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In vitro evaluation of homeopathic medicines at different concentrations against the mycelial growth of *Phomopsis vexans*

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Abstract

This study evaluated the efficacy of various homeopathic medicines (Sulphur, *A. montana*, Phosphorus, and *S. terra*) at different concentrations (0.1% and 0.2%) against the mycelial growth of *Phomopsis vexans* *in vitro*. Results showed that all homeopathic treatments inhibited mycelial growth to varying extents compared to controls (inoculated and untreated). *A. montana* at 0.2% exhibited the highest inhibition over control (inoculated: 74.45%, untreated: 73.38%), followed by Sulphur at 0.2% (72.66% and 71.52%). Higher concentrations (0.2%) of homeopathic medicines generally showed greater inhibition of mycelial growth. The findings suggest that homeopathic medicines can be used as a safer and more sustainable alternative to chemical fungicides for managing *Phomopsis vexans* in brinjal cultivation. This study provides a promising approach for the management of *Phomopsis vexans* and promotes environmentally friendly agricultural practices.

Keywords: Homeopathic medicines, *Phomopsis vexans*, mycelial growth inhibition, *in vitro* evaluation, Sulphur, *A. montana*, Phosphorus, *S. terra*

1. Introduction

Brinjal (*Solanum melongena* L.), also known as eggplant, is one of the most significant vegetable crops cultivated worldwide, particularly in tropical and subtropical regions (FAO, 2020) ^[1]. It is a staple in many cuisines, valued for its nutritional benefits and versatility. Brinjal is rich in vitamins, minerals, and antioxidants, contributing to its health-promoting properties (Khan, 2009) ^[2]. Globally, brinjal is grown in large quantities, with countries like India, China, and the United States being major producers (USDA, 2022) ^[10]. The increasing demand for brinjal has led to intensified cultivation practices, but this has also heightened the risk of disease incidence, impacting yield and quality. One of the significant pathogens affecting brinjal is *Phomopsis vexans*, which causes leaf spot and fruit rot diseases (Singh *et al.*, 2015) ^[9]. This pathogen can lead to substantial yield losses if not managed properly. The disease is prevalent in warm and humid conditions, making it a challenge for farmers in tropical regions (Pun *et al.*, 2018) ^[6]. The symptoms of *Phomopsis vexans* infection include small, circular, brown spots on leaves and fruits, which can merge to form larger lesions, leading to defoliation and fruit decay (Kumar *et al.*, 2017) ^[3]. Traditionally, the management of *Phomopsis vexans* has relied heavily on chemical fungicides. While these chemicals are effective in controlling the disease, their indiscriminate use has led to environmental pollution, development of resistance in pathogens, and health hazards for consumers and farmers (Singh & Singh, 2019) ^[8]. The residues of these chemicals on fruits and vegetables also pose a risk to human health, making it essential to explore safer and more sustainable alternatives. Given the concerns associated with chemical fungicides, there is a growing interest in exploring eco-friendly alternatives for managing plant diseases. Homeopathic medicines have emerged as a promising option due to their natural origin and minimal environmental impact (Sharma *et al.*, 2020) ^[7]. These remedies are considered safe for human health and the environment, making them an attractive choice for sustainable agriculture. Homeopathic medicines can stimulate plant growth, enhance resistance against

pathogens, and promote overall plant health (Majumdar *et al.*, 2018) [4]. Several studies have demonstrated the potential of homeopathic treatments in controlling various plant diseases, including fungal infections (Pathak *et al.*, 2019) [5]. This study aims to evaluate the efficacy of selected homeopathic medicines (Sulphur, *A. montana*, Phosphorus, and *S. terra*) against the mycelial growth of *Phomopsis vexans* under *in vitro* conditions. By assessing the inhibitory effects of these homeopathic medicines on the pathogen, this research seeks to identify potential alternatives for disease management in brinjal cultivation. The findings of this study could contribute to the development of eco-friendly and sustainable disease management strategies, reducing the reliance on chemical fungicides and promoting environmentally friendly agricultural practices.

2. Materials and Methods

2.1 Experimental Site

The present investigation was carried out during 2023-24 and 2024-25 in the laboratory of Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, 208002 (Uttar Pradesh).

2.2 Collection of infected plant sample

Brinjal plant showing typical blight symptoms were first identified and then collected from Student Instructional Farm (SIF), Chandra Shekhar Azad University of Agriculture and Technology Nawabganj, Kanpur. Infected leaf with sporulating lesions were taken from the field and washed in sterilized water. The sample were placed in between the fold sterilized blotter paper and preserved at 4-6°C in refrigerator for further study. Thus samples were later used for isolation and purification of pathogen.

2.3 Preparation of culture media

The pathogen *Phomopsis* blight of brinjal caused by *Phomopsis vexans* can properly grow on Potato Dextrose Agar Media. Therefore, preparation of PDA media is prerequisite for isolation of the fungus.

Requirements

Peeled Potato: 200 g

Agar-agar: 20 g

Dextrose: 20 g

Distilled water: 1000 ml

2.4 PDA preparation procedure

Potato was peeled and chopped into the required amount of small pieces after 30 minutes of boiling in 500 ml of distilled water; it was filtered through muslin cloth. Then 500 ml of boiling water was added to 20 gm of agar-agar and 20 gm of dextrose. The boiling mixture was then supplemented with potato extract, which was thoroughly mixed using a glass rod. After boiling for a short while, it was transferred to each 500 ml flask, which held about 200 ml and the tops were sealed with non- absorbent cotton. The medium was autoclaved for 20 minutes at 15 psi (121.6°C) with a pH adjustment of 7.0±0.1.

2.5 Isolation and Purification of Pathogen

The pathogen *Phomopsis vexans* was isolated from infected brinjal plants showing typical symptoms of leaf spot and fruit rot. The infected plant parts were surface sterilized with 1% sodium hypochlorite solution for 1-2 minutes, rinsed thrice with sterile distilled water, and dried on sterile filter paper. The

sterilized plant parts were then placed on potato dextrose agar (PDA) medium amended with streptomycin (50 µg/mL) to inhibit bacterial growth. The plates were incubated at 25 ± 2 °C for 7-10 days. The fungal colonies that developed were subcultured on fresh PDA medium to obtain pure cultures. The purified cultures were maintained on PDA slants at 4°C for further studies.

2.6 Identification of Pathogen

The isolated pathogen was identified based on its morphological and cultural characteristics. The fungal structures, such as pycnidia, conidia, and conidiophores, were examined under a microscope. The pathogen was identified as *Phomopsis vexans* based on the characteristics described in the literature.

2.7 Homeopathic Medicines

The homeopathic medicines used in this study were Sulphur, *A. montana*, Phosphorus, and *S. terra*. These medicines were procured from a reputable homeopathic pharmacy in Raina market, Nawabganj road, Kanpur. and prepared according to the guidelines of the Homeopathic Pharmacopoeia.

2.8 Evaluation of Homeopathic medicine with suitable concentration against mycelial growth of *Phomopsis vexans*

The efficacy of various homeopathic medicines (*Phosphorus*, *Arnica montana*, *Sulphur*, and *Silicea terra*) at different concentrations (0.1%, 0.20%) was evaluated in PDA medium using Poisoned Food Technique against different pathogens in the laboratory. The homeopathic medicine was tested with three replications per treatment. The fungus was grown on PDA medium for 10 days before setting up the experiment. The PDA medium was prepared and melted, and homeopathic medicine was added to the melted medium at the each required concentrations. The suitable check was maintained without the addition of homeopathic medicine (control). A mycelial disc of 5 mm was taken from the periphery of the old colony. The actively growing hyphal tip was removed by cork borer and placed in the center of poisoned petriplates incubated at 25 ± 2 °C until the control plate was full. The radial growth of the fungus on the poisoned medium was measured on different days. All experiments were carried out in triplicate and the per cent inhibition of mycelia growth over control was calculated using the following formula of Horsfall.

2.9 Data Collection and Analysis

The radial growth of the mycelium was measured, and the percent inhibition was calculated using the formula:

$$\text{Percent inhibition} = [(\text{Control growth} - \text{Treatment growth}) / \text{Control growth}] \times 100.$$

The data were analyzed using analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test (DMRT) at a 5% level of significance.

2.10. Experimental Design

The experiment was conducted in a completely randomized design (CRD) with three replications for each treatment.

3. Results and Discussion

The *in vitro* evaluation of homeopathic medicines against *Phomopsis vexans* revealed that data presented in table-2 that all the tested medicines significantly inhibited the mycelial growth of the pathogen. The highest inhibition was observed in the

treatment with *A. montana* at 0.2% concentration, which recorded 74.45% inhibition over control (inoculated) and 73.38% inhibition over control (untreated), followed by Sulphur at 0.2% concentration, which recorded 72.66% inhibition over control (inoculated) and 71.52% inhibition over control (untreated). The inhibitory effect of *A. montana* and Sulphur on the mycelial growth of the pathogen can be attributed to their antifungal properties. These findings are in agreement with previous reports on the efficacy of homeopathic medicines against fungal pathogens (Sharma *et al.*, 2020; Majumdar *et al.*, 2018) [7, 4]. The use of homeopathic medicines as an alternative to chemical fungicides can help reduce the environmental pollution and health hazards associated with the use of synthetic chemicals (Singh & Singh, 2019) [8]. Moreover, homeopathic medicines are considered safe for humans, animals, and the environment, making them a promising option for sustainable agriculture (Pathak *et al.*, 2019) [5].

The mechanism of action of homeopathic medicines in controlling fungal pathogens is not fully understood, but it is believed that these medicines stimulate the plant's defense mechanisms, making it more resistant to infection (Khan, 2009) [2]. Similar findings were reported by Pun *et al.* (2018) [6], who observed that homeopathic medicines can induce systemic resistance in plants against fungal pathogens. Further studies are needed to understand the mode of action of homeopathic

medicines and to develop effective disease management strategies. The results of this study have significant implications for the management of *Phomopsis vexans* in brinjal cultivation, and the use of homeopathic medicines can provide a safer and more sustainable alternative to chemical fungicides (USDA, 2022) [10]. Overall, the findings of this study suggest that homeopathic medicines have potential as a biocontrol agent against *Phomopsis vexans* and could be used as part of an integrated disease management strategy, thereby reducing the reliance on chemical fungicides and promoting environmentally friendly agricultural practices (FAO, 2020) [1].

Table 1: Use of Homeopathic medicine at different concentration

Treatments	Treatments details	Concentration
T ₁	<i>Silica terra</i>	0.1%
T ₂	<i>Arnica montana</i>	0.1%
T ₃	Sulphur	0.1%
T ₄	Phosphorus	0.1%
T ₅	<i>Silica terra</i>	0.2%
T ₆	<i>Arnica montana</i>	0.2%
T ₇	Sulphur	0.2%
T ₈	Phosphorus	0.2%
T ₉	Control (inoculated)	
T ₁₀	Control (untreated)	

Table 2: Effect of Homeopathic medicines on mycelial growth of *Phomopsis vexans* at different days interval

Treatments	Treatments details	Mycelial growth(mm/days)			Percent inhibition over control (inoculated)	Percent inhibition over control (untreated)
		4th day	6th day	8th day		
T ₁	Sulphur@0.1%	25.00	37.62	59.10	35.76	33.08
T ₂	<i>A.montana</i> @0.1%	23.00	36.02	45.50	50.54	48.48
T ₃	Phosphorus@0.1%	26.00	41.19	62.00	32.61	29.80
T ₄	<i>S. terra</i> @0.1%	25.00	40.12	61.52	33.13	30.34
T ₅	Sulphur@0.2%	12.65	17.28	25.15	72.66	71.52
T ₆	<i>A.montana</i> @0.2%	12.65	15.04	23.51	74.45	73.38
T ₇	Phosphorus@0.2%	18.32	23.71	30.80	66.52	65.13
T ₈	<i>S.terra</i> @0.2%	16.00	21.92	27.78	69.80	68.55
T ₉	Control(inoculated)	32.00	58.79	92.00	0	-4.17
T ₁₀	Control(untreated)	29.00	56.65	88.32		0
CD at 5%		2.422	3.555	6.293		
SE(m)		0.821	1.227	2.133		

4. Conclusion

In conclusion, the present study demonstrates the potential of homeopathic medicines as a biocontrol agent against *Phomopsis vexans*, a fungal pathogen causing leaf spot and fruit rot disease in brinjal. The results of the *in vitro* evaluation showed that *A. montana* and Sulphur at 0.2% concentration were the most effective in inhibiting the mycelial growth of the pathogen. These findings suggest that homeopathic medicines can be used as a safer and more sustainable alternative to chemical fungicides for managing *Phomopsis vexans* in brinjal cultivation. Overall, this study provides a promising approach for the management of *Phomopsis vexans* and promotes environmentally friendly agricultural practices.

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