In-Vitro Antimycotic Potential Of *Thuja-occ* against *Curvularia-lunata* causing Phaeoyphomycosis In Human

Abstract

Various potencies of a homoeopathic drug, *Thuja-occidentalis*–Q 30, 200, 1M, 10M and 50M and Ketoconazole (a synthetic dioxolane imidazole compound–a broad spectrum antifungal) as a positive control were evaluated by following Poison food method. *Thuja* 30 and 200 was found highly effective even more than positive control and vehicle. Linear diametric growth, sporulation and exudation were taken as parameters of assessment.

Introduction

Curvularia is a common soil saprophyte and plant pathogen that has been isolated from cases of mycotic keratitis, endocarditis and PhaeHoyphomycosis 1, 6, 7,10,11,13,16,17, 19, 20, 24. Mycotic infarcts were voided in the heart, spleen, left kidney and right cerebral hemisphere. *Curvularia geniculata* were grown from all infected material. Harris and Dawnham described a disseminated case involving *Curvularia* that occurred in a football player[5], Rohwedder et al. recorded a 25 year old immuno-competent man with a 10 year history of progressive disseminated infection caused by *Curvularia lunata*[14]. Phillips et al reported on sinusitis caused by *Curvularia lunata*[12]. Monte et al. also described a disseminated infection of *Curvularia*. In experimental infection[8], Whitecomb et al. found that *Curvularia lunata* was capable of producing infection in mice, but other species of *Curvularia* were non-infective23. In mice infected with *Curvularia lunata*, lesions were found in liver and spleen. The drugs amphotericin B, miconazole and ketoconazole were found effective but toxicity and treatment duration are limiting factor for their use.

Thuja-occidentalis, (American Arbor–vita; white cedar) a tall tree belongs to the family of cupressaceae, introduced in India from North America and are being cultivated in plalns of India. *Thuja* is considered as antisycotic homoeopathic drug used mainly for wart like excrescences upon mucus and cutaneous surface, vegetative condylomata and spongy tumors. *Thuja* is known to contain oil of thuja, a thujol, flavone glycoside thujin and an acid called thujin. £-Thujone, d-isothujone and l-fenchone are the major constituents of the essential oil from leaves and terminal branches. Others include £ and â pinene, sabinene, myrcene, £ phellandrene, terpinene, limonene, 1, 8 – coneole, ___ terpinene, p-cymene, terpinolene, £ fenchylacetate, camphor, linalool, isothujyle acetate, terpinene-4-ol and borneol. Antifungal efficacy of *Thuja-occidentalis* against *Candida albicans, Trichophyton-rubrum, Trichophyton-mentagrophytes, Aspergillus-flavus* and *Aspergillus-niger* has been reported[2]. Present paper explores in-vitro antifungal potential of *Thuja-occidentalis* in Q, 30, 200, 1M, 10M, 50M potencies against *Curvularia-lunata* known to cause Phaeohyphomycosis (check MS for the correct spelling) in human.

MATERIALS AND METHODS: FUNGAL ISOLATE

Curvularia-lunata was isolated from a patient complaining of hyperpigmented, dry, scaly skin lesions on face, neck, and forearms since 6 months. MIC value

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of Ketoconazole calculated for *Curvularia-lunata* was 0.25 mg/ml by following the method of Wahab et al[21-22]

MEDICINE

Thuja-occidentalis in various potencies viz. Q, 30, 200, 1M, 10M, 50M was used in the present study. Quantity of drug was standardised to be 0.5ml/petridishes.

CONTROLS

Three controls were taken, one of sterile water (autoclaved), second of vehicle i.e. rectified spirit (90% v/v) and third of Ketoconazole (Ranbaxy, India) as positive control. Quantity of Ketoconazole was taken as per MIC calculated.

EXPERIMENTAL PROTOCOL

Antimycotic activity of homoeopathic drug *Thuja-occidentalis* Q, 30, 200, 1M, 10M, 50M prepared in rectified spirit was tested by poison food technique (because of mycelial fungi)[18,19] against *Curvularia lunata*. 0.5 ml of each drug was mixed with 10 ml of Sabourauds dextrose agar (Himedia B No 8h181– Mycological peptone 10 g/l, dextrose 40.01 g/l, Agar 15.0 g/l, PH 5.6) supplemented with Chloramphenicol (Makers laboratories Ltd. India) to prevent bacterial growth just before solidification in 5 cm diameter Petri dishes. SDA plates with 0.5 ml sterile water, 0.5 ml rectified spirit and 0.5 ml containing 0.5 mg Ketoconazole (200 mg Tab, dissolve in 20 ml water) as per MIC of *Curvularia lunata*, were kept as controls. All the plates including controls were inoculated centrally with 1.5 mm diameter of disc of *Curvularia lunata* aseptically from 8 to 10 days old inoculum – original isolate of patients. All the experimental Petri dishes were used in triplicates and incubated at 37°C ± 1°C. The linear diametric growth of colonies were measured (in cm.) on 3rd, 6th and 9th days of post inoculation. Percent growth inhibition was calculated as per formula.

$$\text{Percent Growth Inhibition} = \frac{dc - dt}{dc} \times 100$$

dc = Colony diameter of control dt = Colony diameter of treated plate

RESULTS

Antimycotic potential of homoeopathic drug *Thuja-occidentalis* Q, 30, 200, 1M, 10M and 50M against *Curvularia-lunata* have been presented in table-1 and plate 1. *Thuja* 30 and 200 were found to inhibit the growth of *Curvularia-lunata* by 93.33 percent in each as compared to control 1.91.03 percent in each as compared to control II (RS), but if it is compared by control I, Control II and Control III *Thuja 30, Thuja 200* and *Thuja 10*M showed significant inhibition in linear diametric growth of drug treated colonies of *Curvularia-lunata* (Table-1, Plate 1 – Figures 5, 6 and 8).

Percent inhibition in the linear growth of *Curvularia-lunata* in Ketoconazole was 66.66 percent as compared to control I and 56.52 percent compared to Control II. It is highly significant that *Thuja 30* and 200 have shown very high antifungal potential even more than the positive control – ketoconazole (Figured 10 and 11). *Thuja* 10 M was little higher to the positive control but *Thuja* 50M was almost equal to ketoconazole. Sporulation and exudation have shown no significant variations on the basis of naked eye observation. There was slight reduction in the spore count of *Thuja Q* but exudation (observed by change in colour of media) was prevented in all the petridishes except control I (Plate I – figure 1) and positive control Ketoconazole (Plate 1 – figure 3). There was no considerable changes in the morphology of fungal mycelium and spores size.

DISCUSSION

Mycoses particularly opportunistic infections have significantly increased[9,11,18] in the recent past. Eukariotic nature of fungal cells gives a barrier towards the development of non-toxic antifungal agent. Thus treatment

available may not cause complete cure and recurrence of infection often takes place. *Curvularia sp* is one of the common soil saprophyte that has been isolated from several patients having the history of local injury or surgery. The genus has rarely been reported fatal[6]. Among all the reported species, *Curvularia lunata* has been found capable to produce infection in mice[23]. Keeping this in view we have tried to explore *In-vitro* potential of homoeopathic drug. *Thuja-occidentalis* against *Curvularia lunata*. In our previous papers, activity of *Thuja-occidentalis* was reported against *Aspergillus-flavus* and *Aspergillus-niger,* Dermatophytes and Penicillium[2,3,4,16]. The most controversial aspect of Homoeopathic dilution is that succussion and trituration actually increases the power of a medicine. Clinical efficacy of homoeopathic drug in human is well established and need not be discusses here but in fungal system there is no increase in activity of higher potency against *Curvularia-lunata.* As it is shown in the results that *Thuja* 30 and 200 showed high antifungal efficacy but 50M which is expected to show higher activity has not shown better effects. Thus vital force (unknown factor) of homoeopathic drugs to all living groups (Micro-organisms to higher animals and plants) are different and even varies from genera to genera and species to species. Thus the present finding does not support the concept of high dilution and high energy. Similar results were also observed by Singh and Gupta[15] who reported *in-vitro* antiviral activity of homoeopathic drugs against animal viruses. In conclusion *Thuja* 30, 200 and 10M are very effective against pathogenic isolate *Curvularia-lunata* however other dilutions are not much effective. It is evident from Table –1 that no definite co-relation exists between various potencies of the same drug with regards to their antimycotic properties. Due to lack of experimental animals in our laboratory, *in-vivo* experiment could not be conducted which are needed for further investigations.

REFERENCES

1. Gupta G, Srivastava AK, Gupta N. Mycoses: An update over clinical cure with Homoeopathic drugs. Asian Homoeopathic Journal 1997;7(4): 67-96
2. Gupta G, Srivastava AK. *In-vitro* activity of *Thuja-occidentalis* Linn. Against human pathogenic aspergilli. Homoeopathic Heritage 27(1):5-12.
3. Gupta G, Srivastava AK. (2002) Antimycotic potential of *Thuja-occidentalis* against Dermatophytes in-vitro studies: Homoeopathy for all 3(26) 42-46.
4. Gupta G, Srivastava AK.(2001) Antimycotic potential of Homoeopathic drug *Thuja-occidentalis* against Penicillium sp. Causing onychomycosis in human presented in 12th annual conference of Asian Homoeopathic Medical League on 6th and 7th October, 2001 in Brisbane – Australia.
5. Harris JJ, Downham TF. Unusual fungal infections assoicated with immunological hyporeactvity. *Int J Dermatol* 1978;17:323-330.
6. Kaufman SM, *Curvularia* endocarditis following cardiac surgery. *Am J Clin Pathol* 1971;56:466-470.
7. Lampert RP, Hutto JH et al. Pulmonary ad cerebral mycetoma caused by *Curvularia-pallescens. J Pediatr* 1977;91:603-605.
8. Monte SM, Dela, Hutchens GM. Disseminated *Curvularia* infection. *Arch Pathol Lab Med* 1985;109:872-874.
9. Nair MV, Gupta S, Srivastava AK (eds). Environmental biopollutants and human health pp. 199. New Delhi; Anmol Publication Pvt Ltd. 1996
10. Nityananda KP, Sivasubramanian. et al. A case of mycotic keratitis by *Curvularia geniculata. Arch Opthamol* 1964; 71:456-458.
11. Olsen JH, Dragsted L, Autrup H. Cancer risk and occupational exposure to aflatoxins in Denmark. *Br J Cancer* 1998; 58:392-396.
12. Phillips PM, Rinaldi, Holt GR, Graybill JR, Phaeohyphomycosis sinusitis caused by *Curvularia lunata.* ASM 1986, p, 403 F36.
13. Rippon JW. Medical Mycology – The pathogenic fungi and the pathogenic actinomycetes pp. 797. Philadelphia: WB. Saunders Company.
14. Rohwedder JJ, Simmons JL et al. Disseminated *Curvularia lunata* infection in a football player. *Arch Intern Med* 1979; 139:940-941.
15. Singh LM, Gupta G. Antiviral efficacy of Homoeopathic drugs against animal viruses. *The British Homeopathic Journal* 1985; 74(3): 168-174.
16. Srivastava OP, Srivastava AK, Shukla PK. Advances in Medical Mycology Vol. 2 pp. 182. Lucknow: Evoker Research Perfecting Company.
17. Srivastava AK. Air pollution (Biopollutants in air)

pp. 302. New Delhi: Ashish Publishing House, 1991.
18. Srivastava AK. Mould Allergy – an overview, Advances in Medical Mycology edited by Mukherji et al. pp 63-80. New Delhi: Aditya Books, 1992.
19. Wadhwani K, Srivastava AK. Fungi from Otitis media of agricultural field workers. Mycopathologia 1984;88:155.
20. Wadhwani K, Srivastava AK, Some cases of onychomycosis from North India in different working environment. Mycopathologia 1985;92:149.
21. Wahab S, Tandon RN, Jacob Z, Sagar P, Srivastava OP, *In vitro* activity of a phytochemical alantolactone from *Inula racemosa* Hook f. against some pathogenic and opportunistic fungi. *J Indian Bot* 1981; Soc., 60:278-281.
22. Wahab S, Srivastava OP, Singh NB, Gupta SK, comparative *in vitro* and *in vivo* evaluation of toliciclate, tonaftate, miconazole, clotrimazole and undecylenic acid against *Trichophyton mentagrophytes*. Indian J Exp Biol 1978; 16: 1200.
23. Whitcomb MP, Jeffries CD et al. *Curvularia lunata* in experimental phaeohyphomycosis. Mycopathologia 1970;75:81-88.
24. Wind CA, Pollock FM. Keratomycosis due to [illegible] *Am J Opthamol* 1970; 84:694-696.

Table-1: Antimycotic potential of Homocopathic drug on linear diametric growth and sporulation of *Curvularia lunata*.

Homocopathic drugs	Potency	COLONY DIAMETER (CM)			SPORULATION		
		After 3 days	After 6 days	After 9 days	After 3 days	After 6 days	After 9 days
Sterile Water	Control 1	0.4	1.9	3.00	-	++++	++++
Vehicle (Rectfied Spirit)	Control 2	0.3	1.4	2.30	-	+++	++++
Ketoconazole	Positive Control	0.2	0.6	1.00	-	+++	++++
Thuja occidentalis	Q	0.8	1.1	1.30	-	++	+++
Thuja occidentalis	30	0.1	0.2	0.20	-	-	-
Thuja occidentalis	200	0.1	0.2	0.20	-	-	-
Thuja occidentalis	1M	0.4	0.8	1.50	-	++	++++
Thuja occidentalis	10M	0.3	0.6	0.80	-	+++	++++
Thuja occidentalis	50M	0.2	0.9	1.20	-	+++	++++

+ = upto 25%, ++ = upto 50%, +++ = upto 75%, ++++ = above 75%

* = Based on microscopic observations in relation to controls (Plate-1)

EXPLANATION OF FIGURES:

Plate 1: *In vitro* evaluation of antifungal potential of homocopathic drugs *Thuja occidentalis* on linear diametric growth and sporulation of *Curvularia lunata* on 9th day of post inoculation.

Figure 1: Control (Sterile Water)
Figure 2: Rectified Spirit (vehicle)
Figure 3: Positive control (Ketoconazole)
Figure 4: *Thuja-occidentalis* Q
Figure 5: *Thuja-occidentalis* 30
Figure 6: *Thuja-occidentalis* 200
Figure 7: *Thuja-occidentalis* 1M
Figure 8: *Thuja-occidentalis* 10M
Figure 9: *Thuja-occidentalis* 50M
Figure 10: Percent growth inhibition and linear diametric growth of *Curvularia-lunata* recorded on 9th day of post inoculation based on control 1 (SW)
Figure 11: Percent growth inhibition and linear diametric growth of *Curvularia-lunata* recorded on 9th day of post inoculation based on control II (RS)