E-ISSN: 2618-0618
P-ISSN: 2618-060X

www.agronomyjournals.com
2024; 7(10): 124-126
Received: 09-08-2024
Accepted: 12-09-2024

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In vitro exploration of phytoextracts against stem rot of white onion (*Allium cepa* L.) caused by *Sclerotium rolfsii* Sacc.

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DOI: https://doi.org/10.33545/2618060X.2024.v7.i10b.1727

Abstract
The fungus, *Sclerotium rolfsii* Sacc. associated with naturally infected white onion plant was isolated and its pathogenicity was proved. Six phytoextracts *viz*., *Azadirachta indica*, *Allium sativum*, *Sapindus mukorossi*, *Ocimum tenuiflorum*, *Lantana camara* and *Calotropis gigantea* were evaluated against *Sclerotium rolfsii* by following poisoned food technique at 10 and 20 percent concentration *in vitro*. Among phytoextracts tested *Sapindus mukorossi* was most effective both at lower (10%) and higher (20%) concentration with 47.22% and 52.22% inhibition over control, respectively followed by *Azadirachta indica* (23.14% & 30%) and *Allium sativum* (15% & 38.33%).

Keywords: Stem rot, white onion, *Sclerotium rolfsii*, phytoextracts

Introduction
Onion (*Allium cepa* L.) often referred to as "Queen of the Kitchen" is an important vegetable crop grown worldwide. The white onion traditionally grown in the Alibaug region of Maharashtra using only genuine seeds, has been cultivated for generations. This variety is known for its distinctive taste and color attributed to the unique soil texture in which it thrives. Unlike perfectly spherical onions, the white onion has an appealing shape, tapering slightly towards the bottom. Stem rot of white onion caused by *Sclerotium rolfsii* Sacc. is one of the hazardous soil borne diseases, which hinter the onion production in *Konkan* region. The pathogen can remain dormant in soil as sclerotia for several years. Considering the losses (both quantitative and qualitative) and regular incidence of *S. rolfsii* on white onion in recent past years in the *Konkan* region created interest to isolate and study *S. rolfsii* infected on white onion. In the present investigation, an attempt was made to evaluate phytoextracts from locally available plants against *S. rolfsii*. These phytoextracts are affordable for low-income farmers and have the potential for agricultural use, especially with the growing demand for organically produced crops and promote a healthy ecosystem.

Materials and Methods
Isolation of the causal pathogen
White onion plants naturally infected with stem rot disease were collected from the white onion research field of Department of Agronomy, College of Agriculture, Dapoli, and taken to the laboratory of Department of Plant Pathology, College of Agriculture, Dr. B. S. K. K. V., Dapoli for further investigation. The fungus associated with the infected plant tissues was isolated using the standard tissue isolation method on potato dextrose agar (PDA) medium under sterile conditions.

Mass multiplication of pathogen
Sand-maize meal medium (Biswas and Sen, 2000) [3] was used for mass multiplication of the test pathogen. The medium was prepared in 250 ml flasks by combining 10 g of maize meal, 90 g of washed sand and 15 ml of distilled water followed by sterilization at 15 lbs pressure for 45 minutes in autoclave.

Once cooled to room temperature, the sterile sand-maize meal medium was inoculated with 5-8 mycelial plugs each 5 mm in diameter taken from a week-old culture of the test fungus grown on PDA plates and incubated at room temperature for two weeks.

Proving of pathogenicity of isolated fungus

Pathogenicity of *Sclerotium rolfsii* was carried out on disease susceptible white onion Cv. Alibaug local by employing sick soil method. For this purpose, autoclaved and cooled potting mixture of soil, sand and FYM in 2:1:1 ratio was filled into disinfected pots. The mass multiplied inoculum (sand-maize meal medium) was then incorporated at a rate of 60 g/Kg of potting mixture, mixed thoroughly in top 5-10 cm layer, watered lightly and maintained to allow the test pathogen proliferate and make the potting mixture sick with *S. rolfsii*. The fungus was re-isolated from artificially infected plants and it was compared with the characteristics (cultural and morphological) of the original fungus to fulfill Koch's postulates.

Preparation of phytoextract

Following the procedure outlined by Bhatti (1998) [2], aqueous phytoextracts of six botanicals *viz., Azadirachta indica, Allium sativum, Sapindus mukorossi, Ocimum tenuiflorum, Lantana camara* and *Calotropis gigantea* were prepared. Initially, 100 g of fresh plant material was thoroughly washed using sterilized distilled water. The plant material was then blended in a grinder with 100 ml of sterilized distilled water. The resulting mixture was filtered using a double layered muslin cloth and centrifuged at 4000 rpm for 5 minutes. After centrifugation, the supernatant was collected and the pellet was discarded. To prevent bacterial contamination, the supernatant was filtered through Whatman's filter paper No.1, yielding a standard phytoextract with 100% concentration.

***In vitro* efficacy of phytoextracts**

Efficacy of phytoextracts were evaluated *in vitro* at higher and lower concentration against *S. rolfsii* by applying Poisoned Food Technique (Nene and Thapliyal, 1993) [5] in a Completely Randomized Design with three replications. Observations on radial mycelial growth was recorded in all of the replicated treatments after seven days of inoculation. The efficacy of phytoextracts were expressed as percent inhibition of mycelial growth over control and that was calculated by using the formula given by Vincent (1947) [8].

$$\text{Percent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of test fungus in untreated control plate.

T = Growth (mm) of test fungus in treated plate.

Results and Discussion

***In vitro* efficacy of phytoextracts**

Six phytoextracts were tested for their efficacy against *S. rolfsii* at two different concentrations, 10% and 20% by applying "Poisoned Food Technique" (Nene and Thapliyal, 1993) [5] with three replications in Completely Randomized Design. The results obtained on the effect of various phytoextracts on mycelial growth inhibition of test fungus were recorded and are depicted in Table 1, Plate I a & b and Fig. 1.

Data depicted in Table 1, Plate I a & b and Fig. 1 revealed that all the phytoextracts evaluated were effective in inhibiting the mycelial growth of stem rot causing fungus *Sclerotium rolfsii*. Among phytoextracts tested *Sapindus mukorossi* was most effective both at lower (10%) and higher (20%) concentration with 47.22% and 52.22% inhibition over control, respectively. The study also revealed that phytoextract *Azadirachta indica* at lower concentration of 10% showed 23.14 percent inhibition but when concentration increased to 20% showed 30 percent inhibition over control. Also *Allium sativum* at lower concentration of 10% showed 15 percent inhibition but when concentration increased to 20% showed 38.33 percent inhibition. *Ocimum tenuiflorum* at lower concentration of 10% showed 12.03 percent inhibition but when concentration increased to 20% showed 16.85 percent inhibition. *Calotropis gigantea* at lower concentration of 10% showed least (3.7%) inhibition but when concentration increased to 20% increased inhibition to 11.11 percent. *Lantana camara* at lower concentration of 10% were least effective with 1.67 percent inhibition and when increased to 20% concentration showed 20.92 percent inhibition over control.

The results of present investigation are in close conformity with earlier report of Gour *et al*. (2010) [4], recorded maximum inhibition of colony growth of *S. rolfsii* by neem leaves with 27.89% inhibition. The results are also in close proximity to Parvin *et al*. (2016) [6] who tested *in vitro* efficacy of five phytoextracts against *S. rolfsii* and the highest inhibition rate (25.56%) was observed with garlic. Similar results were also reported by Abd-Elghany *et al*. (2021) [1] who recorded that highest inhibition of 45.6% showed by neem oil followed by garlic and onion extracts with percent growth inhibitions of 26.7 and 20, respectively. The results are also in accordance with Thomas *et al*. (2022) [7] who reported that neem leaf extract showed highest percent growth inhibition (29.81%) against *S. rolfsii* followed by tulsi leaf extract (29.07%).

Table 1: *In vitro* efficacy of phytoextracts against *S. rolfsii*

Tr. No.	Phytoextract used	Conc. (%)	Mean Colony dia. (mm)	Percent inhibition	Conc. (%)	Mean Colony dia.(mm)	Percent inhibition
T_1	*Azadirachta indica*	10	69.17	23.14	20	63.00	30.00
T_2	*Allium sativum*	10	76.50	15.00	20	55.50	38.33
T_3	*Sapindus mukorossi*	10	47.50	47.22	20	43.00	52.22
T_4	*Ocimum tenuiflorum*	10	79.17	12.03	20	74.83	16.85
T_5	*Lantana camara*	10	88.50	1.67	20	71.17	20.92
T_6	*Calotropis gigantea*	10	86.67	3.70	20	80.00	11.11
T_7	Control	-	90.00	-	-	90.00	-
	S.E. m ±		0.42			0.42	
	C.D. at 1%		1.78			1.76	

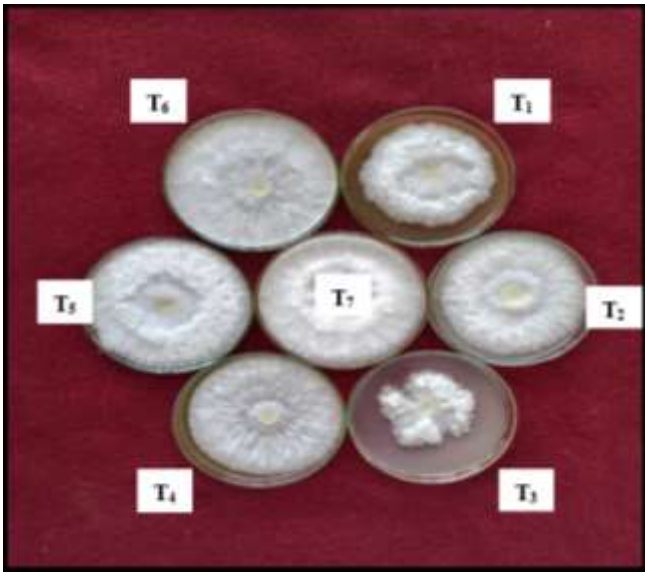

Plate I (a): *In vitro* efficacy of phytoextracts against *S. rolfsii* at lower concentration

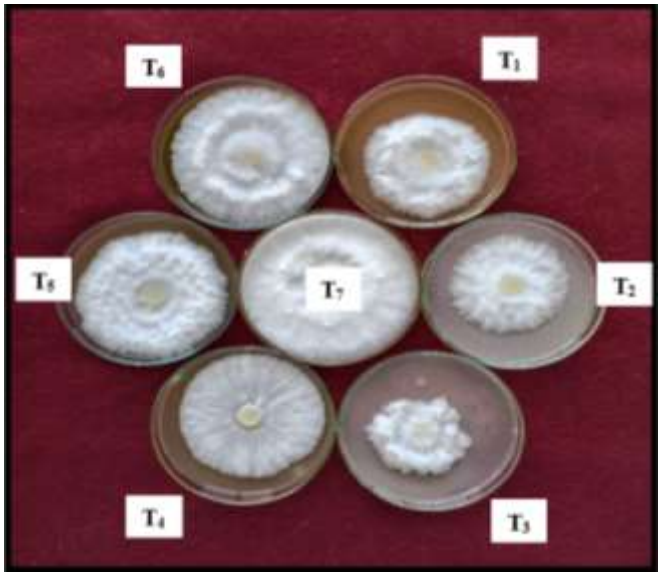

Plate I (b): *In vitro* efficacy of phytoextracts against *S. rolfsii* at higher concentration

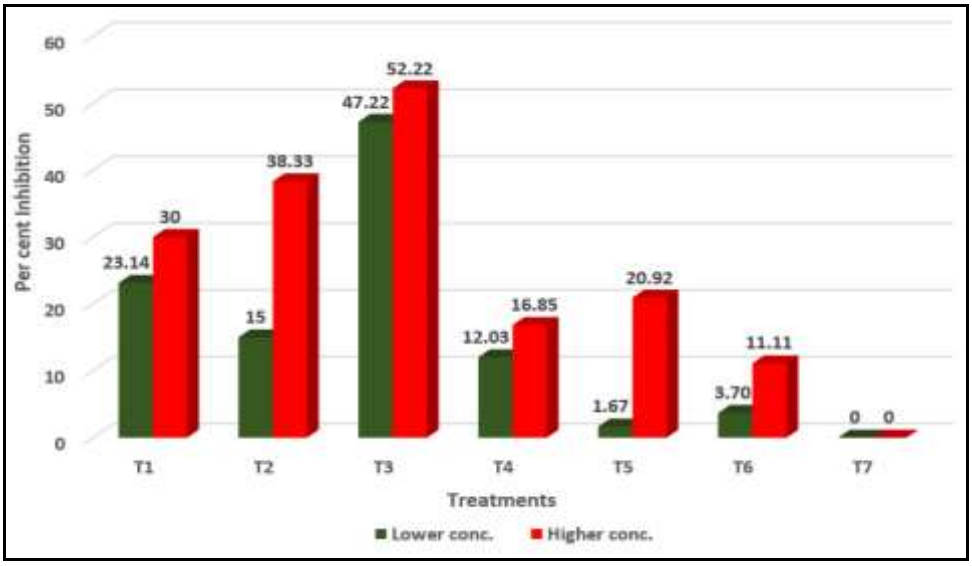

Fig 1: *In vitro* efficacy of phytoextracts against *Sclerotium rolfsii* Sacc.

Conclusions

The obtained result indicated that among six biocontrol agents evaluated *in vitro* against *S. rolfsii*, *Sapindus mukorossi* was significantly effective against *S. rolfsii* showing 47.22% inhibition at the lower (10%) concentration and 52.22% inhibition at the higher (20%) concentration.

Acknowledgement

Authors are thankful to the Department of Plant Pathology, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, (M.S.), India for providing necessary facilities.

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