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EFFICACY OF BOTANICAL EXTRACTS AGAINST *FUSARIUM OXYSPORUM* SCHLECHT CAUSING MULBERRY ROOT ROT–AN *IN VITRO* EVALUATION

Manmohan, M. S. & Govindaiah
Department of Sericulture/Life Science, Plant Protection Laboratory, Jnanabharathi campus, Bangalore University, Bangalore – 560 056, Karnataka, INDIA
Corresponding Author's Email: manmohan.mana@gmail.com

ABSTRACT
Mulberry root rot disease which is caused by *Fusarium oxysporum* Schlecht is one of the most serious disease, because mulberry is perennial crop and the pathogen is soil borne in its habitat. Twenty locally available botanicals were opted to screen against the pathogen in laboratory conditions namely *Amorphophallus campanulatus* Blume., *Artocarpus heterophyllus* Lamk., *Azadirachta indica* A. Juss., *Calotropis gigantea* R.Br., *Calotropis procera* (Ait) R.Br., *Carica papaya* Linn., *Cassia auriculata* Linn., *Cathranthus roseous* (L.) G. Don., *Citrus limon* (Linn.) Rurm.f., *Eucalyptus globulus* Labill., *Euphorbia hirta* Linn., *Gliricidia maculata* (Kunth) Kunth., *Lawsonia inermis* Linn., *Michelia champaca* Linn., *Muntingia calibura* L., *Pongamia pinnata* (Linn.) Merr., *Psidium guajava* Linn., *Syzygium cumini* (Linn.) Skeels., *Tagetes patula* L., and *Terminalia catappa* L. The aqueous extracts of the plant materials were prepared to test their efficacy against *F. oxysporum* by 'spore germination method' and 'poison food technique'. All the aqueous plant extracts showed inhibition in spore germination and mycelial growth in varying degrees excluding *A. heterophyllus* (it enhances the growth of the pathogen). Some of them were very effective, while others are moderate and less effective. *P. guajava* has recorded maximum inhibition in both spore germination and in mycelia growth with 96.3% and 86.5% respectfully, followed by *L. inermis* with 95.7% and 85.1% in spore germination and mycelia growth respectively. Where as, *A. indica* showed 90.7% inhibition in spore germination and 67.7% in mycelia growth. *P. pinnata* showed 81.1% spore and 63.4% mycelia growth inhibition and is followed by *C. procera* with 61.1% inhibition in spore germination and 67.3% in mycelia growth. Further, the promising results obtained from some botanicals during *in vitro* evaluation will be tested in field conditions.

KEY WORDS: Mulberry, root rot, *F. oxysporum*, botanical extracts, spore germination and mycelial growth.

INTRODUCTION

Mulberry (*Morus alba* L.) belongs to the family moraceae, a fast growing deciduous and perennial plant. It is the sole food plant of the silkworm (*Bombyx mori* L.) for silk production. So, mulberry cultivation and silk production together comprises sericulture, which is an important agro based labor intensive cottage industry with greater employment opportunities in rural areas. It is usually cultivated for years together as a mono-crop in the same field and its production is restricted to 70 days/crop, thus completing 5 crops/year (Dandin *et al.,* 2003). The major constraints for successful cultivation of mulberry and quality leaf production are insufficient water, poor agrochemical inputs, weeds and the outbreak of pests and diseases (Govindaiah *et al.,* 2005). Mulberry is affected by number of diseases caused by fungi, bacteria, mycoplasmas, viruses and nematodes. Fungal diseases cause the greater recognizable damage to crop productivity. Due to repeated harvesting of leaf, the soil nutrient gets depleted and makes the plant vulnerable to the soil borne diseases. Among them, root rot disease caused by *Fusarium oxysporum* Schlecht is the major one (Mallikarjuna. B. *et al.,* 2010). Root rot disease is a major problem for mulberry cultivation in SouthIndia. The disease incidence ranges from 10 to 14% in hot spot areas (Philip *et al.,* 1995). The affected plants showsudden withering of leaves followed by death of plants along with decaying and rotting of roots and stem cuttings. The disease is soil borne in nature and spreads fast primarily through contaminated soil, irrigation, diseased saplings farm implements etc. due to its epidemic nature and potential to kill the plants completely, root rot is the deadliest disease of mulberry. Chemical control of disease is the most common and widely accepted method in the agricultural crops; however, the use of these pesticides and fungicides is very dangerous due to the residual effect on silkworm. Therefore the use of botanicals has been assumed special significance for eco-friendly management of the disease.

MATERIALS AND METHODS

Preparation of aqueous plant/leaf extracts

About twenty aqueous extracts of locally available plants such as *Amorphophallus campanulatus* Blume., *Artocarpus heterophyllus* Lamk., *Azadirachta indica* A. Juss., *Calotropis gigantea* R.Br., *Calotropis procera* (Ait) R.Br., *Carica papaya* Linn., *Cassia auriculata* Linn., *Cathranthus roseous* (L.) G. Don., *Citrus limon* (Linn.) Rurm.f.,

Eucalyptus globulus Labill., *Euphorbia hirta* Linn., *Gliricidia maculata* (Kunth) Kunth., *Lawsonia inermis* Linn., *Michelia champaca* Linn., *Muntingia calibura* L., *Pongamia pinnata* (Linn.) Merr., *Psidium guajava* Linn., *Syzygium cumini* (Linn.) Skeels., *Tagetes patula* L., and *Terminalia catappa* L. were prepared by w/v method. The healthy leaves were collected from the selected plants and surface sterilized with 0.1% HgCl2 and subsequent washings have done with sterile water for two-three times. The excess water content was removed by keeping them under shade for few minutes till they become semi dried (Parveez *et al.,* 2009). These semi dried leaf materials were chopped aseptically and homogenized in clean mixer grinder using sterile distilled water at the rate of 1:1 ratio. The homogenized extracts were filtered through double layered sterile muslin cloth. The filtrates prepared were centrifuged at 2000rpm for 20min. and the clear supernatants were collected by filtering through sterile Whattman No. 42 filter paper discs. The extracts thus obtained were considered as stock or standard and used to prepare desired test concentrations (v/v) of 10%, 20% and 30% using sterile distilled water.

***In vitro* screening of aqueous leaf extracts by spore germination method**

The antifungal effect of leaf extracts on conidial germination of the pathogen were tested using different concentrations of aqueous plant extracts by spore germination method using cavity slides (Reddick and Wallance, 1910 and Maji *et al.,* 2005). Spore suspension of *F. oxysporum* was prepared aseptically from 7 days old pure culture. One drop (50µl) of spore suspension and one drop (50µl) of different concentrations of aqueous leaf extracts were taken on separate cavity slides. One cavity was maintained as control without adding any extract. All treatments were maintained in triplicates. And the cavity slides were incubated at ambient temperature (25±2°C) in moist chambers (in large Petri dishes containing blotting papers blotted with sterile water) for 24hrs. After the incubation period, observations were made under microscope to calculate the percentage inhibition (PI) by counting the number of spore germinated and the total number of spores in different microscopic view by using the formula given by Vincent in 1947.

$$PI = \frac{Gc - Gt}{Gc} X \ 100$$

Where, Gc = Germination in control, Gt = Germination in treatment

***In vitro* screening of aqueous leaf extracts by poison food technique**

Further, all the leaf extracts were subjected to secondary screening to test their efficacy on the growth of the pathogen by poison food technique (Philip *et al.,* 1997 and Sharvelle, 1961). Before poisoning the food, the botanical extracts were kept in water bath for about 20minutes at 45^0 C (Nishitha Naik. 2006). Required concentrations of 10%, 20% and 30% of poison food were prepared by mixing the autoclaved PDA medium with botanical extracts in separate conical flasks. These were taken into 90mm Petri dishes and allowed solidify. Upon solidification, 5mm mycelial discs made by sterile cork borer were taken from the peripheral region of the 7 days old pure culture, and then kept at the center of the plate in aseptic condition. Plates without adding extract were served as control. Triplicates have been maintained. All the plates were incubated for 14 days at room temperature (25±2^0 C). After the incubation period, the effects of extracts were determined by measuring the radial growth of the pathogen in the test plates. This was compared with control to calculate the percentage inhibition of mycelia of the pathogen by Vincent's (1947) formula

$$PI = \frac{Mc - Mt}{Mc} X \ 100$$

Where, Mc = Mycelial growth in control, Mt = Mycelial growth in treatment

RESULTS

All the twenty botanical extracts tested showed varied degree of inhibition over control in the conidial/spore germination (table – 1 and figure – 1) and mycelial growth (table – 2 and figure – 2) of the pathogen *F. oxysporum* at different concentrations. All the results obtained from three different concentrations were interpreted in terms of their mean value. The maximum inhibition of spore germination and mycelial growth was recorded in *P. guajava* with 96.1% and 86.2% respectively. This was followed by *L. inermis* with 94.5% and 85.9%, *A. indica* with 90.2% and 68.5%, *P. pinnata* with 79.9% and 63.2%, *C. gigantea* with 75.5% and 65.6% inhibition in spore germination and inhibition in mycelial growth respectively and thus, these four are highly significant. Significant results were also observed in *C. procera* which showed average results with 60.2% inhibition in spore germination and 63.8% inhibition in mycelial growth, which is followed by *E. globulus* with 64.1% and 42.0%, *M. calibura* with 46.3% and 43.0%, *T. patula* with 54.8% and 32.7%, *E. hirta* with 51.8% and 36.8%, *G. maculata* with 25.5% and 42.3%, *A. companulatus* with 71.9% and 25.3%, *S. cumini* with 43.5% and 43.5% inhibition in spore germination and mycelial growth respectively. Minimum results were observed in *T. catappa* with 24.1% and 41.8%, *C. roseus* with 21.6% and 33.8%, *C. limon* with 5.2% and 17.5%, *C. papaya* with 3.1% and 21.7% inhibition in spore germination and mycelial growth respectively and they are less significant. *A. heterophyllus, C. auriculata* and *M. champaka* are not significant and recorded no inhibition in spore germination at all the concentrations. Where as, inhibition in mycelial growth was observed with those treatments with 3.8%, 8.6% and 18.0% respectively. It was observed that, in most of the treatments there was significant interaction with respect to the concentrations. With the increase in the concentration of the extract, there was corresponding increase in the inhibition of the pathogen.

DISCUSSION

The results obtained are in conformity with the Nidhi Sharma and Trivedi (2002) who have reported that the leaf extract of *L. inermis* was effective against mycelial growth of cumin wilt caused by *Fusarium oxysporum* f. sp. cumini. They have also screened *C. procera* (Ait.) R. Br. against the pathogen and found effective. Maji *et al.,* (2005) have screened *C. gigantea*, *Cassia tora* and *Cassia sophera* against some mulberry foliar pathogens by conidial germination and poison food technique and obtained good results. Nishitha Naik (2006) has screened *C. limon, T. erecta* and *L. inermis* against *F. solani*, another root rot pathogen of mulberry and obtained significant results with *L. inermis* in poison food technique.

The present study reveals that, aqueous extract of *P. guajava* and *L. inermis* gives significant results with maximum inhibition of spore germination and mycelial growth in all the concentrations. This was followed by *A. indica* and *P. pinnata* in higher concentrations. These botanicals will be further evaluated in field conditions.

TABLE -1: *In vitro* screening of aqueous plant extracts against *F. oxysporum* by spore germination method

Sl No.	Treatment	Percentage Inhibition over Control			
		Concentrations			
		10%	20%	30%	Mean
1	Control	1.1	1.6	1	1.2
2	*Amorphophallus campanulatus* Blume.	69.9	70.9	74.9	71.9
3	*Artocarpus heterophyllus* Lamk.	-1.8	-1.6	-1.0	-1.4
4	*Azadirachta indica* A. Juss.	86.8	91.4	92.5	90.2
5	*Calotropis gigantea* R.Br.	72.2	75.5	79.0	75.5
6	*Calotropis procera* (Ait) R.Br.	57.9	60.8	62.1	60.2
7	*Carica papaya* Linn.	0.3	2.0	7.0	3.1
8	*Cassia auriculata* Linn.	-1.8	-1.6	-1.0	-1.4
9	*Cathranthus roseous* (L.) G. Don.	13.0	20.5	31.5	21.6
10	*Citrus limon* (Linn.) Rurm.f.	3.4	3.8	8.4	5.2
11	*Eucalyptus globulus* Labill.	54.9	67.7	69.9	64.1
12	*Euphorbia hirta* Linn.	43.8	52.9	58.9	51.8
13	*Gliricidia maculata* (Kunth) Kunth.	32.9	39.4	44.4	25.5
14	*Lawsonia inermis* Linn.	92.9	93.8	96.9	94.5
15	*Michelia champaca* Linn.	-1.8	-1.6	-1.0	-1.4
16	*Muntingia calibura* L.	24.5	54.6	60.0	46.3
17	*Pongamia pinnata* (Linn.) Merr.	77.8	80.0	82.0	79.9
18	*Psidium guajava* Linn.	92.6	96.2	99.6	96.1
19	*Syzygium cumini* (Linn.) Skeels.	33.7	45.3	51.7	43.5
20	*Tagetes patula* L.	44.3	56.6	63.6	54.8
21	*Terminalia catappa* L.	7.2	24.1	41.1	24.1
	CD @ 5%	1.640	1.477	1.101	

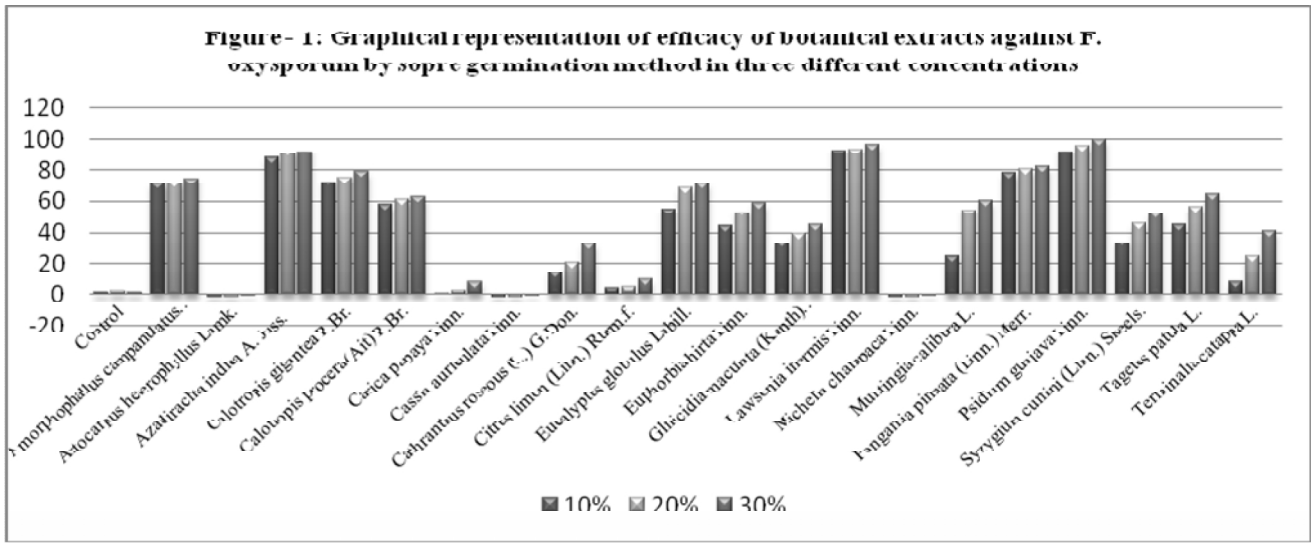
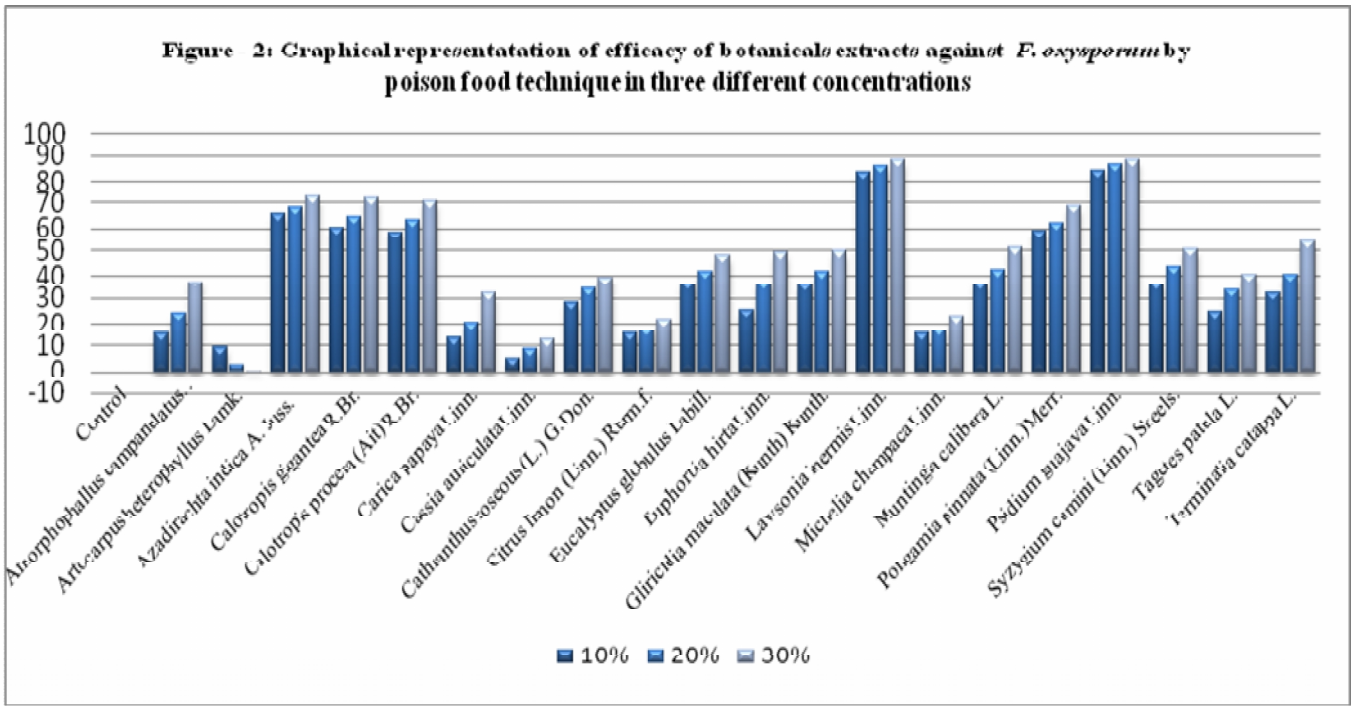


TABLE - 2: *In vitro* screening of aqueous plant extracts against *F. oxysporum* by poison food technique

Sl No.	Treatment	Percentage Inhibition over Control			
		Concentrations			
		10%	20%	30%	Mean
1	Control	0.0	0.0	0.0	0.0
2	*Amorphophallus campanulatus* Blume.	14.5	23.9	37.6	25.3
3	*Artocarpus heterophyllus* Lamk.	9.3	2.3	-0.1	3.8
4	*Azadirachta indica* A. Juss.	65.4	67.9	72.4	68.5
5	*Calotropis gigantea* R.Br.	60.4	64.7	71.9	65.6
6	*Calotropis procera* (Ait) R.Br.	58.2	62.9	70.3	63.8
7	*Carica papaya* Linn.	12.8	20.3	32.2	21.7
8	*Cassia auriculata* Linn.	5.0	8.7	12.1	8.6
9	*Cathranthus roseous* (L.) G. Don.	28.2	34.0	39.4	33.8
10	*Citrus limon* (Linn.) Rurm.f.	14.7	16.5	21.3	17.5
11	*Eucalyptus globulus* Labill.	36.2	41.8	48.0	42.0
12	*Euphorbia hirta* Linn.	25.1	36.4	49.0	36.8
13	*Gliricidia maculata* (Kunth) Kunth.	35.6	41.7	49.6	42.3
14	*Lawsonia inermis* Linn.	83.7	86.0	88.2	85.9
15	*Michelia champaca* Linn.	14.7	16.5	22.8	18.0
16	*Muntingia calibura* L.	36.0	42.2	50.8	43.0
17	*Pongamia pinnata* (Linn.) Merr.	58.6	62.1	69.0	63.2
18	*Psidium guajava* Linn.	83.9	86.5	88.3	86.2
19	*Syzygium cumini* (Linn.) Skeels.	36.1	43.8	50.6	43.5
20	*Tagetes patula* L.	24.4	33.4	40.5	32.7
21	*Terminalia catappa* L.	31.9	40.4	53.3	41.8
	CD @ 5%	**1.574**	**1.291**	**1.310**	



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