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In vitro Evaluation of Leaf Extracts against *Macrophomina phaseolina* in Mulberry through Poisoned Food Technique

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Abstract

Mulberry (*Morus alba* L.) is a valuable tree of immense importance in silk industry due to its foliage, which constitute the chief food for silkworms (*Bombyx mori* L.) the source of fabulous silk. One of the major constraints in the cultivation and production of quality mulberry leaf is the attack of pests and diseases. Among the several diseases, root rot caused by *Macrophomina phaseolina* (Tassi) Goid is becoming a serious problem in many mulberry growing areas of south India. The root rot infected root samples were collected from the field and used for isolation of the pathogen. Cold water extracts of 10 plants species were screened against the mulberry root rot pathogen *M. phaseolina.* Among them, two plants extracts *viz.,* curry leaf (*Murraya koenigii* L.) and Marunthukoorkan (*Coleus forskohlii*) showed the 67.77 percent and 61.10 percent inhibition of mycelial growth over control respectively. Similarly the sclerotial production showed 87.33 percent and 82.15 percent inhibition over control respectively.

1. Introduction

Mulberry (*Morus alba* L.) is a valuable tree of immense importance in silk industry due to its foliage, which constitute the chief food for silkworms (*Bombyx mori* L.) the source of fabulous silk. This perennial tree/ shrub belonging to the family *Moraceae* with about 10 species is found to be distributed in subtropics and temperate zones of both the hemisphere. The total area of mulberry cultivation in India is around 0.216 million hectare. In India, most of the states have taken up sericulture as an important agro-industry (Ravindran *et al*., 1997) and among the states Karnataka, Andhra Pradesh, Tamil Nadu, Jammu and Kashmir and West Bengal are the major contributors in silk production. One of the major constraints in the cultivation and production of quality mulberry leaf is the attack of pests and diseases. Among the several diseases, root rot caused by *Macrophomina phaseolina* (Tassi) Goid is becoming a serious problem in many mulberry growing areas of south India. Recent reports showed that root rot disease caused by *M. phaseolina* was severe in Coimbatore, Annur, and Udumalpet of Tamil Nadu, India. With a view of identifying the cause of root rot disease in mulberry prevalent parts of Tamil Nadu, to find out suitable ecofriendly management strategies the research work had been carried out.

2. Materials and Methods

The root rot infected root samples were collected from the field and used for isolation of the pathogen. The pathogen was isolated from the infected root by tissue segment method, using Potato Dextrose Agar (PDA) medium. The infected root portions were cut into small bits, surface sterilized in 0.1 percent mercuric chloride solution for 30 seconds and washed in repeated changes of sterile distilled water and plated on to PDA medium in sterilized petri dishes. The plates were incubated at room temperature (28±2 °C) for five days and observed for the mycelial growth of the pathogen.

Fresh leaves of selected ten plants (Table 1) were separately washed and ground with sterile water at the rate of one ml g^{-1} of the material. It was filtered through muslin cloth, finally through Whatman No. 1 filter paper and finally through Seitz filter to free from bacterial contaminants. This formed the standard plant extract solution (100%). This was further diluted to required concentrations (Shekhawat and Prasada, 1971).

About 5 ml of the leaf extract was added to 45 ml of sterilized PDA medium and thoroughly mixed just before planting so as to form 10 percent concentration. 15 ml of this mixture was immediately poured into sterilized Petri plate and allowed to solidify. A 10 mm culture disc of *M. phaseolina* was taken and aseptically placed on to the centre of the medium. Then the plates were incubated at 28±2 °C for 10 days. PDA medium without plant extract served as control. Three replications

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Table 1: Plants selected for evaluation against *M. phaseolina*

Scientific name	Common name	Part used	Family
Coleus forskohlii L.	Marunthu Koorkan	Leaf	Labiatae
Murraya koenigii L.	Curry leaf	Leaf	Rutaceae
Abutilon indicum Mill.	Thuti	Leaf	Malvaceae
Ocimum sanctum L.	Tulsi	Leaf	Labiatae
Vitex negundo L.	Notchi	Leaf	Verbinnaceae
Adathoda vasica L.	Adathoda	Leaf	Acanthaceae
Acalypha indica L.	Kupaimeni	Leaf	Euphorbiaceae
Lantana camera L.	Unnimul	Leaf	Verbenaceae
Abrus precatorius L.	Black kundumani	Leaf	Euphorbiaceae
Gymnema sylvestre L.	Chirukurinchanthazhi	Leaf	Aselpiadaceae

were maintained for each treatment. The diameter of mycelial growth was measured after incubation and percent inhibition of the mycelial growth was calculated by following the method of Vincent (1927).

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

Where,

I = inhibition over control;

C = diameter of the mycelial growth in control (cm),

T = diameter of the mycelial growth in treatment (cm).

2.1 Effect of Leaf Extracts on Sclerotial Production of M. phaseolina under in vitro Conditions

The sclerotial production assay was conducted and three replications were maintained for each treatment. The sclerotial production was observed and recorded after 24 h and the per cent inhibition over control was calculated.

3. Results and Discussion

The effect of various plant extracts on mycelial growth of *M. phaseolina* was tested. The results of the experiments are presented in Table 2 and Figure 1. Cold water extracts of 10 plants species were screened against the mulberry root rot pathogen *M. phaseolina.* Among them, two plants extracts *viz.,* curry leaf (*Murraya koenigii* L.) and Marunthukoorkan (*Coleus forskohlii*) showed the 67.77% and 61.10% inhibition of mycelial growth over control respectively. Similarly, the sclerotial production showed 87.33% and 82.15% inhibition over control respectively. Popularization of biopesticides will be an effective solution save environment as Datar (1999) also investigate and recorded the leaf extract of *Polyalthia longifolia* as most effective against *M. phaseolina* causing charcoal rot of sorghum. Bhatnagar and Bansal (2003) reported that garlic completely inhibited the radial growth of *M. phaseolina,* incitant of stem blight disease of cowpea. Sharma and Gupta (2003) stated that ethanol extracts of plants *Ocimum sanctum* were found highly effective in inhibiting the mycelial growth of *R. solani* under *in vitro* conditions. Girijashankar and Thayumanavan (2005) confirmed the antifungal property of *Prosopis juliflora* leaf extracts which exhibited a maximum of 66.6% and 43.0% inhibition by the methanol and cold water extracts, repectively on *M. phaseolina*.

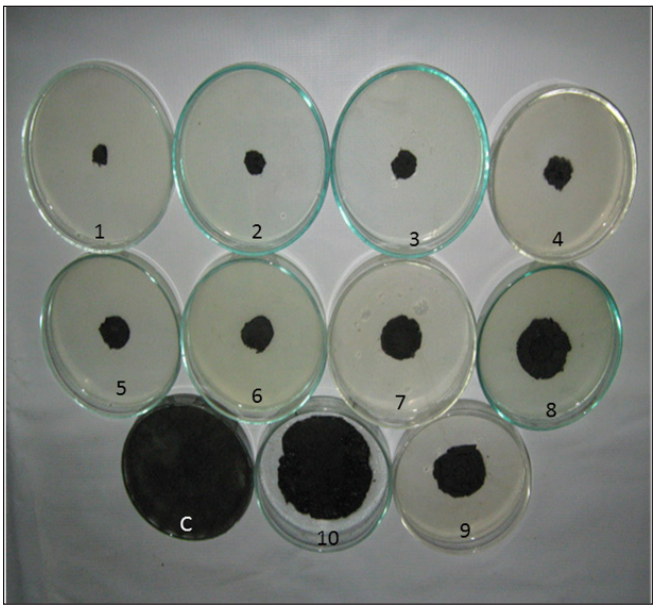

Figure 1: Effect of plant extracts on mycelial growth of M. phaseolina [C = Control; 1 = M. koenigii (10%); 2 = C. forskohlii (10%); 3 = Adathoda vesica (10%); 4 = Abutilon indicum (10%); 5 = Ocimum sanctum (10%); 6 = Vitex negundo (10%); 7 = Acalypha indica (10%); 8 = Lantana camera (10%); 9 = Abrus precatorius (10%); 10 = Gymnema sylvestre (10%)]

4. Conclusion

Extensive and indiscriminate use of pesticides has resulted into several problems like development of resistance in pathogens, food contamination by toxic residue, adverse effect

Table 2: Effect of plant extracts on mycelial growth of *M. phaseolina* (*In vitro*)

Sl. No.	Treatments	Mycelial growth of the pathogen (mm)*	% inhibition over control	Sclerotial production (Nos./disc)*	% inhibition over control
1.	*Coleus forskohlii* L. (10%)	35.0[b]	61.10	27.30[b]	82.15
2.	*Murraya koenigii* L. (10%)	29.0[a]	67.77	19.37[a]	87.33
3.	*Adathoda vasica* L. (10%)	48.0[c]	46.66	32.49[c]	78.76
4.	*Vitex negundo* L. (10%)	68.0[f]	24.44	68.96[f]	54.92
5.	*Ocimum sanctum* L. (10%)	64.0[e]	28.88	56.00[e]	63.39
6.	*Abutilon indicum* Mill (10%)	57.0[d]	36.66	41.52[d]	72.86
7.	*Acalypha indica* L. (10%)	69.0[f]	21.22	69.28[f]	56.18
8.	*Lantana camera* L. (10%)	74.0[g]	17.77	93.16[g]	39.11
9.	*Abrus precatorius* L. (10%)	75.0[g]	16.66	107.8[h]	29.48
10.	*Gymnema sylvestre* L. (10%)	82.0[h]	8.88	108.7[h]	28.30
11.	Untreated control	90.0[i]	-	153.0[i]	-

*Values are mean of three replications means followed by a same letter are not significantly different at the 5% level by DMRT.

on parasitoids and high cost. At this juncture popularization of biopesticides will be an effective solution to save environment.

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