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Evaluation of Certain Plant Extracts and Antagonists Against *Fusarium solani* and *Alternaria tenuissima*, the Incitants of Root Rot and Die-Back Diseases of Mulberry

Seetha Ramulu J.[1,*], Raja Gopal Reddy C.[2] and R. Ramanjaneyulu[3]

[1]*Andhra Pradesh State Sericulture Research and Development Institute, Kirikera - 515211, Hindupur, Andhra Pradesh, India*
[2]*Department of Bio-technology, School of Herbal Studies and Naturo Sciences, Dravidian University, Kuppam, Andhra Pradesh, India*
[3]*Department of Microbiology, Sri Krishnadevaraya University, Anantapur-515003, Andhra Pradesh, India*

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The studies have been conducted to control the soil borne fungal pathogens *viz*, *Fusarium solani* (Mart) Sacc. and *Alternaria tenuissima* the incitants of root rot and die-back diseases on mulberry stem cuttings planted in the mulberry nurseries and also in established mulberry gardens ten plant extracts with 10% concentration except *Lantana camara* (undiluted) were tested through poisoned food technique and four bio-fungicides were also screened by dual culture method under *in vitro* conditions. Plant extract of *Prosopis juliflora* showed the maximum inhibition on the mycelial growth (81.2% over *A. tenuissima* and 80.0% over *F. solani*) and followed by *L. camara* (66.7% over *A. tenuissima* and 68.9% over *F. solani*). Among the antagonists *Pseudomonas fluorescens* and *Trichoderma viride* showed maximum inhibition on the mycelial growth of both pathogenic fungi. The promising plant extracts (*P. juliflora* and *L. camara*) and antagonists (*P. fluorescens* and *T. viride*) were tested against both the pathogenic fungi under *in vivo* conditions along with the existing popular chemical Mancozeb. All the tested plant products and bio-fungicides showed inhibitory effect on both fungi. But the maximum survival percentage of mulberry cuttings was recorded in the treatment with *T. viride* (95% against *F. solani* and 90% against *A. tenuisssima*) followed by *P. fluorescens* (90% against both fungi) and *T. harzianum* (80% against *F. solani* and 85% against *A. tenuisssima*). Incase of the treatments with plant extracts and chemical fungicide the *P. juliflora* (60% against *F. solani* and 55% against *A. tenuisssima*) showed higher survival percentage and followed by *L. camara* (55% against *F. solani* and 50% against *A. tenuisssima*) and Mancozeb (55% against both fungi). In case of control only 10% of survival was recorded in *F. solani* inoculated cuttings and 15% survival in *A. tenuissima* inoculated cuttings.

Key words: Antagonists, *Alternaria tenuissima, Fusarium solani*, Mulberry nursery, Plant extracts, Survivability

Introduction

Mulberry (*Morus alba* L.) is the only food plant of the Silkworm (*Bombyx mori* L.) and is propagated through stem cuttings planted in nurseries or direct planting of cuttings in the main field. Stem cuttings in nurseries were affected by various soil borne fungal pathogens (Gupta *et al*., 1997; Singh and Chohan, 1984; Sukumar *et al*., 1991). Among all, the cutting rot caused by *Fusarium solani* (Mart) Sacc. poses serious problem during initial development of stem cuttings and also causes root rot in established mulberry gardens (Gupta *et al*., 1997; Philip *et al*., 1996; Sukumar *et al*., 1991). The disease incidence of *Alternaria tenuissima* causing die-back of pruned mulberry branches was reported by Reddy *et al*. (2002) and the incidence in nurseries was reported by Seetha Ramulu *et al*. (2008). It causes drying of stems, reduction in the number of lateral branches of the main stem in established gardens and in case of nurseries it leads to the death of the stem cuttings.

*To whom the correspondence addressed
Andhra Pradesh State Sericulture Research and Development Institute, Kirikera - 515211, Hindupur, Andhra Pradesh, India.
Tel: 09908936081; 08556 247381;
E-mail: sithara_jolapuram@rediffmail.com

At present most of the farmers depends on chemicals like Mancozeb and Carbendazim for controlling the soil borne fungal pathogens at nursery stage in mulberry. But these chemicals are effective up to a certain period only and the pathogens will reenter in to the host when these chemicals loose their effectiveness. Another disadvantage is the development of ecological imbalance and increase in soil toxicity due to continuous usage of chemicals. Under this situation there is every necessity to make use of environment friendly biological products that are readily available in nature for combating pests and diseases. In the present study, an attempt was made to evaluate eco-friendly products against these two fungal pathogens of mulberry under *in vitro* and *in vivo.*

Materials and Methods

Screening of plant extracts against *F. solani* and *A. tenuissima* under *in vitro*

Ten plant extracts were tested against the mycelial growth of *A. tenuissima* and *F. solani* through poisoned food technique (McCallan, 1947) under *in vitro* conditions. The plant parts of selected plants were collected and cleaned with fresh water, followed with sterile water and then dried under shade. Individual samples were ground in sterile distilled water (1 ml/1 g) with the help of mortar and pestle. Then this material was taken in a beaker and boiled at 80°C for ten minutes in hot water bath (Awuah, 1989). The material was homogenized for 5 minutes and filtered through muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. This was taken as basic stock solution.

Ten plant extracts were tested against both the fungi at 10% concentration except that of *L. camara* which was used without dilution. For preparation of 10% plant extract, 10 ml of stock solution is mixed with 90 ml of PDA medium and sterilized. The sterilized medium is poured into Petri plates and allowed to set. Actively growing PDA culture discs (5 mm) of *A. tenuissima* and *F. solani* were placed separately at the center of the plate under aseptic conditions. PDA plates without plant extract served as control. Three Petri plates with PDA medium poisoned with Mancozeb for each fungus were maintained for comparison. The plates were incubated at 28°C ± 1°C till the mycelium in the control plates covers the entire plate. The results were expressed in terms of per cent inhibition of mycelium over control (Vincent, 1947).

$$\text{Inhibition}(\%) = \frac{\text{Radial growth of fungus in control(cm)}}{\text{Radial growth of fungus us control}} -$$

$$\frac{\text{Radial growth in the treatment(cm)}}{\text{Radial growth of fungus us control}} \times 100$$

Screening of antagonists against *F. solani* and *A. tenuissima* under *in vitro*

The fungal antagonists *viz*, *T. viride, T. harzianum* and *T. pseudokoningii* and the bacterial antagonist *P. fluorescens* were tested against the *A. tenuissima* and *F. solani* by Dual culture technique (Hung and Hoes, 1976). PDA (20 ml) was poured aseptically in each of sterilized Petri plates and allowed to solidify. Mycelial disc of 4 mm diameter from margin of four-day-old culture of antagonists and test fungi were placed simultaneously at distance 5 cm from each other on PDA medium. The bacterial antagonist *P. fluorescens* was separately streaked on the opposite side of the pathogen. Each treatment was replicated thrice and control (pathogens and antagonists) were maintained. The plates were incubated at 28 ± 1°C till the mycelium in the control plates covers the entire plate. After incubation the growth of antagonist and test fungus was measured by radial measurement. Index of antagonism was determined as ratio of difference between growths in control to that in treatment and multiplies by 100. Per cent inhibition was calculated and analyzed statistically.

Screening of plant extracts and antagonists against *F. solani* and *A. tenuissima* under *in vivo*

To test the efficacy of promising plant extracts and antagonistic fungi against the *A. tenuissima* and *F. solani,* five treatments with two bio-fungicides (*T. viride* and *P. fluorescens*), two plants extracts (*L. camara* and *P. juliflora*) and one chemical fungicide (Mancozeb) were used for conducting the experiment under controlled conditions. Control pots were inoculated with the test fungi separately and left without treatment. All the treatments were given before planting the cutting in to the inoculated soil.

Soil sterilization

The soil was sterilized in hot air oven at 140°C for four hours. After getting it cooled. The soil is divided into two parts and used for inoculation of each test fungus *A. tenuissima* and *F. solani* to each divided part of the sterilized soil in aseptic condition.

Inoculation

The test fungi were grown in corn meal - sand medium for inoculation. (Rangaswamy and Mahadevan, 2001). The 15 days old culture incubated at 29 ± 1°C of *A. tenuissima* and *F. solani* were used for inoculation of sterilized soil. The corn meal-sand culture of the *A. tenuissima* and *F.*

solani was thoroughly mixed with sterilized soil separately at 10% level (w/w - 90 g sterilized soil + 10 g inoculum). Each of the test fungus was mixed with sterilized soil separately and poured in to polythene bags (1 kg inoculated soil / bag). Later twenty cuttings were treated with each test bio-fungicide, plant products and Mancozeb separately and planted in the polythene bags (one cutting/ bag). Twenty cuttings were maintained for each treatment and control. All the bags were watered on alternate days with sterilized water and kept in open condition.

The above explained method was used for preparation of plant extracts. The cuttings were dipped in plant extracts of required concentration (*L. camara* at 100% and *P. juliflora* at 10% concentration) for half an hour and 100 ml of plant extract was added to the inoculated soil in the bag and treated cuttings with plant extract were planted. The talc based formulations of bio-fungicides were used for treatment. The talc based bio-fungicide was mixed with distilled water to form fine slurry and the cleaned mulberry cuttings were dipped in to the slurry for 30 minutes and 10 g of bio-fungicide was added to each bag containing inoculated soil with test fungi and treated cuttings with bio-fungicide were planted in the bags. In case of chemical treatment the cuttings were dipped in 0.1% Mancozeb solution for 30 minutes and 100 ml of 0.1% Mancozeb solution is added to the inoculated bag and cuttings were planted. The disease index was observed and the survival percentage was calculated to each treatment and assessed the efficacy of the bio-fungicides, chemical fungicide and plant products.

Results

In vitro studies

All the tested plant extracts showed the inhibitory effect on mycelial growth of the pathogenic fungi. Among all the plant extracts *P. juliflora* showed highest inhibition on both fungi (81.2% over *A. tenuissima* and 80.0% over *F. solani*) and followed by *L. camara* (66.7% over *A. tenuissima* and 68.9% over *F. solani*) as shown in Table 1. In the present study significant differences in inhibition of mycelial growth was observed in *A. tenuissima* and *F. solani* by the interaction with *T. viride* and *P. fluorescens*. These two antagonists showed high levels of inhibition (80~88%) as shown in Table 2, where as *T. pseudokoningii* and *T. harzianum* showed moderate level of inhibition against both pathogenic fungi.

In vivo studies

All the tested plant products and bio-fungicides showed inhibitory effect on *F. solani*. In the treatment with bio-fungicide the maximum survival percentage was recorded in *T. viride* (95%) followed by *P. fluorescens* (90%) and *T.*

Table 1. Efficacy of plant extracts against the mycelial growth of *A. tenuissima* and *F. solani* under *in vitro* by poisoned food technique

Botanical source	A. tenuissima		F. solani	
	Mycelial growth (cm)	Inhibition (%)	Mycelial growth (cm)	Inhibition (%)
Prosopis juliflora, leaf	1.70	81.17	1.80	80.03
Calotropis procera, leaf	3.80	57.80	3.60	60.03
Annona squaimosa, seed	3.60	60.03	5.90	34.50
Cassia occidentalis, leaf	7.00	22.27	7.60	15.60
Ocimum sanctum, leaf	6.20	31.17	7.40	17.80
Azadiracta indica , seed	3.40	62.30	6.90	23.40
Eucalyptus globules, leaf	7.60	15.60	7.80	13.40
Aloe vera, leaf	7.73	14.13	8.60	4.50
Lantana camara, leaf	3.00	66.70	2.80	68.93
Vinca rosea, leaf	7.90	12.27	4.10	54.50
Mancozeb	0.40	95.56	0.60	93.33
Control	9.00	--	9.00	--
CD ($P \leq 0.05$)	0.29	3.22	0.25	2.75

Each value is an average of three replications.

Table 2. Efficacy of antagonists against the mycelial growth of *A. tenuissima* and *F. solani* under *in vitro* by dual culture method

Name of the antagonist	Mycelial growth Inhibition of *A. tenuissima* (%)	Mycelial growth inhibition of *F. solani* (%)
Trichoderma viride	87.7	88.9
Trichoderma harzianum	64.4	68.9
Trichoderma pseudokoningii	73.3	80.0
Pseudomonas fluorescens	83.3	78.9
Control	0.0	0.0
C D ($P \leq 0.05$)	2.61	2.66

Each value is an average of three replications.

Table 3. Efficacy of plant extracts and antagonistic fungi against the *A. tenuissima* and *F. solani* under simulated conditions (*In vivo)*

Sl. No.	Treatments	No. of cuttings planted	Soil inoculated with *F. solani*			Soil inoculated with *A. tenuissima*		
			No. of cuttings survived	Survival percentage	Percent over control	No. of cuttings survived	Survival percentage	Percent over control
1	Trichoderma viride	20	19	95	+ 85	18	90	+ 75
2	Trichoderma harzianum	20	16	80	+ 70	17	85	+ 70
3	Pseudomonas fluorescens	20	18	90	+ 80	18	90	+ 75
4	Lantana camara	20	11	55	+ 45	10	50	+ 35
5	Prosopis juliflora	20	12	60	+ 50	11	55	+ 40
6	Mancozeb	20	11	55	+ 45	11	55	+ 40
7	Control	20	2	10	0	3	15	0

+ = Increase over control ; Each value is an average of three replications.

harzianum (80%). Incase of the treatments with plant extracts, *P. juliflora* (60%) showed higher survival percentage and followed by *L. camara* (55%). Mancozeb recorded 55% survivability. In case of control only 10% of survivability was recorded. Percent improvement of survivability over control was recorded 85.0%, 70.0%, 80.0%, 45.0%, 50.0% and 45.0% in the treatments with *T. viride, T. harzianum, P. fluorescens, L. camara, P. juliflora* and Mancozeb respectively. The details were presented in Table 3.

All the treatments showed inhibitory effect on *A. tenuissima* under simulated conditions and showed maximum protection against the pathogen than the control. In the treatment with bio-fungicide the maximum survival percentage was recorded in *T. viride* (90%) and *P. fluorescens* (90%) followed by *T. harzianum* (85%). In the treatments with plant extracts *P. juliflora* (55%) gave higher survival percentage and followed by *L. camara* (50%). The chemical treatment with Mancozeb (55%) also showed maximum protection against the pathogen. In case of control only 15% of survivability was recorded. The details of the treatment against *A. tenuissima* were presented in Table 3. Percent improvement of survivability over control was recorded higher with 75.0%, 70.0%, 75.0%, 35.0%, 40.0% and 40.0% in the treatments with *T. viride, T. harzianum, P. fluorescens, L. camara, P. juliflora* and Mancozeb respectively.

Discussion

The present study reported that, the plant extracts of *P. juliflora* and *L. camara* were found to be effective in inhibiting the growth of *A. tenuissima* and *F. solani.* The present findings are agreeing with the earlier reports of Ganesan (1993) with the fungi toxic properties of *P. juliflora*. Antifungal properties of extracts of *P. juliflora* have also been reported by Seshakiran *et al.* (2006) against *Sclerotium rolfsii* and Vadivel *et al.* (2006) against *Alternaria solani*. Muthulakshmi (1990) reported the leaf extract of *P. juliflora* is effective against *A. tenuissima* under *in vitro* by poisoned food technique. Varshney (2001) also reported the anti-fungal properties of *L. camara* against *Drechslera graminea.* Jeewa and Thakore (2005) reported the extracts from *L. camara* were effective in inhibiting the growth of *F. solani.*

In the present study *P. fluorescens* and *T. viride* were found to be effective in controlling *F. solani* and *A. tenuissima.* According to Philip *et al.* (1995) *T. harzianum* is effective against *Fusarium* species. Philip (1996) reported the antagonistic effect of a local isolate of *T. harzianum* against the mulberry root rot pathogen with 74% protection to the mulberry plants. Rajendran (1996) observed the reduced root rot incidence caused by *Rhizocotonia bataticola* with the application of *T. viride* and *T. harzianum* significantly. Bandyopadyay *et al.* (2003) screened some *Trichoderma* strains against major root pathogens. Jha and Jalali (2006) reported the effective inhibition of mycelial growth of *F. solani* by *T. viride*. Sachendra Bohra *et al.* (2005) reported the treatment with *Pseudomonas* significantly reduces the disease severity of *Fusarium* wilt in the nursery. According to Jeevan Ram and Thakore (2005) *T. harzianum* and *P. fluorescens* could suppress the growth of *F. solani,* the storage rot pathogen of ginger. Mostapha (2006) reported *P. fluorescens* have an excellent potential to be used as biocontol agents against mulberry root rot pathogens *viz. B. theobromae*, *F. solani, F. oxysporum* and *R. solani*. All these findings were agreeing with the present findings.

It is finally reveals from the present research findings,

the bio-fungicides *viz, Trichoderma viride* and *Pseudomonas fluorescens* and *Prosopis juliflora* (plant extract) were suggested and can be used as pre-plantation treatment for effective control of cutting rot and die-back diseases of mulberry nursery.

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