

BIOASSAY OF BOTANICAL OILS AGAINST *ALTERNARIA SOLANI*

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ABSTRACT

Objective: *In vitro* antifungal activity of six essential oils of 1. *Ocimum tenuiflorum* (Purple leaves/Krishna Tulsi) 2. *Cymbopogon citrates* (Nimbu ghas), 3. *Origanum majorina* (Sweet majoram), 4. *Ocimum citriodorum* (Nimbu tulsi), 5. *Ocimum basilicum* (Gulal tulsi), and 6. *Ocimum sanctum* (Green leaves/Shree tulsi) were investigated against economically important phytopathogenic fungi, *Alternaria solani*, isolated from infected chilly.

Methods: The experiment was carried out by Whatman paper disc method using Whatman paper No.3 on Potato Dextrose Agar with three replicates. Five concentrations of each essential oils i.e., 20, 40, 60, 80 and 100% were assayed against the test fungus. The experiment was carried out at 27°C and mycelial growth was measured after every third day, up to 15 d using the statistical method.

Results: It is an evident from this study that all the oils used in this study are inhibiting test fungi, oil of *O. basilicum* and *O. sanctum* are completely (100%) inhibiting test fungi.

Conclusion: It may be concluded from the above study that all the taken oils have a good potential to control plant pathogenic fungi and these could be considered for developing a new fungicide.

Keywords: Antifungal activity, Botanical oils, Chilly, *Alternaria solani*, Whatman paper disc method

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INTRODUCTION

Chilly (*Capsicum annum* L.) is an important tropical and subtropical crop on the basis of high consumption, nutritional and cash values to the farmers and consumers, both in developing and developed countries [1]. It is also an important cash crop of India. The world area and production of chilly is approximately 1.5 million hectares and 7 million tonnes, respectively. India accounts for 1.2 million tonnes of production annually, and is the largest producer in terms of international trade, exporting 20 per cent of its total production as dry chilly, chilly powder and oleoresins to over 90 countries [2]. India is also the largest consumer of chilly.

Chilies are rich in vitamins A, B, C, D and E besides minerals like molybdenum, manganese, foliate, potassium, thiamine and copper, it also contains proteins. Chilly contains seven times more vitamin C than an orange. Chilies are important medicinally and used as a tonic to ward off many diseases, it is good in slimming down, stimulates the appetite and digestive system and also helps to clear the lungs. It is also used in beverages [3].

Chilly belongs to the genus *Capsicum*, under the family *Solanaceae*. It is vulnerable to many diseases and pests due to its extreme delicacy and succulence. Diseases caused by fungi and bacteria are major constraints to chilly production. In India, the first report of *Alternaria* sp. associated with chilly disease was made from Delhi by Dutt in 1937 [4]. Mathur and Agnihotri [5] reported fruit rot of chilly caused by *Alternaria* sp. from Rajasthan.

The Deuteromycetes fungal genus *Alternaria* is well known for its notoriously, virulent and destructive plant pathogenic members. The necrotrophic nature of *Alternaria* species typically leads extensive damage to the plant and harvest products. A great number of species were recorded for the genus *Alternaria* infecting different crops causing worldwide economic loss [6].

Alternaria solani is hosted by different species of the family *Cucurbitaceae*, *Brassicaceae*, *Solanaceae* which are having nutritional as well as economical food value [7-10]. It is ubiquitous in nature

and causes early blight of potato and tomato, as well as potato tuber rot and tomato fruit rot etc. besides diseases in chilly [7-10].

A. solani overwinters in the form of mycelium and conidia in plant debris [10]. During vegetation, it spreads by conidia transported by wind and insects. The conidia quickly germinate and infect plants. The optimal temperature for their germination ranges from 28 ° to 30 °C.

For controlling the diseases, numbers of synthetic chemicals are used but indiscriminate use of fungicides may lead to toxic residues, development of fungicide resistance environmental pollution and carcinogenic chilly products [11]. Several effective pesticides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards [12]. In an attempt to modify this condition, some alternative methods of controls have been adopted. Natural products isolated from plants appear to be one of the alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides [13].

In this context, a search for naturally occurring substances with potential biological activity continues in an increasing scale. Plant products are gaining importance in crop protection in view of their selective properties, low cost and safer alternative means to the ecosystem.

Hence botanical oils extracted from plants (which are concentrated liquid with volatile aromatic compounds, hydrophobic in nature) [14] seems to be an effective alternative to synthetic/chemical fungicides against phytopathogenic fungi, which is because of presence of biological active sesquiterpenes, monoterpenes including phenols, carbohydrates, ethers, alkaloids, quinones, flavonoids, aldehydes, ketones, alcohols, sterols, tannins and saponins etc. [15, 16]. Botanical oils are not phytotoxic as well as they are also a significant inhibitor of several fungal pathogens [17, 18].

Besides this many researchers have also reported the significant antifungal activity of essential oils, hence they could be used as

effective antifungal agents [19-21]. In the present study, the selected essential oils were assayed against *Alternaria solani* for their antifungal activity.

MATERIALS AND METHODS

Plant material

Aerial parts of selected plants were collected from growing fields from their natural habitats Udaipur, Rajasthan. The plant materials were dried under the shade condition with proper ventilation. Then were subjected to hydrodistillation to extract their essential oils.

Extraction of essential oil

Extraction of essential oil from subjected plants was carried out using Clevenger-type apparatus established by Montes *et al.*, [22]. Air-dried plant material and distilled water were used then extraction was done for 4 h. anhydrous sodium sulfate was used to remove water after extraction. The resulting oil placed into sealed plastic tubes and was stored in a refrigerator at 4 °C [23]. Oils were diluted by acetone to make different concentrations just before they were used for the antifungal assay.

Isolation and identification of *Alternaria solani*

A. solani were isolated from asymptomatic chilly. Fragments of infected tissues were placed on potato dextrose agar (PDA) medium and incubated at 25 °C. It was placed for seven days to allow mycelial growth. The fragments excised from the mycelia developed were transferred onto fresh PDA medium then purified by monospore isolation. *A. solani* identified on the basis of colony morphology and microscopic observation of conidia. The obtained isolates were stored on PDA medium at 4 °C.

Identification of test plant

Test plants were washed blotted dry and the herbarium of three plants was sent to the Botanical Survey of India (BSI), Jodhpur, Rajasthan, for identification. Plant herbarium no.1 was identified as *Ocimum tenuiflorum*, [Voucher no. B. S. J. O.-2533], Plant herbarium

no. 5 as *Ocimum basillicum* [Voucher no. B. S. J. O.-22492], and Plant herbarium no. 6 as *Ocimum sanctum* [Voucher no. B. S. J. O. 5733].

In vitro antifungal activity test

The experiment was carried out by Whatman paper disc method using Whatman paper No.3. Discs of 6 mm impregnated with 10 µl (of various concentrations from 20% to 100%) of different essential oils were placed separately around the 6 mm disc of test fungi (which is placed inverted at centre in the PDA plate). The Petri plates were incubated at 28±1 °C. Mycelial growth was measured in each Petri plate after every third day, up to 15 d. The controls were used for each case by not exposing the fungus to any oil respectively. Qualitative and quantitative analysis of fungicidal activity was done by following the Deans and Svoboda 1990 [24].

$$\text{Inhibition (\%)} = \left[\frac{(C-T)}{C} \right] \times 100$$

Where: C is the diameter of the fungal colony in control plate (mm) and T is the fungal colony diameter in treatment plate (mm).

Statistical analysis

Experiments were performed in triplicates and data were analyzed by taking their mean.

RESULTS AND DISCUSSION

The result of antifungal activity assay of selected essential oils of *O. tenuiflorum*, *C. citrates*, *O. majorina*, *O. citriodorum*, *O. basillicum* and *O. sanctum* had an inhibitory effect on the fungal mycelial growth. All the six essential oils at all the taken concentrations inhibited the growth of test fungi up to various levels. Among all the six oils used against the test fungus, *O. basilicum* and *O. sanctum* inhibited the test fungus completely (100%) at all the taken concentrations.

All the taken species are reported to have significant antifungal activity [24-26].

Result images and details of pathogen mycelium inhibition are shown below in the fig. 1 and table 1 respectively.

Table 1: Mycelial growth inhibition (%) of *Alternaria solani* on 3rd, 6th 9th 12th and 15th Day

Day	<i>O. tenuiflorum</i>	<i>C. citrates</i>	<i>O. majorina</i>	<i>O. citriodorum</i>	<i>O. basillicum</i>	<i>O. sanctum</i>	Control
3 rd Day	100%	100%	100%	100%	100%	100%	NI
6 th Day	90%	100%	100%	100%	100%	100%	NI
9 th Day	84%	100%	90%	84%	100%	100%	NI
12 th Day	70%	90%	70%	78%	100%	100%	NI
15 th Day	55%	78%	58%	64%	100%	100%	NI

Note: NI= No Inhibition

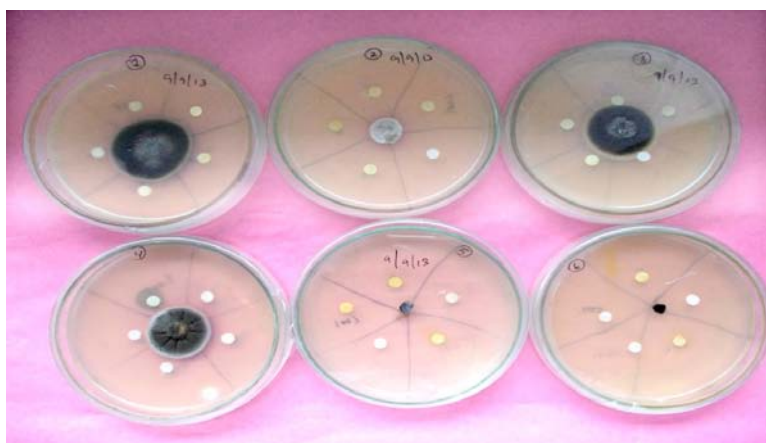


Fig. 1: Radial growth of *Alternaria solani* after 15 d of inoculation with different doses of essential oils: 1. *Ocimum tenuiflorum*, 2. *Cymbopogon citrates*, 3. *Origanum majorina* 4. *Ocimum citriodorum* 5. *Ocimum basillicum*, 6. *Ocimum sanctum*

Note: Inhibition in the mycelial growth of *Alternaria solani* was found to be independent from concentrations i.e. from 20%, 40%, 60%, 80% and upto 100%

CONCLUSION

According to the present investigation, it can be concluded that the essential oils of all the selected plants exhibited significant antifungal activity against the economically important plant pathogenic fungus *A. solani*. Hence it can be concluded that these selected oils could lead to the development of a new environmental friendly antifungal agent, which may be used as a major constituent in the biopesticides against many plants diseases caused by the test fungus after the proper clinical trials.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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