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Chapter 1

Biocontrol of test pathogen *Sclerotium rolfsii*

Indu Kumari[1]* & R. K. Pandey[2]

[1]Assistant Professor, Department of Botany, Nirmala College, Doranda, Ranchi, Jharkhand, India

[2]University Professor, University Department of Botany, Ranchi University, Ranchi, Jharkhand, India

*Corresponding author email: induraj0016@gmail.com

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https://orcid.org/0009-0001-2930-7108

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ABSTRACT:- In the present study, biocontrol of test pathogen *Sclerotium rolfsii* Curzi (ITCC No.-4737) was evaluated. The effect of chloroform and aqueous extracts of selected plants such as *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. against *Sclerotium rolfsii* Curzi were tested. The chloroform extract was more effective than aqueous extract in controlling the test pathogen *Sclerotium rolfsii*. The minimum mycelial growth of *Sclerotium rolfsii* was recorded in seed extract of *Millettia pinnata* L. with 25.7 mm, using chloroform solvent. Significant inhibition was observed in the extract of *Cassia tora* L. seed with 26.3 mm mycelia growth. 30.7 mm mycelial growth was recorded in the bark extract of *Moringa oleifera* Lam. at 10% concentration. Other treatments in chloroform were also effective to control the mycelial growth of the test pathogen *Sclerotium rolfsii*. In aqueous extracts of selected plants, minimum mycelial growth of *Sclerotium rolfsii* Curzi was found in the seed extract of *Millettia pinnata* L. with 33.7 mm. The bark extract of *Millettia pinnata* L. controlled the mycelial growth with 35.3 mm. The seed extract of *Cassia tora* L. showed mycelial growth of *Sclerotium rolfsii* with 40 mm at 10% Concentration. Significant inhibition was also recorded in other prepared extracts of selected plants.

Keywords- Chloroform, aqueous, *Millettia pinnata* L., *Cassia tora* L., *Moringa oleifera* Lam. and *Sclerotium rolfsii*.

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INTRODUCTION

Sclerotium rolfsii is a soil-borne pathogen. It causes Sclerotium wilt disease in different agricultural crops and reduces the quality and quantity of crops. It attacks mostly legumes, crucifers, and cucurbits. Common plants such as maize (*Zea mays* L.), beans (*Phaseolus* sp.), sugar cane (*Saccharum officinarum* L.), tomato (*Lycopersicon esculentum* Mill.), watermelon (*Citrullus vulgaris* Schrad.) are affected by pathogen *Sclerotium rolfsii.* It also infects other plants like banana, cabbage, carrot, radish, lettuce, cauliflower, cucumber, mustard, onion, garlic, ginger, mango, muskmelon, pineapple, potato, tobacco, etc. The symptoms of the disease are the presence of dark-brown lesions on the stem, yellowing, and wilting of the leaves. In favourable condition, it may destroy all affected crops and reduces crop yield[1-5].

Chemical fungicides like hexaconazole, propiconazole, difenconazole, Avatar, Nativo and Vitavax power are used now a day for controlling the test pathogen[6,7]. Chemical Fungicides may be harmful to the environment and to human health.

Few scientists have worked on biocontrol of *Sclerotium* wilt disease caused by the pathogen *Sclerotium rolfsii* Curzi through plant extracts[8-16].

The aim of the present study was to search plant-based chemical fungicides against the test pathogen *Sclerotium rolfsii.* In the present research work, the effects of chloroform and aqueous extracts of selected plants – *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. against *Sclerotium rolfsii* Curzi (ITCC No.-4737) were studied.

Millettia pinnata L., belongs to the family Leguminosae and is one of the most important leguminous trees. It is mainly found in semiarid regions. It is distributed throughout India. It is a rich source of macronutrients and micronutrients like nitrogen, phosphorus, potassium, calcium, magnesium, zinc, copper, and iron. It acts as an excellent fertilizer source in agriculture and acts as a soil binder. *Millettia pinnata* L. is used for cough, cold, leprosy, diarrhea, mental disorders, ulcers, etc. It can be used as fodder, as green leaf manure, as a soil binder, as a soil reclaimer, and as a biofuel. Different Parts of *Millettia pinnata* L. is used in the medical industry as an anti-ulcer, anti-microbial, anti-plasmodial, anti-diarrhoeal, anti-inflammatory, anti-oxidant and antiviral properties.[17-19] Karanj oil contains karanjin -the main active

chemical ingredient. It is also used as insecticide, pesticide, and nematicide in agriculture.

Cassia tora L., plant of Fabaceae family. It is an important legume crop, mostly found in India, Eastern Africa, and Central America. The common name of *Cassia tora* L. is Charota or Chakunda. It is used as herbal medicine worldwide. Leaves and seeds of *Cassia tora* is used in cough, wound healing, head pains, bronchitis, leprosy, colic, dyspepsia, constipation, cardiac disorders, skin diseases, and liver disorders. It contains phenols, flavonoids, tannins, glycosides, steroids, saponins, and alkaloids. It is used as an antibacterial, antifungal, analgesic, and antihelmintic.

Moringa oleifera Lam. is one of the most important plants, widely distributed in India. It is widely used as a nutritional plant, and belongs to the family Moringaceae. It possesses many pharmacological properties such as anti-diabetic, anti-inflammatory, anti-ulcer, anti-microbial, antioxidant, anti-allergic, and anti-cancer activities[20]. The common name of *Moringa oleifera* Lam is munga or Drumstick or horseradish tree. It is a rich source of vitamins A, C, and protein. Different active phytochemicals such as alkaloids, quinine, saponins, flavonoids, steroids, tannin, glycosides, and fats are present. It also contains kaempferol, kaempferitrin, niazinin A, niazinin B, niazimicin A and niaziminin B[21, 22].

Biocontrol of pathogens through plant extracts may be due to the presence of some alkaloids, phenols, tannins, and flavonoids[23]. Antimicrobial activities of some other plants against *Sclerotium rolfsii* Curzi and other pathogen microbes were studied.[24-28]

MATERIALS AND METHOD

COLLECTION OF SELECTED PLANTS

Different parts of Selected plants were collected from different regions of the Ranchi District of Jharkhand and were identified.

COLLECTION OF TEST PATHOGEN

The test pathogen *Sclerotium rolfsii* Curzi (ITCC No.-4737), used in the present study was collected from the Department of Mycology and Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi.

PREPARATION OF PLANT EXTRACT

Different parts of selected plants - *Millettia pinnata* L. *Cassia tora* L. *and Moringa oleifera* Lam. were collected, washed and dried under shade conditions at room temperature. The dried materials were then ground into fine powder and stored in airtight containers at room temperature till extraction[29]. Plant extracts were prepared from the same plants by extracting 1g of dried material with 10 ml of distilled water or chloroform separately. Extracts were filtered through Whatman No 1 filter paper. The samples were then air-dried and dissolved in 10 ml solvent for the antimicrobial test.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of chloroform and aqueous extracts of different parts of selected plants - *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. at different 1.0 %. 2.5 %, 5.0% and 10.0% concentrations were evaluated against the test pathogen *Sclerotium rolfsii*. Evaluation of antimicrobial activity was done by poison food technique against *S. rolfsii*[28, 30]. The diameter of the mycelial growth of *Sclerotium rolfsii* was calculated after the period of incubation.

RESULT AND OBSERVATION

Biocontrol of test pathogen *Sclerotium rolfsii* Curzi (ITCC No.-4737) was done through plant extracts of *Millettia pinnata* L., *Cassia tora* L., and *Moringa oleifera* Lam. using chloroform and aqueous solvents. Effects of chloroform and aqueous extracts of selected plants such as *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. were given in **Table 1 and Table 2**.

Effects of chloroform extracts of different parts of *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. on mycelial growth of *Sclerotium rolfsii* Curzi at different concentration were recorded in Table -1. Chloroform extracts of selected plants controlled the growth of mycelia of the test pathogen *Sclerotium rolfsii* Curzi. Among all treatments, seed extract of *Millettia pinnata* L. was most effective in which diameter of the mycelia growth of test pathogen *Sclerotium rolfsii* was 25.7 mm. Significant inhibition was also recorded in seed extract of *Cassia tora* L. with 26.3 mm mycelia growth. Bark extract of *Moringa oleifera* Lam. showed 30.7 mm mycelial growth. The bark extract of *Cassia tora* L. exhibited 32 mm mycelial growth. However, Bark extract of *Millettia pinnata* L. showed 33.3 mm mycelial growth of *Sclerotium rolfsii*. Inhibition was also recorded in leaf and stem extracts of *Millettia pinnata*

Table 1 - Effect of chloroform extracts of different parts of *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. on mycelial growth of *Sclerotium rolfsii* Curzi at different concentration

Plant Extracts	1%	2.5%	5%	10%
Millettia pinnata L.,	Growth (mm)	Growth (mm)	Growth (mm)	Growth (mm)
a) Seed	57.3	48.3	36.7	25.7
b) Bark	63.3	53.7	46.7	33.3
c) Leaf	66	55.3	49.3	34.7
d) Stem	70	62	54.7	49.3
Cassia tora L.				
a) Seed	63.3	55.3	39.7	26.3
b) Bark	70	58	49.3	32
c) Leaf	72	66.3	53.7	38.3
d) Stem	74	73.3	60.7	48.7
Moringa oleifera Lam.				
a) Bark	54.7	48	35.3	30.7
b) Leaf	64.3	55.7	45.7	35.7
c) Stem	72	63.3	60.3	47
Check	90	90	90	90

Table 2 -Effect of aqueous extracts of different parts of *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. on mycelial growth of *Sclerotium rolfsii* Curzi at different concentration.

Plant Extracts	1%	2.5%	5%	10%
Millettia pinnata L.,	Growth (mm)	Growth (mm)	Growth (mm)	Growth (mm)
a) Seed	67.3	60	50.3	33.7
b) Bark	75	58.3	52	35.3
c) Leaf	78	64.7	53.3	47
d) Stem	83.7	65.3	60	56
Cassia tora L.				
a) Seed	69	61.7	54.7	40
b) Bark	78.3	64.7	60	53
c) Leaf	82.7	72	63.7	56.3
d) Stem	84.3	76.3	72.7	58.3
Moringa oleifera Lam.				
a) Bark	68	60	60.7	43.3
b) Leaf	74.3	70.7	66.7	56.3
c) Stem	86.3	81	72	61.7
Check	90	90	90	90

L. with mycelial growth 34.7 mm and 49.3 mm respectively at 10% concentration. Other treatments were also effective to control the mycelial growth of test pathogen *Sclerotium rolfsii.*

Biocontrol of test pathogen *Sclerotium rolfsii* Curzi through plant extracts in water solvent are recorded in Table 2. Minimum mycelial growth of the test pathogen was recorded in an aqueous extract of *Millettia pinnata* L. seed which was 33.7 mm. The bark extract of *Millettia pinnata* L. inhibited mycelial growth. The diameter of mycelial growth was 35.3 mm. In the seed extract of *Cassia tora* L., mycelial growth of *Sclerotium rolfsii* was 40 mm. The bark extract of *Moringa oleifera* Lam. showed 43.3 mm mycelial growth. Significant inhibition was also recorded in aqueous extracts of the leaf and stem of *Millettia pinnata* L. with mycelial growth 47 mm and 56 mm respectively. Bark, leaf and stem extract of *Cassia tora* L. also inhibited the growth of mycelia of *Sclerotium rolfsii* with arange from 53 mm to 58.3 mm. The leaf and stem extracts of *Moringa oleifera* Lam. showed 56.3 mm and 61.7 mm mycelial growth of *Sclerotium rolfsii* at 10% concentration.

CONCLUSION

Biocontrol of test pathogen *Sclerotium rolfsii* through some plant extracts is possible. In chloroform and aqueous solvents, extracts of some plants such as *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. were effective to control the mycelial growth of *Sclerotium rolfsii* Curzi at 10% concentration. Chloroform extracts of selected plants were more effective in comparison to aqueous extracts against *Sclerotium rolfsii.* Seed extract of *Millettia pinnata* L. was found to be excellent in inhibiting the mycelial growth of the test plant pathogen at 10 % concentration. From the present research work, it may be concluded that *Millettia pinnata* L., *Cassia tora* L. and *Moringa oleifera* Lam. can be utilized for the control of diseases caused by *Sclerotium rolfsii*. It may be due to the presence of some secondary metabolites like phenols, tannins, saponins, glycosides, flavonoids, steroids and alkaloids.

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