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Antifungal potential of the spices and medicinal plant extracts on the fungal growth of *Fusarium oxysporum*

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Abstract
Soil-borne plant pathogen is one of the most threatening and destructive diseases infecting various vegetable crops worldwide. Among the several diseases, Fusarium wilt causes heavy losses in the tomato-growing regions of the world. A study was undertaken to manage wilt diseases using plant products. The infected samples were collected from wilt-infected tomato plants and Fusarium isolates were found to be virulent. Based on morphological and molecular studies the virulent isolates were identified as *Fusarium oxysporum* f. sp. *lycopersici*. Antifungal screening of plant extract was tested against the fungal growth of the pathogen by Agar well diffusion. Among the plant samples tested, the neem extract recorded the maximum inhibitory effect (75.61 percent) on the mycelial growth of *F. oxysporum* f. sp. *lycopersici.* The extract of onion exhibited 50.65 percent inhibition of the mycelial growth of the pathogen. Conclusively, the neem extract could be explored for managing wilt disease in vegetable crops as it possesses antifungal activity against the growth of the pathogen.

Keywords: Plant extract, Fusarium wilt, antifungal activity

Introduction

Tomato (*Solanum lycopersicum* (L.) Karst.) is a vegetable crop that belongs to the family Solanaceae, widely cultivated around the world due to its rich source of vitamins and fiber, particularly, Vitamin C and A. The red color of tomato fruit is due to the presence of lycopene (antioxidants), ascorbic acid, and β-carotene with a great source of minerals like Ca, Fe, Mg, K, and Zn. Tomato is cultivated in an area of about 7.78 lakhs/ ha with a total production of around 193.97 lakhs tonnes in the year 2019 in India (NHB, 2019, www.nhb.gov.in). Andhra Pradesh, Madhya Pradesh, Karnataka, Tamil Nadu, Odisha, Bihar and West Bengal are the major states producing tomatoes. Soil-borne plant pathogens, are a major constraint in tomato production and are affected by several diseases like wilt caused by *Fusarium oxysporum* (Srivastava, 1972) [9], Early blight late, Septoria leaf spot, Late blight, Verticillium wilt, Anthracnose, Buckeye rot, and Southern blight (Sanoubar *et al*., 2017; Praveen *et al*., 2021) [8, 7]. Among the diseases, soil-borne pathogens such as damping off caused by *Pythium* sp, *Rhizoctonia solani* and wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* are the important diseases that cause a yield loss of 10-60%. Chemical control of such plant pathogens disturbs the environment, subverts ecology, changes soil structure and productivity and affects water resources. The interest in biological control of plant pathogens has increased over the past decade due to the importance of environment-friendly usage and is also considered to be the best alternative to the extensive use of chemical pesticides for combating crop diseases (Ongena and Jacques, 2008) [5]. Most species from the genus *Trichoderma* sp., *Pseudomonas* sp., and *Bacillus* sp., are considered safe beneficial microorganisms and possess remarkable abilities to synthesize many substances that have been successfully used in agriculture for plant disease management (Stein, 2005) [10]. The secondary metabolites produced by several species of bio-control agents have been found to show antibacterial or antifungal activity against different phytopathogens (Ongena and Jacques, 2008) [5]. In recent years, scientists have realized the need and made a great attempt to use plant metabolites/volatiles as effective alternatives for plant disease management to conventionally used chemicals. The plant-induced metabolite and volatiles express systemic resistance in plant against pathogens and also promotes plant growth. Based on this scientific evidence, the present study was taken up to find out the effect of plant extract on the management of Fusarium wilt of tomato with the following objectives on Isolation and characterization of wilt pathogens of tomato and assessing the efficacy of plant extracts against wilt pathogens under *in vitro* conditions.

Materials and Methods

Isolation of the pathogen

The infected collar portion and root bits associated *Fusarium* pathogen were cut into small pieces and surface sterilized with 0.1% sodium hypochlorite for 30 seconds and subsequently, three washings were given with sterile distilled water. Then, they were placed in sterilized Petri dishes containing Potato dextrose agar (PDA) medium by half plate technique and incubated at the laboratory conditions at 25 ± 2 °C for 7 days. The mycelial growth, colony characters, and spore characters were recorded seven days after inoculation (DAI).

Preparation of plant extracts

Plant extracts were prepared by taking economic plant parts and macerated with mortar and pestle using sterile distilled water. The macerated biomass was centrifuged at 10, 000 RPM for 5 min, filtered the extract, and kept overnight in culture tubes for the exudation of bio-metabolites at 4 °C. The biomass was filtered using a muslin cloth, followed by the filtration method using Whatman No. 1 filter paper, sterilized, and stored at 4 °C as stock solutions.

Management using plant products

In vitro, plant extract screening was carried out using onion, garlic, turmeric, ginger, and neem at 50, 100, 250, and 500ppm equipped with agar well dilution technique. The product's efficacy was assessed by measuring the area of the inhibition zone (mm) after 5 days of incubation at 25±2 °C. The efficacy of the plant extract against the mycelial growth of the pathogen was assessed by measuring the area of the inhibition zone in mm.

Results and Discussion

Morphological characters of *Fusarium* sp.

The colony color of Fusarium isolates varied from initially white to pinkish at the 5 days of incubation and finally turned pinkish appearance. The margin of the colony was radially smooth to irregular. The mycelial topography was cottony and fluffy. Fusarium produced two types of conidia *viz.*, micro and macro-conidia. Micro conidia were small, oval-shaped, hyaline, and single or bi-celled and produced larger in counts. The size of micro-conidia ranges from 6.50 to 8.09 µm in length and 2.40 to 4.9.00 µm in width. Macro-conidia were less in number, sickle-shaped hyaline with more or less two to three septate and ranges from 15.34 to 29.53 µm in length and 2.69 to 6.28 µm in width. Abundant chlamydospores were also observed terminally and intercalary after 9 days of incubation. Based on the morphological evidence it is identified as *Fusarium* (Fig 1.). A similar assessment was supported by Mathur (1972) [3] described the cultural and morphological characteristics of *Fusarium*. Microconidia are hyaline, unicellular, ovoid, ellipsoid, or reniform (2.7 to 8.04 µm). Macroconidia are formed in sporodochia, they are borne on simple conidiophores. Conidia are stout, fusiform, falcate, gradually tapering at both ends, pedicellate with rostrate apex and delicate walls and septation (12.8-31.7 µm). Chlamydospores are terminal or intercalary, spherical or globose, smooth, one-celled.

Antifungal Efficacy of Plant Extract

The plant extract was screened for anti-fungal activity against *Fusarium oxysporum*. The results indicated that the extract of garlic exhibited 16 percent mycelial growth inhibition followed by mint extract and ginger extract at 500 ppm (Fig. 2). The area of inhibition was due to exposure to the activity of garlic extract which was significantly superior to all the other treatments used in the study. However, a comparison of the efficacy between garlic and mint extract was similar in comparison to control plates. (Fig. 3). The antagonistic effect of garlic extract against *Fusarium* fungus has already been reported by several workers. Kutawa *et al*. (2018) [2] found that plant extract from garlic inhibited the growth of FOL under *in vitro* and *in vivo* conditions. Parveen *et al*. (2014) [6] reported that *in vitro* screening of selected plant extract inhibited the growth of *Fusarium oxysporum*, *Alternaria alternata*, and *Penicillium expansum*. Suleiman *et al*. (2014) [11] reported that the percent inhibition was significantly higher in plates exposed to garlic extract against control plates wherein the full growth of *Fusarium oxysporum*, *Candida albicans*, *Aspergillus*, *Curvularia* was recorded. Benkeblia (2004) [1] reported that the extract from onion and garlic exhibited a high level of inhibition against *Fusarium oxysporum*, this study negatively correlated with the present findings when exposed to onion extract against the pathogen.

A B C

Fig 1. Morphological characteristics of *Fusarium oxysporum.* A. Mycelial growth of the pathogen, B. Microscopic images of *Fusarium* mycelium, C. Micro and macro-conidial of the pathogen.

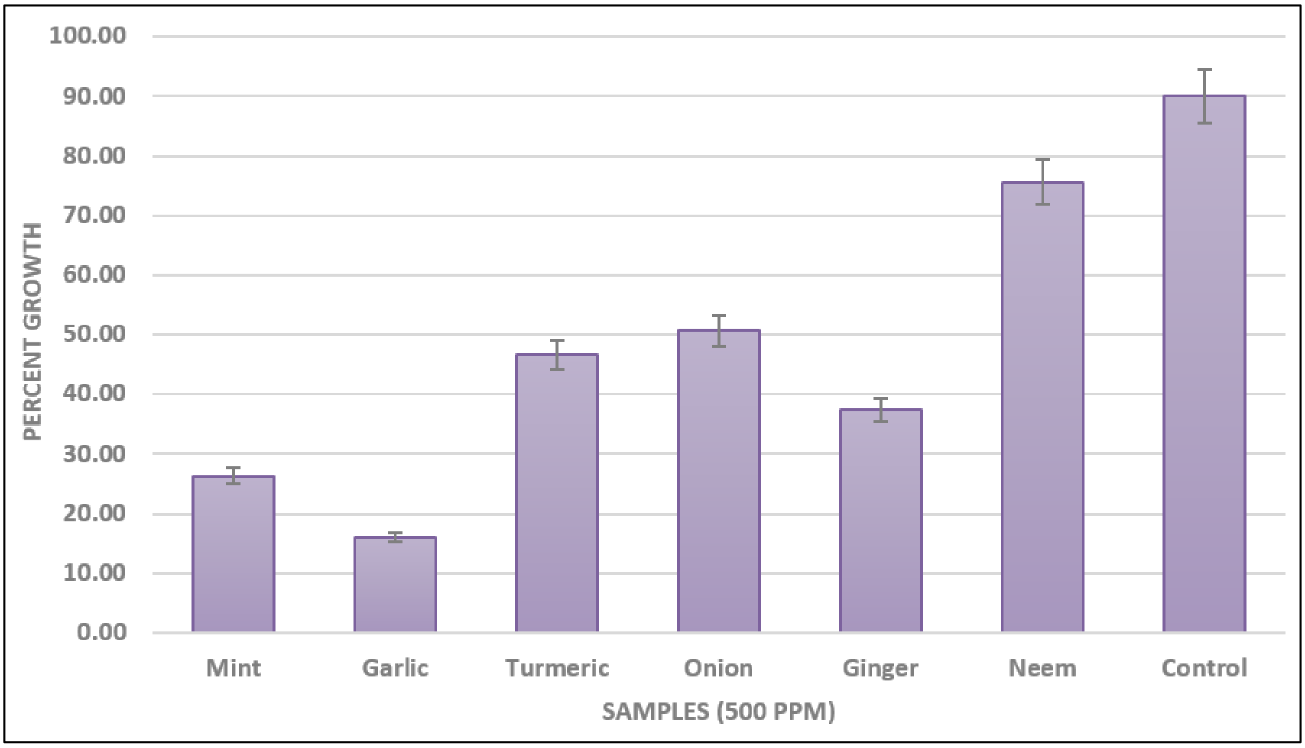
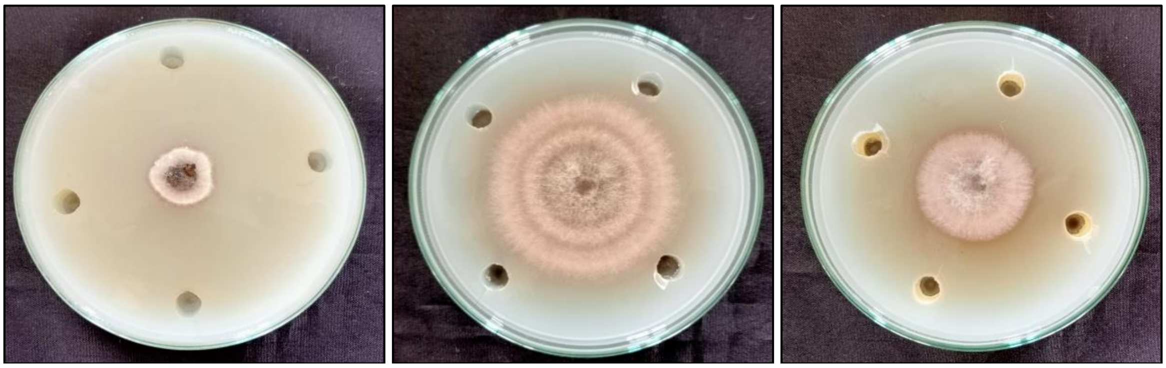


Fig 2. Effect of plant extract on fungal growth of *Fusarium oxysporum*



A B C

Fig 3. Antifungal effect of plant extract on fungal growth of *Fusarium oxysporum* by Agar well diffusion method. A. Garlic exposed plate, B. Mint exposed plate, C. Control plate

Conclusion

The present study concluded that the antifungal activity of garlic extract was shown to exhibit higher percent inhibition against the fungal growth of the pathogen. The garlic extract has the maximum antifungal properties which support in control of the fungal growth of pathogens. The present research could be explored for assessing the antifungal properties and development of novel formulations from the garlic for management of phytopathogens.

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