EVALUATION OF MYRRH (*COMMIPHORA MOLMOL*) ESSENTIAL OIL ACTIVITY AGAINST SOME STORAGE FUNGI

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Abstract

The antifungal activity of Myrrh (*Commiphora molmol*) essential oil was tested against three storage fungi, *Aspergillus flavus*, *A. niger* and *Penicillium citrinum*. Results showed inhibitory effect of the oil against tested fungi with the increasing concentrations. Minimum inhibitory concentration (MIC) using agar dilution method was 4% (v/v) for *A. niger* and *A. flavus* and above 4% (v/v) for *P. citrinum*. Concentration (0.5-2)% (v/v) of oil also affected the sporulation of these fungi causing reduction or complete inhibition of sporulation process. Effect of Myrrh essential oil was also noticeable on spores germination, concentration of 4% (v/v) of oil inhibited complete germination of *A. niger* spores whereas this concentration caused 90% inhibition of *A. flavus* spores. Activity of vapour of Myrrh essential oil was investigated using a Petri dish atmosphere. Results showed inhibitory effect of the vapour with the increasing volume of essential oil added. MIC of vapour was (100) µl or slightly higher for all fungi.

Introduction

Myrrh is the hardened sap that oozes from the stem of *Commiphora molmol* (Family Burseracea) a tree that is native to desert areas of northern Africa and the middle East, collected from natural cracks or man-made cuts in the tree bark (1, 2). Myrrh had a number of uses throughout recorded history, valued as a fragrance as well as a medicinal agent by the early Egyptians and the ancient chines. Myrrh was well-known and used extensively during Bibilical times, it was used in foods and drinks as a flavoring agent, in perfumes and other cosmetics as a fragrance, in incense as a part of religious ceremonies and in embalming as a preservative (2). Medicinally, Myrrh was taken orally to treat arthritis, digestive complaints, schistosomiasis and respiratory infection; it was also taken to treat leprosy and syphilis (3, 4). Topically, Myrrh was also applied to bacterial and fungal skin infections (1, 5). Since fungi are significant destroyers of foodstuffs during storage retarding their nutritive value and sometimes by producing mycotoxins, storage fungi are common controlled by synthetic chemicals which almost creat several side effects to human health (6, 7). Therefore some alternative control had been applied recently including many plant extracts and essential oils obtained from various plant parts (8, 9, 10) to replace synthetic chemicals. The present work was dealing with the possibility of using Myrrh essential oil to control growth of some storage fungi including applicability of Myrrh essential oil as fumigants.

Materials and Methods

-Organisms

Single isolates of *Aspergillus flavus* (afaltoxin producing isolate), *A. niger* and *Pencillium citrinum* were obtained from the culture collection of the Department of Biology, College of Education Ibn Al-Haitham, Baghdad Univ. Cultures were maintained on potato Dextrose Agar medium (PDA).

-Essential oil

Myrrh was obtained from the local market as resin pieces. Extraction of Myrrh oil was done in our laboratory using a "Quick-fit" essential oil distillation apparatus.

-Effect of Myrrh essential oil on growth of the tested fungi

1-Agar dilution method

Agar dilution method was used to determine the antifungal activity of Myrrh essential oil (10). A series of two fold dilution of oil ranged from 4% (v/v) to 0.25% (v/v) was prepared in PDA medium after autoclaving. A final concentration of 0.5% (v/v) tween-80 was incorporated into the agar medium to enhance oil solubility. Plates

inoculated with (2) µl spot containing approximately 10^4 spore/ml in the center of the plate. PDA medium with 0.5% (v/v) tween-80 but no oil was used as a positive growth control. Inoculated plates were incubated at (25) ºC for (5) days.

Fungal colony diameters in control as well as in treatment sets were recorded and the results were expressed as percentage of mycelial inhibition. MIC was determined as the lowest concentration of oil inhibiting the visible growth on the agar plates.

2- Effect of Myrrh essential oil on spores germination

The method of (11) was used, spore suspension of *A. flavus* and *A. niger* was prepared from 1-2 weeks old slant cultures on PDA medium, harvested by covering the culture with sterile distilled water and brushing gently with an inoculation loop. The spore suspension was filtered through (4) layers of sterile muslin to remove hyphae. The spore suspension was collected and counted in a haemocytometer, adjusted to $2x10^5$ spore/ml. Germination experiment was performed using (5) ml of autoclaved PD broth medium in a vial, different concentrations of Myrrh essential oil was added. A final concentration of 0.5% (v/v) tween-80 was incorporated into the liquid medium to enhance oil solubility. Vials were inoculated with (0.5) ml of spore suspension and incubated at (25) ºC. Samples (loop full drop) were taken from the vials and examined microscopically after (18) hr. of inoculation to asses spore germination. Spores were recorded as having germinated when an emerging germ tube was perceptible. Germination counts were calculated from observation of 50-100 spores.

3- Effect of vapour (volatile phase) of Myrrh essential oil on tested fungi:-

The technique used in this experiment was the microatmosphere method described by (12). Pyrex glass Petri dishes were used with exactly the same shape (9 cm diameter, 1.5 cm high).

An exact volume of (22) ml of PDA medium was poured into each dish, leading to an identical internal atmosphere volume in all the dishes. Each dish was inoculated with (2) µl spot containing approximately 10^4 spore/ml in the center of the plate. The plates were left for about 5-10 min until the water of the inoculum was absorbed within the medium. The Petri dishes were turned upside down and a 2-cm diameter filter paper disc was put in the middle of the cover and soaked with variable amount of essential oil ranged from (25-150) µl. Control Petri dishes were prepared under the same condition except soaking the filter paper with (50) µl ethanol, the Petri dishes were incubated in reversed position for (5) days at (25) ºC. Results were recorded as colony diameters at different concentrations of the oil. The minimal amount of oil able to completely inhibit the growth of fungi can be also determined under experimental conditions.

Results and Discussion

Myrrh essential oil using agar dilution method Fig.(1) was inhibitory to the growth of the tested fungi. Gradual inhibition of growth with the increasing concentration was obvious. MIC for *A. niger* and *A. flavus* was 4% (v/v) whereas for *P. citrinum* it was rather above 4% (v/v). In this respect this result is an accord with (10) in which the MIC of Myrrh against *Candida albicans* was > 2% (v/v) using agar dilution method. The effect of essential oil was also obvious on sporulation of the tested fungi especially with *A. flavus*, 0.5% (v/v) inhibited complete sporulation of the fungus leading to a very dense and compact mycelial growth Fig. (2).

Effect of Myrrh essential oil on spore germination of *A. flavus* and *A. niger* was also noticeable Fig.(3). Concentrations (0.25, 0.5, 1, 2, 4) reduced spore germination gradually for *A. flavus* reaching to 10% germination at 4% (v/v) oil, whereas at this concentration complete inhibition of *A. niger* spores germination was shown and concentration 2% gave 5% germination, which indicate higher sensitivity of *A. niger* over *A. flavus*. Since there has been very little recognized measure to express the antimicrobial activity by gaseous contact and because essential oils are highly volatile at room temperature. In this respect (13) reported that volatile oils may have an important role in preservation of foodstuffs against filamentous fungi, as well as the gaseous studied and analyzed by (17), the

result phase of various essential oils are used now a days as ethical medicine for colds (14, 15) and inhalation therapy of essential oils has been used to treat acute and chronic bronchitis and acute sinusitis. Additionally, cinnamon bark oil was reported to have antifungal activity against respiratory tract mycoses (16), therefore the vapour effect of Myrrh essential oil was introduced in this investigation to evaluate its activity against three storage fungi. The results of exposing tested fungi to various amounts of Myrrh essential vapour in a Petri dish atmosphere Fig. (4) showed inhibitory activity of the vapour with the increasing concentrations of the oil. Volume (100) µl inhibited the growth of *A. niger* completely whereas at this concentration *A. flavus* and *P. citrinum* showed almost similar degree of growth, which indicate that the MIC of the vapour of Myrrh essential oil against the previous two fungi was slightly higher than (100) µl.

The antifungal activity of Myrrh essential oil on tested fungi is attributed to its constituents. The essential oil of Myrrh was showed the content of 15 compound, the main constituents is furanoeudesma-1, 3-diene. In this respect also (5) extracted, purified and characterized 8 sesquiterpene fractions from Myrrh, they focused their attention on a mixture of furanodiene-6-one and methoxyfuranoguais-9-ene-8-one, which showed antibacterial and antifungal activity with MIC ranging from 0.18-2.8 µg/ml. Additionally, (18, 19) found that Myrrh essential oil contains terpenes (C10 compounds), sesquiterpenes (C15compounds), esters, cinnamaldehyde, cumin aldehyde and eugenol among other ingredients. The sesquiterpenes fraction contains furanosesq-uiterpenes (18). Several of these components have been previously tested for biological activity and found to be active against numbers of bacteria and fungi (9, 13).

In conclusion, despite the small number of storage fungi tested in this work, Myrrh has proven to be an effective in controlling growth of storage fungi and usefulness as fumgants. Further studies are needed to test the vapour activity on wider atmosphere.

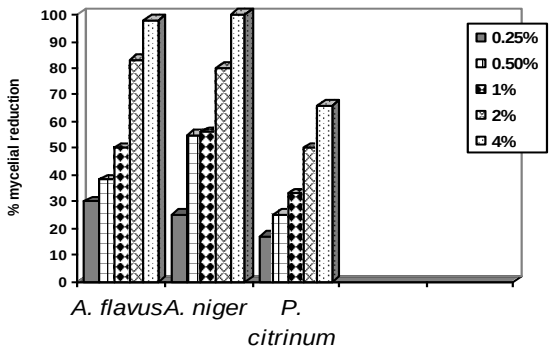

Fig. (1): Effect of Myrrh essential oil on radial growth of tested fungi (percentage of mycelial reduction).

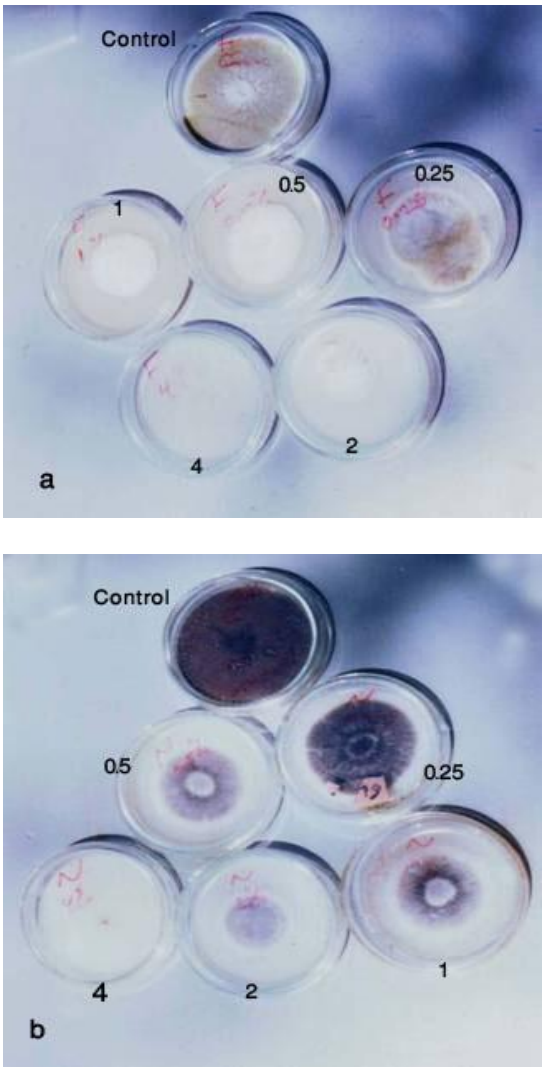

Fig. (2): Effect of Myrrh essential oil on growth and sporulation of a *A. flavus* and b- *A. niger*.

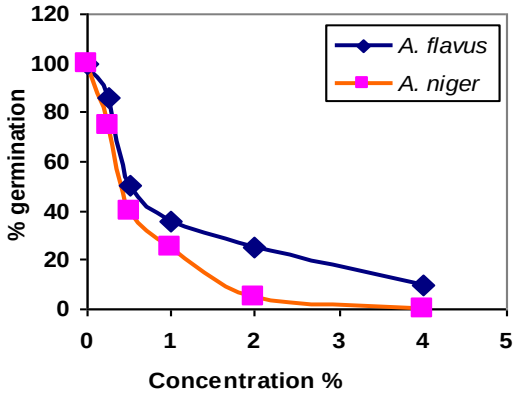

Fig. (3): Effect of Myrrh essential oil on growth and sporulation of a- *A. flavus* and b- *A. niger*.

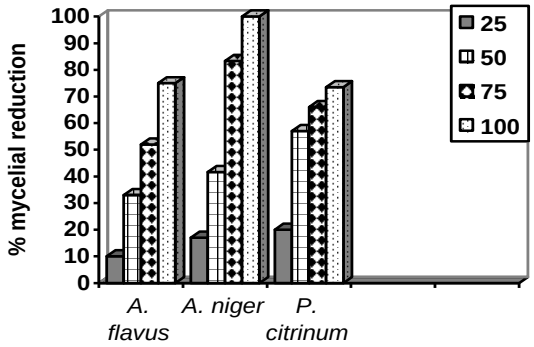

Fig. (4) : Effect of vapour of Myrrh essential oil (µl) on radial growth of tested fungi (percentage of mycelial reduction).

References

[1] Drug Digest. (2006). www. drugdigest. org/DD/DVH/Herbswho/O,3923,552,251,237.7c6umt myrrh.OG htmi.

[2] Marshall,S. (2004). Myrrh: Magi, medicine and mortality. The pharmaceutical J. 273: 919-921.

[3] Massoud, A.; Elsisi, S.; Salama, O. and Massoud, A. (2001). Preliminary study of the therapeutic efficacy of a new Fasiolicidal drug derived from *Commiphora molmol* (Myrrh). Am. J. Trop. Med. Hyg., 65 (2): 96-99.

[4] Sheir, Z.; Nasr, A.; Massoud, A.; Salama, O.; Badra, G.; E-Shennawy, H.; Hassan, N. and Hammad, S. (2001). A safe effective, herbal antischistosomal therapy derived from Myrrh. Am. J. Trop. Med. Hyg.; 65 (6): 700-704.

[5] Dolara, P.; Corte, B.; Ghelardini, C.; Pugliese, A.M.; Cerbai, E.; Menichetti, S. and Lonostro, A. (2000). Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from Myrrh. Planta. Med., 66 (4): 356-358.

[6] Bajaj, B.S. and Ghosh, A.K. (1975). Antifungal antibiotics in perspective, In: Raychand huri, S.F.; Verma, A.; Bhargava, K.S. and Mchrotra, B.S. (ed.). Advances in mycology and plant pathology. Sagar Printers, New Delhi, India.

[7] Beye, F. (1978). Insecticides from vegetable kingdom. Plant Res. Dev., 7: 13-31.

[8] Mishra, A.K. and Dubey, N.K. (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. Appl. and Env. Microbiol., 60 (4): 1101-1105.

[9] Elgayyar, M.; Draugham, F.A.; Golden, D.A. and Mount, J.R. (2001). Antimicrobial activity of essential oils form plants against selected pathogenic and saprophytic microorganisms. J. Food Prot., 64 (7): 1019-1024.

[10] Hammer, K.A.; Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol., 86 : 985-990.

[11] Ali, B.Z. (1984). A comparative study of growth regulation in fungi, Ph. D. Thesis. College ofScience, Durham Univ., U.K., pp. 202.

[12] Benjlali, B.; Elaraki, A.A.T.; Ayadi, A. and Ihlal, M. (1984). Method to study antimicrobial effects of essential oils: Application to the antifungal activity of six moroccan Essences. J. Food protection, 47 (10): 748-752.

[13] Deans, S.G. and Svoboda, K.P. (1990).The antimicrobial properties of Marjoram (*Origanum marjorana* L.) volatile oil. Flavour and Fragrance J. (5): 187-190.

[14] Federspil, P.; Wulkow, R. and Zimmermann, T. (1997). Effect of standardized Mytrol in therapy of acute sinusitis-Results of a double blind, randomized muticenter study compared with placebos. Laryngo- Rhino-Otologic, 76 : 23-27.

[15] Boyd, E.M. and Sheppard, E.P. (1970). Nutmeg oil and camphene as inhaled expectorants. Archieves of Otolarynglogy. 92 : 372-378.
[16] Singh, H.B.; Srivastava, M.; Singh, A.B.; and Srivislata, A.K. (1995). Cinnamon bark oil, a potent fungitoxicant against fungi causing respiratory tract mycoses. Allergy, 50 : 995-999.
[17] Tian, J. and Shi, S. (1996). Constituents of essential oil of imported Myrrh and gum opoponax. Zhongguo Zhong Yao Za Zhi: 21 (4): 235-237 (Abstract Article in Chinese).
[18] Eavn, W.C. (2002). Trease and Evans pharmacognosy (15th ed.) London, W.B. Saunders.
[19] Barnes, J.; Anderson, L.A. and Phillipson J.D. (2002). Herbal medicines (2nd ed.) London-Pharmaceutical Press.

الخلاصة

تم إختبار الفعالية المضادة للفطريات للزيت الأساس المستخلص من مادة المر تجاه ثلاث أنواع من فطريات الخزن، تضمنت *Aspergillus flavu*، *A. niger*، *Penicillium citrinium*. أظهرت النتائج تأثيراً مضاداً للزيت بزيادة التراكيز، وكان التركيز المثبط الأدنى للزيت بإستخدام طريقة التخفيف بالأكار بقيمة 4% للنوعين *A niger* و *A flavus* وأكثر من ذلك للفطر *P citrinum*. أظهرت التراكيز (0.5–2)% تأثيراً واضحاً على عملية إنتاج الابواغ من قبل الفطريات المدروسة . إذ أدت هذه التراكيز أما إلى إنخفاض واضح للأبواغ او منع تام لعملية التبويغ. كما أظهرت نتائج دراسة تأثير تراكيز مختلفة من الزيت الأساس تجاه إنبات الأبواغ للنوعين *A. flavus* و *A. niger* تأثيراً واضحاً للزيت في عملية الإنبات إذ أدى التركيز 4% إلى منع تام لإنبات أبواغ الفطر *A. niger* وخفض بنسبة 90% لإنبات أبواغ الفطر *A. flavus*. تم إختبار تأثير الزيت الأساس للمر بإستخدام الطبق الزجاجي . أظهرت النتائج كذلك فعلاً مضاداً للبخار بزيادة حجم الزيت المضاف وكان التركيز المثبط الأدنى (100) مايكروليتر أو أكثر قليلاً لجميع الفطريات المدروسة.