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Dimethyl Sulfoxide (DMSO) has an Additive Effect and Alters Minimal Inhibitory Concentrations of Antifungal Drugs

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Abstract

Background: **Dimethyl sulfoxide (DMSO) is commonly used as a solvent for anti-fungal drugs. It has been reported to possess anti-fungal activity by itself so may interfere in the evaluation and comparison of anti-fungal drugs. DMSO 1% and below are usually considered to possess insignificant effect on the growth of fungi. The present study was aimed to determine any additive/synergistic effect of DMSO (1%) with anti-fungal drugs.**

Methods: **The effect of DMSO (1%) was determined on the colonial growth of *Trichophyton rubrum, and Microsporum canis* along with clotrimazole, griseofulvin, ketoconazole and thymoquinone (an active principle of *Nigella sativa*). Similarly, the ability of DMSO (1%) to enhance the effect of amphotericin-B and thymoquinone was observed on the growth of *Aspergillus niger*. The fungi were grown in three sets of plates of dermasel agar for each drug containing: (a) serial dilutions of the drug alone; (b) serial dilutions of the drug plus DMSO 1% in each dilution and (c) dermasel agar alone, as control.**

Results: **DMSO (1%) lowered the MICs of all drugs tested against the fungi used, except amphotericin-B against *Aspergillus niger*. Presence of DMSO (1%) in serial dilutions of drugs also significantly shifted the growth curves of fungi towards right.**

Conclusion: **DMSO, as a solvent, is one of the important factors that can alter the results of antifungal drugs.**

Key words: **DMSO, addition/synergism, antifungal drugs, dermatophytes, *Aspergillus niger*, dermasel agar**

Introduction

Dimethyl Sulfoxide (DMSO) is frequently used as a solvent for antifungal drugs in various studies for the determination of their MICs[1-5]. The stock solutions of anti-fungal drugs are usually prepared in 100% DMSO and then serial dilutions are made in the culture media, which changes the concentration of DMSO from higher to lower, in different sets of plates or tubes.

The influence of various concentrations of DMSO (2, 1 and 0.5%) on the growth of yeast (Candida species) has been investigated in one study, reporting that 2% DMSO significantly slowed the growth and lowered the growth curve in all 8 species of Candida tested while 1% and below had insignificant effect on the kinetics of growth[6]. Therefore, DMSO 1% and below are usually considered as safe. However, recently, we have reported the inhibitory effect of DMSO on the growth of some dermatophytes even below 1%[7].

The present study was aimed to investigate how far DMSO 1% could affect the activity of antifungal drugs in terms of alterations in their MICs and overall growth in their serial dilutions.

Materials and Methods

a. Source of DMSO, drugs and dermasel agar:

DMSO, clotrimazole, griseofulvin, ampotericin-B and thymoquinone were obtained from SIGMA-USA and ketoconazole from Spectrum Chemical Manufacturing Corporation USA. The dermasel agar (containing mycological peptone 1%, glucose 2% and agar 1.45%) and dermasel agar with a supplement of chloramphenicol and cycloheximide were obtained from OXOID, England.

b. Fungi

Trichophyton rubrum and Microsporum canis were obtained from skin scrapings of patients with clinical diagnosis of dermatophytosis. They were initially inoculated on dermasel agar with a

supplement of chloramphenicol and cycloheximide, incubated at 30° C for 7-10 days and identified by their colonial morphology and microscopic examination of lactophenol cotton blue mounts from the colonies. Subcultures were then prepared on dermasel agar plates for 7-10 days for use in the experiment.

Table 1. Percentage inhibition of the growth of *T. rubrum* and *M. canis* by various concentrations of clotrimazole, ketoconazole, griseofulvin and thymoquinone on day 14, alone and in the presence of DMSO 1%.

a. *T. rubrum*

Clotrimazole	(µg//ml)	5	2.5	1.25	0.62	0.31	0.15	0.07	
	Alone	100	91.6	87.4	85.2	77.1	63.3	50.6	
	With DMSO 1%	100	100	92.2	88.6	82.8	74.4	63	
Griseofulvin	µg/ml	10	5	2.5	1.25	0.62	0.31	0.15	0.075
	Alone	82.4	69.3	63.5	58.1	51.4	44	31.6	21
	With DMSO 1%	89.1	81.2	70.8	66.6	62.3	57.8	49.8	39.8
Ketoconazole	(µg/ml)	10	5	2.5	1.25	0.62	0.31	0.15	0.075
	Alone	81.9	76.5	69.4	59.5	53.3	36.9	24.4	9.5
	With DMSO 1%	86.9	80.9	75.9	67.9	61.9	54.8	45.3	37.8
Thymoquinone	mg/ml	0.2	0.1	0.05	0.025	0.012			
	Alone	100	46.5	22.6	14.4	9.1			
	With DMSO 1%	100	100	59.3	37.9	23			

b. *M. canis*

Clotrimazole	(µg//ml)	0.62	0.31	0.15	0.075	0.037	0.018	0.009	
	Alone	84.8	75.8	64.8	45.9	31.6	20.9	13.52	
	With DMSO 1%	100	82.4	75.8	57.4	40.2	26.6	19.3	
Griseofulvin	µg/ml	10	5	2.5	1.25	0.62	0.31	0.15	
	Alone	100	81.3	79.8	76.7	71.8	64.5	57.3	
	With DMSO 1%	100	100	84	80.9	76.7	72.5	63	
Ketoconazole	(µg/ml)	4	2	1	0.5	0.25	0.125	0.062	0.031
	Alone	80.5	66.9	51.9	39.1	31.2	25.6	22.6	18.8
	With DMSO 1%	87.2	80.8	65	50.8	38	35	33.9	32.7
Thymoquinone	mg/ml	0.25	0.125	0.062	0.031	0.15			
	Alone	100	100	30	4.4	0.9			
	With DMSO 1%	100	100	100	33.5	25.6			

Standard strains of Aspergillus niger were obtained from UK, initially inoculated on dermasel agar with a supplement of chloramphenicol and cycloheximide and then sub-cultured on dermasel agar plates for 3-4 days.

c. Preparation of dilutions of drugs

Stock solutions of antifungal drugs were prepared in a solvent other than DMSO (alcohol) and serial dilutions made in the dermasel agar in two sets: (a) without DMSO and (b) containing 1% DMSO in each dilution.

d. Antifungal susceptibility test

The isolates of various fungi were sub-cultured on three set of culture media containing: Set (a) serial dilutions of drug in dermasel agar; Set (b) serial dilutions of drug and 1% DMSO and Set (c) dermasel agar only (controls). Four plates were used for each dilution and the controls.

Colonial discs of 5 mm in diameter were cut from the periphery of 7-10 days old cultures of dermatophytes, as well as from 3-5 days old cultures of Aspergillus in dermasel agar, aseptically inoculated onto different sets of media and then incubated at 30^{O} C. The cultures were examined on day 2nd and 4th for Aspergillus and day 7th and 14th for dermatophytes. Diameter of the growth of fungi was measured in each plate and the results of day 4th for Aspergillus and day 14th for dermatophytes were interpreted by the measurement of the mean of four plates at each level of dilution[8].

e. Analysis of results:

From the mean growth of fungus at each concentration level of the drug, percentage inhibition of the growth was determined in the absence as well as in the presence of DMSO1%, taking that of controls as 100%. MIC of each drug tested was taken as the concentration causing more than 80% inhibition. Linear graphs were plotted taking concentration of the drug in the X-axis and mean growth in the Y-axis, for both in the absence as well presence of DMSO 1%.

Results:

The percentage inhibition of the growth of T. rubrum and M. canis with clotrimazole, ketoconazole, griseofulvin and thymoquinone alone and in the presence of DMSO 1% is given in Table 1 and that of A. niger with amphotericin-B and thymoqunone in Table 2. There was a dose related inhibitory effect on the growth of fungi by all drugs tested.

Table 2. Percentage inhibition of growth of *A. niger* by various concentrations of amphotericin-B and thymoquinone on day 4; alone and in the presence of DMSO 1%.

Ampotericin B	(mg/ml)	1	0.5	0.25	0.125	0.062	0.031	0.015
	Alone	73.8	70.9	68.7	64.1	58.2	51.9	42.6
	With DMSO1%	76.3	74.3	68.4	63.7	57.8	52.3	45.6
Thymoquinone	(mg/ml)	1	0.5	0.25	0.125	0.062	0.031	0.015
	Alone	100	87.9	62.7	52.7	46.6	34.2	9.3
	With DMSO1%	100	100	73.9	59.4	50.9	42.5	34.2

Table 3. MIC of various antifungal drugs alone and in the presence of DMSO 1% against *T. rubrum, M. canis* and *A. niger.*

a. *T. rubrum* and *M. canis*

	T. rubrum		*M. canis*	
	Alone	**With DMSO 1%**	**Alone**	**With DMSO 1%**
Clotrimazole	1.25	0.31	0.62	0.31
Griseofulvin	10	5	0.31	0.075
Ketoconazole	10	5	4	2
Thymoquinone*	2	1	0.25	0.062

* (mg/ml)

b. *A. niger*

Drug (mg/ml)	Alone	With DMSO 1%
Amphotericin-B	74% inhibition with 1 mg/ml	74% inhibition with 0.5 mg/ml

Thymoquinone	1	0.5

MICs of clotrimazole, griseofulvin, ketoconazole and thymoquinone against T. rubrum in the absence of DMSO were 1.25µg/ml, 10µg/ml, 10µg/ml and 2mg/ml; while in the presence of DMSO 1% MICs were 0.31µg/ml, 5µg/ml, 5µg/ml and 1mg/ml, respectively. Similarly MICs of clotrimazole, griseofulvin, ketoconazole and thymoquinone against M. canis in the absence of DMSO were 0.62µg/ml, 0.31µg/ml, 4µg/ml and 0.25mg/ml; while in the presence of DMSO 1% were 0.31µg/ml, 0.075µg/ml, 2µg/ml and 0.062mg/ml, respectively (Table 3a).

There was no change in the MIC of amphotericin-B with DMSO 1% while it reduced the MIC of thymoquinone from 1mg/ml to 0.5mg/ml (Table 3b).

Linear graphs for the concentration of the drug versus growth, both in the absence as well presence of DMSO 1% are shown in figures 1 to 10. There was a consistent shift of the growth curves towards right with all drugs in the presence of DMSO 1%, except amphotericin-B against A. niger.

Discussion

In the comparative evaluation poor agreement between methods for antifungal drug susceptibility testing has been reported[9]. One factor might be the effect of DMSO on the growth of fungi, commonly used as a solvent.

Fig 1. Growth curves of *T. rubrum* in the presence of different concentrations of clotrimazole alone and the same concentrations of Clotrimazole plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

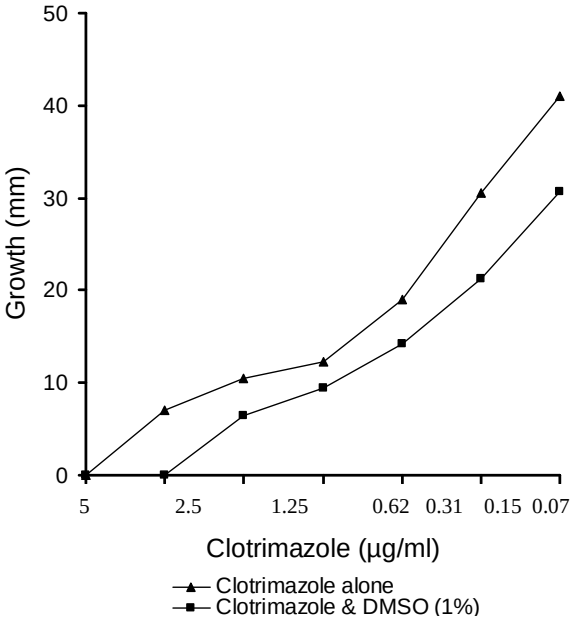


Fig 2. Growth curves of *M. canis* in the presence of different concentrations of clotrimazole alone and the same concentrations of clotrimazole plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

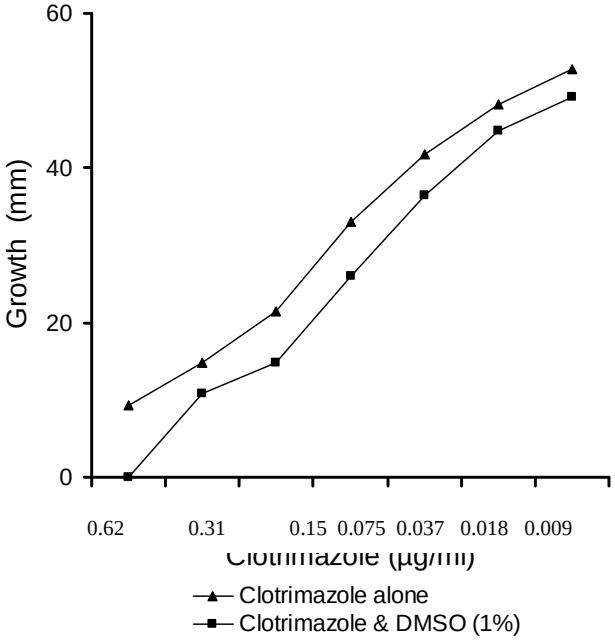


Fig 3. Growth curves of *T. rubrum* in the presence of different concentrations of griseofulvin alone and the same concentrations of griseofulvin plus DMSO 1%. A shift towards right of the latter shows an

additive effect of DMSO.

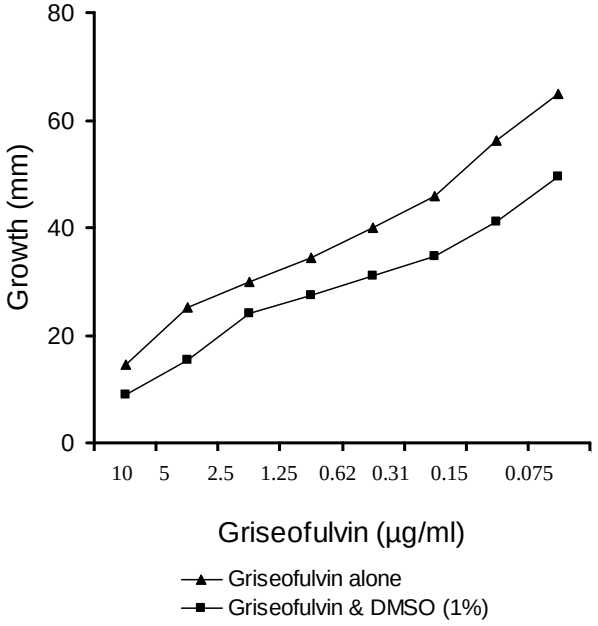


Fig 4. Growth curves of *M. canis* in the presence of different concentrations of griseofulvin alone and the same concentrations of grseofulvin plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

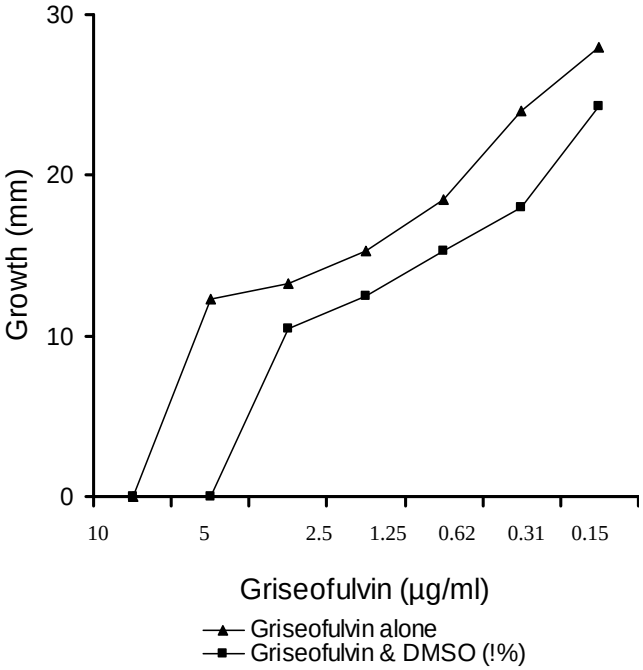


Fig 5. Growth curves of *T. rubrum* in the presence of different concentrations of ketoconazole alone and the same concentrations of ketoconazole plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

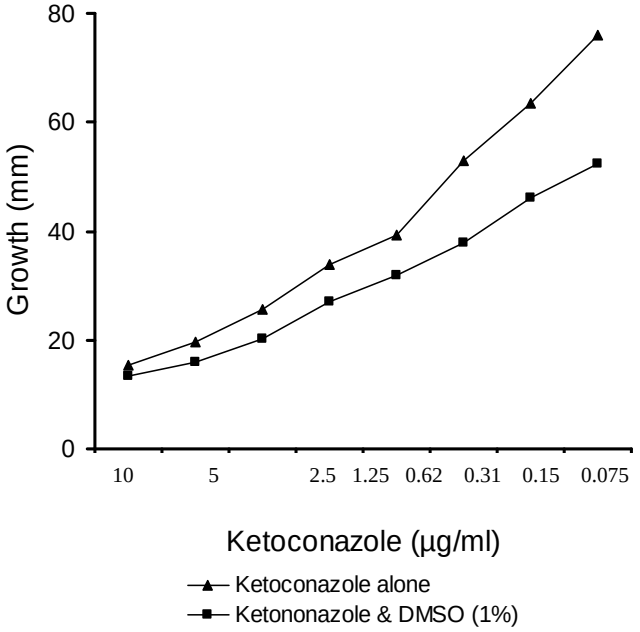


Fig 6. Growth curves of *M. canis* in the presence of different concentrations of ketoconazole and the same concentrations of ketoconazole plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

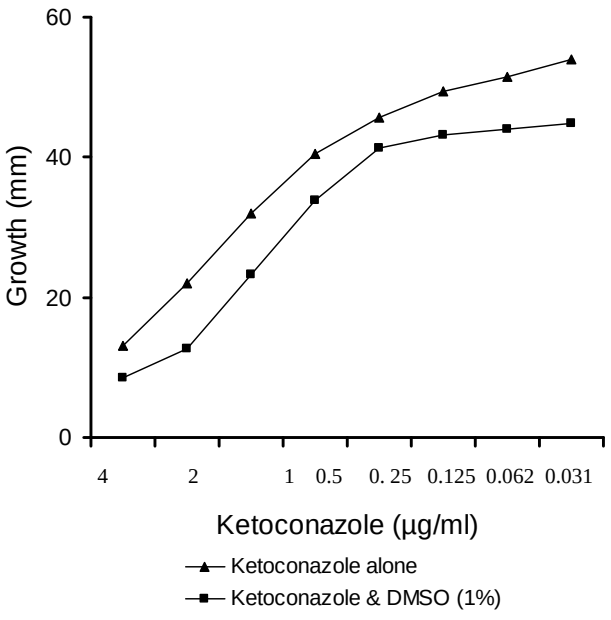


Fig 7. Growth curves of *T.rubrum* in the presence of different concentrations of thymoquinone alone and the same concentrations of thymoquinone plus

DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

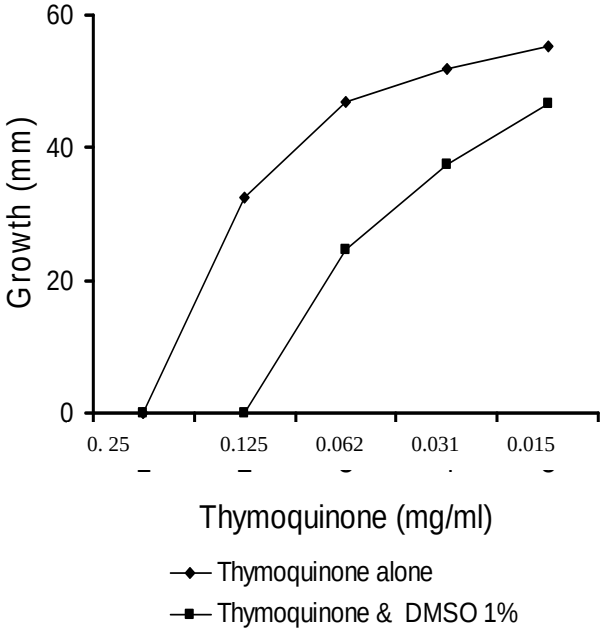


Fig 8. Growth curves of *M. canis* in the presence of different concentrations of thymoquinone alone and the same concentrations of thymoquinone plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

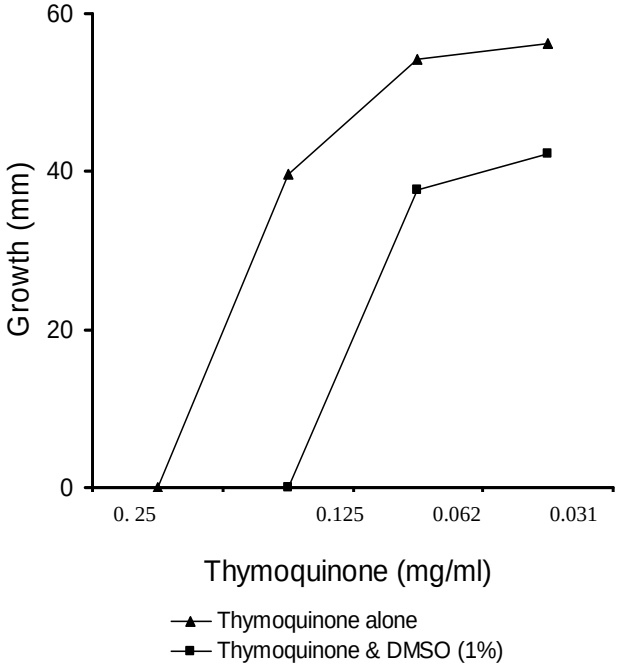


Fig 9. Growth curves of *A. nigers* in the presence of different concentrations of thymoquinone alone and the same concentrations of thymoquinone plus DMSO 1%. A shift towards right of the latter shows an additive effect of DMSO.

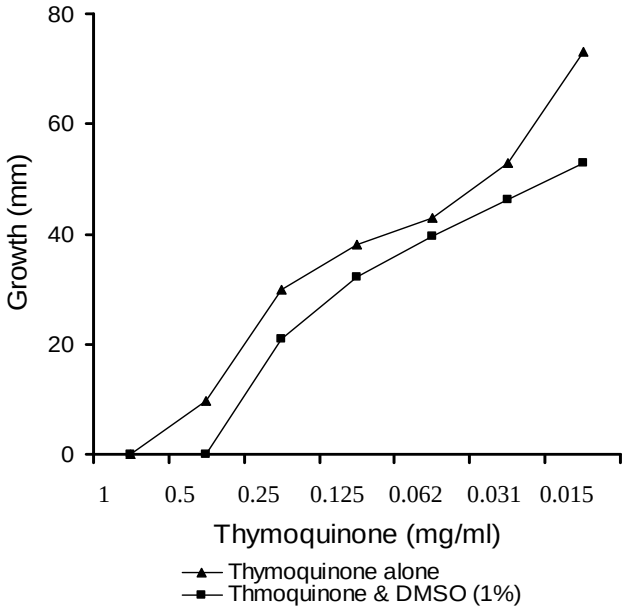
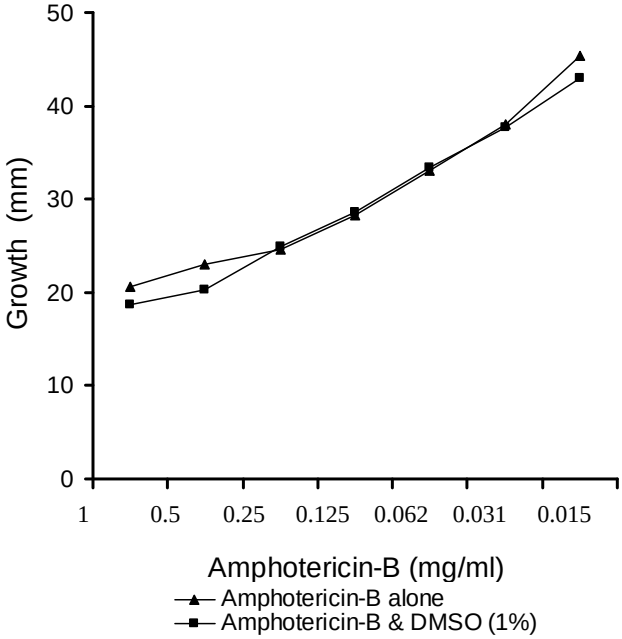


Fig 10. Growth curves of *A. niger* in the presence of different concentrations of amphotericin-B alone and the same concentrations of amphotetricin-B plus DMSO 1%.



In the present study DMSO 1% significantly decreased MICs of clotrimazole, griseofulvin,

ketoconazole and thymoquinone against T. mentagrophytes and M. canis. There was concentration dependant inhibition of the growth of fungi by these drugs and the presence of DMSO 1% shifted the concentration related growth curves of the fungi towards right, showing an additive effect with them. Thymoquinone, an active principle of N. sativa, has previously been shown to inhibit dermatophytes in a dose related fashion[10].

Azoles and griseofulvin are inactive against Aspergillus[11-13], therefore, we used amphotericin-B, which is widely used against Aspergillus infection. Failure of amphotericin-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[14-15]. We also observed higher MIC (1mg/ml showing only 74 % inhibition) of amphotericin-B against A. niger. Thymoquinone was used because in a previous study we found thymoquinone inhibits A. niger comparable to amphotericin-B[16].

Conclusions

DMSO 1% causes an additive effect with antifungal drugs, can lower their MICs against dermatophytes and shifts their growth kinetics. Therefore, it is an important factor in the variability of results for the evaluation of antifungal drugs.

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