

## FUNDAMENTAL RESEARCH

### In-vitro activity of *Thuja occidentalis* Linn. against human pathogenic aspergilli

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#### ABSTRACT

In-vitro antifungal potential of homoeopathic drug *Thuja occidentalis* Q, 30, 200, 1M, 10M, 50M against *Aspergillus flavus* causing cutaneous aspergillosis and *Aspergillus niger* causing otomycosis in Human are evaluated by following food poisoning method. Thuja Q, 30, 200 are found highly potent against *Aspergillus flavus* and 50M against *Aspergillus niger*. Percent growth inhibition, sporulation and exudation are taken as parameters for assessment. Results are reported for the first time and are discussed in relation to homoeopathic concept "Higher dilution and high energy".

Keywords: Antifungal drugs, *Thuja occidentalis*, *Aspergillus flavus*, *Aspergillus niger*, Aspergillosis

#### INTRODUCTION

Aspergilli are wide spread in the environment and are common inhabitants of soil. It is readily recognized that very large number of people, both adults and children are potentially exposed to such indoor and outdoor contaminants<sup>1,2,3</sup>. Several studies have provided evidence for the association of cancer in human with inhalation of aflatoxin contaminated dust<sup>4,5</sup>. It is now recognized that there are basically three categories of disease involving Aspergilli viz. Allergic aspergillosis, colonizing aspergillosis and invasive aspergillosis<sup>7,8,9</sup>. Treatment of aspergillosis with an antifungal drug such as amphotericin B is the first step of management but in neutropenic patients it is unsuccessful. Side effects and treatment duration may not be overlooked and is suggestive of finding a nontoxic safe remedy<sup>9,10</sup>.

*Thuja occidentalis*, a tall tree belongs to the family Cupressaceae. 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. Antifungal action of *Thuja occidentalis* against *Candida albicans*, *Trichophyton rubrum*, and *Trichophyton*

mentagrophytes is reported<sup>11</sup>. The present paper explores in-vitro antifungal potential of homoeopathic drug *Thuja occidentalis* Q, 30, 200, 1M, 10M and 50M against human pathogenic aspergilli.

#### MATERIALS AND METHODS FUNGAL ISOLATES

A) *Aspergillus niger*: A clinical isolate of patient complaining of chronic ear discharge for several years. Patients registered himself in GCCHR for the management of disease. MIC of ketoconazole for *Aspergillus niger* was standardized as 0.75 mg/ml by following the method of Jacob et al.<sup>12-13</sup>.

B) *Aspergillus flavus*: Isolated from skin of the patient at GCCHR showing hyperpigmentation with severe itching. MIC of ketoconazole for *Aspergillus niger* was standardized as 0.50 mg/ml by following the same method as above<sup>12-13</sup>.

#### MEDICINES

*Thuja occidentalis* in various potencies like Q, 30, 200, 1M, 10M and 50M was purchased from manufacturer. Quantity of drug was standardised as 0.5 ml.

#### CONTROLS

Three controls were taken, one of sterile water, second of vehicle i.e. rectified spirit and third control of Ketoconazole as positive control. Quantity of drug was taken as per their MIC calculated.

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## 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<sup>12-13</sup> against test fungi *Aspergillus niger* and *Aspergillus flavus*. 0.5 ml of each drug was mixed with 10 ml of Sabouraud's dextrose agar just before solidification in 50 mm diameter petridishes. SDA plates with 0.5 ml sterile water, 0.5 ml rectified spirit and 0.5 ml containing 5 mg ketoconazole (200mg tablet dissolved in 20 ml water) for *Aspergillus niger* and 0.75 ml for *Aspergillus flavus* were kept as controls. All the plates including controls were inoculated centrally with 1.5 mm diameter of disc of test fungi aseptically from 8 to 10 days old SDA plates culture. All the experimental petridishes were used in triplicates and incubated at 37°C. The linear diametrical growth of colonies were measured (in cm.) on 3rd, 6th and 9th days of post inoculation. The results were presented on an average of three plates on 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

Antifungal efficacy of homoeopathic drug *Thuja occidentalis* Q, 30, 200, 1M, 50M have been presented in Tables - 1 and 2 against *Aspergillus flavus* and *Aspergillus niger*. *Thuja* 30, 200 are found to inhibit the linear diametric growth of *Aspergillus flavus* equally on 9th day of post inoculation. *Thuja* 1M showed no effect, the growth of *Aspergillus flavus* was almost equal to control I (SW) and more than control II (RS) (Table - 1). Other potencies showed less sporulation in comparison to controls but *Thuja* 30 and 200 showed no sporulation (Plate - I; figs : 5 - 6). Percent growth inhibition of *Aspergillus flavus* on 9th day of post inoculation are presented in figs. 10 and 11. Fig. 10 is based on control I (SW) and fig. 11 is based on control II (RS). It is evident from the figs. 10 - 11 that *Thuja* 30 and *Thuja* 200 showed maximum inhibition i.e. 77.77% in each as compared to control I and 64.28% in each as compared to control II. Percent growth inhibition of positive control i.e. ketoconazole was 93.33 and 89.28 as compared to control I and control II respectively. *Thuja* 30 and *Thuja* 200 was less by 15.56 percent in each as compared to ketoconazole based on control I and 25 percent in each as compared ketoconazole based on control II.

*Thuja* 50 M was very effective against *Aspergillus niger*. Colony diameter observed was 2.2 mm al-

Table 1 : Antifungal potential of Homoeopathic drugs on linear diametric growth and Sporulation of *Aspergillus flavus*

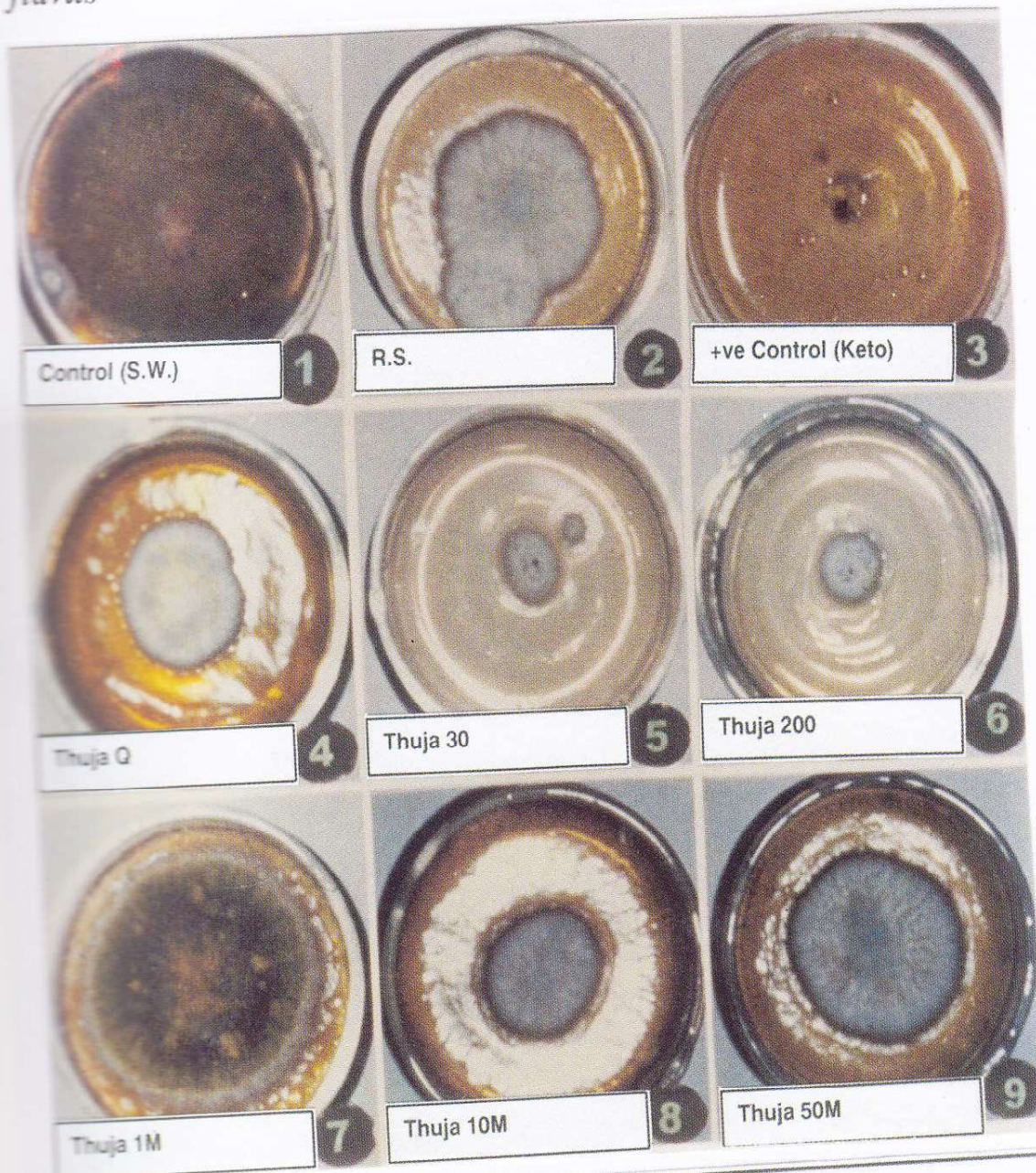
Homoeopathic drugs	Potency	COLONY DIAMETER (CM)			SPORULATION*		
		After 3 days	After 6 days	After 9 days	After 3 days	After 6 days	After 9 days
1. Sterile Water	Control 1	1.0	2.0	4.5	+++	+++	+++
2. Vehicle (Rectified Spirit)	Control 2	-	1.3	2.8	+	++	++
3. Ketoconazole	Positive Control	-	-	0.3	-	-	+
4. <i>Thuja occidentalis</i>	Q	-	1.4	2.0	-	-	++
5. <i>Thuja occidentalis</i>	30	-	.03	1.0	-	-	-
6. <i>Thuja occidentalis</i>	200	-	.04	1.0	-	-	-
7. <i>Thuja occidentalis</i>	1 M	1.2	2.4	4.2	++	+++	+++
8. <i>Thuja occidentalis</i>	10 M	-	1.0	2.0	-	+	++
9. <i>Thuja occidentalis</i>	50 M	-	1.4	3.1	-	+	++

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

\* = Based on naked eye observations (Plate - I)



**IN-VITRO EVALUATION OF ANTIFUNGAL POTENTIAL OF HOMOEOPATHIC DRUG – *Thuja occidentalis* LINN. AGAINST HUMAN PATHOGENIC FUNGI – *Aspergillus flavus***

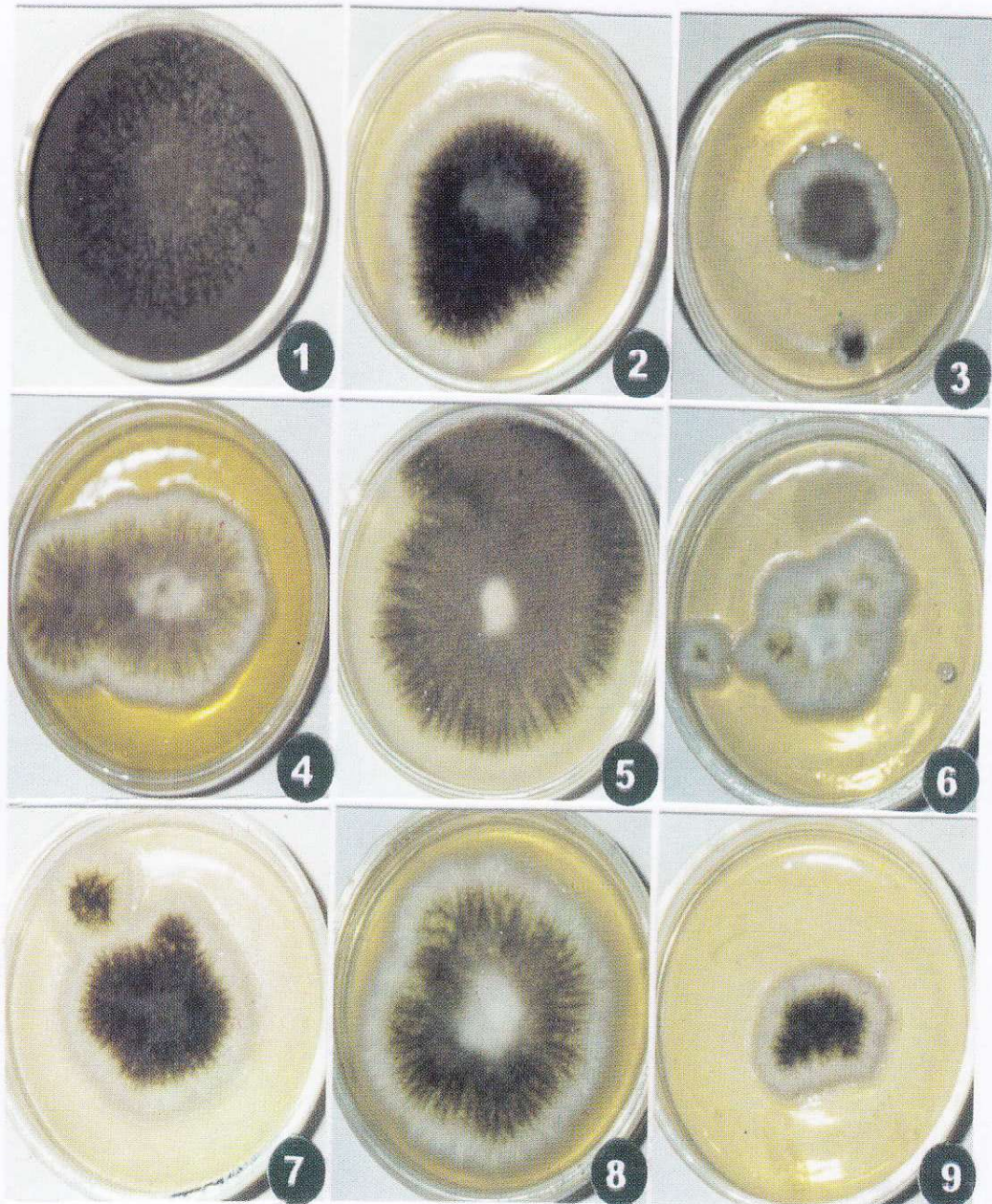


1.) Control (Sterile Water) 2.) Rectified spirit (Vehicle) 3.) Positive Control (Ketoconazole) 4.) Thuja occidentalis Q 5.) Thuja occidentalis 30 6.) Thuja occidentalis - 200 7.) Thuja occidentalis 1M 8.) Thuja occidentalis 10 M 9.) Thuja occidentalis 50 M

**PLATE I**



**IN-VITRO EVALUATION OF ANTIFUNGAL POTENTIAL OF HOMOEOPATHIC DRUG – *Thuja occidentalis* LINN. AGAINST HUMAN PATHOGENIC FUNGI – *Aspergillus niger***



- 1.) Control (Sterile Water) 2.) Rectified spirit (Vehicle) 3.) Positive Control (Ketoconazole) 4.) *Thuja occidentalis* Q 5.) *Thuja occidentalis* 30 6.) *Thuja occidentalis* - 200 7.) *Thuja occidentalis* 1M 8.) *Thuja occidentalis* 10 M 9.) *Thuja occidentalis* 50 M



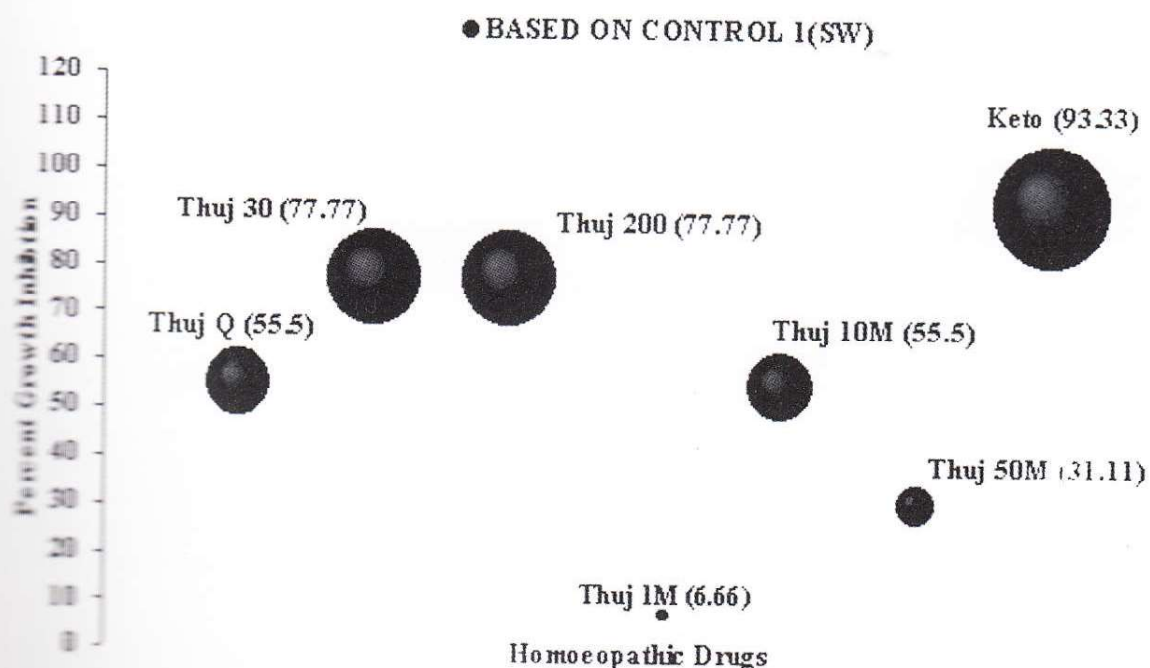
Table : 2 : Antifungal potential of Homoeopathic drugs on linear diametric growth and Sporulation of *Aspergillus niger*

Homoeopathic drugs	Potency	COLONY DIAMETER (CM)			SPORULATION <sup>+</sup>		
		After 3 days	After 6 days	After 9 days	After 3 days	After 6 days	After 9 days
1. Sterile Water	Control 1	2.2	3.5	4.6	+++	+++	+++
2. Vehicle (Rectified Spirit)	Control 2	-	2.6	3.6	+	+++	+++
3. Ketoconazole	Positive Control	-	1.8	2.1	-	+++	+++
4. <i>Thuja occidentalis</i>	Q	-	2.2	3.0	-	+++	+++
5. <i>Thuja occidentalis</i>	30	1.5	3.5	4.0	+++	+++	+++
6. <i>Thuja occidentalis</i>	200	-	2.0	2.4	-	-	+
7. <i>Thuja occidentalis</i>	1 M	-	3.6	4.0	-	+++	+++
8. <i>Thuja occidentalis</i>	10 M	-	1.5	2.8	-	+++	+++
9. <i>Thuja occidentalis</i>	50 M	-	1.8	2.2	-	+++	+++

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

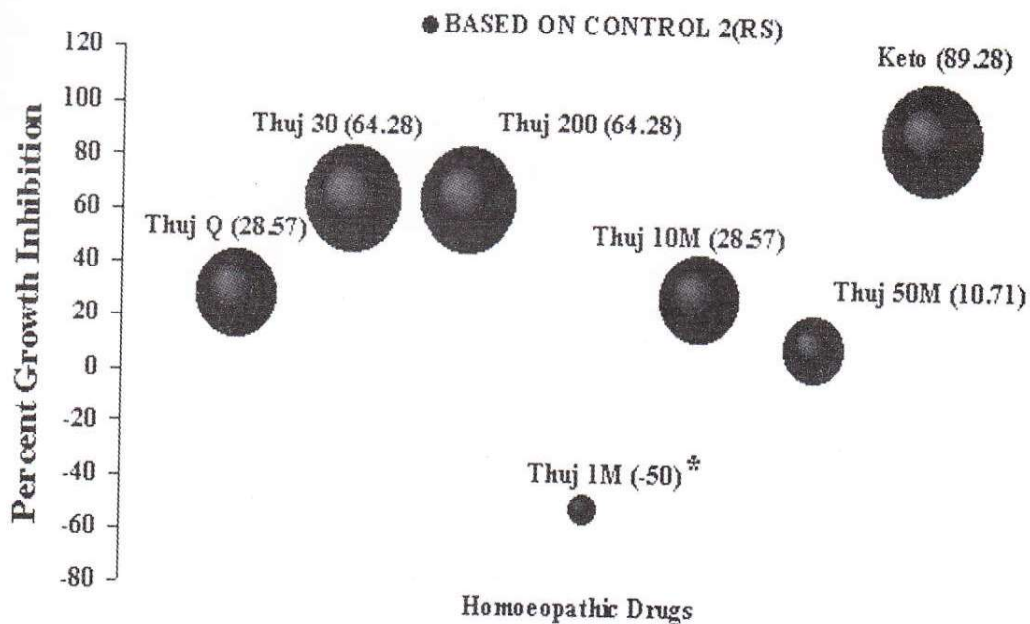
\* = Based on naked eye observations (Plate - II)

### PERCENT GROWTH INHIBITION OF *Aspergillus flavus* RECORDED ON 9TH DAY OF POST INOCULATION



SW = Sterile Distilled Water, Thuj = *Thuja occidentalis*, Keto = Ketoconazole

PERCENT GROWTH INHIBITION OF *Aspergillus flavus*  
RECORDED ON 9TH DAY OF POST INOCULATION



\* = Represent growth more than control, Thuj = *Thuja occidentalis*, RS = Rectified Spirit (Vehicle), Keto = Ketoconazole

most equal to Ketoconazole (2.1 mm) on 9th day of post inoculation (Table - 2). However, no significant effect on sporulation was noted (Plate II; Figs: 12 - 20). Thuja 200 showed effect on sporulation but linear diametric growth of colony was insignificant. Percent inhibition growth was presented in figs. 21 - 22. Fig 21 is based on sterile water (Control I) and fig 22 is based on rectified spirit (Control II). As it is evident from figs. 21 - 22, Thuja 50M showed maximum percent growth inhibition 52.17 of *Aspergillus niger* as compared to control I (SW) and 38.89 as compared to control II (RS). Thuja 50 M is equally effective as the allopathic drug "Ketoconazole". Observations taken on 3rd and 5th day of post inoculation are shown just to observe linear growth pattern of test fungi thus not discussed in details however mentioned in table 1 and table 2.

DISCUSSION

Infection due to species of *Aspergillus* have become common and often prove fatal on systemic dissemination in compromised host<sup>14,15,16,17</sup>. Compromisation of the patient results from Tuberculosis, Cancer, Bronchiectasis, Lymphoma, Diabetes, Sarcoidosis, Silicosis and Collagen vascu-

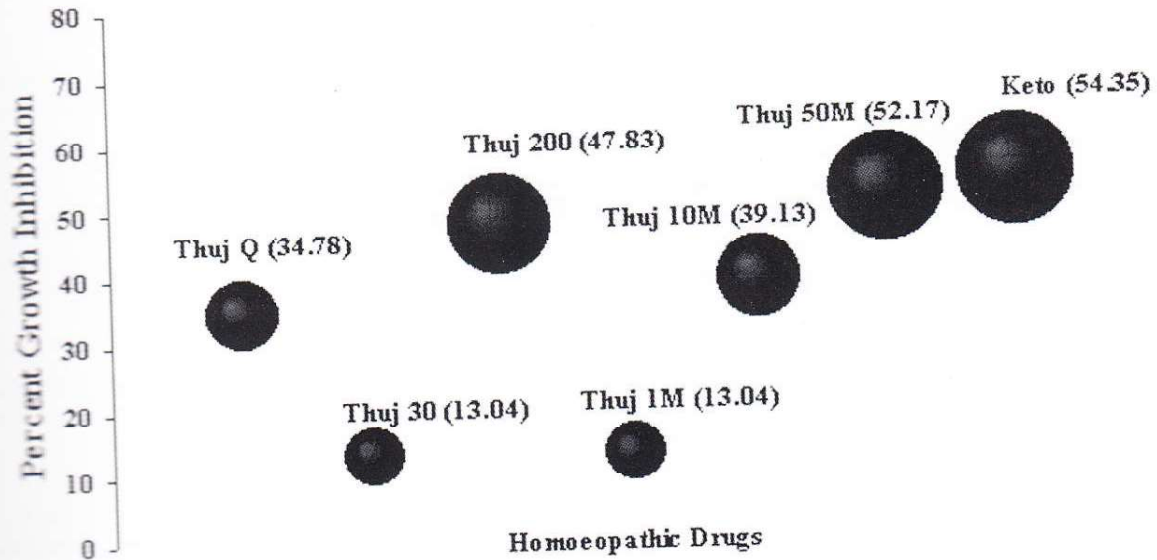
lar diseases, tissue barrier breaks, accidental infections, renal or cardiac transplant and frequent use antibiotics, cortisone and cytotoxins. Treatment available for the Aspergillois is not very safe in terms of drug toxicity<sup>18-19</sup>. Thus in the present investigation homoeopathic drug *Thuja occidentalis* is evaluated in vitro for its antifungal potential. Thuja 30 and 200 was found promising against *Aspergillus flavus* whereas Thuja 50M was found effective against *Aspergillus niger*. Mode of action as to how homoeopathic drug works is not evaluated in this study. How some potency of homoeopathic drugs enhanced the growth of fungus is the subject of further study. It is well established from the present investigation that Thuja 30 and 200 is very effective against *Aspergillus flavus* causing cutaneous aspergillois in human and Thuja 50M is effective against *Aspergillus niger* known to cause otomycosis. Due to limitation of experimental animal facilities in our Laboratory, in - vivo experiments could not be done but are suggested for further investigation.

The most controversial aspect of homoeopathic dilution is that succussion and trituration actually in-



**PERCENT GROWTH INHIBITION OF *Aspergillus niger* RECORDED ON 9TH DAY OF POST INOCULATION**

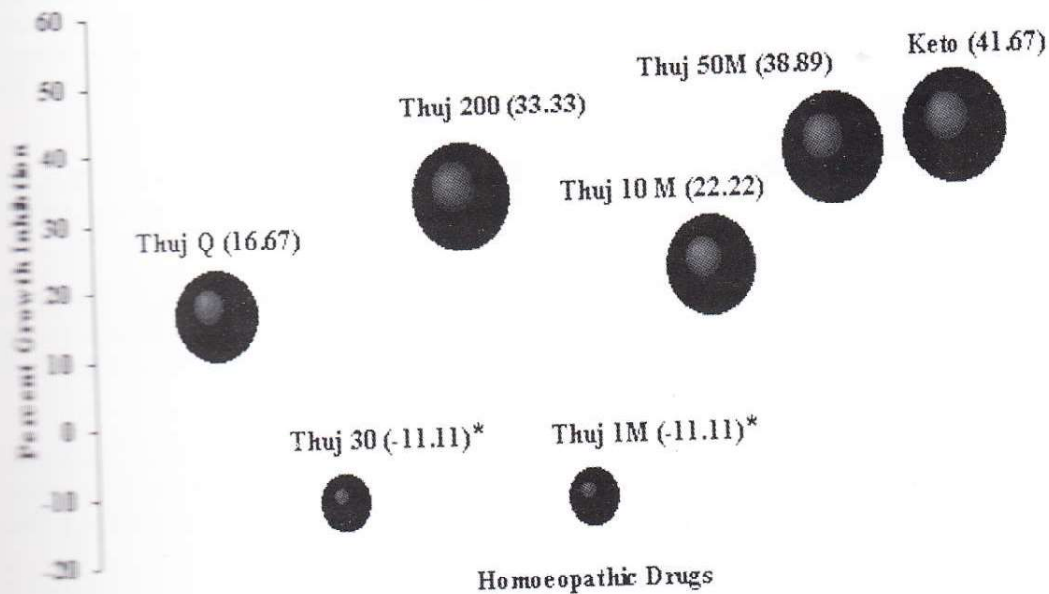
● BASED ON CONTROL 1(SW)



SW = Sterile Distilled Water, Thuj = *Thuja occidentalis*, Keto = Ketoconazole

**PERCENT GROWTH INHIBITION OF *Aspergillus niger* RECORDED ON 9TH DAY OF POST INOCULATION**

● BASED ON CONTROL 2(RS)



\* = Significant growth more than control, Thuj = *Thuja occidentalis*, RS = Rectified Spirit (Vehicle), Keto = Ketoconazole

crease the power of remedy. Clinical efficacy of high dilution is well established in homoeopathic science. But in the present investigation it is proved that there is no increase in the activity of higher dilution. Thuja 30 and 200 is effective against *Aspergillus flavus* however higher dilution 1M, 10M and 50M is not effective which contradicts theory of high dilution and high energy. The present investigation constitute the first report for proving in vitro action of homoeopathic drug *Thuja occidentalis* Q, 30, 200, 1M, 10M, 50M against human pathogenic fungi viz. *Aspergillus flavus* and *Aspergillus niger*. The findings also disqualify the concept of higher energy and high dilution in the system of fungi - a eukarotic organism. Similar results were also observed by Singh and Gupta et al.<sup>20,21,22</sup> and proved antiviral efficacy of homoeopathic drugs against plant and animal viruses. Sharma 1998 suggested vital force is certainly a form of Sharmon-composed "basic substance" which mediates the molecular mechanisms underlying vital functions of health and disease and also potentization of medicines through dynamization processes as human cells<sup>23</sup>. Vital force concept requires updation in the term of energy quanta, their measuring parameters and constitutional suitability of potency to the patients and their disease.

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