

## *In-vitro* evaluation of different plant extracts on mycelial growth of *Sclerotium rolfsii* the cause of root rot of sugar beet

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### Abstract

Aqueous extract of twenty different plant species were invitro evaluated for their inhibitory effect on mycelial growth of *Sclerotium (Athelia) rolfsii* causing southern *sclerotium* rot in sugar beet, at pathology laboratory of Sugar Crops Research Program, National Agricultural Research Centre, Islamabad, Pakistan. Generally, all plant species inhibited mycelial growth of the pathogen but maximum inhibition was recorded by *Azadirachta indica* (73.8%) followed by *Cassia fistula* (73.5%) and *Cannabis sativa* (67.1%). The minimum inhibition was showed by *Trigonella foenumgraecum* (34.3%) and *Cassia angustifolia* (36.3%).

**Key words:** Root rot, sclerotium rot, sugar beet.

### Introduction

Different soil borne fungal pathogens are responsible for poor establishment and stand loss in sugar beet (Harveson and Rush, 1998; Kiewnick *et al.*, 2001; Weiland and Sundsbak, 2000). Yield and sugar contents are also condensed due to these pathogens (Harveson and Rush, 2002; Windels and Lamey, 1998). *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) a soil-borne plant pathogenic fungi incitant of southern sclerotium rot in sugarbeet. The pathogen is pervasive in warm temperate and subtropical regions of the world and have a broad host range (Punja, 1985) The fungus produce differentiated sclerotia and sterile mycelium like other sclerotium producing fungi. which are characterized by small tan to dark-brown or black spherical sclerotia having internally differentiated rind, cortex and medulla (Punja & Rahe 1992). The most distinctive effect of this pathogen is rotting of affected tissues that are directly attacked by the fungus. However, the mass of mycelium it produce, secretes oxalic acid as well as pectinolytic, cellulolytic, and some other enzymes which kill and disintegrates tissues before it actually penetrates the host. Once established in the plants, the fungus progress and generate both mycelium and sclerotia quite rapidly, especially at high moisture and high temperature i.e. between 30 and 35°C (Agrios, 2005).

Management of the plant diseases incited by soil borne pathogens is not achievable chemically, due to the widespread host range, abundant growth of the pathogen and its capability of producing excessive sclerotia that may persist in soil for several years (Chet & Henis, 1972; Punjor, 1985). Therefore, plant extracts may be used as an alternative source for controlling soil-borne diseases since they comprises a rich source of bioactive substance (Wink, 1993). Plant extracts are eco-friendly, display structural diversity and complexity and infrequently contain halogenated atoms. (Duke *et al.*, 2000). Protective, curative and antagonistic activity of different plants against variety of diseases has been reported by several workers (Kandasamy *et al.*, 1974; Hale & Mathers, 1977; Rahber-Bhatti, 1986; Kalo & Taniguchi, 1987). The present study was ascertain to investigate the inhibitory effect of leaf extracts of various plant species on the mycelial growth of *S. rolfsii* under invitro conditions.

### Materials and Methods

#### Isolation of *Sclerotium rolfsii*

Infected beet root was collected, washed thoroughly in order to free them from soil. Then cut the infected portion in to small pieces of about

3-5 mm thick and sterilized with 1 % sodium hypochlorite solution for two minutes, rinsed thrice in sterilized distilled water (SDW) and dried on sterilized filter paper at room temperature. The tissue sections were then placed on potato dextrose agar (PDA) and incubated at 25°C for seven days. Ultimately, the pure culture of the pathogen was isolated and subsequently maintained on PDA.

#### Preparation of Plant Extracts

Fresh leaves (20 gm) of twenty different plant species (Table 1.) were collected and surface sterilized with 0.02 percent HgCl<sub>2</sub> and repeatedly washed with sterilized water. The surface sterilized leaves along with 20 ml of distilled water were macerated to pulp. The sap thus extracted was first passed through four layers of muslin cloth and then filtered through Whitman's filter paper No.41 in 50 ml Pyrex flasks. The flasks were tightly wrapped with aluminum foil and autoclaved at 15 psi for 20 minutes and kept in UV light for one hour.

#### In vitro assay

Inhibitory effect of plant extracts was assessed using poisoned food technique. A volume of 2 ml of each plant extract was aseptically poured into the petriplates followed by the addition of Potato dextrose agar medium (18 ml). The petriplates were kept swirling while adding media so as to get a thorough mixing of the contents. Each treatment was replicated thrice and allowed for solidification.

After one hour, 5 mm disc from actively growing margins of *S. rolfisii* colony was placed in the centre of each of the petriplate. Control was maintained without adding extracts. The plates were incubated at 25±2°C. The colony diameter in each treatment was measured on the fifth day after inoculation and the percent inhibition of each isolates was calculated and the data was analyzed.

$$\% \text{age inhibition} = \frac{\text{Diameter of colony of control} - \text{Diameter of colony in treatment}}{\text{Diameter of colony of control}} \times 100$$

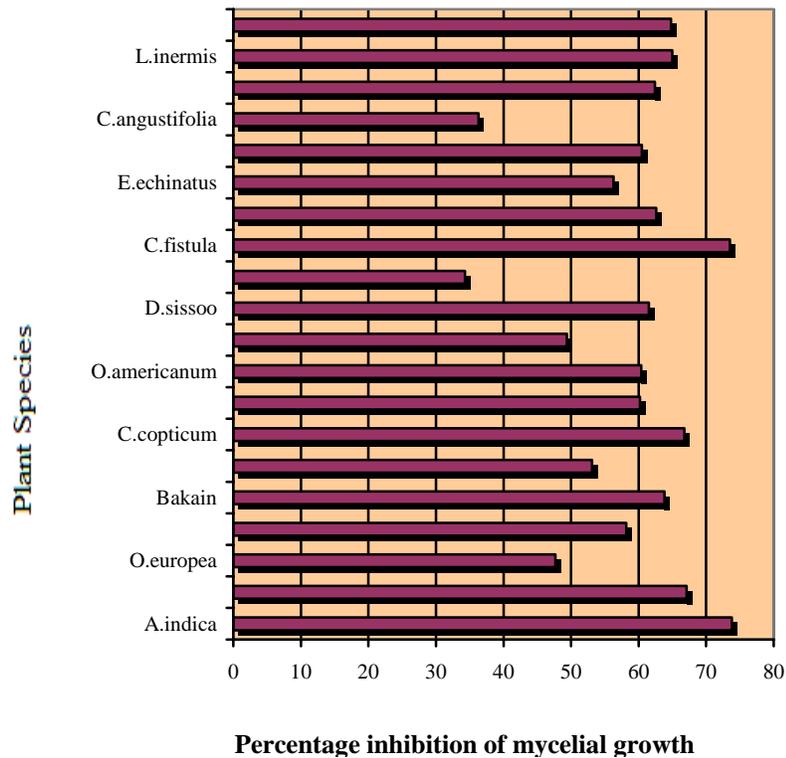
#### Results and Discussion

The effect of differences in plant leaf extracts on mycelial growth of *S. rolfisii* was significant. When compared with the control, the leaf extracts of all plant species were significantly more effective in inhibiting the mycelial growth of *S. rolfisii* under *in vitro* conditions. In general, all the leaf extract were better than control in reducing the mycelial growth of *S. rolfisii* (Fig 1). Maximum inhibition was recorded by *Azadirachta indica* followed by *Cassia fistula* and *Cannabis sativa*.

The minimum inhibition was showed by *Trigonella foenumgraecum* followed by *Cassia angustifolia*. The significant decrease in mycelial growth of the fungus treated with various plant leaf extracts was probably due to presence of antifungal compounds or ingredients in plant leaf extracts. It is also possible that the extracts inhibited or altered the mode of action of pathogen's biological chemicals. Inhibitory effects of plant leaf extracts have also been observed on viruses and other soil fungi (Baker, 1981; Kuc & Shain, 1977). Jalal and Ghaffar (1992) studied antifungal characteristics of *Ocimum sanctum* L. and found that its leaf extract completely inhibited the growth of *S. rolfisii* and other fungi. Use of barks and corks of commonly grown trees and shrubs is also an economical and feasible way of controlling major plant diseases. Leaf decoction of *Acacia nilotica*, *Calotropis procera*, *Datura stramonium*, *Dodonea viscosa* and *Rhazya stricta* were found to be effective in processing urediospore germination on detached leaves of wheat (Rahber-Bhatti, 1988). Volatile fraction of two medicinal plants; *Azadirachta indica* and *Eucalyptus globules* were more effective in suppressing the sclerotial germination of *Macrophomina phaseolina* than non-volatile fractions (Dubey & Kishore, 1990). Leaf extract of *Datura stramonium* reduced the development of rust pustules on the leaves of wheat (Hussain *et al.*, 1992). Herbaceous plants have been widely used in various ways against the fungal diseases. Aqueous leaf extract of *Allium sativum*, *Datura alba* and *Withana somnifera* inhibited the growth of *Alternaria alternata*, *A. brassicola* and *Myrothecium roridum* (Mughal *et al.*, 1998). Shahzad and Ghaffar (1988) reported that *Paecilomyces lilacinus* (a fungal parasite) was effective to inhibit growth of sclerotial fungi i.e. *Macrophomina phaseolina*, *Rhizocotinia solani* and *Sclerotium oryzae*, causing root rot in many plants. Jalal and Ghaffar (1992) used aqueous plants extracts of *Allium cepa* (onion), *Calotropis procera* (Akk), *Chenopodium album* (Bathu), *Chenopodium murale* (Karund), *Azadirachta indica* (Neem) and *Cannabis sativa* (Bhang) for antifungal activity against *Macrophomina phaseolina*, *Alternaria radicina*, *Helminthosporium tusricum* and *Ascochyta rabiei*. Leaf extracts of some medicinal plants in our study also exhibited significant effect against *S. rolfisii*. On the basis of present study, it is concluded that leaf extract of the plants which have been found effective against collar rot can be recommended against the disease after in field trials.

**Table 1:** List of plant species, used for studying the effect of their leaf extracts on mycelial growth of *Sclerotium rolfsii*.

S. No.	Common Names of Plants	Scientific Names of Plants
1	Neem	<i>Azadirachta indica</i>
2	Bhang	<i>Cannabis sativa</i>
3	Olive	<i>Olea europea</i>
4	Dharek	<i>Melia azadirach</i>
5	Bakain	<i>Melia azadirach</i>
6	Kaner	<i>Nerium oleander</i>
7	Ajwain	<i>Carum copticum</i>
8	Aak	<i>Calatropis procera</i>
9	Niazboo	<i>Oscimum basilicum</i>
10	Saunf	<i>Foeniculum vulgare</i>
11	Tahli	<i>Delbergia sisso</i>
12	Methi	<i>Trigonella foenumgraecum</i>
13	Amaltas	<i>Cassia fistula</i>
14	Soya	<i>Anethum graveolens</i>
15	Unt-Katara	<i>Echinops echinatus</i>
16	Gul-Babuna	<i>Matricaria chamomilla</i>
17	Senna-Makki	<i>Cassia angustifolia</i>
18	Parthenium	<i>Parthenium hysteropharous</i>
19	Mahndi	<i>Lawsonia inermis</i>
20	Tobacco	<i>Nicotiana tabacum</i>



**Fig. 1:** Efficacy of different plant extracts on inhibition of mycelial growth *S. rolfsii*.

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