**Short Communication**

# Anti-Fungal Activity of Thymoquinone and Amphotericine B Against *Aspergillus Niger*

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**Abstract:**

Activity of *Nigella sativa* oil, ether extract and some of its active principles have been reported in the literature against a number of bacteria and *Candida albicans*. In the present study the effect of thymoquinone, an active principle of *N. sativa* and amphotericine B was determined against *Aspergillus niger* ATCC 16404. The organism was grown on dermasel agar containing 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of thymoquinone and dermasel agar alone as a control. There was 16.7, 36.2, 47.3, 67.8, 90.6 & 100% inhibition of growth of *Aspergillus niger* with these concentrations after 96 hours of incubation. Growth on the control plate after 96 hours was considered as 100%. Similarly there was 52.3, 65.1, 76.7, 81.6, 84.7, 85.6, 90.7, 92 and 93.8% inhibition of growth with 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 and 2.0mg/ml of amphotericin B.

**Key words**: *Aspergillus niger*, *Nigella sativa*, thymoquinone, amphotericin B and mycoses

## Introduction:

*Nigella sativa*, called as Habbah Al-Sauda in Arabic countries, is commonly used as a natural remedy for many ailments over 2000 years and is frequently added to bread and prickles as a flavouring agent[(1)]. Many active principles including thymoquinone, have been isolated from *N. sativa* seed [(2-4)]. Activity of *N.sativa* oil, ether extract and its active principles has been reported in the literature against a number of bacteria (including *Staphylococcus aureus*, *Pseudomonas aeruginosa* & *Escherichia coli*) and a yeast, like *Candida albicans* [(5-8)]. More recently ether extract of *N. sativa* and thymoquinone have been reported to possess antifungal activity against dermatophytes[(9)].

*Aspergillus* species are the most common mold causing severe infections[(10-12)]. *Aspergillus niger* is a filamentous mold. Even though filamentous molds are ubiquitous in the environment, only over the past two decades have such saprophytic fungi emerged as a major threat in patients with compromised host defenses, such as those with hematologic malignancies, bone marrow transplant recipients and HIV infection and is reported to cause cutaneous infection, paranasal aspergilloma and osteitis of middle ear in such patients [(13-16)]. *Aspergillus niger* is also reported to cause endocarditis after heart surgery and infection of exenterated orbit even in immunocompetent patients [(17, 18)].

In this paper we report the findings of the study of antifungal activity of thymoquinone, an active principle of *Nigella sativa*, and amphotericin B against *Aspergillus niger*.

## Materials and Methods

### a. *Growth and identification of Aspergillus niger*

A standard strain of *Aspergillus niger*, ATCC 16404, was cultured on dermasel agar (Oxoid). The plates were incubated at $30^0$C for 96 hours. The growth was identified as *Aspergillus niger* by colonial morphology and by microscopy after staining with lactophenol cotton blue.

### b. *Preparation of Reagents & Media*

Thymoquinone (Aldrich, USA) was dissolved in 5 ml of ethanol and then mixed with sufficient amount of pre-sterilized dermasel agar to obtain serial dilutions containing 2.0, 1.0, 0.5, 0.25, 0.125, & 0.062 mg/ml of thymoquinone. Similarly amphotericin B (SIGMA, USA) was dissolved in 5

ml of DMSO and then serially diluted in dermasel agar to give final concentrations of 2.0, 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015 & 0.0075 mg/ml. Four plates were used for each concentration of thymoquinone and amphotericin B. Four plates of dermasel agar alone were prepared as a control containing corresponding amounts of solvents.

### *C. Susceptibility Testing*

Susceptibility testing was carried out as described Ali-Shtayeh [19]. A 5 mm in diameter mycelial disc of *Aspergillus niger*, cut from the periphery of 48-72 hours old culture in dermasel agar was aseptically inoculated onto each set of above mentioned plates. The inoculated plates were incubated at $30^0$C for 96 hours. The growth was examined after 48 & 96 hours of inoculation and results were interpreted by measurement of the mean diameter of the growth. The percentage inhibition of *Aspergillus niger* with different concentrations of thymoquinone and amphotericin B was then calculated by taking its growth on non-drug dermasel agar as 100%. Minimum inhibitory concentration (MIC) of thymoquinone and amphotericin B was determined as their minimum concentration showing ≥90% inhibition of growth of the fungus.

## Results

There was 16.7, 36.2, 47.3, 67.8, 90.6 & 100% inhibition of growth of *Aspergillus niger* with 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of thymoquinone, giving MIC of 1.0 mg /ml when growth recorded after 96 hours. The growth on the control plates after 96 hours was considered as 100%. Similarly, there was 52.3, 65.1, 76.7, 81.6, 84.7, 85.6, 90.7, 92 & 93.8% inhibition of growth with 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 & 2.0 mg/ml of amphotericin B, giving MIC of 0.5mg/ml. Results are shown in table 1.

**Table (1)**

**Percentage inhibition of growth of Aspergillus niger ATCC 16404 with different concentrations of thymoquinone and amphotericin B after 96 hours.**

| | **Concentrations of drugs used (mg/ml)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2.0 | 1.0 | 0.5 | 0.25 | 0.125 | 0.062 | 0.031 | 0.015 | **0.0075** |
| **Thymoquinone** | 100 | 90.6 | 67.6 | 47.3 | 36.2 | 16.7 | NT* | NT | NT |
| **Amphotericin B** | 93.8 | 92 | 90.7 | 85.6 | 84.7 | 81.6 | 76.7 | 65.1 | 52.3 |

***NT: Not tested** (Virtually no inhibition observed in initial experiments).

**Discussion**

*Aspergillus* species are the most common mold causing severe invasive infections in immunocompromized individuals [10-12]. Fluconazole and ketoconazole are inactive against *Aspergillus* [20-22]. Currently amphotericin B is most widely used against aspergillus infection, but failure of ampoterricin B treatment against invasive aspergillosis has also been reported. Overall, the response to amphotericin B remains poor, with a favourable outcome in only 30–40% of treated patients [23, 24]. So a newer effective drug is required for invasive aspergillosis.

Successful validation of amphotericin B testing against Aspergillus species has been problematic [24]. In the present study more than 80% inhibition was obtained with amphotericine B at a concentarion of 0.062 mg/ml and more than 50% inhibition was shown by 0.0075 mg/ml, which also seems very high.

In the present study we observed a dose related anti-aspergillus effect of thymoquinone. Previous reports also showed concentration-dependent inhibition of growth of Gram-positive & Gram-negative bacteria, and yeasts by *N. sativa* seed and hexane-extracted *N. sativa* oil [7, 25]. Thymoquinone showed compareable activity against *Aspergillus niger* although in relatively higher concentrations. However, thymoquinine at lower concentrations did not show compareable activity.

Possibly our study will promote further investigations to determine usefulness of thymoquinone and related compounds against aspergillosis and other opportunistic mold infections.

## References


1. El-Kadi A, Kandil O. Effect of *Nigella sativa* (the black seed) on immunity. Proceeding of the 4th International Conference on Islamic Medicine, Kuwait. Bulletin of Islamic Medicine, 1986; 4: 344-348.
2. El-Dakhakhany M. Studies on the chemical constitution of Egyptian *Nigella sativa* seeds. Planta Medicine, 1963; 1(4): 465-470.
3. Ata-ur-Rahman, Malik S, Cun-Hung H, Clardy J). Isolation and structure determination of nigellicine, a novel alkaloid from seeds of *Nigella sativa*. Tetrahedron Lett, 1985; 26: 2759-2762.
4. Kumara SS, Huat BT Extraction, isolation and characterization of anti-tumor principle, alpha-hedrin, from seeds of *Nigella sativa*. Planta Med, 2001; 67(1): 29-32.
5. El-Fatatry. Isolation and structure assignment of an anti-microbial principle from volatile oil of *Nigella sativa* L seeds. Pharmazie, 1975; 30(2): 109-111.
6. Topozada HH, Masloum H, El-Dakhakhany M). The antibacterial properties of *Nigella sativa* seeds: Active principle with some clinical application. J Egyp Med Assoc, 1965; 48(suppl): 187-202.
7. Hanafi MS, Hatem ME. Studies on the antimicrobial activity of *Nigella sativa* seed (Black cumin). J Ethnopharmacol, 1991; 34(2-3): 275-278.
8. Morsi. Antimicrobial effect of crude extract of *Nigella sativa* on multiple antibiotic resistant bacteria. Acta Microbiol, 2000; 49(1): 63-74.
9. Salih Hamad Mohamad Aljabre, Mohammad Akram Randhawa, Naem Akhtar, Omar Mohamad Alaklobi, Abdulrahman Mohamad Alqurashi, Ali Aldossary. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. Journal of Ethnopharmacology, 2005, 101, 116-119.
10. Anaissie EJ, Bodey GP, Rinaldi MG. Emerging fungal pathogens. Eur J Clin Microbiol Infect Dis, 1989; 8:323-330
11. Kontoyiannis DP, Bodey GP. Invasive aspergillosis in 2002: an update. Eur J Clin Microbiol Infect Dis, 2002; 21:161-172
12. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis, 2002; 34:909-917
13. Johnson AS, Ranson M, Scarffe, JH, Morgenstern GR, Shaw AJ, Oppenheim BA. Cutaneous infection with *Rhizopus oryzae* and *Aspergillus niger* following bone marrow transplant. J Hosp Infect, 1993, 25 (4): 293-296
14. Arnaud MV, Moraes MA, Nobrega P. 2 cases of paranasal aspergilloma due to *Aspergillus niger*. Rev Soc Bras Trop, 1994, 27 (1): 43
15. Lesueur A, Salmon Ceron D, Corlieu P, Ginsburg C, Sicard D. *Aspergillus niger* osteitis of the middle ear in a patient with HIV infection. Presse Med 1997, 26 (23): 1098
16. Itoh K, TakahashiM, Yagasaki F, Endoh K, Wakao D, Kawai N, Tominaga K, Kusumoto S, Fukuda M, Bessho M, Enomoto H. A neutropenic acute myeloid

leukemica patient complicated with chronic otitis media due to *Aspergillus niger* and yeast-like fungi caused by superinfection. Kansenshogaku Zasshi, 1999, 73: 618-622

17. Kreiss Y, Vered Z, Keller N, Kochva I, Sidi Y, Gur H. *Aspergillus niger* endocarditis in an immunocompetent patient: an unusual course. Post grad Med J, 200, 76 (892): 106-106
18. Ugurlu,S, Maden A, Sefi N, Sener G, Yulug N. *Aspergillus niger* infection of exenterated orbit. Ophthl Plast Reconstr Surg, 2001, 17 (6): 452-453
19. Ali Shtayeh MS, Abu-Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. Mycoses, 1999 42(11-12):665-672.
20. Sutton DA, Sanche SE, Revankar SG, Fothergill AW, Rinaldi MG. *In vitro* amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. J Clin Microbiol*,* 1999; **37**: 2343–2345.
21. Moore1 CB, N. Sayers, J. Mosquera, J. Slaven and Denning DW. Antifungal Drug Resistance in Aspergillus. Journal of Infection*,* 2000; 41: 203–220
22. Ana Espinel-Ingroff. Comparison of the E-test with the NCCLS M38-P Method for Antifungal Susceptibility Testing of Common and Emerging Pathogenic Filamentous Fungi. J Clin Microbiol, 2001, 39; 4: 1360-1367.
23. Mahomed-Yunus S. Moosa, George J. Alangaden, Elias Manavathu and Pranatharthi H. Chandrasekar. Resistance to amphotericin B does not emerge during treatment for invasive aspergillosis. J Antimicrob Chemother, 2002; 49: 209-213
24. Johnson EM, Oakley KL, Radford SA *et al*. Lack of correlation of *in vitro* amphotericin B susceptibility testing with outcome of in a murine model of Aspergillus infection. J Antimicrob Chemother*,* 2000; 45: 85–93.
25. El-Sayed MM, El-Banna HA, Fathy FA. The use of *Nigella sativa* oil as a natural preservative agent in processed cheese spread. Egyptian J Food Science, 1994, 22(3): 381-396.

(بحث مختصر)

# نشاط الثيموكينون والأمفوترسين (ب) المضاد للنوع الأساسي للسبرجيلوس نيجر

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**الملخص:**

أصبح استعمال زيت النيجلا ساتيفا (والمسماة بالحبة السوداء في العربية) كعلاج لكثير من الأمراض في بلدان الشرق الأوسط شائعا. فكثيراً ما تتم إضافتها إلى الخبز و المخللات كمحسن للطعام والرائحة. و لقد سجلت الدوريات الطبية نشاط زيت النيجلا ساتيفا المستخلص أو الشق النشط منه ضد عدد من البكتيريا وفطريات الكانديدا البيكانز.( كانديدا البيضاء).

ومن ثم كانت فكرة البحث لتتركز على تقدير فائدة زيت النيجلا ساتيفا أو أحد مشتقاتها النشطة على العدوى من الطحالب العابرة كالأسبرجيلوس نيجر (الأسود) والتي قد تحدث في الأشخاص ذوي الأضطراب المناعية. فتمت دراسة نشاط الثيموكينون والأمفوترسين (ب) ضد الأسبرجيلوس نيجر (الأسود) ATTCC16404.

و لقد أثبتت الدراسة زيادة تصاعدية لوقف نمو الأسبرجيلوس الأسود النوع ATTCC16404. (١٦,٧ و ٣٦,٢ و ٤٧,٣ و ٦٧,٨ و ٩٠,٠٦ و ١٠٠٪) مع التركيزات المتزايدة للثيموكينون (٠,٦٢ و ٠,١٢٥ و ٠,٢٥ و ٠,٥ و ١و ٢ جرام/مللي على التوالي).و لقد أتخذ البحث النمو على الأطباق الضابطة بعد ٩٦ساعة محتسباً النمو ١٠٠٪ عيارياً.

كما أثبتت الدراسة زيادة تصاعدية لوقف نمو الأسبرجيلوس نيجر (الأسود) النوع ATTCC 16404.

( ٥٢,٣ و ٦٥,١ و ٧٦,٧ و ٨١,٦ و ٨٤,٧ و ٧٥,٦ و ٩٠,٧ و ٩٢ و ٩٣,٨٪) مع التركيز المتزايدة لأمفوترسين (ب) (٠,٠٠٧و ٠,٠١٥ و ٠,٠٣١ و ٠,٠٦٢ و ٠,١٢٥ و ٠,٢٥ و ٠,٥و١ و ٢ مجم /مللى على التوالي).