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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 5879-5881

www.thepharmajournal.com
Received: 16-12-2022
Accepted: 20-01-2023

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Antifungal activities of industrial clove waste against *Aspergillus flavus* and *Sclerotium rolfsii*

Ranapratap Raut, Lalit Mahatma and Ravikumar Vaniya

Abstract
Industrial Clove Waste (ICW) is the waste by product of the clove processing industry, and which is available in abundant quantity in India and which contains various chemical compounds that may possess significant antifungal, insect-repellent, and antimicrobial properties. In present investigation, the antifungal activities of industrial clove waste were tested against two major pathogens *viz*., *Aspergillus flavus* and *Sclerotium rolfsii* under *in vitro* condition. The results of *in vitro* studies revealed that, all the tested concentrations (1.0 and 2.0%) of industrial clove waste were found most effective against both the pathogens tested. There was cent per cent inhibition of mycelial growth of *Aspergillus flavus* and *Sclerotium rolfsii* at 1.0 and 2.0% concentrations of industrial clove waste.

Keywords: Industrial clove waste, *Aspergillus flavus*, *Sclerotium rolfsii*, antifungal activities

Introduction
Aspergillus flavus is a saprotrophic and pathogenic (Machida and Gomi, 2010) [8] fungus with a cosmopolitan distribution (Ramírez *et al*, 2014) [10]. It is best known for its colonization of cereal grains, legumes, and tree nuts. Postharvest rot typically develops during harvest, storage, and/or transit. *A. flavus* infections can occur while hosts are still in the field (pre harvest), but often show no symptoms (dormancy) until postharvest storage or transport. In addition to causing pre harvest and postharvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are toxic to mammals (Agrios, 2005) [1].

Sclerotium rolfsii is indeed a soil-borne pathogen that commonly occurs in warm regions, including the tropics and subtropics, and is capable of infecting over 500 plant species, including many agricultural and horticultural crops (Aycock, 1996) [1]. It is a destructive pathogen that can cause significant crop losses, especially during the rainy season when temperatures are high.

The continuous use of chemical fungicides to manage soil-borne pathogens such as *Sclerotium rolfsii* can lead to various problems, including pollution of air, water, and soil; residual toxicity; and the development of resistance in the pathogen. In addition, pesticides can also harm beneficial organisms and their toxic forms may persist in soil, potentially increasing the incidence of resistance among pathogens towards synthetic chemicals (Cakir *et al*., 2005) [4].

Plant extracts or bio-pesticides are increasingly being used as an alternative to chemical pesticides for controlling soil-borne diseases because they are a rich source of bioactive compounds and are generally eco-friendly. These extracts have protective, curative, and antagonistic activities against many diseases. As plants produce numerous secondary metabolites that are not crucial for growth and development, they can act against microbial pathogens based on their toxic nature (Rosenthal *et al*., 1991 and Schafer *et al*., 2009)) [11, 12]. The use of plant-based products is gaining popularity over other chemical pesticides due to concerns over the environment and human health

Clove (*Syzygium aromaticum* (L.) Merril. & Perry) is one of the most ancient and valuable spices of the Orient. It is used as a spice and it has medicinal properties. The oil has many industrial applications and is used extensively in perfumes, soaps, and as a clearing agent in histological work. Due to the wide industrial usage of clove oil, substantial quantities of the clove are processed for the extraction of oil. Processing also generate abundant industrial clove waste which does not have any significant value and difficult to dispose. Industrial clove waste is a plant-origin-based byproduct of the clove processing industry, which contains different chemical compounds might have significant antifungal, insect-repellent, and antimicrobial activities. Recently the use of plant based products are being preferred over other chemical pesticides. Therefore, the finding of new antifungal agents that are plant based, safe or less toxic to the environment and human health would be extremely valuable. Reports are available only on antifungal activities of essential clove oil but there is no such information available on industrial clove waste. The present study aimed to investigate the antifungal activities of industrial clove waste against *Aspergillus flavus* and *Sclerotium rolfsii.*

Materials and Methods

Location of work

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

Isolation of the pathogens

The diseased samples were collected from the university farm and isolation of fungi 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.

In vitro evaluation of industrial clove waste against *Aspergillus flavus* and *Sclerotium rolfsii*

Two concentrations (1 and 2%) of concentration of industrial clove waste were evaluated under *in vitro* conditions by Poisoned Food Technique (PFT) against *Aspergillus flavus* and *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.

Percent inhibition of mycelial growth=C/T×100

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

Results and Discussion

The results of the mycelial growth and per cent growth inhibition were recorded seven days of inoculation. The results obtained are presented in table 1, photo 1 and 2. The results showed that the both the concentrations (1.0 and 2.0 per cent) of industrial clove waste showed cent per cent inhibition of *Aspergillus flavus* and *Sclerotium rolfsii.*

The present findings were in accordance with the earlier studies of Darvin (2014) [5]. 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) [6] against *F. moniliformae, H. oryzae* and *R. Solani*. Similarly, Rahman *et al* (2020) [7] 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) [7] 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. However, sweet flag at 10% inhibited 83.00% and 71.59% of mycelial growth and sclerotia production of the fungus respectively followed by wild sage at 10% inhibited 82.33% of mycelial growth of the fungus. Bhutia *et al* (2015) [3] 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 industrial clove waste against *Aspergillus flavus* and *Sclerotium rolfsii*

Tr. No.	Treatment details	Conc. (%)	Mycelial growth (mm)	
			A. flavus	*S. rolfsii*
T_1	Control	-	90.0*	90.0
T_2	Industrial Clove waste	1.0	0.00	0.00
T_3	Industrial Clove waste	2.0	0.00	0.00

*Average of five replications

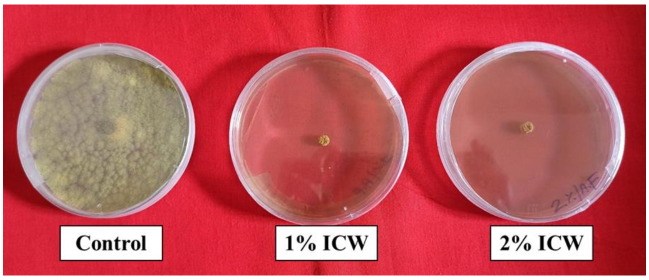

Plate 1: *In vitro* evaluation of industrial clove waste against *Aspergillus flavus*

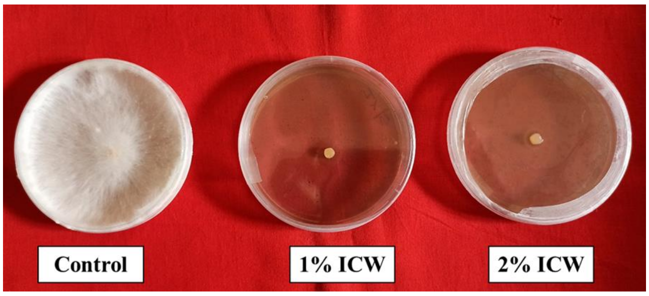

Plate 2: *In vitro* evaluation of industrial clove waste against *Sclerotium rolfsii*

Conclusion

Industrial clove waste is highly effective against *Aspergillus flavus and Sclerotium rolfsii* under laboratory condition at 1.0 per cent and above concentrations.

Future thrust

Bio-pesticide in emulsifiable concentration (EC) formulation can be prepared from industrial clove waste for plant disease management.

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