidis* and *Syzygium aromaticum*. The minimum inhibitory concentration result of all tested plant extracts by broth dilution technique were showed antifungal activity against nine plant pathogenic fungi with MIC values ranging from 0.25 to 4mg/m

. The finding of the present investigation could be an important step towards the possibilities of using natural plant products as biocontrol agents for plant diseases caused

by different fungal species: *F* spp., *R. solani*, *A.* spp and *P.* spp as eco-friendly antifungal agents. However further studies are needed to isolate and identify the most effective antifungal compounds of these promising plants

Plants produce a broad range of bioactive chemical compounds via their so called secondary metabolites. Plant extracts may be an attractive alternative for the use of natural product to control phytopathogenic fungi avoiding chemical fungicide application. Further purification, extraction and photochemical analysis of the active compounds of those plants would give a strong antifungal activity comparable to synthetic fungicides.

Qualitative results obtained from preliminary phytochemical screening of essential oils and TLC separation of methanolic extracts of 15 medicinal plants showed the presence of various secondary metabolites like carbohydrate, protein, phenols, saponins, tannins, terpenoids and flavanoids. Other phytoconstituents like alkaloids and fixed oil in high amounts, which may be responsible for the antifungal efficacy of these medicinal plants.

As a pilot investigation, we tried to determine effectiveness of selected essential oils of 15 Egyptian medicinal plants, which showed good antifungal activity *in vitro*. Chemical analysis and identifications of different bioactive compounds from selected oils were performed by using GC-mass technique and showed that a number of antifungal compounds of diverse skeletal patterns have been found in the plants. These compounds belong mainly to six broad chemical groups, such as terpenoids, phenolics and phenolics acids, saponins, tannins, flavonoids and iso-flavonoids, Terpenoids and steroidal alkaloids. The GC/MS result revealed that presence of variable levels of one / or more of interesting bioactive compounds, like: Eucalyptol, Limonene, D-Limonene , Linalool, Zingiberene, thymol , p-cymene, Eugenol, Alpha- Farnesene, Carveol, propyl

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trisulphide, methyl propyl trisulphide, Menthol, Estragole, Octadecadienoic acid, n-Hexadecanoic acid, methyl ether, Alpha- Phellandrene, Menthone, Alpha- Terpir, Gamma- Terpineol, piperitone, Palmitic acid, Lavandulyl acetate, Caryophyllene, Oleuropein, Verbascoside, Geranyl acetone and Methyl hexadecanoate which are found to have plenty of pharmacological properties responsible for the antifungal activity of these plants. Further studies are needed on these extracts in order to isolate, identify, characterize and elucidate the structure of these compounds.

On the basis of obtained results, it can be **concluded that** the important and potential role of the selected herbal and medicinal plants as biocontrol agents against the investigated phytopathogenic fungal isolates and the finding of the present investigation is very encouraging and could be an important step towards great potentials in the control of plant and storage fungal diseases using naturally occurring substances that are both humanly and environmentally friendly and at the same time affordable at less cost to the users than the procurement and use of chemically formulated fungicides

The ultimate conclusion of this study supports the traditional uses of different medicinal plant extracts in safe controlling of different plant diseases caused by pathogenic fungi. It also suggests that a great attention should be paid to herbal and medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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المخلص العربي

تعد الفطريات المسببة للأفات النباتية بمثابة عامل العدوي الرئيسي في النباتات، مما تسبب تغيرات خلال مراحل النمو خلال مرحلة ما قبل الحصاد وما بعد الحصاد للمحاصيل والخضار والفواكه و من بين أكثر الكائنات الدقيقة أصابة للحبوب والفواكه الفطريات و التي تهيمن سلالات من الفطريات الرشاشيات والفيوزاريوم و حيث يتم فقد في كمية و جودة الغذاء نتيجة الاصابات الفطرية أو سمومها. و لتقلل حجم الخسائر الناجمة عن الأصابات الفطرية للنبات قبل و بعد الحصاد خلال مراحل تخزينها بعد حصادها لفترات طويلة يمكن ان تنخفض اذا ما تم معاملة البذور بالمبيدات الفطرية. و بالنظر الى الآثار الضارة للمبيدات الفطرية العضوية و المستخدمة حاليا على الصحة العامة والبيئة مما قلل من الاقبال عليها و أستخدامها. الامراض الناجمة عن استهلاك الاغذية الملوثة بالبكتيريا لالمرضة او سمومها مصدر قلق كبير للصحة العامة. السيطرة على الكائنات الدقيقة المرضية المسببة لأمراض يمكن ان نقلل من انتشارها عبر الاغذية لتأكيد الاستمرار في الاستهلاك ليكن امانا و صحيا.

ومن الممكن مكافحة تلك الأمراض الفطرية المدمرة للنباتات باستخدام المبيدات الكيميائية المتاحة و الفعالة في مكافحة المرض و لكن وجد عدم رغبة في استخدام تلك المبيدات الكيميائية نظرا لتأثيرها السلبي على صحة الانسان و الحيوان و النبات و أمن البيئة

ذلك و حيث تهدف تلك الدراسة إلى استبدال المبيدات الفطرية العضوية غير مرغوب فيها و غير الآمنة مع مصادر طبيعية و آمنة للمكافحة البيولوجية الفعالة للأمراض الفطرية النباتية المدمرة. وكان الهدف الرئيسي من هذه الدراسة للتحقيق في الخصائص المضادة للفطريات من النباتات البرية والطبية من المستخلصات والزيوت الأساسية ضد الفطريات الممرضة للنبات و المدمرة للمحاصيل الاقتصادية، والخضروات والفواكه. و من بين تلك الفطريات ما يلي:-

Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigatus, Penicillium chryogenum, Penicillium commune, Fusarium oxysporum, Fusarium brachygibbosum, Fusarium solani, and Rhizoctonia solani,

و قد تم التأكيد على الصفات المورفولوجية التقليدية للفطريات المعزولة و ذلك من خلال التعرف الجزيئي باستخلاص الحمض النووي لمختلف العزلات التسعة وتكبير جزء من ITS من

خلال تقنية PCR تفاعل البلمرة المتسلسل و باستخدام بادئات خاصة بالجزء المكبر (ITS1)، ITS4) وينقي PCR الناتج و الذى بدوره يتم تحليل التتابع النيكلوتيدى له وفق قاعدة البيانات المتعارف عليها و وفقا لبنك الجينات للتحديد والتفريق بين العزلات الفطرية محل الدراسة بالإضافة الى تحليل شجرة النشوء والتطور و التى تمدنا بالمعلومات الوراثية الكافية للكشف والتصنيف المحكم وللتفريق بين التسع أنواع من الفطريات بشكل موثوق. تحديد دقيق وتشخيص لمسببات الأمراض النباتية خلال المراحل المبكرة من العدوى مما يساعد كثيرا في تحسين إدارة الأمراض. هناك صعوبة فى التعرف على مسببات الأمراض النباتية التى منشأها التربة أثناء المراحل الأولى من العدوى و لكن يمكن أن يتم الكشف عنها بنجاح بواسطة الأساليب الجزيئية.

و قد اوضحت النتائج النهائية لمحاولة تحديد فعالية الزيوت الطبيعية المختارة للخمس عشرة من النباتات الطبية المصرية، والتي أظهرت نشاط مضاد جيد في المختبر. تم إجراء التحليل الكيميائي وتحديد الهوية من المركبات النشطة بيولوجيا المختلفة من الزيوت المختارة باستخدام تقنية كمية لفصل الكروماتوجرافى للغازات GC-MS فأظهرت أن عددا من المركبات المضادة للفطريات متنوعة فى الانماط التركيبية تم العثور عليها فى النباتات. هذه المركبات تنتمي أساسا إلى ست مجموعات كيميائية واسعة، مثل تيربينويدس، الفينول وأحماض فينولية، الصابونين والتانينز والفلافونيدات وايزو-فلافونيدات، تيربينويدس وقلويدات الستيرويدية. كشفت نتيجة تحاليل GC-MS أن وجود مستويات مختلفة من واحد / أو أكثر من المركبات النشطة بيولوجيا و الغاية فى الأهمية، مثل: يوكالبيتول، الليمونين، D-الليمونين، لينالول، Zingiberene، الثيمول، ف سيمين، الأوجينول، سداسي Farnesene، Carveol، ثالث كبريتيد بروبييل، ثالث كبريتيد ميثيل بروبييل، المنثول، Estragole، حمض Octadecadienoic، حامض Hexadecanoic، الأثير الميثيل، سداسي فيلاندرين، Menthone، سداسي Terpir، غاما تريينول، piperitone، وحامض البالمتيك، Lavandulyl، خلات، كاريوفيلين، Oleuropein، Verbascoside، Geranyl، الأستون وميثيل هيكساديكانات التى توجد لديها الكثير من الخصائص العلاجية المسؤولة عن

النشاط الحيوى لهذه النباتات، وهناك حاجة لدراسات إضافية على هذه المقتطفات من أجل عزل وتحديد وتوصيف وتوضيح بنية هذه المركبات.

و حيث ان النباتات تنتج مجموعة واسعة من المركبات الكيميائية النشطة بيولوجيا عبر ما يسمى نواتج تفاعلاتها الثانوية. و قد تكون المستخلصات النباتية بديلا جذابا لاستخدام المنتجات الطبيعية للسيطرة على الفطريات الممرضة للنبات من أجل تجنب المبيدات الفطرية الكيميائية. يحتاج إلى مزيد من التنقية، واستخراج وتحليل الضوئي من المركبات النشطة من تلك النباتات تعطي النشاط مضاد قوي مشابه لمبيدات الفطريات الاصطناعية.

وأظهرت النتائج التي تم الحصول عليها من الفحص الكيميائي النباتي الأولي من الزيوت الأساسية والفصل الكروماتوجرافي على الشرائح الرقيقة حيث تم فصل مستخلص من الميثانولي للخمسة عشر نباتات طبييا و قد وجد العديد من المركبات الايضية الثانوية مثل الكربوهيدرات والبروتين والفينول، الصابونين والتانين وتيربينويدس و flavanoids. phytoconstituents الأخرى مثل قلويدات وزيت ثابت بكميات عالية، والتي قد تكون مسؤولة عن فعالية المضادة للفطريات من هذه النباتات الطبية

و قد استهدفت الدراسة الحالية: تقييم دور الزيوت الطبيعية و المستخلصات المائية و الكحولية (الميثانول) لعدد خمسة عشر نبات بين النباتات الطبية و هى حصالبان و الجنزبيل -الثوم- البصل- الشمر- الحبة السوداء- الزعتر- سنا (السنامكى) -النعناع- القرنفل- الشبث- الكافور-اللافندر- الزيتون-و السدر فى المكافحة الحيوية لبعض الفطريات النباتية التى عزلت من بعض المحاصيل المصرية و عرفت جزئيا و الفطريات هى:- اسبرجيلس فلافز اسبرجيلس اوريذا اسبرجيلس قيوميجاتس و بنسيليم كريوجينم و بنسيليم كومون و الفيوزاريوم اوكسيسبورم و فيوزاريوم براكيجبسوم و الفيوزاريوم سولانى و يزوكتونيا سولانى.

و اشتملت الدراسة على تقييم تأثير ثلاث تركيزات مختلفة لكل مستخلص من المستخلصات الزيتية و المائية و الكحولية على كل فطر على حدى و ذلك بقياس مدى التثبيط لنمو الفطر عند تلك التركيزات

و تحديد النباتات الاكثر فاعلية فى التثبيط للفطر و اشتملت الدراسة ايضا على اجراء تحليل وصفى الى لكيمياء النبات لكل نبات على حده مما اوضح وجود العديد من مركبات الايض الثانوية مثل الفلافونويدات و الالكاتويدات و الفينولات و التانين و الصابونيين و التيربينويدات بتركيزات مختلفة فى كل نبات و التى تم التعرف على العديد من مركباتها و التى لها تاريخ بحثى كمضادات فطرية و ذلك باستخدام جهاز الفصل الكروماتوجرافى للغازات و من تلك المركبات الليمونول- لينالول- ايجنول- مينثول- ليمونين- زينجيرين.

قد تم إجراء التحليل الكيميائي وتحديد مجموعة من المركبات النشطة بيولوجيا مختلفة من الزيوت المختارة باستخدام تقنية GC-MS الشامل وأظهرت أن عددا من المركبات المضادة للفطريات تنتمي أساسا إلى ست مجموعات كيميائية واسعة، مثل تيربينويدس، الفينول وأحماض فينولية، الصابونين والتانين والفلافونيدات و ايزو-فلافونيدات، تيربينويدس وقلويدات الستيريودية.

كشفت نتائج تحليل ال وجود مستويات مختلفة من واحد / أو أكثر من المركبات النشطة بيولوجيا محل الاهتمام، مثل: أيوكالينتول، الليمونين، D-الليمونين، ينالول، زينجيرين، الليمونول، بى سيمين، الأوجينول، سداسي Carveol، Farnesene، بروبيل ثالث كبريتيد، بروبيل ميثيل ثالث كبريتيد، المنثول، Estragole، حمض Octadecadienoic، ن Hexadecanoic حامض، الأثير الميثيل، سداسي فيلاندرين، Menthone، سداسي Terpir، غاما ترينينول، piperitone، وحامض البالمتيك، Lavandulyl خلات، كاريوفيلين، Oleuropein، Verbascoside، Geranyl الأستون وميثيل هيكساديكانات التي توجد لديها الكثير من الخصائص الدوائية المسؤولة عن نشاط حيوى لهذه النباتات. هناك حاجة لدراسات إضافية على هذه المقتطفات من أجل عزل وتحديد وتوصيف وتوضيح بنية هذه المركبات.

وبناء على ما تقدم ذكره من النتائج التي تم الحصول عليها، فإنه يمكن استنتاج أن الدور الهام والمحتمل من الأعشاب المختارة أو النباتات الطبية وقدرتها على مقاومة العزلات الفطرية الممرضة للنبات تحققت

والنتيجة التي توصلت إليها الدراسة الحالية أمر مشجع للغاية ويمكن أن يكون خطوة هامة نحو إمكانات كبيرة في مكافحة الأمراض الفطرية النباتية والتخزين باستخدام المواد الطبيعية التي هي على حد سواء إنسانيا وبيئيا ودية وفي نفس الوقت بأسعار معقولة بتكلفة أقل للمستخدمين من شراء واستخدام مبيدات الفطريات كيميائيا و قد خلصت الدراسة الى ضرورة دعم الاستخدامات التقليدية لمختلف مستخلصات النباتات الطبية فى السيطرة الآمنة للأمراض النباتية المختلفة التي تسببها الفطريات المسببة للأمراض. وتقتصر أيضا

أن ينبغي إبداء اهتماما كبيرا للنباتات العشبية والطبية التي تتميز بالعديد من الخصائص الدوائية التي قد تكون بما فيه الكفاية أفضل عند النظر في المكافحة الأمنة للأفات الفطرية التي تصيب النباتات.

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للنباتات"

رسالة

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كمطلب جزئى للحصول على درجة دكتوراة الفلسفة فى العلوم

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"الأستخدام الأمثل للنباتات الطبية البرية كمورد طبيعى فى المكافحة الحيوية للفطريات
الممرضة للنباتات"

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قسم الدراسات البيئية

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