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Evaluation of Antifungal Activities of different Clove Extracts against *Sclerotium rolfsii* Sacc. causing Stem Rot of Indian Bean

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ABSTRACT: The study evaluated the antifungal activities of different clove extracts against *Sclerotium rolfsii,* the causative agent of stem rot of Indian bean. The extraction of bioactive compounds from clove poses a significant challenge. To overcome this challenge, various organic solvents were employed during the extraction process. To prepare the extracts, clove powder was soaked in different solvents including acetone, propanol, mustard oil, castor oil, and water for a period of 48 hours. The *in vitro* evaluation was done using the Poisoned Food Technique, where a 1.0 per cent concentration of each extract was used. The results showed that propanol and acetone clove extracts showed cent per cent inhibition of *S. rolfsii* compared to the untreated control. Propanol alone showed 14.67 mm of mycelium growth and 83.70 per cent inhibition. This study highlights the potential of clove extracts as an alternative to chemical fungicides in managing stem rot caused by *S. rolfsii* in Indian bean.

Keywords: Solvents, Clove extract, *Sclerotium rolfsii*, *in vitro.*

INTRODUCTION

Clove (*Syzygium aromaticum* (L.) Merril. & Perry) has been treasured as a valuable spice in the Orient for centuries. Not only is it commonly used in cooking, but it is also employed in traditional medicine due to its many medicinal properties. The primary active compounds found in clove oil and extracts are eugenol, eucalyptol, and beta-caryophyllene. These compounds have been shown to possess insecticidal, antimicrobial, and nematicidal activities against various plant pathogens, as demonstrated in several studies (Bakkali *et al.,* 2008; Barakat, 2014; Hamini *et al*., 2014; Kacániová *et al.,* 2021; Elnabawy *et al*., 2022).

The Indian bean, also known as the mung bean, is indeed an ancient and important pulse crop in India. It is vulnerable to various biotic and abiotic factors, including fungal diseases, which can significantly reduce crop yield. Indian bean growers in South Gujarat face a significant threat from stem rot, a devastating disease caused by the fungal pathogen *Sclerotium rolfsii* Sacc. *Sclerotium rolfsii* is a soilborne plant pathogen that is responsible for causing stem rot and other diseases in various crops worldwide, including legumes, vegetables, and ornamental plants. The fungus produces sclerotia, which are compact masses of mycelium that can remain viable in soil for several years (Dhingra and Sinclair 1995). The disease caused by *S. rolfsii* is characterized by wilting, yellowing, and stem rot, which can result in plant death (Cao *et al.,* 2020). The fungus is known to infect plants at any growth stage, but young plants and seedlings are most vulnerable (Lahlali *et al*., 2014). According to a study by Bhattacharya *et al.* (2019), *S. rolfsii* was responsible for 15-20% yield losses in Indian bean crops in West Bengal, India. Similarly, a study by Shahnawaz *et al*. (2018) reported yield losses of up to 40% in Indian bean due to stem rot caused by *S. rolfsii* in the state of Jammu and Kashmir, India. It causes significant yield losses up to 60 percent (Panchal *et al*., 2015; Raja *et al*., 2020).

The use of chemical fungicides to control soil-borne pathogens such as *Sclerotium rolfsii* can have detrimental effects, including soil, water, and air pollution, residual toxicity, and the development of pathogen resistance. Moreover, these pesticides can harm beneficial organisms and their toxic residues can persist in soil, increasing the incidence of resistance among pathogens towards synthetic chemicals.

Plant extracts or bio-pesticides are gaining popularity as an eco-friendly alternative to chemical pesticides for controlling soil-borne diseases due to their rich source of bioactive compounds. These extracts exhibit protective, curative, and antagonistic activities against various diseases. The secondary metabolites produced by plants, which are not essential for growth and development, can act against microbial pathogens based on their toxic nature (Rosenthal, 1991; Schafer and Wink 2009). The use of plant-based products is gaining popularity over other chemical pesticides due to

concerns over the environment and human health. Given the hazardous effects of fungicides, the present study aims to investigate the antifungal activity of various clove extracts against *Sclerotium rolfsii* Sacc., causing stem rot in Indian bean.

MATERIALS AND METHODS

Location of work. Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat.

Isolation of the pathogen. The diseased sample was collected from the university farm and isolation of fungus was done by following standard tissue isolation technique. The infected pieces were cut into small bits and then washing in running water. These bits were surface sterilized with 0.1 % Mercuric chloride ($HgCl_2$) solution for one minute followed by washing in distilled sterile water, then aseptically transferred to PDA (Potato Dextrose Agar) plate and incubated at room temperature for seven days. From this fungal growth hyphal tip was used for further purification. The pure cultures that obtained were further stored in refrigerator for further studies.

Preparation of clove extracts using different solvents. The study used five solvents (acetone, propanol, mustard oil, castor oil, and water) to prepare clove extracts. Ten grams (10 g) of clove powder were soaked in 100 ml of each solvent for 48 hours to extract the main active biological compounds. After the soaking period, the extract was filtered through Whatman filter paper and the final extract was 10 per cent of the clove extract.

In vitro* evaluation of different prepared clove extract against *Sclerotium rolfsii. One per cent (1%) concentration of each prepared clove extracts and solvents alone were evaluated under *in vitro* conditions by Poisoned Food Technique (PFT) against *Sclerotium rolfsii*. Observation on mycelial growth and per cent growth inhibition were recorded after seven days of inoculation and inhibition of mycelial growth (%) was calculated using the formula.

$$\text{Inhibition of mycelial growth (\%)} = \frac{C - T}{C} \times 100$$

Where,

C = Radial growth in control, T = Radial growth in treatment

RESULTS AND DISCUSSION

The study evaluated the antifungal activity of various solvent-based clove extracts and solvents alone against *Sclerotium rolfsio in vitro*, along with a control. Mycelial growth and per cent growth inhibition were measured seven days after incubation, and the results were presented in Table 1, Plate 1, and Fig. 1. The findings revealed that the Propanol clove extract (T_1) and Acetone clove extract (T_2) exhibited complete inhibition of fungal growth. Mustard oil clove extract (48.33 mm) demonstrated 46.30% inhibition, followed by castor oil clove extract (62.00 mm) with 31.11% inhibition, and water clove extract (66.83 mm) with 25.74% inhibition. Among the various solvents, propanol demonstrated the least mycelial growth (14.67 mm), followed by acetone (67.50 mm) with 83.70% and 25.00% inhibition, respectively, and mustard oil (78.00 mm) with 13.33% inhibition over the control.

The present findings were in accordance with the earlier studies of Darvin (2014). He reported that among several plant extracts tested *in vitro*, clove extract of garlic was most effective against *S. rolfsii* recording lowest mycelial growth (0.0 mm) and highest per cent inhibition (100%).The antifungal activity of the clove oil has also been reported by Kaur *et al*. (2019) against *F. moniliformae, H. oryzae* and *R. Solani*. Similarly, Rahman *et al.* (2020) evaluated different plant extracts against *S. rolfsii* and they reported that, garlic clove extract (96.67%) showed highest growth inhibition of *S. rolfsii* followed by Allamonda (51.12%) over untreated control. Konjengbam *et al.* (2021) tested aqueous plant extracts (1:1 w/v) of five locally available botanicals against growth and sclerotia production of *Sclerotium rolfsii* Sacc. Causing white rot of onion in Manipur. They found that garlic extract was observed to be most effective and completely inhibited mycelial growth as well as sclerotia production of the fungus at all the tested concentrations. Bhutia *et al.* (2015) reported that *Zingiber officinale, Polyalthia longifolia* and *Clerodendrum inerme* leaf extracts exhibited more than 80.00 per cent inhibition of against mycelial growth of *Colletotrichum musae*.

Table 1: *In vitro* evaluation of different clove extracts against *S. rolfsii*.

Tr. No.	Treatment Details	Mycelial growth (mm)	Per cent growth inhibition
T_1	Propanol clove extract	0.707 (0.000)	100.0
T_2	Acetone clove extract	0.707 (0.000)	100.0
T_3	Mustard oil clove extract	6.987 (48.33)	46.30
T_4	Castor oil clove extract	7.904 (62.00)	31.11
T_5	Water clove extract	8.000 (66.83)	25.74
T_6	Only propanol	3.894 (14.67)	83.70
T_7	Only acetone	8.244 (67.50)	25.00
T_8	Only mustard oil	8.857 (78.00)	13.33
T_9	Only castor oil	9.513 (90.00)	0.000
T_{10}	Control	9.513 (90.00)	-
SEm±		0.082	
CD at 5%		0.245	
CV(%)		2.221	

Figures outside the parentheses are +0.5 transformation values where in parentheses are original values

Plate 1: *In vitro* evaluation of different clove extracts against *S. rolfsii.*

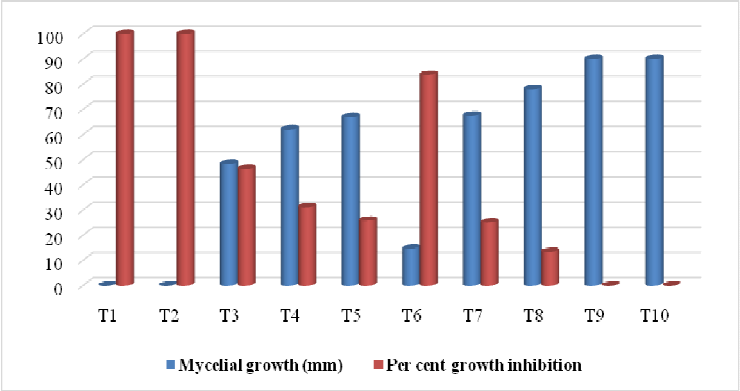

Fig. 1. *In vitro* evaluation of different clove extracts against *S. rolfsii.*

CONCLUSIONS

Organic solvents such as propanol and acetone are more effective in extracting the antifungal components present in clove compared to water and oil. Among the different solvent based clove extracts, Propanol clove extract and Acetone clove extract showed complete inhibition of *S. rolfsii* at 1.0 per cent concentration.

FUTURE SCOPE

Further studies can be conducted to isolate and identify the specific antifungal compound present in clove extract. This information can be useful in developing new and effective fungicides for controlling *S. rolfsii*. Additionally, understanding the mode of action of these antifungal compounds will provide a better understanding of how to control soil-borne diseases in a more sustainable and environmentally friendly manner.

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