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In- vitro effect of neem (*Azadirachta indica*) and lantana (*Lantana camara*) leaf extract on the growth of *Sclerotium rolfsii*

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Abstract
In this study, effect of neem and lantana leaf extract on the growth *Sclerotium rolfsii* was evaluated under *in- vitro* condition on Potato Dextrose Agar (PDA) medium. Results showed the growth inhibition of *Sclerotium rolfsii* to the extent of 34.2% and 62.2% with neem leaf extracts and 0% and 0% growth inhibition with lantana leaf extracts, @ 10 and 40% concentrations, respectively. Extract of neem leaf exhibited antagonistic activity and lantana had synergistic effect on the growth of *Sclerotium rolfsii*. Mycelium growth of *Sclerotium rolfsii* in the PDA plates supplied with 40% lantana leaf extracts were more thick and profuse when compared plates with 10% leaf extract, in comparison with control plates. However, in 15 days old culture plates number of sclerotia produced were found maximum in plates with 10% lantana leaf extract and very less sclerotia in plates with 40% lantana leaf extracts, compared to control.

Keywords: Neem, lantana, inhibition, antagonistic and synergistic

Introduction
Damping-off is a disease caused by *Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp., *Sclerotium* spp. and *Colletotrichum* spp. Seeds fails to germinate in case of pre-emergent damping-off and young seedlings get infected, weakened and get killed, in case of post- emergent damping-off. High soil moisture and prevailing cool weather conditions favors the damping-off disease development and can cause 60-75% damage (Koenning, 2001) [4].
Rhizoctonia spp., *Pythium* spp., *Fusarium* spp., and *Sclerotium* spp are the plant pathogens known to be associated with damping-off and are the common soil dwelling pathogens and can stay alive in soil as resting spores and pathogenically on alternate hosts and weeds. The pathogens are dispersed through irrigation water and contaminated soil on equipment during soil working and intercultural operations. In post-emergent damping-off, pathogen attacks the collar region of seedlings on the surface of soil. Water soaked lesions appear on the hypocotyls and roots after the plant emerge and make the plant dull, collapse, wilt and die. Damping-off disease exhibits intermittent patches or gaps in the plant population in both nursery and field conditions.
Sclerotium rolfsii Sacc, is a soil borne fungal plant pathogen exhibiting diverse host range and ubiquitous in soils worldwide, able to cause root rot and damping-off disease in tomato, cucurbits, potato, pepper, coriander, cantaloupe, celery, okra, carrot, cauliflower, cabbage, bean, eggplant, and groundnut, including several crops having economic importance. *Sclerotium rolfsii* being an aerobic basidiomycete fungus. Optimum growth for this fungal development is 27-30 ºC both under *in-vitro* and field conditions. At this temperature *Sclerotium rolfsii* produces abundant white fluffy mycelium and sclerotia, at the later stage. Sclerotia are the reproductive propagules produced by *S. rolfsii* and are not truly spores, but are composed of highly compacted, melanized hyphae. Sclerotia are the hard, round structures, dark in color, produced from the main fungal hyphal strands about 7 days after infection and measures from 0.3 mm to 2.0 - 3.0 mm in diameter. Sclerotia which are initially white, turns yellow, tan, or brown in color as they mature. During sclerotium development, drops of clear watery exudates are produced at the surface on the PDA media under *in-vitro* conditions. Nutrient composition of mature sclerotia are rich in amino acids, fatty acids, sugars and lipids (Liu and Wu, 1971) [5]. However, droplets do not appear on the sclerotia surface in soil (Christias, 1980) [1].

Controlling soil borne disease in general and damping-off disease in particular is often a difficult task. Chemical fungicides give an excellent control, but have far reaching consequences on environment and ecological entities. Exploiting naturally occurring plant substances for managing plant diseases is a safe and eco-friendly approach.

In this present study, efforts have been directed to know the antifungal properties in neem and lantana leaf extracts. Investigating the inhibitory activity of leaf extracts of neem and lantana on the *Sclerotium rolfsii* 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 ± 2ºC 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. The purified fungus cultures were maintained on PDA slants in test tubes for further studies. 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 pathogen identification and was identified as *Sclerotium rolfsii* Sacc (Current name: *Athalia rolfsii* (Curzi) C.C. Tu and Kimbr.).

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 ± 2ºC. 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

$$\% FG = Dc-Dt / Dc \times 100$$

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 *Sclerotium rolfsii* and resulted in the inhibition to the level of 34.2 and 62.2 % at 10 and 40% concentration, respectively, in comparison to control which has 0% inhibition (Plate.1). 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) [2]. 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) [6, 7, 8]. Whereas, leaf extract of lantana showed synergetic effect on the growth of *Sclerotium rolfsii*. There was 0% inhibition noticed at 10% and 40% concentration of lantana leaf extract, in comparison to control which showed 0% inhibition (Plate 2). Mycelium growth of *Sclerotium rolfsii* in the PDA plates supplied with 40% lantana leaf extracts were more thick and profuse when compared plates with 10% leaf extract, in comparison with control plates. However, in 15 days old culture plates number of sclerotia produced were found maximum in plates with 10% lantana leaf extract and very less sclerotia in plates with 40% lantana leaf extracts, compared to control. This synergism can be attributed to the phytochemical constituents of the plant (Joo *et al*., 2005) [3].

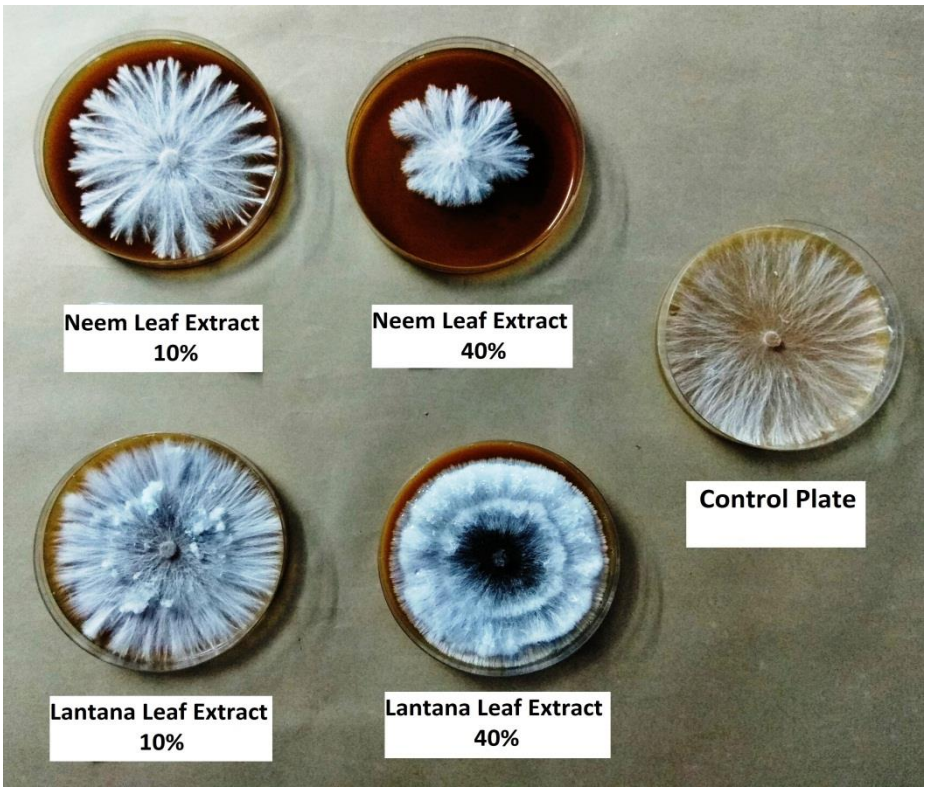

Plate 1: Effect of neem and lantana leaf extract on growth of *Sclerotium rolfsii*

Plate 2: Sclerotial formation in 15 days old lantana extracts plates

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