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Effect of Neem (*Azadirachta indica*) and Lantana (*Lantana camara*) Leaf Extract on the Growth of *Fusarium solani* under *in-vitro* Conditions

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A B S T R A C T

In an experiment, effect of neem and lantana leaf extract on the growth of damping-off pathogen, *Fusarium solani* was evaluated under *in- vitro* condition on Potato Dextrose Agar (PDA) medium. Results showed the growth inhibition of 45.2% and 66.1% with neem leaf extracts and 0% and 0% growth inhibition with lantana extracts, @ 10 and 40% concentrations, respectively. Extract of neem leaf exhibited antagonistic activity and lantana had synergistic effect on the growth of *Fusarium solani* under *in-vitro* condition. This experiment was conducted only at the lowest and highest concentration to ascertain effect of leaf extract on growth of fungi. Based on this preliminary experiment effective dosage or concentration may be determined.

Introduction

Damping-off is a complex disease caused by *Pythium* spp., *Fusarium* spp. (Schlecht), *Rhizoctonia* spp. *Sclerotium* spp. and *Colletotrichum* spp. that kill or weaken seeds or seedlings before or after the seed germination. Damping-off is most prevalent in wet and cool conditions (Koenning, 2001).

During kharif season the disease results in about 60-75% damage. High soil moisture and moderate temperature, high humidity favours the disease development. Pathogens associated with damping-off are the common soil dwelling pathogens and can stay alive in soil as resting spores and pathogenically on alternate hosts and weeds. Damping-off pathogens are easily dispersed through irrigation water and contaminated soil on equipment. The infected plants reveal two types of symptoms *viz*., pre-emergence damping-off and post-emergence damping-off. The pathogen attacks the collar region of seedlings on the surface of soil. Water soaked, greasy lesions appear on the hypocotyls and roots after the plant emerge and make the plant to collapse and wilt. *Fusarium solani* (Current name: *Neocosmospora solani* (Mart.) L. Lombard and Crous) is a soilborne fungal plant pathogen found in agricultural soils

marking a worldwide distribution. It infects several plant species of diversified plant families. Chief host plants among them are potato, tomato, chilli, onion, brinjal, bean, pea and all the members of cucurbitacea family such as water melon, musk melon, cucumber, bottle guard, ridge guard and squash guard. *Fusarium solani* induces plants to undergo damping-off, root rot, stem rot, surface rot and corn rot. *F. solani* produces scarce to plentiful white to cream colored mycelium on potato dextrose agar (PDA) medium. Colonies of *F. solani* are fast growing, develops variable color and texture, sometimes granular to fluffy, rose-red color, purple, or lavender color. Colony starts out as white, cottony growth that gradually turns dark with maturity (Zaccardelli *et al.*, 2008).

Fungicides known to inhibit fungal plant pathogens but at the cost of polluting environment and by affecting non target entities. Several plants have known to bear inhibitory effect on the growth of fungal pathogens in an eco-friendly way. Previous literatures suggests that neem and lantana leaf extracts known to have antifungal activity against only certain plant pathogens, due to the naturally occurring phytochemical and antifungal substances. Investigating the inhibitory activity leaf extracts of neem and lantana on the *Fusarium solani* under *in- vitro* condition is a primary step towards achieving the botanical management of plant disease.

Materials and Methods

Collection and isolation of fungi

Studies were carried out at the Department of Botany, Faculty of Science, B. N University, Udaipur district of Rajasthan. Damping-off diseases affected onion seedling samples were randomly collected from nursery and the adjoining field from Udaipur region. The diseased plants were collected in the polythene bags and were transported to the laboratory for the purpose of isolating damping-off pathogens from the infected root bits. Infected root bits of samples were gently washed under tap water for about a minute to remove any dirt and soil particles. The root pieces (0.5 cm) were dipped in 0.01% $HgCl_2$ for about 15 seconds and then passed from three washes of distilled sterile water for 2-3 minutes each to remove the traces of $HgCl_2$. The treated root pieces were dried completely in the aseptic condition and then transferred to Petri plates containing sterilized potato-dextrose agar (PDA) medium at the rate of 5-6 pieces/ Petri plate. All the Petri plates were kept at 25 ± 2^{o}C for 7 days. The colonies which were showing distinct mycelial growth habit were segregated by hyphal tips and transferred on to the fresh potato dextrose agar (PDA) medium (Plate.1). The purified fungus cultures were maintained on PDA slants in test tubes for further studies (Plate.1). The growth is sub-cultured/multiplied whenever needed during the entire study. Isolated fungal pathogens were sent to Agharkar Research Institute, Pune for the purpose of morphological identification and were identified as *Fusarium solani* (Current name: *Neocosmospora solani* (Mart.) L. Lombard and Crous).

Neem and lantana leaves extract preparation and poison bait technique

Matured leaves of neem (*Azadirachta indica*) and Lantana (*Lantana camara*) were collected from the plant/tree grown in the B. N. University campus. Collected leaves were washed in the clean tap water and followed by washing leaves in the sterile distilled water. The leaves were shade dried at 27°C, weighted 25 gm and finely ground using pistal and mortar. Fine paste was collected and added into 100 ml distilled water in 250 ml beaker and manually stirred for half an hour and allowed to stand for an hour. The supernatant was filtered using muslin cloth to get leaf

extract and the resulting filtrate was considered as 100% (Achimu and Scholsser, 1992). Autoclave sterilized PDA media were prepared bearing 10% and 40% neem and lantana leaf extracts separately in it and poured 20 ml in the sterilized disposable Petri plates of 90mm. PDA plates without neem or lantana leaf extract served as control. Each of the plates were inoculated with 5 mm mycelial disc of freshly grown 7 days old pure culture of test pathogens and incubated at 25 ± 2°C. Three replications were maintained for each treatment. Percent inhibition of test fungus by neem leaf extract and lantana leaf extract were separately examined under *in- vitro* and percent inhibition was calculated using the following formula

% FG = Dc-Dt / Dc x100

Where:

% FG = % inhibition of fungi growth
Dc = diameter of control
Dr = diameter of test

Results and Discussion

In this present investigation, neem leaf extract exhibited inhibitory activity against *Fusarium solani* and resulted in the inhibition of 45.3 and 66.1 % at 10 and 40% concentration, respectively, in comparison to control which has 0% inhibition (Plate.2). This inhibitory activity of neem leaf extract can be mainly attributed to the presence of various bioactive compounds such as alkaloids, cardiac glycosides, flavonoids, phlobatannins, saponins, tannins and terpenoids (Jeyasakthy *et al.,* 2012). Previous reports also suggest that neem leaf extract inhibited the growth and sporulation in many fungi *viz.*, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans, Microsporum gypseum, Pythium aphanidermatum* and *Colletotrichum gloeosporides* under *in- vitro* conditions at the different concentration of 5, 10, 15 and 20% concentration (Mahmoud *et al.,* 2011; Natarajan *et al.,* 2003 and Saseed, *et al.,* 2008).

Whereas, leaf extract of lantana showed synergetic effect on the growth of *Fusarium solani* and has resulted in the fast and profuse growth of fungus at the higher concentration (40%) when compared to lower concentration (10%). There was 0% inhibition noticed at 10% and 40% concentration of lantana leaf extract, in comparison to control with the 0% inhibition (Plate.2). This synergism can be attributed to the phytochemical constituents of the plant (Joo *et al.,* 2005).

Plate.1 Pure culture of *fusarium solani* in Petri plate and slants

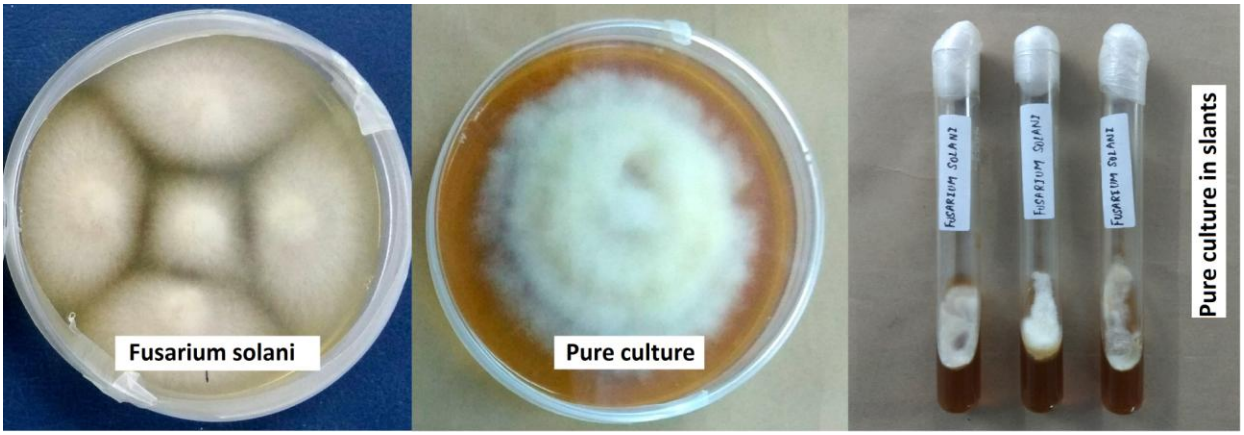
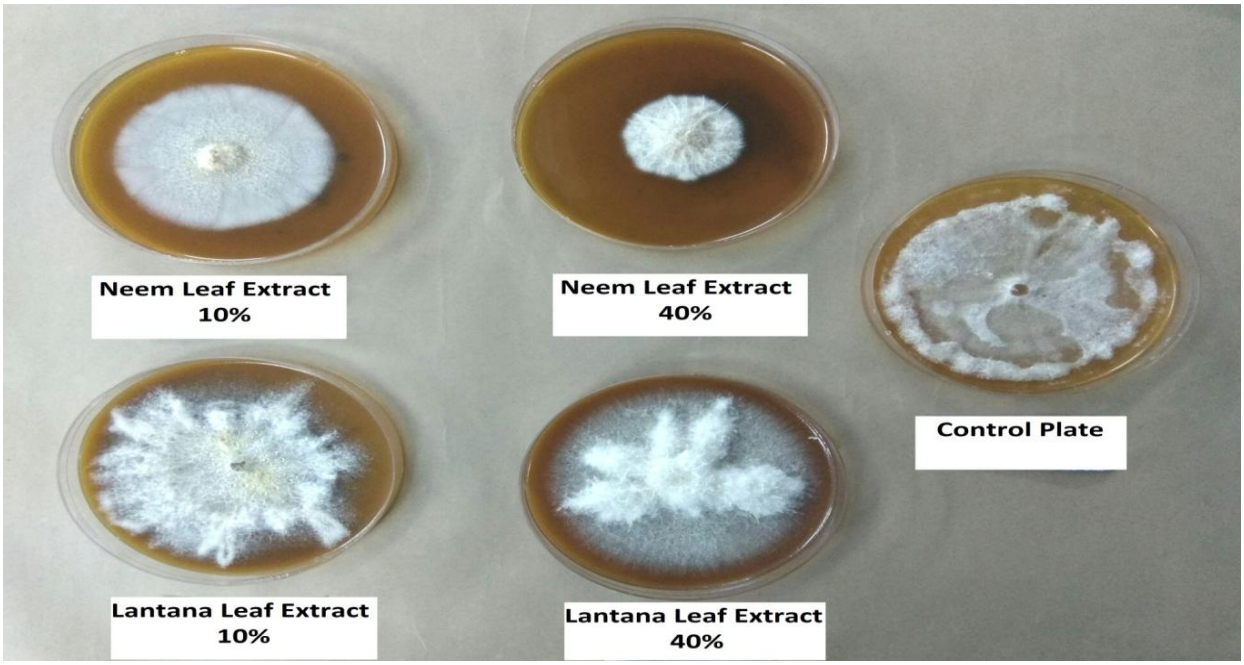


Plate.2 Effect of neem and lantana leaf extract on the growth of *Fusarium solani*



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