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In Vitro Screening of Plant Extracts, Nutrients and Organic Amendments for Management of Mulberry Root ROT (*Macrophomina phaseolina* (Tassi.) Goid)

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Abstract: ***Ten plant species were screened under in vitro conditions for the management of mulberry root rot (Macrophomina phaseolina (Tassi.) Goid.) Among them, two plants extracts viz., curry leaf (Murraya koenigii L.) and Marunthu koorkan (Coleus forskohlii) showed the 67.77 percent and 61.10 per cent inhibition of mycelial growth over control respectively. Six nutrients were screened against the M. phaseolina. The experimental results showed that Zinc sulphate (0.1 %) inhibited the mycelial growth and sclerotial production to a tune of 68.81 per cent and 70.19 per cent respectively over control. Five organic amendments were screened against M. phaseolina under in vitro condition. The results showed that Jatropha cake (5%) was found best with 84.72 and 84.20 per cent inhibition of mycelial growth and sclerotial production over control respectively, over control***

Keywords: Mulberry, Plant extracts, Nutrients, organic amendments, *Macrophomina phaseolina.*

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. Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is becoming a serious problem in many mulberry growing areas of south India. Application of oilcakes *viz.,* neem, castor, groundnut and mahua at 0.2 and 1 per cent (w/w) reduced the population of recoverable propagules of *M. phaseolina* cotton (Dwidevi and Singh, 1986). Zinc sulphate 50 kg/ha reduced the root rot incidence (*M. phaseolina*) on cowpea, blackgram and greengram (Latha *et al*., 1997). Saini and Kumar (1997) reported that silicon and tin possess remarkable fungicide effect on *M. phaseolina.* Cobalt and copper at the concentration of 400 ppm inhibited the growth of *M. phaseolina* of sesame (Gabr *et al.,* 1998). Organic substances used @ 5 % level were more effective as compared to 3 % or 1 % levels. Use of organic amendments rich in or supplemented with N has been found to be effective in the control of *Macrophomina* infection (Ghaffar *et al*., 1969). Organic substances used @ 5 % level were more effective as compared to 3 % or 1 % levels. Use of organic amendments rich in or supplemented with N has been found to be effective in the control of *Macrophomina* infection (Ghaffar *et al*., 1969). Soil amended with dry fragments of alfalfa, clover, wheat and cotton reduced the population of sclerotia of *M. phaseolina* in soil.

2. Materials and Methods

The efficacy of the following ten botanicals, six nutrients and five organic amendments were evaluated for their antifungal activities against management of *M. phaseolina* under *in vitro* conditions.

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 vesica 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.	Chirukurinchan thazhi	Leaf	Aselpiadaceae

Nutrients

1. Zinc sulphate (0.1%)
2. Calcium nitrate (0.1%)
3. Magnesium sulphate (0.1%)
4. Calcium carbonate (0.1%)
5. Calcium chloride (0.1%)
6. Ferrus sulphate(0.1%)

Organic amendments

1. Neem cake (5%)
2. Jatropha cake (5%)
3. Seribed waste (5%)
4. Groundnut cake (5%)
5. Gingelly cake (5%)

Fresh leaves of selected ten plants were separately washed and ground with methanol 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 fiter 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 so as to form 10 per cent concentration.

About 100 mg of nutrients and 5 g of organic amendments were added to 100 ml of sterilized PDA medium so as to form 0.1 and 5 per cent concentration of nutrients and organic amendments. 15 ml of these three mixtures were 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 ± 2^0 C for 10 days. PDA medium without plant extract, nutrients and organic amendments were served as control. Three replications were maintained for each treatment. The diameter of mycelial growth was measured after incubation and per cent inhibition of the mycelial growth was calculated by following the Vincent's method (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)

3. Result and Discussion

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 Marunthu koorkan (*Coleus forskohlii*) showed the 67.77 percent and 61.10 per cent inhibition of mycelial growth over control respectively. Similarly the sclerotial production showed 87.33 per cent and 82.15 per cent inhibition over control respectively. The present results are in agreement with those reported by Girijashankar and Thayumanavan (2005). They confirmed the antifungal property of *Prosopis juliflora* leaf extracts which exhibited a maximum of 66.6 per cent and 43.0 per cent inhibition by the methanol and cold water extracts, respectively on *M. phaseolina.* Datar (1999) in his investigation recorded the leaf extract of *Polyalthia longifolia* as most effective against *M. phaseolina* causing charcoal rot of sorghum. Such pathogens are even more important in mulberry IDM as leaves are fed to silkworms. 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. Gautam *et al.,* (2003) screened 24 botanicals (Family: Asteraceae) the maximum inhibition of *M. phaseolina* was caused by *Eclipta alba* at 24 hrs but it got decreased at 72 hrs time interval. At 24 hrs the maximum inhibition of *Chrysanthemum coronarium* was less when compared to *Eclipta alba* but it is increase at 72 hrs time interval.

Six nutrients were screened against the *M. phaseolina*. The experimental results showed that Zinc sulphate (0.1 %) inhibited the mycelial growth and sclerotial production to a tune of 68.81 per cent and 70.19 per cent respectively over control followed by Calcium nitrate and Calcium carbonate (0.1%) recorded 50.36 per cent and 41.80 per cent inhibition of mycelial growth over control respectively. With regard to sclerotial production recorded 54.42 per cent and 45.52 per cent inhibition over control respectively (Table 2). Findings by Lakpale *et al.* (1997) also reported that Zinc sulphate 500 ppm concentration retarded the mycelial growth and dry weight of *R solani.* The disease reduction is most often attributed to improved nutrition that boosts host defense or directly inhibits fungal growth and its activity (Huber, 1989).

Five organic amendments were screened against *M. phaseolina* under *in vitro* condition. The results showed that Jatropha cake (5%) was found best with 84.72 and 84.20 per cent inhibition of mycelial growth and sclerotial production over control respectively, followed by neem cake and seribed waste (Table 3). Regarding the use of concentration of organic amendments, Ghaffar *et al.* (1969) reported that Organic substances used @ 5 % level were more effective as compare to 3 % or 1 % levels and also the use of organic amendments rich in or supplemented with N has been found to be effective in the control of *Macrophomina* infection. Amendment of seeds powder of local trees not only enhanced the plant growth but also reduced the infection of pathogenic fungi like *M. phaseolina, Fusarium* spp. and *R. solani* present in soil which cause root rot disease in plants Ahmed *et al.* (2009).

Table 1: Effect of plant extracts on mycelial growth of *M. phaseolina* (*In vitro*)

SI. No	Treatments	Mycelial growth of the pathogen (mm)*	per cent inhibition over control	Sclerotial production (Nos./disc)*	per cent 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 vesica* 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.

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

SI. No	Treatments	Mycelial growth of the pathogen (mm)*	per cent inhibition over control	Sclerotial production (Nos./disc)*	per cent inhibition over control
1.	Zinc sulphate (0.1%)	29.00[a]	68.81	45.00[a]	70.19
2.	Calcium nitrate (0.1%)	46.16[b]	50.36	69.10[b]	54.42
3.	Calcium carbonate (0.1%)	54.12[c]	41.80	82.26[c]	45.52
4.	Magnesium sulphate (0.1%)	62.05[d]	33.00	93.53[d]	38.05
5.	Calcium chloride (0.1%)	65.60[d]	30.53	105.36[e]	30.22
6.	Ferrus sulphate (0.1%)	69.96[e]	24.77	108.42[e]	28.19

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

Table 3: Effect of organic amendments on mycelial growth of *M. phaseolina* (*In vitro*)

SI. No	Treatments	Mycelial growth of the pathogen (mm)*	per cent inhibition over control	Sclerotial production (Nos./disc)*	per cent inhibition over control
1.	Jatropha cake (5%)	13.50[a]	84.72	23.42[a]	84.20
2.	Neem cake (5%)	34.00[b]	61.52	52.26[b]	64.76
3.	Seribed waste (5%)	42.64[c]	51.74	67.48[c]	54.49
4.	Groundnut cake (5%)	68.35[d]	22.69	96.69[d]	33.45
5.	Gingelly cake (5%)	86.00[e]	8.64	131.0[e]	11.66
6.	Untreated control	88.36[e]	-	148.3[f]	-

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

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