

Comparison of Antifungal Activity of Thymoquinone and Amphotericin B Against *Fusarium solani* in-vitro.

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Abstract:

The activity of thymoquinone, an active principle of *Nigella sativa*, and amphotericin B was compared against a clinical isolate of *Fusarium solani*. The organism was isolated from a nail lesion of an immunocompetent adult male in routine fungal culture on dermasel agar. For susceptibility testing the organism was grown on two sets of dermasel agar containing, 1.0, 0.5, 0.25, 0.125, 0.062 & 0.031 mg of thymoquinone and amphotericin B/ml. It was grown on dermasel agar alone as control. The growth on 10th day of inoculation was recorded as % inhibition taking growth of control as 100%.

There was 0, 2.0, 3.0, 18.3, 59.3, and 100% inhibition of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone /ml on 10th day of incubation. At similar concentrations of amphotericin B, there was 6.2, 24.5, 31.2, 39.7, 54.4 and 72.4% inhibition of growth. There was no complete inhibition of growth at any concentration of the drug.

The study shows that at higher concentrations thymoquinone, giving a steep dose-effect relationship, more effectively inhibited the growth of a clinical isolate of *Fusarium solani* as compared to amphotericin B which gave shallow dose-effect relationship. However, amphotericin B was better at lower concentrations.

Key words: *Nigella sativa*, thymoquinone, amphotericin B, *Fusarium solani*

Introduction:

Nigella sativa called as Habbah Al-Sauda in Arabic, is commonly used as a natural remedy for many ailments over 2000 years and is frequently added to bread and pickles as a flavouring agent (Al-Kadi & Kandil, 1986). Many active principles have been isolated from *N. sativa* seed including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellidine, nigellimine-N-oxide and alpha-hedrin (Al-Dakhkhany, 1963; Ata-ur-Rahman et al., 1985; Ata-ur-Rahman & Malik, 1995; Kumara & Huat, 2001). Besides many other pharmacological effects, activity of *N. sativa*

volatile oil, ether extract and its active principle thymohydroquinone has been reported in the literature against a number of bacteria (including *Staphylococcus aureus*, *Pseudomonas aeruginosa* & *Escherichia coli*) and fungi like *Candida albicans* & *Aspergillus niger* (Topozada et al. 1965; El-Fataty, 1975; Hanafi & Hatem, 1991; Morsi, 2000; Al-Jabre et al., 2003).

Fusarium solani 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 and bone marrow transplant recipients (Anaissie et al., 1989; Marr et al., 2002; Kontoyiannis & Bodey, 2002). *Aspergillus* is by far the most common mold causing severe infections. However, *Fusarium* spp., have been increasingly recognized as lethal pathogens in these patients after invasive aspergillosis (Anaissie et al., 1989; Marr et al., 2002; Nelson et al., 1994; Martino et al., 1994; Boutati & Anaissie, 1997; Girmenia et al., 2000).

The skin and respiratory tract are the primary portals of entry for *Fusarium* infection (Nelson et al., 1994; Guarro & Gene, 1995; Musa et al., 2000). Localized skin and nail infections have also been associated with subsequent dissemination of *Fusarium* species when the patient becomes neutropenic during the course of immunosuppressive treatment (Gupta et al., 2000). Hospital water distribution systems have recently been implicated as sources of nosocomial fusariosis (Anaissie et al., 2001).

Keeping in view the antibacterial and anti-aspergillus activity we thought that *N. sativa* or some of its active principles might have useful activity against *Fusarium solani*, a relatively resistant opportunistic fungus. In this study, the activity of thymoquinone against *Fusarium solani* was compared to that of amphotericin B *in vitro*.

Materials and Methods:

Fusarium solani was isolated from a nail clipping from an adult immunocompetent male with clinical diagnosis of onychomycosis. The specimen was cultured on dermasel agar (Oxoid) in the Department of Microbiology, College of Medicine, King Faisal University, Dammam, Saudi Arabia. The plates were incubated at 30°C for 10 days. The growth was identified as *Fusarium solani* by colonial morphology and by microscopy after staining with lactophenol cotton blue.

Preparation of Reagents & Media:

Thymoquinone (Sigma, USA) and amphotericin B (Sigma, USA) were separately dissolved in 4 ml of dimethyl sulphoxide (DMSO) (Sigma, USA) and then serially diluted in dermasel agar to give final concentrations of 1.0, 0.5, 0.25, 0.125, 0.06 & 0.031 mg/ml. Four plates of each concentration were prepared. Four plates of dermasel agar containing the same concentrations of DMSO as in the treated plates, alone were used as a control.

Susceptibility Testing:

Susceptibility testing was carried out as previously described (Ali-Shtayeh & Abu-Ghdeib, 1999). A mycelial disc of clinical isolate of *Fusarium solani*, 5 mm in diameter, cut from the periphery of 7 days old culture in dermasel agar was aseptically inoculated onto each set of above mentioned plates. The inoculated plates were incubated at 30°C for 10 days. The growth was examined on 4th and 7th days and finally reported on 10th day of inoculation and results interpreted by measurement of the mean diameter of the growth. The percentage inhibitions of *Fusarium solani* with different concentrations of thymoquinone and amphotericin B were then calculated by taking its growth on non-drug dermasel agar as 100%.

Statistical Analysis:

The results of thymoquinone and amphotericin B were compared statistically using students "t" test and P-values determined for the differences between the means \pm se of corresponding concentrations of thymoquinone and amphotericin B.

Results:

Percentage inhibitions of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone and amphotericin B /ml on 10th day of incubation are shown in table 1. Growth on the control plates on respective days was considered as 100%. There was complete inhibition of growth at 1.0 mg thymoquinone/ml and there was no complete inhibition of growth at any concentration of amphotericin B. At higher concentrations (0.5-1.0 mg/ml), thymoquinone, giving a steep dose-effect relationship, more effectively inhibited the growth of a clinical isolate of *Fusarium solani* as compared to amphotericin B, which gave a rather shallow dose-effect relationship. However, amphotericin B was better at lower concentrations (0.031-0.25mg/ml).

Table (1)
Percentage inhibition of growth of *Fusarium solani* with different concentrations of thymoquinone and amphotericin B after 10 days of incubation.

Concentrations (mg/ml)	% inhibition of growth		*P-values from students "t" test
	Thymoquinone (Mean \pm se)	Amphotericin B (Mean \pm se)	
0.031	0 \pm 0	6.2 \pm 0.76	<0.01
0.062	2.0 \pm 1	24.5 \pm 0.92	<0.001
0.125	3.0 \pm 0.25	31.2 \pm 0.65	<0.02
0.25	18.3 \pm 1.45	39.7 \pm 1.22	<0.001
0.5	59.3 \pm 3.93	54.4 \pm 2.72	>0.1
1.0	100 \pm 0	72.4 \pm 2.12	<0.001

*P-values for the differences between the means \pm se of corresponding concentrations of thymoquinone and amphotericin B.

Discussion:

N. sativa has been used for many ailments in ancient Greek, Indian and Arabic medicines for treatment of warts, collar-studd abscesses, ringworm infections and gastrointestinal infections(Al-Jishi, 2000). In the present study we observed a dose related anti-fusarium activity of thymoquinone, an active principle of *N. sativa*. There were 0, 2.0, 3.0, 18.3, 59.3, and 100% inhibitions of growth of *Fusarium solani* with 0.031, 0.062, 0.125, 0.25, 0.5, 1.0 mg thymoquinone/ml on 10th day of incubation. Previous reports also showed concentration-dependent inhibitions of growth of Gram-positive & Gram-negative bacteria, and *Candida albicans* by *N. sativa* seed and hexane-extracted *N. sativa* oil (Hanafi & Hatem, 1991; Al-Syed et al., 1994).

In the present study amphotericin B showed activity against *F. solani* but there was no complete inhibition at any concentration of the drug tested. Lewis et al. have also reported shallow concentration-effect curve and low efficacy of amphotericin B against *F. solani* (Lewis et al., 2005). The amphoteric properties and high protein binding of this drug may partially explain this effect (Bekersky et al., 2002). The relatively steep concentration-effect curve of thymoquinone against *F. solani* resembles to that of voriconazole reported in another study (Lewis et al., 2005). Perhaps, the kinetic properties of thymoquinone resemble to voriconazole but needs further investigations.

Fusarium is one of the most resistant fungi to the arsenal of modern antifungal agents. Current therapeutic approaches for fusariosis are suboptimal, resulting in exceedingly high mortality rates (Anaissie, 1989; Martino, 1994; Boutati & Anaissie, 1997; Girmenia *et al.*, 2000; Torres & Kontoyiannis 2003). The mainstay in the treatment of fusariosis has traditionally been amphotericin B. However, the *in vitro* susceptibility of *Fusarium* species to amphotericin B is, at best, mediocre (Anaissie, 1989; Boutati & Anaissie, 1997). The activity of amphotericin B in animal models of fusariosis is also limited (Anaissie *et al.*, 1992; Guarro *et al.*, 1999). In fact, only high doses of liposomal amphotericin B have been shown to be active against *Fusarium* species in animal models using immunocompetent mice (Ortoneda *et al.*, 2002).

Further investigations of usefulness of *N. sativa* and its active principles in the treatment of opportunistic fungal infections like fusariosis should be considered.

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مقارنة النشاط المضاد للفطريات للثيموكينون كمادة فعالة في الحبة السوداء (النجيلا ساتيفا) الامفوتيرسين بي ضد ميكروب الفيوسريم سولاني

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الملخص :

الهدف من البحث:

أجريت البحث لمقارنة نشاط اليموكينون التي تكون الشق النشط للحبة السوداء أو ما تسمى بحبه البركة (النجيلا ساتيفا) ضد ميكروب الفيوسريم سولاني المعزول سريريا مع نشاط الامفوتيرسين بي على نفس الميكروب

طريقة البحث :

تم فصل ميكروب الفيوسريم سولاني المعزول سريريا من إصابة بالظفر في ذكر يافع ولا يعاني من اعتلال بالمناعة خلال مزرعة فطرية روتينية على الدرماسيل الاجار. تم تحديد الميكروب عن طريق الفحص الظاهري للمستعمرات الميكروبية والفحص المجهرى. وللحساسية ضد المضادات التالية من اليموكينون والامفوتيرسين بي : ١ و ٠.٥ و ٠.٢٥ و ٠.٠٦٢ و ٠.٠٣١ ملي جرام لكل ميلي لتر. كما تمت على مجموعة أخرى بدون مضادات كمجموعة ضابطة. تم قياس النمو في اليوم العاشر من التلقيح كنسبة مئوية لمنع النمو

النتائج :

أثبتت الدراسة ان اليموكينون قد أوقف نمو الميكروب بنسب ٢.٠ و ٣ و ١٨.٣ و ٥٩.٣ و ١٠٠٪ تبعاً للتركيزات التالية من الثيموكينون ٠.٢١ و ٠.٠٦٢ و ٠.١٢٥ و ٠.٠٢٥ و ٠.٥ و ١ ملي جرام لكل ملي لتر مقارنة بالامفوتيرسين بي الذي منع نمو الميكرو بنسب مئوية كما يلي ٦.٢ و ٢٤.٥ و ٣١.٢ و ٣٩.٧ و ٥٤.٤ و ٧٢.٤ لنفس التركيزات من الامفوتيرسين. وقد لوحظ أيضا عدم منع كامل للنمو في أي تركيز من الامفوتيرسين بي .

الاستنتاج :

أثبتت الدراسة أن اليموكينون التي تكون الشق النشط للحبة السوداء أو ما تسمى بحبة البركة (النجيلا ساتيفا) قد أوقفت نمو ميكروب الفيوسريم سولاني المعزول سريرياً مقارنة بالامفتيرسين بي على نفس الميكروب.