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Antifungal Activity of Tamarind (*Tamarindus indica* Linn.) Leaf Extract Against *Colletotrichum gloeosporioides*

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Abstract *- Anthracnose (Colletotrichum gloeosporioides)* is considered as most important and destructive disease of mango. Use of natural plants is recommended because it is cost-effective and safe. Plant extracts like tamarind shown to have antifungal activity. The experiment was conducted in Pampanga, Philippines following two factor-factorial Complete Randomized Design to determine the antifungal effect of tamarind against anthracnose, specifically to: determine zones of inhibition in *C. gloeosporioides* as affected by different solvents in young and mature leaf extracts; determine interaction effect between different solvents and type of leaf used; determine the most effective tamarind extract against *C. gloeosporioides.* Results reveals that young tamarind leaf extract at 1:1 ratio significantly affected the production of zone of inhibition of C. *gloeosporioides* while the mature tamarind leaf extract did not inhibit the test organism. The ethanol extract using young leaves were the most effective against the test organisms. Findings showed that the use of young tamarind leaf extract using different solvents at 1:2 ratio produced strong effect against *anthracnose* in terms of inhibitory activity. The use of young leaf ethanol extract against *anthracnose* was effective and can be considered as good biofungicide because its efficacy is comparable to Mancozeb.

Keywords **-** Biofungicide, Tamarind Leaf Extract, Anthracnose

INTRODUCTION

Tamarindus indica L. commonly known as tamarind belongs to the family Ceasalpiniaceae (Fabaceae) which is indigenous to South East Asia but is widely planted and distributed in tropical and subtropical regions (Little and Wadsworth,1964).

Tamarind is a large, long-lived usually evergreen tree which commonly grows to a height up to 25 m, with stem diameters of up to 150 cm, are characterized by a dense, spreading, rounded crown, a low-branching habit, paripinnate leaves, and thick, gray, deeply fissured bark (National Academy of Sciences, 1979).

As regards its uses, the pulp of the tamarind fruit is widely used for food and beverage like syrup, juice, concentrates and exotic food products like chutneys, curries, pickles and meat sources (Ishola, 1990). Fruit pulp is also used to quench thirst. It is also a useful drink to persons recovering from sickness (Morton, 1987).

In terms of its nutritive value, tamarind is an excellent source of tartaric acid, citric acid, vitamin C and sugars (Nyadoi and Abdullah, 2004).

Diseases caused by *Colletotrichum* species occur on a wide range of plant species and have been recorded worldwide as both pre and post-harvest causes of crop loss (Jeffries, Dodd, Jeger, 1990).

Colletotrichum gloeosporioides commonly known as anthracnoseis considered to be the most important and destructive disease of mango. This disease can infect almost 100% of fruits produced under wet or very humid conditions (Fitzell and Peak,1984). In addition, it caused a significant yield reduction up to 80% under favorable condition (Agostin *et al.*, 1992). The symptom appears as irregularly shaped, black nurotic spots on both sides of the mango leaf. Conidia of this organism germinate on the surface of leaves and form appressoria and remain as quiescent infection.

Generally, application of fungicide is the most common method to control anthracnose. However, frequent fungicide application leads to fungal resistance and environmental hazards. (Dodd *et al., 1989*).

Some plant extracts have been recently shown to have antifungal activity. One of these plants which is tamarind. According to Neetu and Bohra(2003), crude ethanolic and aqueous extract from tamarind leaves, stems, fruit, pulp, seeds and bark were found toxic against *Aspergillus flavus* and *Fusarium oxysporum* in vitro.

The study is important and timely because the findings can help solve the problem of mango farmers including consumers. Controlling anthracnose, a major disease in mango, is the most common problem of farmers in mango production. On the other hand, consumers are demanding less chemical residue on produce mangoes

of health concerns.

At present, the use of natural plants as an alternative control method that is both safe to farmers and consumers is highly recommended since it helps reduce environmental risks brought about by too much application of chemicals on crops and fruit trees. In addition, the proper use of these plants can help boast consumer confidence on the purchased product.

Furthermore, the results of the study may strengthen the importance of natural plants in the country that have antifungal property.

OBJECTIVES OF THE STUDY

The general study aimed to determine the antifungal effect of tamarind against *Colletotrichumgloeosporioides.*

Specifically, it aimed to:

1. determine the zones of inhibition in *C.gloeosporioides* as affected by different solvents of young and mature tamarind leaf extracts;
2. determine the interaction effect between different solvents and type of leaf used;
3. determine the most effective tamarind extract against *C.gloeosporioides.*

MATERIALS AND METHODS

The experimental set-up was laid out following the two factor-factorial Complete Randomized Design (CRD). Each treatment was replicated three times. The following are the factors used in the study.

Phase 1. (using 1:1 ratio)

Factor A. (Types of Leaves)

L_1 – Young leaves

L_2 – Mature leaves

Factor B. (Types of Extraction Solvents)

S_1 - Distilled water(negative control)

S_2 - Tamarind leaf extracts(water bathed)

S_3 - Ethanol extracts

S_4 –Mancozeb(positive control)

S_5 – Tamarind leaf extracts(not water bathed)

Phase 2. (Young tamarind leaf extract using 1:2 ratio)

(Types of Extraction Solvents)

S_1-Distilled water (negative control)

S_2-Tamarind leaf extracts(water bathed)

S_3-Ethanol extracts

S_4-Dithane(positive control)

S_5-Tamarind leaf extracts(not water bathed)

Phases of the Experiment

The study is composed of bioassay experiment divided in to phases, namely:

(1) extraction technique using different solvents and type of leaf using 1:1 ratio (2) extraction technique using different solvents in young tamarind leaf using 1:2 ratio. The solvent and type of leaf with significant results in phase one was used in phase two of the study.

Collection of Plant Specimen

The test organism that was used is the *Colletotrichum gloeosporoiodes*. This fungus was isolated from a diseased mango leaf showing anthracnose symptoms-a black spot on the leaves of mango. The leaf specimen was cut into small pieces and disinfected with sodium hypochlorite for 1-2 minutes and rinsed twice in separate distilled water for 1-2 minutes.

To maintain pathogenicity of the isolated fungus, periodic reisolations were carried out to come up with pure culture. A 7-10 day old pure culture of *C.gloeosporoiodes* was used. The organism was properly identified.

Preparation and Sterilization of Culture Media

Potato Dextrose Agar (PDA) was prepared by weighing 200g potato, 20g agar, 20g sucrose, and 1000 ml of distilled water. The potatoes were washed, peeled and sliced into cubes and boiled in 1000 ml distilled water. Boiled potatoes were strained using a cheesecloth. Afterwhich, sucrose and agar were thoroughly mixed with the potato broth was subjected to sterilization using electric autoclave for about 15 minutes at 121°C.

Sterilization of Laboratory Glasswares

All laboratory glasswares that were used were washed and sterilized for 15 minutes at 15 psi (121^0C) using an autoclave. The inoculating loop was sterilized by dipping it in 95% ethanol and allowing it to glow to redness over an alcohol before each use (Munir et al., 2008).

Preparation of the Tamarind Leaf Extract

Three hundred grams of young and matured tamarind leaf was collected and washed. The leaf was air dried for about 24 hours and pulverized. The leaves were soaked for 24 hours. Immediately after soaking, the leaves were extracted and filtered using cheesecloth and whatman and were stored in separately in tightly covered bottles, ready for the experiment.

A. **Aqueous Tamarind Leaf Extract**

Three hundred grams of tamarind leaves (young and matured) were washed using tap water. The leaves were mixed with 300 ml of distilled water. The mixture was heated to boiling point and allowed to boil for 15 minutes over low flame. It was cooled and filtered through a cheese-cloth and was stored separately in tightly covered bottles in a refrigerator, ready for the bioassay experiment (Satish *et al.*, 2007).

B. **Ethanol Extract**

The dried leaf was pulverized into a fine powder and 300/g of powdered leaves was soaked with ethanol (300/ml) until the leaves were fully submerged inside a 1L Beaker. The container was then closed with a carbon paper or foil and was set aside for 48 hours. The mixture was filtered using cheese cloth. After filtration, filtrates were evaporated to dryness using a water bath.

Preparation of the Inoculants

Test microorganism from the pure culture was transferred to the non-inoculated agar plates to produce the working culture for the tamarind leaf extract. A flattened needle was sterilized by heating the tip of the needle in an alcohol lamp until it became glowing red. Portions of the mycelia radiating from the tissue section of the pure culture obtained using the sterile flattened needle was dissolved in small amount of distilled water (ml). The dissolved culture was transferred and mixed with the molten agar in petri plates. The side of the inoculated agar plates was heated to prevent contamination of other microorganisms. The agar was allowed to solidify before placing the discs.

Preparation of the Filter Paper disc

The filter paper disc was prepared by cutting about 1 cm in diameter of Whatman # 42 filter paper. This was wrapped in an aluminium foil and was autoclaved for 15/min at 15 psi (121^0C) (Munir *et al.*, 2008).

Paper Disc Diffusion Assay

The sterilized filter paper disc was immersed into the prepared leaf extracts. The excess liquid was allowed to drain. Filter paper was placed at the center of the petri

plates previously inoculated with fungal culture. The plates were incubated at 37ºC for about 20-24 hours in an upside down position to prevent the inoculated agar plates from contamination by moisture generated during the incubation period (Munir *et al.*, 2008).

Parameters Gathered:

Zones of inhibition

This was recorded using a millimetre ruler placed on the surface of the bottom plate without removing the cover.

Fungal Inhibition Test

This was done by referring to determine the antifungal activity, a standard measurement was used to compare.

The standard measurement in determining antifungal activity was utilized to compare with the results and identify its effectiveness. This standard was based on the work of Florendo*et al.,* (2008).

Zone of Inhibition	Inhibitory Activity
>17	+++ = strong
12-16	++ = moderate
7-11	+ = weak
6 or 0	- = negative

RESULTS AND DISCUSSIONS

Results of the study showed that the use of different solvents on young tamarind leaf extract significantly affect the production of zone of inhibition on the test organism (*Colletotrichumgloeosporioides*). The S_4 (mancozeb) obtained the widest zone of inhibition giving a strong inhibitory activity. On the other hand, among the tamarind leaf extracts used, S_3 (ethanol extract) was found to be the most effective in producing zone of inhibition (Table 1)

Meanwhile, S_2 (tamarind leaf extracts – water bathed) did not differ significantly from S_5 (tamarind leaf extracts – not water bathed) both having weak inhibitory effect but still gave significant result compared to that of S_1 (distilled water).

Table 1. Zone of inhibition of different solvents of young tamarind leaf extract against anthracnose

Treatments	*Mean (zone of inhibition)	Inhibitory activity
S_1(Distilled water)	0^{d}	Negative
S_2(tamarind leaf extract water bathed)	11.33^{c}	Weak
S_3(Ethanol extract)	20^{b}	Moderate
S_4(Mancozeb)	56.83^{a}	Strong
S_5(tamarind leaf extract not water bathed)	11.17^{c}	Weak

* Means with the same letter (a-d) are not significant different at 5% (HSD) level

Table 2 presents the findings on the antifungal activity of tamarind leaf extract using different solvents at 1:1 ratio on the test organism, 24-48 hours after application.

Compared to young tamarind leaf extract, the use of mature leaf extract showed negative inhibitory effect against *Colletotrichumgloeosporioides*. Although zones of inhibition were observed, the presence of secondary growth indicates that the treatments had negative effects and could not completely inhibit the test organism.

Moreover, no significant effect was observed in treatments S_2 (tamarind leaf extracts –water bathed), S_3 (ethanol extract) and S_5 (tamarind leaf extracts –not water bathed). Inaddition, their performance in producing zone of inhibition is comparable with that of S_1 (distilled water). On the other hand, S_4 (mancozeb) was found to produce the most significant zone of inhibition.

Table 2. Zone of inhibition of different solvents of mature tamarind leaf extracts against *Colletotrichumgloeosporioides*(mm)

Treatments	*Mean(zone of inhibition)	Inhibitory activity
S_1(Distilled water)	0^{e}	Negative
S_2(tamarind leaf extract water bathed)	4.90^{de}	Negative

S_3(Ethanol extract	3.75[de]	Negative
S_4(Mancozeb)	57.07[a]	Strong
S_5(tamarind leaf extract not water bathed)	5.50[d]	Negative

* Means with the same letter (a-e) are not significant different at 5% (HSD) level.

Results of the data reveal that regardless of the type of leaf, all solvents used were significantly different to each other (Table 3). Furthermore, it can be observed that S_4 (mancozeb) had the widest zone of inhibition followed by S_3(ethanol extract). Meanwhile, S_2 (tamarind leaf extracts - water bathed) and S_5(tamarind leaf extracts - not water bathed) had the same zone of inhibition on the fungus but was found to be more effective compared to that of S_1(distilled water). The S_1 showed no inhibition effect on the test organism.

On the other hand, regardless of the type of solvents used for extraction, results from the statistical analysis showed that there is significant difference between young and mature leaf extract in terms of inhibition. Extract from young tamarind leaf produced the widest zone of inhibition. This finding was possibly due to the chemical composition of the tamarind leaf. According to Lewis et al., 1961, some active components in tamarind such as tartaric acid was responsible for this effect.

It can be observed that the type of tamarind leaf extracts and different solvents greatly affected the production of zone of inhibition thus, significant interaction was present.

The data further reveals that among the tamarind extracts and type of leaf used, the S_3 (ethanol extract) using L_1 (young leaf) was the most effective in inhibiting the test organism.

On the other hand, both S_5 (tamarind extracts – not water bathed) and S_2 (tamarind leaf extracts – water bathed) of L_1(young leaf) was comparable to each other in relation to the production of zone of inhibition while the effectiveness of S_5, S_3 andS_2 in L_2(mature leaf)in suppressing the test organism is similar to that of control.

Table 3. The interaction effect of zone of inhibition of young and mature tamarind leaf extract as affected by different solvents against *Colletotrichum gloeosporioides* (mm)

Solvents	Type of Leaves		*Mean(zone of inhibition)	Inhibitory activity
	L_1	L_2		
S_1(Distilled water)	0^{E}	0^{E}	0^{d}	Negative
S_2(tamarind extract water bathed)	11.33^{C}	4.90^{DE}	8.12^{c}	Weak
S_3(Ethanol extract)	20.00^{B}	3.75^{DE}	11.88^{b}	Weak
S_4(Dithane)	56.83^{A}	57.07^{A}	56.95^{a}	Strong
S_5(tamarind extract not water bathed)	11.17^{C}	5.50^{D}	8.33^{c}	Weak
Mean**	19.87^{x}	14.24^{y}	17.06	

* Extract means with having the same letter (a-d) are not significantly different at 5% (HSD) level.

** Type of leaf means with having different letter (x-y) are significant at 5% (HSD) level.

*** Type of leaf x extract means having the same letter (A-E) are not significantly different at 5% (HSD) level.

Results from analysis of variance showed that the use of different tamarind extracts at the ratio of 1:2 significantly differ to each other in terms of zone of inhibition against the test organism (Table 4).

All the tamarind extracts, S_2 (tamarind leaf extracts-water bathed) and S_3 (ethanol extract) except S_5 (tamarind leaf extracts-not water bathed) significantly produced a strong inhibitory effect and its efficacy against *Colletotrichum gloeosporioides* was found to be comparable with that of S_4 (mancozeb). Figuratively speaking, S_5 (not water bathed) appeared to be less effective when compared to treatments S_2, S_3 and S_5 was found to have similar and negative inhibitory activity with that of S_1 (distilled water).

Table 4. The production of zones of inhibition on young tamarind extract as affected by different solvents using 1:2 ratio against *Colletotrichum gloeosporioides* (mm)

Treatments	*Mean (zone of inhibition)	Inhibitory activity
S_1(Distilled water)	0^b	Negative
S_2(tamarind leaf extract water bathed)	57.67^a	Strong
S_3(Ethanol extract	56.27^a	Strong
S_4(Mancozeb)	57.30^a	Strong
S_5(tamarind leaf extract not water bathed)	0.65^b	Negative

*Means having the same letter are not significantly different at 5% (HSD) level.

This study was delimited on the antifungal effect of young and mature tamarind leaf extract using different solvents against *Colletotrichum gleoesporoiodes* commonly known as mango anthracnose using bioassay experiment.

CONCLUSIONS

From the result gathered in the study, the following conclusions are drawn:

1. There is a significant difference on the zone of inhibition in *Colletotrichum gloeosporioides* as affected by different solvents in young and matured tamarind leaf extracts.
2. There is an interaction effect between the different solvents and type of leaf used. The use of young tamarind leaf extract is more effective as an agent against *Colletotrichum gloeosporioides* compared to mature tamarind leaf extract.
3. The use of young leaf in tamarind S_3(ethanol extract) against *Colletotrichum gloeosporioides* was effected to be a good biofungicide and its efficacy comparable to S_4 (mancozeb).

RECOMMENDATIONS

In view of the conclusions drawn from the foregoing findings, the following recommendations are forwarded.

1. Further study should be done on young tamarind leaf extract using ethanol as solvent for extraction technique with emphasis on the different concentrations.
2. The use of young and matured leaf extract of tamarind using different solvents in other species of fungi.
3. The use of tamarind leaf extracts using different solvents as antibacterial.
4. Young tamarind leaf extract should be used directly on the fruits to test its effectiveness.

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