

Evaluation of different botanicals against *Sclerotium rolfsii* causing collar rot disease of lentil

Abstract

Lentil (*Lens culinaris* Medik.) is an important pulse crop in semiarid regions of Iran, India, Turkey and Canada and originated in the fertile crescent of the Near East and dates back to the beginning of agriculture itself. Lentil suffer from attack of number seed borne diseases such as vascular wilt, collar rot, root rot, stem rot, rust, powdery mildew and downy mildew, which are caused by *Fusarium oxysporum* f.sp. *lentis*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Uromyces fabae*, *Erysiphe polygoni* and *Peronospora lentis*, respectively. Among the diseases, foot and root rot of lentil caused by *Sclerotium rolfsii* are common and the most serious disease. The fungi can attack the crop during any time from seedling to flowering stage and are comparatively more destructive at the seedling stage. The effect of phyto extracts of nine plant species were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *Sclerotium rolfsii*. Significantly minimum mycelium growth was recorded in *Curcuma longa* (39.25 mm) while maximum mycelium growth was observed in *Ricinus communis* (90.00 mm).

Formatted: Justified

Comment [NM1]: severe

Comment [NM2]: at any stage?

Introduction

Lentil (*Lens culinaris* Medik.) is an important pulse crop. Lentil is utilized for human consumption as an edible protein resources and also is a best source of vitamin A and provides potassium, fiber, iron and vitamins B. (Kochhar, 2009)

Comment [NM3]: Separate the ideas

Lentil is used for human consumption as an edible protein resource. It is also a good source of vitamin A, potassium, fiber, iron, and vitamins B. (Kochhar, 2009).

Among the fungal diseases, collar rot of lentil caused by *S. rolfsii* (Dey *et al.*, 1993) are generic and the most solemn disease. *Sclerotium rolfsii* can invade the crop during seedling to flowering stage. The pathogen *S. rolfsii* relatively more severe at the early phase. The fungus is soil-borne and produces survival structure sclerotia, which can survive very long time in the soil. *S. rolfsii* affects the lower stem and roots of lentil at or near the soil line. During infection whitish mycelia growth of the fungus can be seen at the junction of the branch with the stem close to the soil level, which is the most favorable point of attack. In advanced stage of disease, a white mycelial web dispersion over the soil and the basal plant part of the plant and sclerotia of mustard seed size are observed on the diseased area. In its advanced stage infection becomes prominent in the root system and subsequently the entire shoot withers and falls and finally the plant die.

Comment [NM4]: The pathogen *S. rolfsii* is more severe in the early stages.

The use of plant extracts has been shown to be ecofriendly and effective against many plant pathogens (Thobhunluepop, 2009; Duru and Onyedineke, 2010). Botanicals are of

Comment [NM5]: The plant eventually die

natural origin, biodegradable and non-toxic to environment. Considering the nature safety, cost effective and host target specificity, present investigation was carried. From the above facts, these type of research work are needed in Madhya Pradesh. So the present study was carried out to assess antifungal potential of some more plant extracts against *in-vitro* growth of *S. rolfsii*.

Material and Method

The experiment was carried out at Department of Plant Pathology, College of Agriculture, Gwalior. The effect of botanicals of various plant species as listed in Table-1 were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *S. rolfsii*. The experiment was laid out in complete randomized design and replicated four times. Healthy fresh plant parts were taken, washed thoroughly with fresh water and finally raised with sterilized water and air dried on blotter paper. Fifty gram of fresh plant parts were cut into small pieces and minced with help of grinder and 50 ml distilled water was added. The botanical extracts were filtered through double layered muslin cloth in 150 ml conical flasks and plugged with non absorbent cotton. The filtered extracts were autoclaved at 15 lb persquare inch pressure for 20 minutes. Autoclaved extracts were individually added into molten sterilized potato dextrose agar @ 20 per cent mixed thoroughly at time of pouring in the previously sterilized Petri dishes. The Petri dishes were incubated aseptically after solidification by placing 5 mm diameter mycelia disc at the centre. Ten days old pure culture of *Sclerotium rolfsii* was used. The plate without phyto extract served as control. The Petri dishes were incubated at $25\pm 2^{\circ}\text{C}$. The observation was recorded at 120 and 168 hrs of inoculation. The per cent growth inhibition of the pathogen was worked out by using formula given by Vincent (1947).

Comment [NM6]: Which parts specifically ...the leaves, the roots ??????????

Comment [NM7]: Old reference please replace with new one

$$\text{PGI} = \frac{\text{C}-\text{T}}{\text{C}} \times 100$$

Where,

PGI= Per cent growth inhibition

C= Growth in control

T=Growth in botanicals

Formatted: Left

Table-1 List of botanicals

S.No.	Botanicals	Plant part used	Per cent / 100 ml	Common name	Family	Constituents
1.	<i>Curcuma longa</i>	Powder	20 %	Haldi	Zingiberaceae	Curcumenol, curdione, curcumin, isocurcumenol, curcumol, stigmasterol, zingiberene and curcumene
2.	<i>Michelia champaca</i>	Leaf	20 %	Champaca	Magnoliaceae	Phenylacetoneitril, phenylethyl alcohol, alpha + beta ionone, methyl, methyl anthranilate, indole and methyl linoleate.
3.	<i>Ricinus communis</i>	Leaf	20 %	Castor	Euphorbiaceae	Alkaloid ,ricinoleic acid, stearic,linoleic, palmatic acid, sitosterol, squalene tocopherols and stearic acid
4.	<i>Nerium oleander</i>	Leaf	20 %	Kaner	Apocynaceae	Oleandrin, digitoxigenin and gitoxigenin
5.	<i>Ficus religiosa</i>	Leaf	20 %	Pipal	Moraceae	Phenols, tannins, steroids, alkaloid and flavonoids, beta-sitosteryl-D-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol.

Comment [NM8]: Write in full

Comment [NM9]: Why not percent but per cent ?????????

6.	<i>Pisidium guajava</i>	Leaf	20 %	Guava	Myrtaceae	Guajanic acid, beta- sitosterol, uvaol, oleanolic acid and ursolic acid.
7.	<i>Duranta erecta</i>	Leaf	20 %	Sky flower	Verbenaceae	coumarinolignoids, (E)-cinnamic acid, (E)- p-methoxycinnamic acid, and lamiide, β -sitosterol, naringenin, acteoside, lamiide, sucrose, and raffinose
8.	<i>Saraca asoca</i>	Leaf	20 %	Ashok	Fabaceae	quercetin, quercetin-3-O- α -Lrhamnoside, kaempferol 3-O- α Lrhamnoside, amyirin, ceryl alcohol and β sitosterol
9.	<i>Tridax procumbens</i>	Leaf	20 %	Tridax	Asteraceae	Alkyl esters, sterols, pentacyclic triterpenes, fatty acid and polysaccharides

Results and Discussion

Effect of plant extracts on mycelial growth of *Sclerotium rolfsii*

Fungicidal ability of nine botanicals viz., *Curcuma longa* powder, *Michelia champaca* (champa), *Ricinus communis* (castor), *Nerium oleander* (kaner), *Ficus religiosa* (pipal), *Tridax procumbens*, *Psidium guajava*, *Duranta erecta* (hedge) and *Saraca asoca* (Ashoka) were tested against the growth of *S. rolfsii* at 20% concentration and was recorded at 3, 5 and 7 days after inoculation.

Significantly lowest mycelium growth (7.75 mm) was recorded in case of *C. longa*, where inhibition was 74.17 at 3 days after inoculation. The next best botanical found was *N. oleander* with mean mycelium growth of 11.25 mm followed by *M. champaca* (19.00 mm), *S. asoca* (20.25 mm), *P. guajava* (21.50 mm) and *D. erecta* (24.00 mm). Mycelial growth was highest in case of untreated control (30.00 mm); there was no inhibition of mycelial growth. Among the ten phytoextracts tested, *C. longa* extract gave higher level of inhibition of mycelial growth (70.52 %) at 20 per cent concentration and recorded minimum mycelial growth (18.50 mm) which was statistically similar to *N. oleander* (21.50 mm) at five days after inoculation. The next best botanical found was *S. asoca* (41.50 mm) followed by *P. guajava* (46.00 mm), *D. erecta* (48.50 mm) and *M. champaca* (52.00 mm). Maximum mycelial growth (62.75 mm) was recorded in untreated control which was found at par with *R. communis* (60.75 mm) and *F. religiosa* (60.25 mm). Average radial mycelial growth of *S. rolfsii* in all the botanicals was varied from 30.00 mm to 90.00 mm at seven days after inoculation. However, significantly lowest mycelial growth was recorded in *C. longa* (39.25 mm). This was followed by *N. oleander* (43.75 mm), *S. asoca* (61.50 mm) and *P. guajava* (78.50 mm). Whereas, the botanicals named *R. communis* (90.00 mm) was found statistically similar with untreated control and recorded maximum mycelial growth followed by *F. religiosa* (86.00 mm), *D. erecta* (80.75 mm), *M. champaca* (80.50 mm) and *T. procumbens* (79.25 mm) (Figure 1).

Comment [NM10]: Unit..?

Comment [NM11]: can you be consistent in writing % or (percent) choose one

Comment [NM12]: be consistent choose if you use numbers in words or not

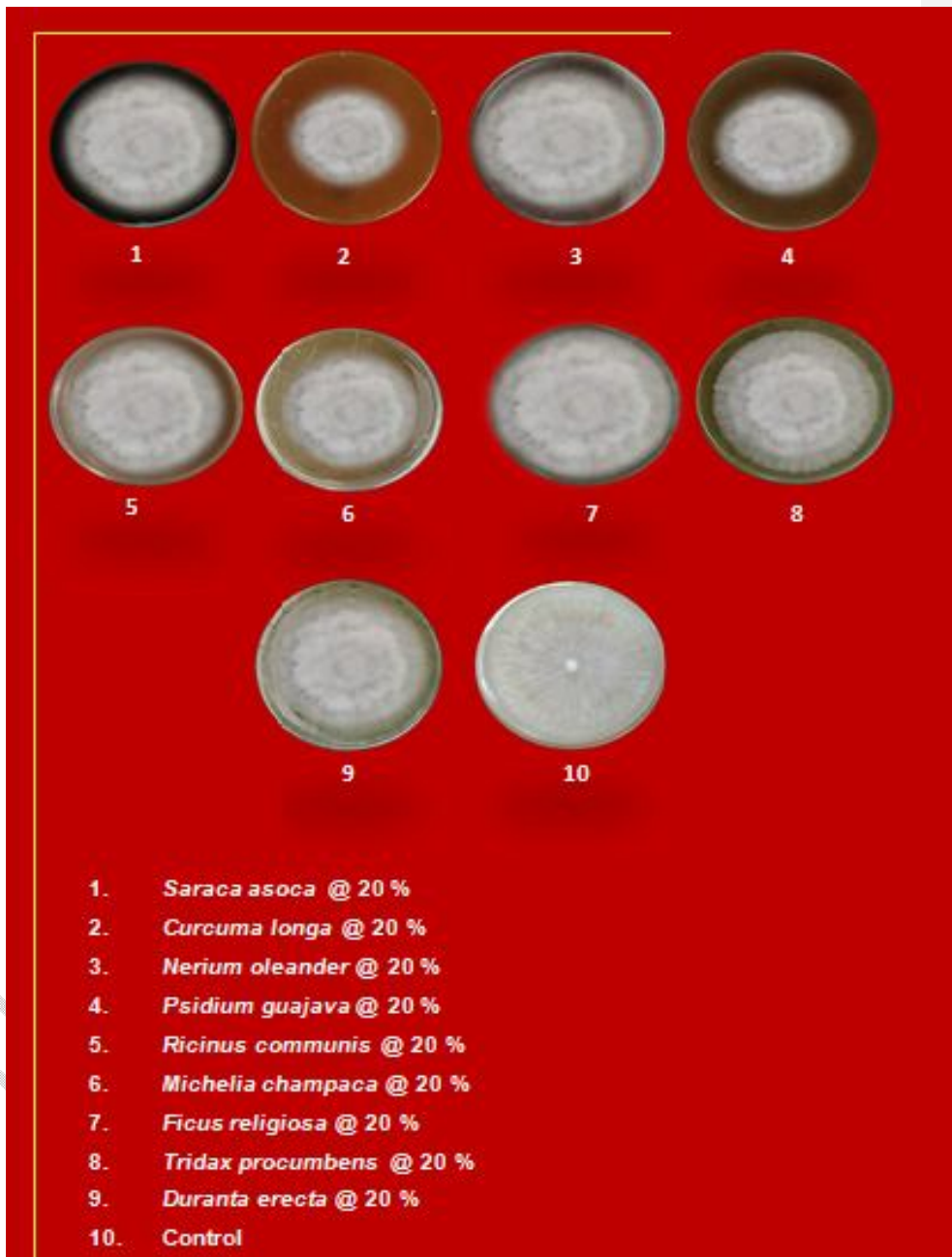


Plate 1: Evaluation of different botanicals against *Sclerotium rolfsii* in vitro.

Table 2: *In-vitro* evaluation of botanicals against *Sclerotium rolfsii*

Botanicals	3 DAI		5 DAI		7 DAI	
	Mycelium growth (mm)	Per cent inhibition	Mycelium growth (mm)	Per cent inhibition	Mycelium growth (mm)	Per cent inhibition
<i>Michelia champaca</i>	19.00	36.67	52.00	17.13	80.50	10.56
<i>Curcuma longa</i>	7.75	74.17	18.50	70.52	39.25	56.39
<i>Ficus religiosa</i>	26.50	11.67	60.25	3.98	86.00	4.44
<i>Nerium oleander</i>	11.25	62.50	21.50	65.74	43.75	51.39
<i>Tridax procumbens</i>	26.50	11.67	53.00	15.54	79.25	11.94
<i>Saraca asoca</i>	20.25	32.50	41.50	33.86	61.50	31.67
<i>Ricinus communis</i>	27.50	8.33	60.75	3.19	90.00	0.00
<i>Duranta erecta</i>	24.00	20.00	48.50	22.71	80.75	10.28
<i>Pisidium guajava</i>	21.50	28.33	46.00	26.69	78.50	12.78
Control	30.00	0.00	62.75	0.00	90.00	0.00
SEm±	0.63	-	1.07	-	0.80	-
C.D. at 5 %	1.83	-	3.08	-	2.30	-

Comment [NM14]: %
 Comment [NM15]: %
 Comment [NM16]: %

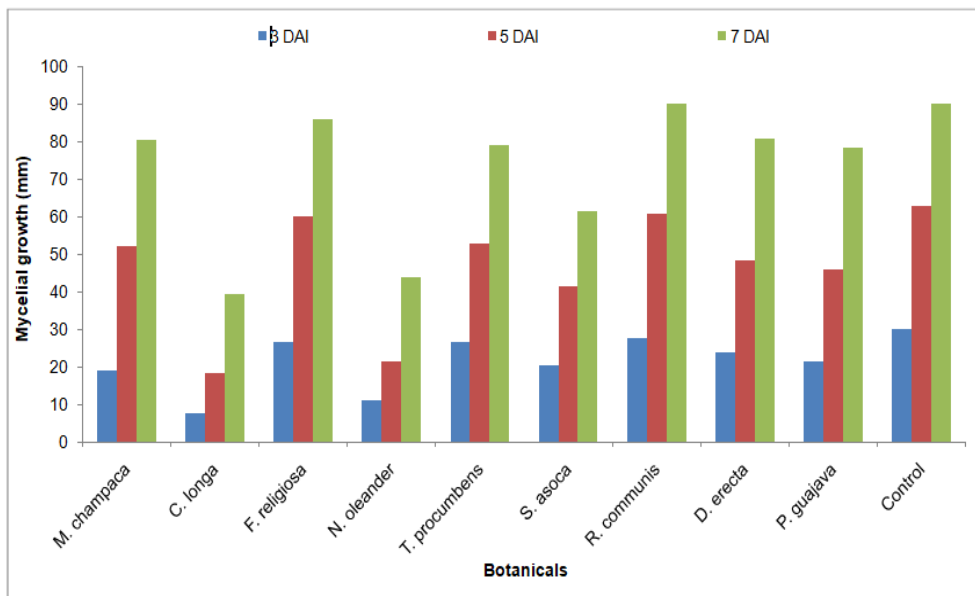


Fig.-1: *In-vitro* evaluation of botanicals against *Sclerotium rolfsii*

Comment [NM17]: Put your figure in order

Discussion

Botanical metabolites and plant-based fungicides is one of the good alternatives because plant extracts are expected to have minimum environment effect and hazards to consumers in contrast to the synthetic fungicides (Varma and Dubey, 1999). Active principles from medicinal plants are being tried as replacements of synthetic fungicides in management of plant diseases in organic farming system. Continuous use of fungicides and chemicals causes pollution in environment and there is a required to minimize the amount of fungicides application to the soil. Thus, plant extracts and bio agents can be used for management of disease as an alternative source. Plants extracts are eco-friendly, protective, curative and antagonistic to many diseases (Kalo and Taniguchi, 1987).

Fungicidal ability of nine botanicals was tested against the growth of *S. rolfsii* at 20% concentration. Significantly least mycelial growth was recorded with *C. longa* followed by *N. oleander*, *S. asoca* and *P. guajava*. Whereas, the botanicals named *R. communis* was found statistically similar with untreated control and recorded maximum mycelial growth. Similar experiment conducted by Bharathi and Benagi, (2018) and reported that minimum mycelial growth was noticed in the combination of garlic bulb extract, rhizome extract of turmeric and black tulsii leaf extract (1:1:1). Srawani and Chandra (2020) evaluated botanical against *S. rolfsii* by using food poison technique. Lemon grass leaf (*Cymbopogon citrates*) showed greater effect in reducing the pathogen growth followed by Datura leaf (*D. stramonium*) and Calotrophis leaf (*C. procera*). Farooq *et al.* (2010) reported that maximum inhibition of *S. rolfsii* was recorded by *Azadirachta indica* (73.8%). Singh *et al.* (2007) reported that foliar extract of neem followed by that of ashoka, caused maximum inhibition of mycelial growth, sclerotial production and viability of *S. rolfsii* causing collar rot of lentil.

Madhavi *et al.* (2011) conducted experiment for *in vitro* evaluated eight different plant extracts against *S. rolfsii*. Neem leaf extract caused maximum inhibition of mycelial growth (80.74%). Sclerotial production was inhibited to an extent of 11% and the inhibition caused

Comment [NM18]: Old reference replace with recent

Comment [NM19]: Please read and rephrase

Comment [NM20]: Too old replace

Comment [NM21]: Spelling check

was maximum with neem extract, followed by *Polyalthia longifolia* [Ashoka]. Singh and Dwivedi (1989) found that *Emblica officinalis*, *A. indica*, ginger extracts and turmeric reduced dry-weight of mycelium and sclerotial production *in vitro* in *S. rolfsii*.

Comment [NM22]: Too old

Conclusion

A laboratory experiment was carried out for evaluation of different botanicals against *S. rolfsii*. Fugicidal ability of nine botanicals were tested against the growth of *S. rolfsii* at 20% concentration. Significantly least mycelial growth was recorded with *C. longa* followed by *N. oleander*, *S. asoca* and *P. guajava*. Whereas, the botanicals named *R. communis* was found statistically similar with untreated control and recorded maximum mycelial growth.

Comment [NM23]: This conclusion does not cap everything any recommendations?

Referances

- Bharathi, A.R. and Benagi, V.I. (2018) *In vitro* evaluation of botanicals and bio-agents against *Sclerotium rolfsii* Sacc. incitant of wilt complex disease of betelvine (*Piper betle* L.). *The Phar. Inno. J.*; 7(6): 334-336
- Dey, T.K.; Ali, M.S. and Chowdhury, N. (1993). Vegetative growth and sporangia production in *Phytophthora colocaseae*. *Indian J. Root crops*, 17 (2): 142-146.
- Duru, C.M. and Onyedineke, N.E., 2010. In vitro study on the antimicrobial activity and phyto- chemical analysis of ethonalic extracts of the mesocarp of *Voacanga africana*. *An. J. plant Physiol.*, 5: 163-169.
- Farooq, M. A.; Iqbal, U.; Iqbal, Sh. M. ; Afzal, R. and Rasool, A. (2010). *In-vitro* evaluation of different plant extracts on mycelial growth of *sclerotium rolfsii* the cause of root rot of sugar beet. *Mycopathology* 8(2): 81-84

Comment [NM24]: Replace

Kalo, F. and Taniguchi, T. (1987). Properties of a virus inhibitor from spinach leaves and mode of action. *Ann Phytopath Sec Japan*, 53:159-167.

Comment [NM25]: Replace

Kochhar, S. L. (2009). Economic Botany in the Tropics. MacMillan India Ltd., Daryaganj, New Delhi. p.658.

Madhavi, G. B.; Bhattiprolu, S.L. and Reddy, V.B. (2011) Effect of various plant extracts on dry root rot of chillies caused by *Sclerotium rolfsii* *J. Hortl. Sci.* 6(2):156-158.

Singh, R.K. and Dwivedi, R.S. 1989. Effect of plant parts and products on morphology and growth of *Sclerotium rolfsii* a foot root rot pathogen of barley. *Acta Bot. Indica*, 17:125-127

Comment [NM26]: Replace

Singh, S., Prajapathi, R.K.; Srivastava, S.S.L.; Pandey, R.K. and Gupta, P.K.2007. Evaluation of different botanicals and non target pesticides against *Sclerotium rolfsii* causing collar rot of lentil. *Ind. Phytopath.*, 60:499-501

Sravani, B. and Chandra, R. (2020) Fungicidal effect of plant extracts on the growth of *Sclerotium rolfsii*, the incitant of collar rot of chickpea . *Int. J.Chemi. Stud.*; 8(1): 389-392.

Thobhunluepop, P., 2009. Implementation of biofungicides and seed treatment in organic rice cv. KDML 105 farming. *Pakistan J. Biol. Sci.* 12, 1119-1126.

Varma, J. and Dubey, N.K. (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. *Curr Sci.*, 76:172-179.

Comment [NM27]: Replace

Vincent JM. 1947. Distortion of fungal hyphae in presence of certain inhibitors. *Nature* 154: 850.

Comment [NM28]: Replace