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Antifungal properties of indigenous phytoextracts against *Fusarium* spp. causing root rot in mulberry (*Morus* spp.)

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Abstract

This study aimed to evaluate the antifungal properties of selected indigenous plant species as potential alternative strategy to manage root rot in mulberry *(Morus* spp). Bioassays of 12 phytoextracts indicated varying fungicidal activity. The *in vitro* experiment suggests that *Bangbangsit* (*Lantana camara* L.), Banaba (*Lagerstroemia speciosa* (L.) Pers.), *Eucalyptus* (*Eucalyptus globulus* Labill.), *Hagonoy* (*Chromolaena odorata* (L.) R.M.King & H.Rob.), and *Neem* (*Azadirachta indica* A.Juss) have fungicidal properties that inhibited the growth of *Fusarium* spp *in vitro*. Greenhouse trials showed that, *bangbangsit* (*Lantana camara* Linn) extract was superior over *banaba*, *eucalyptus, neem* and *hagonoy* extracts in reducing root rot infection at 30 and 60 days after planting (DAP), respectively. These plants could be utilized to access their effectiveness in field condition. It will help in the formulation of ecofriendly control measures, which is cheap and can be recommended for the sericulture farmers against the root rot disease in mulberry.

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Introduction

In the Philippines, mulberry root rot, caused by a soil born fungus, *Fusarium* spp. (Telan and Gonzales, 1998), was first observed in mulberry plantation of the Sericulture experimental area in La Union province, spread to Ilocos Sur in 1994. The disease is rampant and alarming in India [Biswas, (1992) and Teotia and Sen (1994)]. In 2008, the disease caused 30%-40% field infestation to the mulberry area in the region, that lead to immediate uprooting and burning infected plants to avoid wide spread outbreaks and to save the healthy plants. In any high value crops, chemical pesticides are most commonly used for controlling diseases in the field. However, their adverse effects on soil beneficial microorganisms and the environment cannot be ignored. Continuous use of potentially hazardous chemicals is posing an increased threat to environment. At present, chemical control measures, such as sapling treatment with Dithane M 45 and Benlate drenching of soil before planting at recommended rate to control the disease is the normal practice to eliminate the disease.

The use of locally available plants in the control of pests is an ancient technology that has been used in many parts of the world. Some plants viz. *Derris, Nicotiana* and *Ryania* were used to combat agricultural pests during prehistoric period. In 1940's, such botanical pesticides were partly displaced by synthetic pesticides that at the time seemed easier to handle and longer lasting. In different countries, the use of synthetic pesticides has undoubtedly resulted in achievement of green revolution, though increased crop production. However, due to detrimental effects of the use of chemicals, to environment, an attempt to other eco-friendly botanical pesticides may be recommended strategy in the management of mulberry root rot. In fact, these alternatives will help in the formulation of ecofriendly control measures, which is cheap and can be recommended for the farmers against the disease as reported by Sultana *et al.* (2011) and Saqib, Malik., Shinwari, and Shinwari (2011). Considering the need for an alternative approach to control the phytopathogens, it was believed to be worthwhile to screen the antifungal effects of locally available flora (Bhardwaj, 2012).

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish, Mohana, Ranhavendra and Raveesha (2007).

At present, no data on ecologically sound management strategy using botanical fungicides against the disease is established, thus this attempt. Further, the use of plant resources for its antifungal activity is an attractive avenue for the development of sustainable mode of sericulture, hence, indigenous and locally available, need to be explored for their antifungal property. The study aimed to investigate the antifungal properties of 12 phytoextracts against *Fusarium* spp. causing root rot in mulberry, in vitro and potential phytoextracts *in vivo*.

Materials and methods

Selected twelve indigenous plants locally used for medicinal purposes based on their abundant availability during the cropping season were evaluated for their fungicidal properties *in vitro* and *vivo* against *Fusarium* spp. causing root rot in mulberry.

Collection of Plant Species

Collection of plant species was done at the DMMMMSU- SRDI and neighboring towns yield a total of 12 plant species, i.e., Acapulco (*Cassia alata* L.), Bangbangsit (*Lantana camara* L.), Banaba (*Lagerstroemia speciosa* (L.) Pers., Eucalyptus (*Eucalyptus globulus* Labill.), Garlic vine (*Mansoa alliacea* (Lam.) A.H.Gentry, Hagonoy (*Chromolaena odorata* (L.) R.M.King & H.Rob.) Lemon Grass (*Cymbopogon marginatus* (Steud.) Stapf ex Burtt-Davy), Tubli (*Derris elliptica* (Wall.) Benth.), Madre Cacao (*Gliricidia sepium* (Jacq.) Walp.), Neem tree (*Azadirachta indica* A.Juss.), Oregano (*Origanum vulgare* L.) and Yellow Ginger (*Curcuma longa* L.).

In vitro Bio assay

Fungal and spore seeded methods were used to evaluate the efficacy of 12 plants under laboratory condition**.** For fungal plug method, twelve (12) locally available plants, which are known for their medicinal values were tested for their fungicidal properties against *Fusarium* spp., the plant pathogen causing mulberry root rot.

Phytoextracts of plant leaves and rhizomes were prepared after yhe method of Telan *et al.*, 1998. The fresh plant parts were taken and washed with running tap water followed by sterile distilled water. It was then ground with sterile distilled water at the rate of 1.0 ml per gram of plant tissue (1:1 v/w) with mortar and pestle and filtered through double layered white muslin cloth.

The filtrate so obtained formed the standard plant extract solution, i.e., 100%. The plant extracts thus prepared were tested *in vitro* against the mycelial groth of Fusarium spp by adding the different phyto extracts to potato dextrose agar (PDA). The one (1) liter phyto-extract decocsion was prepared by adding with 39 grams commercially available potato dextrose agar (PDA) and was poured into sterile petri plates and allowed to set. Three replications were maintained for each Phytoextracts. The petriplates were planted with mycelium plug (4mm in diameter) of the test fungus, taken from the margin of five-day old pure culture. The mycelium disc inoculated on PDA with no plant extract but with only sterile water serves as control plates. The whole set up was incubated in inverted manner at 26 ± 1°C in incubator for six days. A 4-mm diameter mycelia disc of the test organism was inoculated on each pre mixed agar plate. Inoculated plates were incubated at 25±1°C and growth was measured along the perpendicular lines. Daily radial growth (in mm) of test fungus in all of the test extracts was recorded for six days. Each treatment was replicated thrice with appropriate untreated control. Each treatment was replicated thrice. All the culture plates were incubated in dark condition. The mycelia growth of fungus was measured after 1,3 and 6 days of incubation. The percent inhibition was calculated by measuring the diameter of mycelial growth in control plates less the diameter of mycelial growth in treated colony divided by the diameter of mycelial growth in control plates. The percent inhibition was calculated using the formula:

$$\% \text{ of inhibition} = \frac{\text{Diameter of control colony} - \text{Diameter of treated colony}}{\text{Diameter of control colony}} \times 100$$

For the spore seeded-method, same preparation of decocsion was done, however the pathogen was inoculated using suspension. With the aid of sterilized pipette, two ml of spore or mycelial suspension were seeded into sterilized petri plates. Ten (10) ml of melted PDA with decocsion of phyto materials (at 40-45°C) were aseptically poured into the plates. The plates were rotated gently to fully incorporated and evenly distribute the spore mycelia with the agar and allowed to congeal at room teperature. Data on fungal growth and development was obtained and statistically analyzed to ascertain significant differences.

Greenhouse Trial/ In vivo Test

Greenhouse or in vivo tests were carried out at the nursery and greenhouse experimental area of the SRDI experimental area for two consecutive trials. The effective plant extracts that showed high percentage of inhibiton *invitro* test were evaluated under greenhouse condition. Five per cent (5%) of *Bangbangsit (Lantana camara* Linn), *Banaba* (*Lagerstroemia speciosa* Linn), *Eucalyptus* (*Eucalyptus globus),* Hagonoy (*Chromolaena odorata),* and Neem tree (*Azadirachta indica)* were further prepared to evaluate their effect *in-vivo* condition. Systemic fungicide at recommended rate and plain water was used as positive and negative controls, respectively.

Preparation of Rice Grain-hull substrate for Fusarium spp

Bottles (1liter capacity) were filled with previously soaked rice grains were stoppered with aluminum foil secured with rubber bands and sterilized at 15 psi for two (2) hours. The substrated were allowed to cool after sterilization. The substrate bottles were then aseptically inoculated with pure culture of Fusarium spp using a transfer needle. The inoculated substrate bottles were incubated at room temperature for two- three weeks.

Preparation of Planting medium

The prepared grain ricehull sbustrate in bottles inoculated with *Fusaium* spp were thoroughly mixed with previously sterilized soil. The incorporation was done late in the afternoon and covered with banana leaves to avoid sudden stressful environmental condiuton.

Preparation of Cuttings

Alfonso cuttings were incubated for 5 days at shaded place and watered regularly to enhance root germination. Incubated cuttings were soaked to the different phyto extracts solutions overnight before planting. The nursery beds were inoculated and incorporated with previously mass produced *Fusarium* spp., two (2) days before the planting of mulberry cuttings. A susceptible mulberry variety (cv Batac) cuttings were planted in Randomized Complete Block Design (RCBD) in 1×3m² plots with three replications for each treatment. The cuttings were dipped into the different plant extracts for one (1) hour before planting in the designated nursery beds. The excess plant extract solutions were further drenched to the nursery beds planted with cuttings previously soaked according to the designated treatment. In control plot, cuttings were dipped with fungicides at recommended rate (RR) and with plain water, respectively.

Data on disease incidence, and plant height (cm) were recorded at 30, 60 days after planting (DAP). Diseased incidence was gathered by getting the total number of saplings infected with *Fusarium* rot divided by the total number of saplings assessed multiplied by 100. Plant height (in cm) was gathered by measuring the plant from the base to the tip of the tallest leaf. Disease incidence was gathered by counting the plants observed to be infected with MRR divided by the total number of plants observed multiplied by 100 calculated as per the formula given below:

Plant disease incidence (%) =

$$\frac{\text{Total number of plants infected by MRR}}{\text{Total number of plant assessed}} \times 100$$

Results and discussion

Laboratory/In vitro bioassay

Of the total number of bioassayed for fungicidal efficacy, all the twelve plants or phyto - extracts tested showed varied degree of inhibition in plug and spore seeded methods over control in the conidial/ spore germination and and mycelial growth, respectively.

The results as presented in Table 1 show that the phytoextracts were effective in significantly reducing the growth of mycelia as compared with control plates.

Phyto extracts of *Bangbangsit* (*Lantana camara* Linn), *Banaba* (*Lagerstroemia speciosa L*inn), *Eucalyptus (Eucalyptus globus), Hagonoy* (*Chromolaena odorata*), and *Neem* tree (*Azadirachta indica)* plants showed promising effect as shown by zone of inhibition of *Fusarium* spp.

The growth of *Fusarium spp* was totally inhibited by *Bangbangsit (Lantana camara Linn*), *Banaba (Lagerstroemia speciosa Linn), Eucalyptus (Eucalyptus globus)* and *Hagonoy (Chromolaena odorata)* with the corresponding zero (0.0) percent growth compared with control with corresponding 100% growth in fungal plug and spore seeded methods, respectively. The plant extract of *Madre de cacao* (*Gliricidum sepium), Oregano (Origanum vulgare),* and *Lemon grass (Cymbopogon marginatus),* showed comparatively slightly low activity against *Fusarium spp*., while garlic vine (*Mansoa alliance), neem tree (Azadirachta indica)* and *Tubli (Derris elliptica)* showed inhibitory effect ranging from 88.77%-82.67%.

The effect of different phyto extracts showed statistical difference. This might be due to the varying effectiveness of the plant extracts concentrations. But it is interesting to note that the performance of phyto extracts against *Fusarium* spp did not vary in either plug or spore seeded methods.

Table 2 shows the performance of phyto extracts against *Fusarium spp*. under greenhouse condition. Treated saplings with different plant extracts showed significant differences in disease incidence (DI) % and plant height (cm) as compared with saplings treated with *Fusarium spp* without treatment. *Bangbangsit* (*Lantana camara)* and *Hagonoy (Chromolaena odorata) s*howed significantly lowest percent disease incidence of only 13.75% and 14,08%, respectively among the materials tested, i.e., *banaba, eucalyptus, neem* and commercial fungicide, 30 days after planting (DAP). Moreover, same results are observed 60 DAP. It may be noted from the results that among the phyto extracts, *bangbangsit* extract was superior over *banaba, eucalyptus, neem* and *hagonoy* extracts to control root rot infection in 30 and 60 DAP, respectively.

The mulberry saplings in nursery beds, when treated with *bangbangsit* showed a very low disease incidence, 13.75% and 17.81% infection in mulberry plants compared to untreated naturally infested soil with root rot incidence of 38.54% and 41.81%. Moreover, 67.32% and 57.40% reductions of root rot infection of mulberry plants were achieved in the greenhouse trials. The phytoextracts treated saplings are as vigorously growing as those saplings treated with fungicide at recommended rate, therefore, no phytotoxicity reactions were observed. These phytoextracts are rich source of bioactive secondary metabolites of wide variety such as tannins, terpenoids, saponins, flavonoids, and other compounds, reported to have in vitro antifungal properties against *Fusarium* spp, a pathogen causing FRR disease. Furthermore, these phyto-extracts are siginificantly effective in inhibiting the mycelial growth and spore germination of *Fusarium* in vitro and on the development of FRR in mulberry in-vivo when used as a pre-planting treatment.

The above results corroborate with the earlier reports of Singh (2010) which indicated that phytoextracts like neem products were effective in reducing several fungal diseases of crop plants under field conditions. Further, they concluded that such an antifungal activity of neem extract might be due to the presence of active chemicals like azadirachtin, nimbidin, nimbinin, nimbolidin, nivasin etc. contained in the extract. Moreover, in many study conducted, plant extracts act as a soil treatment against soil borne fungi like *Phytium aphanidermatum* and *R solani* (Guttierrez, Shew,. and Melton (1997); Mathew and Gupta, 1996; Sachin , Upamanyu, Gupta and Shyam 2004), *Fusarium oxysporum* (Singh, Frisvad, Thraneand Mathur (1991) and Singh, Shukla, Prakash, Kumar Singh Mishra and Dubey (2010) and *Colletotrichum atramentarium* (Kannaiyan and Prasad, 1981). Some plant extracts acts as contact fungicides and found effective in disrupting cell membrane integrity at different stages of fungal development, while others interfere with metabolic process. Sunita Chandel and Manica Tomar (2008) disclosed that clove extract inhibited the growth of *Rhizoctonia solani*, *R. oryzae, R. oryzae-sativa* by 100%; while neem leaf, rosemary and pelargenium extracts were considered to be potential phytoextracts to control the tested soilborne phytopathogens.

In addition, *in vivo* experiment showed that mulberry cuttings treated with *P. juliflora* and and *L. camara* showed 60% and 55% survival percentage, respectively. This is in connection with the report of Suprakash Manoranjanand Chatterjee. (2012) of which in their *in vivo* experiements suggest that these phyto-extracts are good materials and can be used as pre-plantation treatment for effective management of FRR disease fusarial wilt of tomato and brinjal in India . Further, Srivastava and Lal. (1997) disclosed that several higher plants and their constituents have been an effective against plant disease and yet proven to be harmless and non-phytotoxic unlike chemical fungicides. Also, the results are in consonance with the works that several plant species possess fungal and antibacterial properties.

Table 1. Mycelium growth inhibition effect of different phyto extracts *in vitro against Fusarium* spp causing mulberry root rot., 2015[2].

Source of phyto-extracts	Fungal plug method[2]			Spore seeded method[2]		
	1 DAI[3]	3 DAI	6 DAI	1 DAI	3 DAI	6 DAI
No Treatment	-	0.0 e	0.0 e	-	0.0 e	0.0 e
Acapulco (*Cassia alata* Linn.)	-	71.0 c	68.6 c	-	68.3 c	38.3 d
Bangbangsit (*Lantana camara* L.)	-	100.0 a	100.0 a	-	100.0 a	100.0 a
Banaba (*Lagerstroemia speciosa* L)	-	100.0 a	100.0 a	-	96.6 a	66.6 c
Eucalyptus (*Eucalyptus globus)*	-	100.0 a	100.0 a	-	100.0 a	100.0 a
Garlic vine (*Mansoa alliacea)*	-	72.0 c	69.0 c	-	68.3 c	78.3 c
Hagonoy (*Chromolaena odorata*)	-	100.0 a	100.0 a	-	86.6 c	76.6 d
Lemon grass (*Cymbopogon marginatus)*	-	70.3 c	67.3 c	-	56.6 c	26.6 d
Tubli (*Derris elliptica)*	-	82.6 bc	79.6 bc	-	85.0 b	65.0 c
Madre Cacao (*Gliricidum sepium)*	-	48.3 d	45.3 d	-	58.3 c	28.3d

Neem tree (*Azadirachta indica)*	-	95.6 a	97.6 a	-	98.3 a	76.0 b
Oregano (*Origanum vulgare*)	-	79.3 c	76.3 c	-	60.0 d	55.0 e
Yellow Ginger (*Curcuma longa*)	-	88.6 b	85.6 b	-	100.0 a	77.3 b

[1]- means followed by the same letter in a column are not significantly different at 5% level of significance, LSD.

[2]- in mm, [3]- DAI – days after inoculation; 1DAI- no growth observed

Table 2. Disease incidence (%) and plant growth (cm) performance at 30, and 60 days after planting (DAP) of mulberry saplings treated by different phytoextracts.

Source of Phytoextracts	Disease Incidence (%) at 30DAP	Plant Height (cm) at 30DAP	Disease Incidence (%) at 60DAP	Plant Height (cm) at 60DAP
Bangbangsit (*Lantana camara* L.)	13.75 d	27.52 a	17.81c	40.31a
Banaba (*Lagerstroemia speciosa* L.)	26.85 b	23.88 a	29.93 b	38.20b
Eucalyptus (*Eucalyptus globus*)	21.14 b	13.09 b	34.14 ab	2.07c
Hagonoy (*Chromolaena odorata*),	14.08 d	12.42 b	22.19 bc	39.16b
Neem (*Azadirachta indica)*	19.74 bc	28.14 a	25.02 bc	42.65a
No treatment	38.54 a	15.55 b	41.81 a	27.16b
Fungicide at Recommended Rate	19.04 bc	29.14 a	25.69 bc	45.65a

[1] - means followed by the same letter in a column are not significantly different at 5% level of significance, LSD

Conclusion

The study aimed to investigate the antifungal properties of 12 phytoextracts against *Fusarium* spp. causing root rot in mulberry. Phyto extracts of *Bangbangsit* (*Lantana camara* Linn), *Banaba* (*Lagerstroemia speciosa* Linn), *Eucalyptus* (*Eucalyptus globus) Hagonoy* (*Chromolaena odorata*), and *Neem* tree (*Azadirachta indica)* plants showed promising effect against *Fusarium* spp. as shown by zone of inhibition, three (3) and six (6) days after inoculation (DAI), respectively. Greenhouse trials further showed that among the phytoextracts tested, *bangbangsit* (*Lantana camara* Linn) extract was superior over banaba, eucalyptus, neem and hagonoy extracts in reducing root rot infection in 30 and 60 DAP. These plants could be utilized to field trials to access their effectiveness in field condition.

It will help in the formulation of ecofriendly control measures, which is cheap and can be recommended for the sericulture farmers against the root rot disease in mulberry.

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