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Evaluation of antifungal activity of plant extracts against collar rot of lentil incited by *Sclerotium rolfsii* (Sacc.)

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Abstract
Lentil (*Lens culinaris* Medik.) is a popular pulse crop grown in semi-arid areas of Iran, India, Turkey, and Canada). Lentil is consumed as loaves, curries and pies in the whole world and in India, it (locally known as Masur) is either consumed whole grain or its split seeds, used as Dal. *Sclerotium rolfsii* (Sacc). is a soil-borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt, foot rot and collar rot. Maximum inhibition of mycelial growth was recorded in *Allium sativum* (61.36%), followed by *Curcuma longa* (28.41%), *Duranta erecta* (25.00%), while minimum inhibition percent of mycelial growth was recorded in treatment control (00%) at the ends of 10% concentration. At 20% concentration maximum inhibition was recorded in *Allium sativum* (81.75%), followed by *Curcuma longa* (49.05%), *Zingiber officinale* (42.97%), *Duranta* erecta (38.03%), *Calotropis gigantean* (33.84%), *Cynodon dactylon* (29.66%), *Allium cepa* (20.92%), *Parthenium hysterophorus* (20.16%), and *Azadirachta indica* (18.25%), while minimum percent inhibition of mycelial growth was recorded in treatment control (0.00%).

Keywords: *Sclerotium rolfsii*, plant extracts, mycelial growth and inhibition

Introduction
Lentil (*Lens culinaris* Medik.) is a popular pulse crop grown in semi-arid areas of Iran, India, Turkey, and Canada. Lentil is consumed as loaves, curries and pies in the whole world and in India, it (locally known as Masur) is either consumed whole grain or its split seeds, used as Dal. In India, main lentil growing states are Madhya Pradesh, Bundelkhand region of Uttar Pradesh and Bihar. Globally, lentil is grown in 6.1 Mha of land with annual production and productivity of 6.3 Mt. and 1038 kg/ha (Choukri *et al.*, 2020) [3]. India is the second largest producer of lentils after Canada, with 3.6 Mt and 0.87 Mt annual production, respectively. The major lentil growing regions of India are U.P., M.P., West Bengal, Bihar, Haryana and Rajasthan. In India Lentil is cultivated over an area of 1.32 ha with production and productivity of 1.18 million tonnes and 894 kg/ha respectively. Uttar Pradesh and Madhya Pradesh are major lentil producer in India occupying nearly 35.17% and 28.79% area to all India respectively (Anon, 2020) [1].

Sclerotium rolfsii (Sacc). is a soil-borne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt, foot rot and collar rot on more than 500 plant species including almost all the agricultural and horticultural crops (Fernando *et al*. 2004; Clarkson *et al*., 2004; Del Rio *et al*., 2007; Sten *et al*., 2017) [9, 4, 7, 16]. Lentil, collar rot or root rot is most destructive disease which is caused by *S. rolfsii* (Smolinska and Kowalska, 2018) [15], occurs in almost every lentil growing region in warm areas with high soil moisture (30-40%) and high temperature (25 ºC) at the seedling stage. The pathogen survives well in soil as sclerotia in the presence of sufficient organic matter even under adverse climatic conditions (Wu *et al*., 2008) [20]. *S. rolfsii* is a non-specialized soil borne fungal pathogen of worldwide importance and has a host range of over 500 species (Xu *et al*., 2008) [19]. It is too difficult to remove the pathogen from infected field due to its diverse nature of survival as sclerotia production and their ability to persist in the soil for several years except use of PGPR those are potential bio-control agent too (Das *et al*., 2017; Singh *et al*., 2012) [6, 17]. The first sign of lentil collar rot was a small amount of paleness on the plant stem near the soil surface, followed by yellowing of the leaves and lack of plant vigor. *S. rolfsii* infection appears as a brown to black discoloration in the collar region of the

lentil. The discoloration progressed 4-6 cm downward and upward, respectively, along the top root and stem. Plant extracts deserve a special attention to develop a strategy for ecologically safe method of plant disease management. Several higher plants and their constituents have shown success in plant disease control and proved to be harmless and non-phytotoxic unlike chemical fungicides (Singh *et al*. 1986, Singh and Dwivedi 1990, Dubey 1991, Bisht and Kamal 1994) [22, 18, 8, 2]. Using extracts from plants containing natural antifungal compounds for plant disease control is considered to be one of the desirable methods for plant protection in agriculture (Kim *et al*. 2002) [10]. The findings will be a document in the research arena. Considering the above facts the present study was, therefore, undertaken to reveal the differences in mycelial growth and inhibition percent among the plant extract against *S. rolfsii.*

Materials and Methods

The experiments were conducted in the, Department of Plant Pathology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Viswa Vidhadlya, Gwalior. The experiments were performed during 2021-22.

In vitro evaluation of aqueous plant extracts

An *in vitro* experiment was conducted for preliminary screening of nine aqueous plant extracts (@ 10 and 20%). Water extracts of leaves of *Azadirachta indica, Cyanodon dactylon, Calotropis gigantean, Duranta* erecta, *Allium sativum, Allium cepa, Curcuma longa, Zingiber officinale and Parthenium hysterophorus* were evaluated *in vitro* against *Sclerotium rolfsii* following poisoned food technique (Nene and Thapliyal 1993) [13] on potato dextrose agar (PDA) medium. To prepare plant extract, healthy parts of selected plant species were washed with sterile distilled water and chopped into small pieces with sterilized sharp knife. Each sample was separately grounded and homogenized in mechanical grinder with equal quantity of sterile distilled water 1:1 (W/V). The homogenate obtained was strained through double layered cheese cloth and the filtrate collected was again filtered through Whatman No. l filter paper. The clear leaf extracts were considered as the stock solution of 100 percent concentration. An appropriate quantity of each leaf, bulb and rhizome extract was mixed separately with melted PDA medium in conical flask (250 ml capacity) to get desired concentrations (10 and 20%) of each extract and autoclaved.

The PDA amended with plant extract were poured separately into sterilized petridishes @ 20 ml per plate. After solidification, the plates were inoculated with 5 mm mycelium block of *Sclerotium rolfsii* cut from 3 days old culture of the pathogen. Plates containing un-amended PDA were also inoculated with the pathogen, which served as control. The colony diameter of the test fungus grown on PDA was recorded when the plates under control were fully covered with the mycelium of the test fungus. The inhibition of mycelium growth was measured based on growth of the fungus on control plate and also that on treatment plate following the formula of Vincent (1927) [23].

Percent inhibition (I) =C−T / C x 100,

Where, C = growth of test fungus (mm) in control plate,
T = growth of test fungus (mm) in treatment plates.

The percent data were converted into arcsine transformation values. All data were analyzed statistically and the means were separated by least significance test (LSD) at p=0.05% level.

Result

Antifungal activity of nine botanical from different plant sources (*Azadirachta indica, Cyanodon dactylon, Calotropis gigantean, Duranta* erecta, *Allium sativum, Allium cepa, Curcuma longa, Zingiber officinale and Parthenium hysterophorus*) were evaluated at 10 and 20% concentrations in the laboratory against *S. rolfsii* using food poisoned technique. All of the tested botanicals significantly inhibited the mycelial growth of *Sclerotium rolfsii* at 10 and 20 percent concentrations compared to the control. The data summarized in table-1 revels that all the treatments were found effective against *S. rolfsii* over control. The fungal growth was recorded at seven days after inoculation. At 10 percent concentration the radial growth varied from 34.00 to 88.00 mm as compared to control. Minimum mycelial growth was recorded in treatment *Allium sativum* (34.00 mm), followed by *Curcuma longa* (63.00 mm), *Duranta* erecta (66.00 mm), *Zingiber officinale* (68.67 mm), *Calotropis gigantean* (75.33 mm), *Azadirachta indica* (78.67 mm), *Parthenium hysterophorus* (80.67 mm), *Cynodon dactylon* (84.33 mm), and *Allium cepa* (86.00 mm), while maximum mycelial growth was recorded in treatment control (88.00 mm). At 10% concentration maximum inhibition of mycelial growth was recorded in treatment *Allium sativum* (61.36%), followed by *Curcuma longa* (28.41%), *Duranta erecta* (25.00%), *Zingiber officinale* (21.97%), *Calotropis gigantean* (14.39%), *Azadirachta indica* (10.61%), *Parthenium hysterophorus* (8.33%), *Cynodon dactylon* (4.17%) and *Allium cepa* (2.27%), while minimum inhibition percent of mycelial growth was recorded in treatment control (00%).

At 20 percent concentration the radial growth it was ranged from 16.00 to 87.67 mm as compared to control. *Allium sativum* (16.00 mm), recorded minimum mycelial growth followed by *Curcuma longa* (44.67 mm), *Zingiber officinale* (50.00 mm), *Duranta* erecta (54.33 mm), *Calotropis gigantean* (58.00 mm), *Cynodon dactylon* (61.67 mm), *Allium cepa* (69.33 mm), *Parthenium hysterophorus* (70.00 mm) and *Azadirachta indica* (71.67 mm), while maximum mycelial growth was recorded in treatment control (87.67 mm). At 20% concentration maximum inhibition was recorded in *Allium sativum* (81.75%), followed by *Curcuma longa* (49.05%), *Zingiber officinale* (42.97%), *Duranta* erecta (38.03%), *Calotropis gigantean* (33.84%), *Cynodon dactylon* (29.66%), *Allium cepa* (20.92%), *Parthenium hysterophorus* (20.16%), and *Azadirachta indica* (18.25%), while minimum percent inhibition of mycelial growth was recorded in treatment control (0.00%).

Table 1: Effect of botanicals on radial growth and inhibition percent of *S. rolfsii in vitro.*

Botanicals	Radial growth (mm)			
	Concentration			
	10 percent	% Inhibition	20 percent	% Inhibition
Cynodon dactylon	84.33 (66.65)	4.17	61.67 (51.72)	29.66
Allium sativum	34.00 (35.65)	61.36	16.00 (23.56)	81.75
Azadirachta indica	78.67 (62.42)	10.61	71.67 (57.81)	18.25
Calotropis gigantean	75.33 (60.20)	14.39	58.00 (49.58)	33.84
Allium cepa	86.00 (68.00)	2.27	69.33 (56.35)	20.92
Curcuma longa	63.00 (52.51)	28.41	44.67 (41.92)	49.05
Zingiber officinale	68.67 (55.95)	21.97	50.00 (44.98)	42.97
Duranta erecta	66.00 (63.89)	25.00	54.33 (47.47)	38.03
Parthenium hysterophorus	80.67 (69.70)	8.33	70.00 (56.76)	20.16
Control	88.00 (71.00)	-	87.67 (69.41)	-
SeM±	0.72	-	0.73	-
C.D. @ 5%	2.14	-	2.17	-

*Mean of three replication

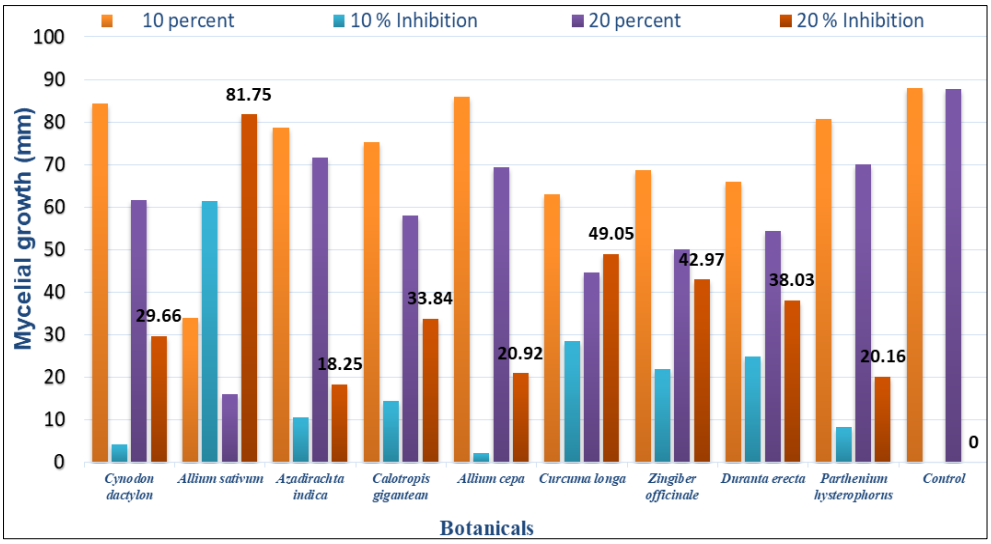
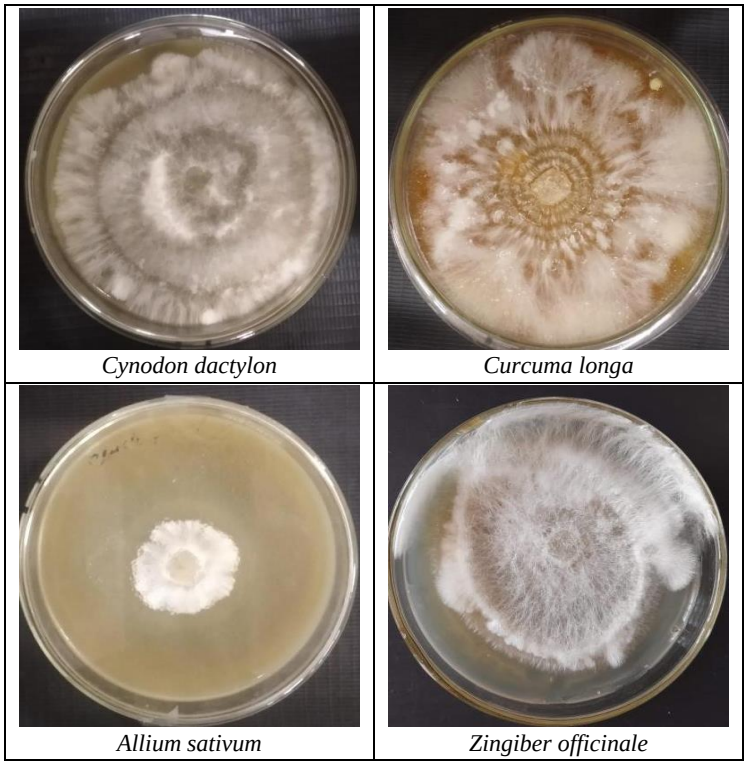


Fig 1: Effect of botanicals on radial growth and inhibition percent of *S. rolfsii in vitro.*



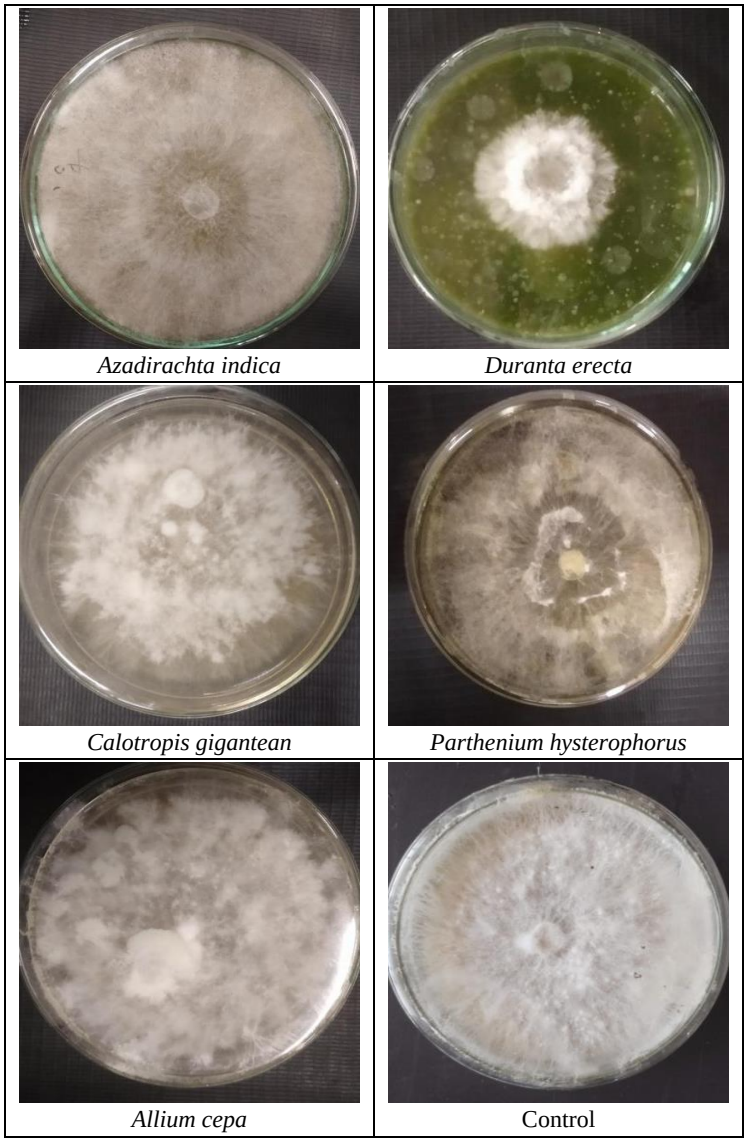


Plate 1: *In vitro* evaluation botanicals against *sclerotium rolfsii*

Discussion

In view of environmental pollution and associated health hazards as well as the development of fungicidal resistant strains of the pathogen. The application of plant extract for the managements of plant disease increased in recent years because these cause no health hazard or pollution and proved to be very effective against plant disease. To find out possibilities of replacing fungicides with other ecofriendly product, plant extract of various plants was used for management of plant diseases. Use of plants extract or liquid preparation is an age old practices in India intended to provide cheap and effective options to the farmer in the country. In present study nine botanicals were evaluated at two different concentrations @ 10 and 20% against *S. rolfsii* under *in vitro* condition. Twenty percent concentration minimum mycelial growth was recorded in treatment *Allium sativum*, followed by *Curcuma longa*, *Zingiber officinale, Duranta* erecta, *Calotropis gigantean*, *Cynodon dactylon*, *Allium cepa*, *Parthenium hysterophorus*, and *Azadirachta indica*, while maximum mycelial growth was recorded in treatment control. At 20% concentration maximum inhibition was recorded in *Allium sativum*, followed by *Curcuma longa*, *Zingiber officinale, Duranta* erecta, *Calotropis gigantean*, *Cynodon dactylon*, *Allium cepa*, *Parthenium hysterophorus*, and *Azadirachta indica*, while minimum percent inhibition of mycelial growth was recorded in treatment control. The present result is in confirmation with previous workers (Prithiviraj *et al.*, 1998; Yoshida *et al.*, 1999; Kurucheve and Padmavathi, 1997) [14, 21, 11,]. The presence of antimicrobial components like Allicin-E and Zajoene-iso-E-10-devinylajoene are responsible for antifungal properties of Garlic. Similarly, phytochemicals like terpenoids, saponins, flavonoids, tannins and alkaloids are responsible for the antifungal properties of Parthenium (Devkota and Sahu, 2017) [5].

Conclusions

The use of phytoextracts obtained from garlic have shown promising results towards controlling the collar rot of lentil. They can be used as green alternatives for harmful chemicals. This will help in decreasing the environmental degradation caused by the pesticides. Moreover, the residual effects of the pesticides in the crop will also be eliminated posing no threat to humanity. At 10 and 20% concentration maximum inhibition of mycelial growth was recorded in treatment *Allium sativum* (61.36% and 81.75%), while minimum inhibition percent of mycelial growth was recorded in treatment control (00%).

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