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Study on the Role of Four Homeopathic Drugs on the Growth of *Microsporum gypseum*

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ABSTRACT

The present study aims to evaluate the antifungal potential of four homeopathic drugs on the growth of a fungus, *Microsporum gypseum.* Mother tinctures of four homeopathic drugs, namely *aconite*, *arnica*, *arsenic*, and *agaricus*, were screened at five concentrations (1%, 2%, 3%, 4%, and 5%) for their antifungal properties against *M. gypseum.* Amongst the four drugs tested, *aconite* was found to be the most effective drug, followed by *arnica*, *arsenic*, and *agaricus*. No fungal growth was observed at 5% concentration of any of the homeopathic drugs tested.

Keywords: Anti-fungal activity, *Aconite, Agaricus*, *Arnica*, *Arsenic*, Homeopathy, *Microsporum gypseum*

INTRODUCTION

The keratinophilic fungi are a group of highly specialized fungi that degrade most non - degradable keratins and use this protein for their growth and metabolism and *Microsporum gypseum* is one of them (Sowjanya and Vardhini, 2023; El-Dawy *et al*., 2024). These keratinophilic fungi survive in diverse ecological conditions (Rathore and Bhadauria, 2024; Sowjanya and Vardhini, 2024). In recent times, the study of homeopathy has gained considerable attention for its potential to control infections caused by pathogenic bacteria and fungi (Prajapathi *et al*., 2017; Pannek *et al*., 2018). It is believed that homeopathy addresses the root cause of a disease and cures the disease by neutralizing the causative microbe. Moreover, homeopathic drugs are generally reported to have minimal side effects and have been employed in the treatment of fungal infections (Nwabudike, 2012). It was observed that a number of homeopathic drugs inhibited the spore germination of fungal pathogens causing post-harvest diseases of fruits and vegetables (Castro *et al*., 2013; Dua *et al*., 2014). The homeopathic drug known as *Thuja* extracted from *Thuja occidentalis* at different concentrations *viz*., 30 C *Thuja*, 200 C *Thuja*, 1 M *Thuja*, 10 M *Thuja* and 50 M *Thuja* was found to inhibit the growth of various fungal isolates like *Fusarium* spp, *Aspergillus flavus*, *Bipolaris* spp, *Exserohilum* spp and *Curvularia* spp (Asha *et al*., 2014).

In humans, the hyphae of *Microsporum* spp. invade the scalp and result in hair loss. *Microsporum gypseum* is a keratinophilic fungus capable of causing skin diseases called tinea or ringworm especially in human beings. The infection results in the release of pus and formation of kerion. Pus formation leads to discharge from the affected hair follicles in the hands and feet. It is also capable of degrading avian feathers which will affect the very life of the birds as flying is the main function of most birds. The objective of the present investigation was therefore to evaluate the efficacy of four homeopathic drugs, namely *aconite*, *arnica*, *arsenic*, and *agaricus*, in controlling the growth of *M. gypseum*. All four homeopathic drugs in this study were capable of controlling the growth of *M. gypseum.*

MATERIALS AND METHODS

Four homeopathic drugs were used in this study: *aconite* (*Aconitum napellus* — monkshood or wolf's bane), *arnica* (*Arnica montana* — leopard's bane), *arsenic* (*Arsenic album*), and *agaricus* (*Agaricus muscarius* — Amanita). Their inhibitory effects on *M. gypseum* were evaluated at five concentrations (1%, 2%, 3%, 4%, and 5%) according to the methods described in the Homoeopathic Pharmacopoeia of India (1974). These concentrations were obtained by adding appropriate amounts of homeopathic drugs to

the Sabouraud glucose agar (SDA) medium using poisoned food technique.

Sabouraud Dextrose Agar (SDA) is a non-selective isolation medium commonly used for the growth and maintenance of *M. gypseum*. It was prepared by dissolving 65 g in one litre of distilled water and autoclaving at 121°C for 15 minutes. The medium was prepared in accordance with EN ISO 11133 guidelines for microbiological examination. SDA was prepared according to the harmonized methods described in the United States Pharmacopoeia (USP), European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP) for examination of *Microsporum gypseum* in non-sterile products.

Preparation of *Aconite*

The mother tincture of *aconite* was prepared with ethanol (45% v/v) using 100 g of whole fresh flowering plant *Aconitum napellus* L. The tincture was prepared at a 1:10 drug strength. The pure ethanol was diluted with purified water to exactly 45% v/v. The moist magma (plant mass comprising solids and plant moisture) constituted 1/10 of the total drug weight. 1%, 2%, 3%, 4% and 5% of aconite were prepared from the mother tincture of *aconite*.

Preparation of *Arnica* (*Arnica montana* - Leopard's bane)

The mother tincture of *arnica* was prepared by the ethanolic extractions (45% v/v) of the 100 gm whole fresh flowering plant and the ethanolic extracts of the underground parts of *Arnica montana*. The tincture was prepared at a (1:10) drug strength. The pure ethanol was diluted with purified water to exactly 45% v/v. The moist magma (plant mass comprising solids and plant moisture) constituted 1/10 of the total drug weight. 1%, 2%, 3%, 4% and 5% of *arnica* were prepared from the mother tincture of *arnica*.

Preparation of *Arsenic* (*Arsenic album*)

The mother tincture of *arsenic* (*Arsenic album*) was prepared from the white oxide of metallic arsenic. A solution was prepared by diluting the aqueous *arsenic* (one part of arsenic was dissolved in 99 parts of water viz., 1:100). It was diluted till the point wherein there was no trace of arsenic left behind in the solution and hence it was no longer poisonous. 1%, 2%, 3%, 4% and 5% of *arsenic* were prepared from the mother tincture of *arsenic*.

Preparation of *Agaricus* (*Agaricus muscarius* - Amanita)

The mother tincture of *agaricus* was prepared by ethanolic extraction (45% v/v) of 100 g of whole fresh fungus using the potentization method. The tincture was prepared at a (1:10) drug strength. The pure ethanol was diluted with purified water to exactly 45% v/v. The moist magma (plant mass comprising solids and plant moisture) constituted 1/10 of the total drug weight. Working concentrations of 1%, 2%, 3%, 4%, and 5% were prepared from the mother tincture of *agaricus*.

The effect of homeopathic drugs on the mycelial growth was studied under *in vitro* conditions on Sabouraud's glucose agar (SDA) medium. The medium individually supplemented with the above-mentioned homeopathic drugs at five concentrations (1%, 2%, 3%, 4%, and 5%) was poured into Petri plates and inoculated with a 5 mm diameter mycelial disc taken from the actively growing region of 8–10 day-old cultures. Three replicates were set up for each homeopathic drug at each concentration. SDA without homeopathic drug served as control. The inoculated plates were incubated at 28 ± 2 °C for seven days. The diameter of the colony was measured on 3rd, 5th and 7th days. T-test has been performed to compare the effect of different homeopathic drugs with respect to control.

RESULTS

Table 1 and **Figure 1** summarize the effect of different homeopathic drugs on the growth of *Microsporum gypseum* recorded on the 3rd day.

The highest growth inhibition was achieved by *aconite*, with no growth recorded at 3%, 4%, and 5% concentrations. This was followed by *arnica, arsenic* and *agaricus*. Complete growth inhibition was achieved by all four drugs at 5% concentration. The data was subjected to statistical analysis and is expressed in terms of standard error [Mean ± S.E (n=3)]. The effect of different homeopathic drugs with respect to control was compared by performing T-test.

Table 2 presents the effect of different homeopathic drugs on the growth of *Microsporum gypseum* observed on 5th day. *Aconite* caused the maximum fungal growth inhibition and no fungal growth was detected at 4% and 5% concentrations and a minimum growth was recorded at 3% concentration.

Table - 1: Effect of different homeopathic drugs on the growth (in cm) of *Microsporum gypseum* (3rd day)

Homeopathic drugs	Different concentration of the drugs									
	1%	t-value	2%	t-value	3%	t-value	4%	t-value	5%	t-value
Control	2.31±0.044		2.31±0.044		2.31±0.044		2.31±0.044		2.31±0.044	
Agaricus	1.96 ±0.06	4.33***	1.60 ±0.011	15.7***	1.40 ±0.028	17.50***	1.10 ±0.057	32.08***	-	
Arsenic	1.80± 0.028	9.61***	1.40± 0.028	17.5***	1.10± 0.057	32.08***	0.82± 0.030	28.11***	-	
Arnica	1.56 ±0.044	12.09***	1.10 ±0.057	32.08***	0.78 ±0.015	33.26***	-	-	-	
Aconite	1.20±0.028	21.3***	0.80±0.028	29.03***	-	-	-	-	-	
# Values are mean ± S. E										
*,**,*** significant at P≤0.05,0.025,0.010 respectively										

Table - 2: Effect of different homeopathic drugs on the growth (in cm) of *Microsporum gypseum* (5th day)

Homeopathic drugs	Different concentration of the drugs									
	1%	t-value	2%	t-value	3%	t-value	4%	t-value	5%	t-value
Control	2.78±0.016		2.78±0.016		2.78±0.016		2.78±0.016		2.78±0.016	
Agaricus	2.30 ±0.028	15.0***	1.73 ±0.024	43.75***	1.62 ±0.017	50.43***	1.43 ±0.024	67.5***	-	
Arsenic	2.16± 0.044	15.5***	1.73 ±0.024	52.50***	1.32± 0.017	63.40***	0.92± 0.011	97.8***	-	
Arnica	1.71 ±0.016	53.5***	1.33 ±0.024	72.50***	0.88 ±0.016	95.00***	-	-	-	
Aconite	1.58±0.016	60.0***	1.00±0.057	35.60***	0.63 ±0.033	71.60***	-	-	-	
# Values are mean ± S.E										
*,**,*** significant at P≤0.05,0.025,0.010 respectively										

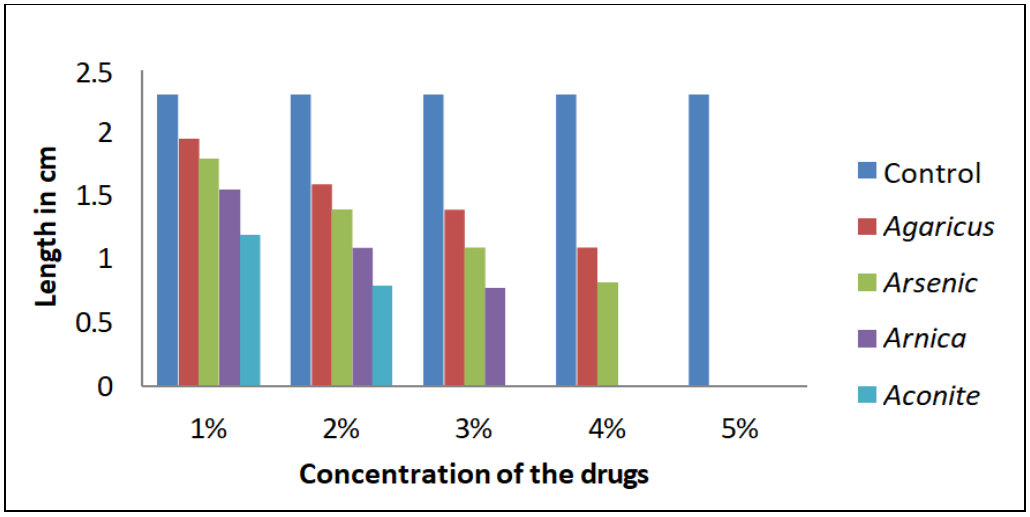


Figure 1: Effect of homeopathic drugs on the growth *of Microsporum gypseum* (3rd day).

The effect of *arnica* was similarly strong, with no fungal growth detected at 4% and 5% concentrations. This was followed by *arsenic* and *agaricus* (**Figure 2**). The results were statistically analyzed and are

expressed in terms of standard error [Mean ± S.E (n=3)]. T-test has been performed to compare the effect of different homeopathic drugs with respect to control.

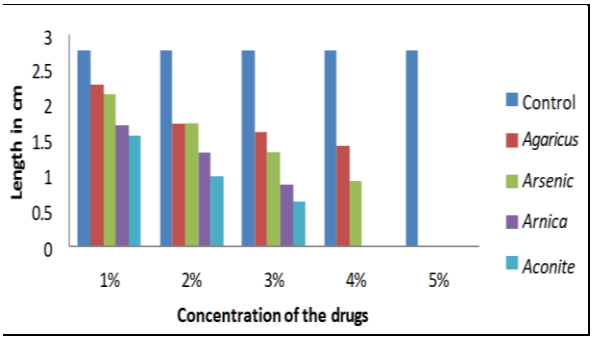


Figure 2: Effect of homeopathic drugs on the growth of *Microsporum gypseum* (5th day).

Table 3 summarizes the effect of different homeopathic drugs on the growth of *Microsporum gypseum* recorded on the 7th day. The results obtained were similar to those observed on 3rd and 5th days. The effect of *aconite* and *arnica* was pronounced followed by *arsenic* and *agaricus* (Figure 3). Statistically analyzed data was expressed in terms of standard error [Mean ± S.E (n=3)]. The Effect of different homeopathic drugs with respect to control was analyzed by performing T-test. Statistically analyzed data was expressed in terms of standard error [Mean ± S.E (n=3)].

Table - 3: Effect of different homeopathic drugs on the growth (in cm) of *Microsporum gypseum* (7th day)

Homeopathic drugs	Different concentration of the drugs									
	1%	t-value	2%	t-value	3%	t-value	4%	t-value	5%	t-value
Control	3.60±0.076		3.60±0.076		3.60±0.076		3.60±0.076		3.60±0.076	
Agaricus	2.53 ±0.016	15.28***	2.35 ±0.170	6.90***	1.75±0.028	23.12***	1.24 ±0.023	29.8***	-	
Arsenic	2.35± 0.076	12.50***	1.88 ±0.016	24.57***	1.46± 0.030	26.75***	1.13± 0.072	24.7***	-	
Arnica	1.83±0.044	22.12***	1.45 ±0.028	26.87***	0.93 ±0.016	38.14***	-	-	-	
Aconite	1.78±0.016	26.00***	1.24±0.023	29.80***	0.75 ±0.028	35.62***	-	-	-	
# Values are mean ± S.E *,**,*** significant at P≤0.05,0.025,0.010 respectively										

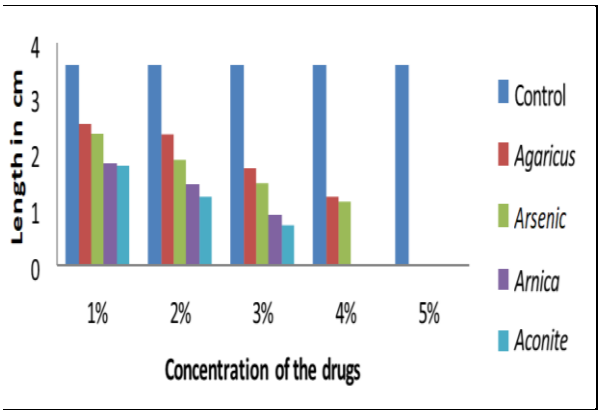


Figure 3: Effect of Homeopathic drugs on the growth of *Microsporum gypseum* (7th day).

DISCUSSION

The diseases caused by certain keratinophilic fungi are causing a lot of alarm and uneasiness among people. *Microsporum gypseum,* a keratinophilic fungus, causes skin diseases in humans, domestic animals, and birds. The different homeopathic drugs viz., *aconite, arnica, arsenic* and *agaricus* inhibited the growth of *M. gypseum* at lower concentration (1-3%) and prevented the proliferation of mycelia at higher concentration (4 to 5%).

Amongst the four homeopathic drugs tested, *aconite* was found to be most effective, followed by *arnica*. While Aconite arrested fungal growth at 3% concentration itself. According to homeopathic theory, diseases in living organisms arise from suppressed mechanisms that disrupt physiological normalcy (Gupta *et al*., 2015). These suppressed mechanisms lead to diseases or malfunctions in plants which ultimately result in reduced growth and yield (Lisboa *et al*., 2005; Toledo *et al*., 2009). However, homeopathic drugs are capable of mitigating these deleterious effects and restoring physiological normalcy in living organisms (Rissato *et al*., 2016). It is well established that homeopathic medicines are devoid of side effects because the dilution of the original homeopathic drug molecule in a dosage form is extremely high. Therefore, homeopathic drugs at the low concentration could be efficient in controlling

pathogenic fungal strains. This preliminary study suggests that homeopathic drugs such as aconite, arnica, arsenic, and agaricus represent safer and more cost-effective alternatives to conventional allopathic antifungal treatments.

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