

“In vitro studies for anti-fungal activity of Homoeopathic medicines against plant fungus *Gibberella fujikuroi*.”

Snehal Ashok Barkund¹, Pashmin Kaur Anand², Mayur Arjun Aswani², Arun Bhargav Jadhav³, Bipinraj Nirichan Kunchiraman⁴ & Chetan Hanamantrao Shinde⁵.

¹Post - Graduate Scholar, Department of Homoeopathic Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Homoeopathic Medical College & Hospital, Katraj – Dhankawadi, Pune, India.

²Department of Microbiology, Bharati Vidyapeeth (Deemed to be) University, Rajiv Gandhi Institute of Information Technology and Biotechnology, Katraj – Dhankawadi, Pune, India.

³Principal, Bharati Vidyapeeth (Deemed to be) University, Homoeopathic Medical College and Hospital, Katraj - Dhankawadi, Pune, India.

⁴Assistant Professor, Department of Microbiology, Bharati Vidyapeeth (Deemed to be) University, Rajiv Gandhi Institute of Information Technology and Biotechnology, Katraj – Dhankawadi, Pune, India.

⁵Associate Professor, Department of Homoeopathic Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Homoeopathic Medical College & Hospital, Katraj – Dhankawadi, Pune, India.

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ABSTRACT

Gibberella fujikuroi is a plant pathogen which affects rice and other plants and causes Bakanae disease. In this anti-fungal study author aimed to evaluate the in vitro effects of Homoeopathic Medicines *Thuja occidentalis*, *Silicea*, *Mercurius Solubilis*, *Lachesis Mutus*, *Calcarea Carbonica* in (6C, 12C, 30C, 200C, 1M, 10M, CM) potencies against *Gibberella fujikuroi*. The Fungus was screened by agar well diffusion method and MIC according to percentage method. It was found that *Thuja occidentalis* (10M), *Silicea* (200C and 1M), *Mercurius Solubilis* (6C, 12C and 10M), *Lachesis Mutus* (6C and 200C), *Calcarea Carbonica* (30C and 200C) inhibits 100% growth of *Gibberella fujikuroi*. These Homoeopathic Medicines can be used as alternative agro-homoeopathic fungicides in the agriculture field.

Keywords: Anti-fungal, Homoeopathic Medicines, *Gibberella fujikuroi*, MIC, Potato Dextrose Broth (PDB).

Introduction -

Gibberella fujikuroi is a plant pathogen fungus which produces gibberellins and secondary metabolites such as carotenoids, bikaverin, fusarin, phytotoxins and mycotoxins. [1,2] This fungus has majorly affected plants, humans and animals. *Gibberella fujikuroi* is a spore killer fungus. When one parent carries a killer element (SK^k) and another carries the sensitive counterpart (SK^s) causes killing. [3]

Gibberella fujikuroi complex (GFC) has more than 50 distinct species or phylogenetic lineages. In this 34 species are described by their morphological characters, 10 species on the basis of sexual fertility and 20 species produces mycotoxins. [4] GFC is a monophyletic taxon. It causes devastating diseases on economically important plants. Consumption of contaminated food can cause hazardous diseases in humans and animals. It lowers the productivity in livestock and death if prolonged exposure occurs. [4] *Gibberella fujikuroi* can produce carcinogenic mycotoxins and cause equine leukoencephalomalacia of horses and donkeys. [5]

Gibberellins are plant growth hormone. [6] The production of gibberellin by *Gibberella fujikuroi* is depending on the nature of carbon and nitrogen sources. It is stimulated by high carbon to nitrogen ratio. [7] This fungus is the industrial source of gibberellic acid. 14 strains of *Gibberella fujikuroi* were screened to select for the most potent strain. [7, 8] *Gibberella fujikuroi* has affected rice and cause Bakanae disease which is first reported in Japan by Hori in 1989. Bakanae is the condition in which “foolish seedling” which means abnormal excessive elongation of the plant occurs. [9] It is known to be a seed born as well as soil born pathogen of rice and it infect the seedlings during germination. [10] Yabuta and Hayashi (1939) reported that gibberellins causes elongation of other plants, i.e. barley, buckwheat, rapeseed, tomato, cucumber, morning glory etc. [6] Bavistin (Carbendazim) is commonly used fungicide to treat *Gibberella fujikuroi* fungus of bakanae disease. [10,11] Bavistin may cause genetic defects and damage fertility or the unborn child. EPA has classified Bavistin as Group C: “Possible Human Carcinogen”. [12]

Homoeopathy is an art and science of healing where ultra-high dilutions are used as medicine. The principle “Law of minimum dose” means lower the dose higher the effectiveness. Homoeopathy can treat humans as well as plants. [13] Homoeopathic medicines namely *Thuja occidentalis*, *Silicea*, *Mercurius*

Solubilis, Lachesis and Calcareo Carbonica were seen to have anti - fungal activities in humans as mentioned in Synthesis Repertory 9.0 and Murphy Repertory. [14, 15] Centesimal scale potencies of above selected homoeopathic medicines viz. 6C, 12C, 30C, 200C, 1M, 10M, CM were used in this research work.

Aim of the present study is to screen the ability of above mentioned Homeopathic medicines to inhibit pathogen *Gibberella fujikuroi* in-vitro and to study the mechanism of inhibition.

Material and Methods -

Media and Chemicals -

All media (Potato Dextrose Broth and Agar) [1] used in the study was procured from Hi Media Mumbai, India. All the reagents and chemicals used in this study were of AR grade. Bavistin (Carbendazim) as a chemical control was purchased from the Agrochemical shop. Dispensing alcohol 90% [16] is used as a vehicle control.

Collection of Organism -

The fungus *Gibberella fujikuroi* (Accession No. - 1321) was procured as active slant from the National Collection of Industrial Microorganism (NCIM) Pune, India. It was used to examine antifungal activity of selected Homoeopathic medicines.

Homoeopathic Medicines -

Homoeopathic medicines namely Thuja occidentalis, Silicea, Mercurius Solubilis, Lachesis and Calcareo Carbonica of liquid potencies 6C, 12C, 30C, 200C, 1M, 10M [14,15] were obtained from GMP (Good manufacturing practices) approved standard Homoeopathic medicines manufacturer.

Slide culture technique (Direct microscopy by lacto-phenol cotton blue stain) -

One drop of Lacto-phenol cotton blue stain added on glass slide with an inoculated cover slip of *Gibberella fujikuroi* culture. This preparation was examined by using the low power (40×) objective lens of the microscope and High-power (100×) objective lens in oil emersion to see the morphological structure of organism.

Agar Well-Diffusion Assay -

Antifungal activity of selected Homoeopathic medicines with various potencies against the *Gibberella fujikuroi* fungus was determined using the agar well diffusion method. [17,18,19] (100µl) suspension of *Gibberella fujikuroi* culture with 0.5 O.D. was spread on potato dextrose agar petri plates and was incubated for 30min at 28°C. Petri plates were punched with sterilized borer and mentioned Homoeopathic medicines with various potencies (20µl) were added in wells. It was incubated at 28°C for 48hrs. Bavistin (20µl) was used as Chemical control. Dispensing alcohol (ethanol 90%) is used as vehicle control. [19] The zone of inhibition around the wells was measured to examine the antifungal activity of *Gibberella fujikuroi*. This assay was carried out in duplicates.

Minimum Inhibitory Concentration according to Percentage -

Eppendorf Tube Dilution Assay -

The Eppendorf tube assay was used to determine MIC value of selected homoeopathic medicines with various potencies. Each eppendorf tube (2ml) was weighted before filling. Each eppendorf tube filled with Potato dextrose broth (500µl), Homoeopathic potency (500µl) and *Gibberella fujikuroi* culture with 0.6 O.D. (500µl) was added. The study was performed with Chemical control (Bavistin), Positive control (broth + culture), Negative control (Potato dextrose broth), Vehicle control (dispensing alcohol) [19] incubated for 24hrs. at 28°C. After incubation eppendorf tubes were centrifuged at 8000 rpm for 10min. Supernatant was discarded and the pallet was kept in the oven at 55°C temp for 24hrs. for drying. Weight of dry pallet and the eppendorf tube was measured for inhibition and the percentage was calculated.

Results -

1) Slide Culture Technique -

Gibberella fujikuroi showed no spores in slide culture technique under 40x and 100x objective lens as a spore killer pathogen.

2) Agar Well-Diffusion Assay -

Selected Homoeopathic Medicines Thuja occidentalis (6C,200C,1M), Silicea (12C, 30C, 200C, 1M, 10M, CM), Mercurius Solubilis (200C, 1M, 10M), Lachesis Mutus (6C, 12C, 10M, CM), Calcareo Carbonica (12C, 200C) showed zone of inhibition around the well. The inhibition zone ranged from 0.5cm to 1.15cm. (Table.1)

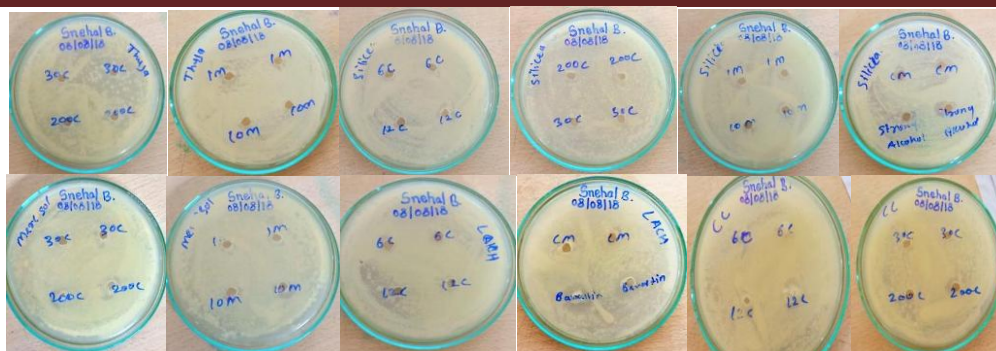


Figure 1 - Antifungal assay of Homoeopathic Medicines by Agar well diffusion method

Table 1: Zone of inhibition of Homoeopathic Medicine by Agar well diffusion method

Name of Medicine	Potency	Inhibition zone (Mean ± Standard Deviation) in c.m.
Thuja occidentalis	6C	0.55 ± 0.07
	12C	0.50 ± 0.00
	30C	0.50 ± 0.00
	200C	0.65 ± 0.21
	1M	0.75 ± 0.35
	10M	0.50 ± 0.00
Silicea	6C	0.50 ± 0.00
	12C	0.70 ± 0.28
	30C	0.65 ± 0.07
	200C	0.65 ± 0.07
	1M	0.55 ± 0.07
	10M	0.70 ± 0.28
Mercurius Solubilis	6C	0.50 ± 0.00
	12C	0.50 ± 0.00
	30C	0.50 ± 0.00
	200C	1.15 ± 0.21
	1M	0.70 ± 0.00
	10M	0.90 ± 0.14
Lachesis Mutus	6C	0.65 ± 0.21
	12C	0.60 ± 0.14
	30C	0.50 ± 0.00
	200C	0.50 ± 0.00
	1M	0.50 ± 0.00
	10M	0.70 ± 0.28
Calcarea Carbonica	6C	0.50 ± 0.00
	12C	0.70 ± 0.28
	30C	0.50 ± 0.00
	200C	0.65 ± 0.21
	1M	0.50 ± 0.00
	10M	0.50 ± 0.00
Chemical control (Bavistin)	0.1%	1.25 ± 0.07
Dispensing alcohol	90%	0.95 ± 0.07

3) Minimum Inhibitory Concentration according to Percentage -

The MIC of Homoeopathic Medicines Thuja occidentalis (10M), Silicea (200C, 1M), Mercurius Solubilis (6C, 12C, 10M), Lachesis Mutus (6C, 200C), Calcarea Carbonica (30C, 200C) showed 100% inhibition in the Eppendorf tube dilution assay. (Table - 2)

Table: 2 MIC according to % of the Homoeopathic Medicines by (Eppendorf Tube Dilution Assay)

Sr. No.	Homoeopathic Medicines	Homoeopathic potency	Inhibition %
1.	Thuja occidentalis	6C	058
		12C	071
		30C	054
		200C	063
		1M	079
		10M	100
		CM	013
2.	Silicea	6C	021
		12C	058
		30C	042
		200C	100
		1M	100
		10M	075
		CM	058
3.	Mercurius Solubilis	6C	100
		12C	100
		30C	008
		200C	033
		1M	029
		10M	100
		CM	083
4.	Lachesis Mutus	6C	100
		12C	033
		30C	075
		200C	100
		1M	021
		10M	058
		CM	042
5.	Calcarea Carbonica	6C	088
		12C	029
		30C	100
		200C	100
		1M	038
		10M	050
		CM	067
6.	Chemical control (Bavistin)	0.1%	042
7.	Alcohol (Dispensing alcohol)	90%	021

Discussion -

The fungus *Gibberella fujikuroi* has harmful effects on plants, animals, and humans. It produces Bakanae disease on rice and causes loss in rice production. It directly affects the economy of the country. Homoeopathic Medicines namely Thuja occidentalis, Silicea, Mercurius Solubilis, Lachesis Mutus, Calcarea Carbonica with their potencies (6C, 12C, 30C, 200C, 1M, 10M, CM) [17,18] used in the study was selected as per their antifungal activity mentioned in Synthesis Repertory 9.0 and Murphy's Homoeopathic Repertory to screen their ability to inhibit the *Gibberella fujikuroi* fungus. Since many years it was a topic of debate that Homoeopathy is a "Pseudoscience" but the results obtained in this study undoubtedly showed the effectiveness of Homoeopathic medicines.

After analyzing the results of present study it was clear that selected Homoeopathic Medicines has an ability to inhibit the fungus *Gibberella fujikuroi* as a zone of inhibition was seen in antifungal assay viz. Thuja occidentalis 6C (0.55 ± 0.07), 200C (0.65 ± 0.21), 1M (0.75 ± 0.35), Silicea 12C (0.70 ± 0.28), 30C (0.65 ± 0.07), 200C (0.65 ± 0.07), 1M (0.55 ± 0.07), 10M (0.70 ± 0.28), CM (0.80 ± 0.14), Mercurius Solubilis 200C (1.15 ± 0.21), 1M (0.70 ± 0), 10M (0.90 ± 0.14), Lachesis Mutus 6C (0.65 ± 0.21), 12C (0.60 ± 0.14), 10M (0.70 ± 0.28), CM (0.60 ± 0.14), Calcarea Carbonica 12C (0.70 ± 0.28), 200C (0.65 ± 0.21) and in Eppendorf tube assay (MIC according to percentage) Homoeopathic Medicines Thuja occidentalis (10M), Silicea (200C, 1M), Mercurius Solubilis (6C, 12C, 10M), Lachesis Mutus (6C, 200C), Calcarea Carbonica (30C, 200C) showed 100% inhibitory action. In this Potencies varied from 6C to 10M showed the inhibition. Partial inhibitory action observed in Thuja occidentalis (6C, 12C, 30C, 200C, 1M), Silicea (12C, 10M, CM), Mercurius Solubilis (CM), Lachesis Mutus (30C, 10M), Calcarea Carbonica (6C, 10M, CM). It signifies that Homoeopathic Medicines has ability to control the growth of *Gibberella fujikuroi* fungus with their potencies.

Conclusion -

It concludes that Homoeopathic medicines Thuja occidentalis (10M), Silicea (200C and 1M), Mercurius Solubilis (6C, 12C and 10M), Lachesis Mutus (6C and 200C), Calcarea Carbonica (30C and 200C) showed 100% inhibitory action and Thuja occidentalis (6C, 12C, 30C, 200C, 1M), Silicea (12C, 10M, CM), Mercurius Solubilis (CM), Lachesis Mutus (30C, 10M), Calcarea Carbonica (6C, 10M, CM) showed partial inhibitory action. So, control the growth of *Gibberella fujikuroi* fungus effectively and thus helpful in maintaining the health of the plant. It can be used as an alternative agro-homoeopathic fungicide on rice as it is effective, safe and economic. Further studies needs to be for understanding mechanism of action of these ultra-high diluted homoeopathic medicines.

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