

Original Research Article

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## Evaluating Eco-friendly Botanicals against *Colletotrichum gloeosporioides* causing Anthracnose of Mango

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### ABSTRACT

#### Keywords

*C. gloeosporioides*;  
Mango anthracnose;  
Plant extract

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The present study was conducted to evaluate the efficacy of aqueous extract and dry extracts of 12 different plant species against *C. gloeosporioides* the causal agent of mango anthracnose under natural disease epidemics. Significant difference was observed among the extracts in their effect on suppressing the mycelial growth of the pathogen under *in vitro* conditions. None of the extracts were able to completely prevent the development of the pathogen. However, most of the extracts significantly reduced disease development over the control. Among the fresh plant extracts, at 7.5% concentration, maximum inhibition was noticed in the *Simarouba glauca* extract with upto 59.41% mycelial inhibition followed by 50.03% by *Lawsonia inermis* and *Azadirachta indica* with 58.84 % mycelial inhibition. Among the dry powder extracts, at 7.5% concentration, *Moringa oleifera* showed a maximum inhibition of 40.81% followed by *S.glauca* with 37.64% inhibition and 32.25% inhibition with *Geranium sanguineum*.

### Introduction

Mango (*Mangifera indica* L.) is one of the most popular fruit crop grown through the tropical and subtropical countries of the world. It is one of the most favoured fruits in the international market because of its attractive fragrance, flavour, excellent taste, sweetness and beautiful colour. However, mango productivity is affected by many problems limiting its production. Among them, the diseases play an important role limiting its production. Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.)

Penz. and Sacc., is by far the most important field and postharvest disease prevalent in all mango growing areas of the world and is often associated with high rainfall and humidity (Arauz, 2000). The pathogen affects young leaves, flower panicles and forms latent infections on the fruit (Dodd *et al.*, 1989). The pathogen is also present in quiescent form in immature fruits and is more significant in the postharvest stage (Spalding and Reeder, 1986). Synthetic fungicides are currently used as the major means for managing pre and post-harvest anthracnose. However, the concern over the environmental

hazards of the chemicals and emergence of new races of the pathogen has diverted research towards finding alternative methods to manage the disease (Yao and Tian, 2005). Plant extracts are considered as a safer alternative to the chemical fungicide to manage the disease (Tripathi and Shula, 2007) as they are considered eco-friendly and safe alternatives.

## **Materials and Methods**

### **Isolation of the pathogen**

The mango leaves infected with anthracnose were initially collected from mango orchard and used for isolation of the fungus. The infected portions along with some healthy parts were cut and surface sterilized using one per cent sodium hypochlorite solution for 60 seconds.

These bits were thoroughly washed in sterile distilled water for three times to remove the traces of sodium hypochlorite if any and then transferred to sterilized Petri plates (3 leaf bits per Petri plates) containing Potato Dextrose Agar (PDA) under aseptic condition under laminar air flow and incubated at room temperature ( $27\pm 1^\circ\text{C}$ ). After 72 hr, colonies which developed from the bits were transferred into fresh PDA medium. Colonies which developed from such culture was periodically observed for mycelia growth and sporulation under microscopic. Mycelial and spore character was used as a means for identification of the pathogen.

### **Identification of the fungus**

The pathogen was identified based on its mycelial and spore characters described by Barnett and Hunter (1972). After identification they were transferred to new PDA slants and incubated at  $27\pm 1^\circ\text{C}$  for further use. The fungus was sub cultured on PDA slants and allowed to grow at  $27 \pm 1^\circ\text{C}$

for 7 days. Such slants were preserved in refrigerator at  $5^\circ\text{C}$  and maintained. Sub culturing was done once in a month, such cultures were used throughout the study.

### ***In vitro* evaluation of botanicals**

Botanicals which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to know the antifungal activity of some aqueous plant extracts against *C.gloeosporioides* using poisoned food technique explained earlier. Design used was factorial CRD and each treatment replicated thrice with three different concentrations (2.5, 5 and 7.5 %). The plant leaf extracts which were used are listed in Table 1. The plant materials were collected and washed in distilled water and leaves were grinded into fine paste and mixed in sterile distilled water in the ratio 1:100 w/v. The suspensions were left to stand for 24 hours at room temperature and then filtered through double layer of cheese cloth, centrifuge at 5000rpm for 10 min. The different concentrations of plant extract were added to PDA media and autoclaved. Later, media was poured into sterile petriplates and allowed to solidify. On the center of the media 5mm mycelial growth of *C. gloeosporioides* was placed using cork borer. Incubate the plates at  $27^\circ\text{C}$  for seven days. The diameter of the colony was measured in three directions and average was worked out. Those Petri plate were also observed for presence or absence of sporulation. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

## Results and Discussion

Among the 12 fresh plant extract tested (Table 2 and Plate 1), all the extracts showed some inhibitory action against the pathogen as compared to the control plate. At 2.5% concentration, maximum mycelial inhibition of 30.32% was seen with *Lawsonia inermis* extract, followed by *Simarouba glauca* with 30.28 % inhibition. Least inhibition of 2.46% was seen with *Ashwagandha somnifera* extract. At 5% concentration, maximum inhibition of up to 52.84% was seen with *Thymus vulgaris* extract followed by *S.glauca* with 49.33% and *L.inermis* with 48.55%. At 7.5% concentration, maximum inhibition was noticed in the *S.glauca* extract upto 59.41% mycelial inhibition followed by 50.03% in *L.inermis* and *Azadirachta indica* with 58.84 % mycelial inhibition.

The botanicals which were collected were dried under shade and the powdered and the extraction was done using the powder. The extraction thus made was used to test the inhibitory activity against the *Colletotrichum*

*gloeosporioides* (Table 3 and Plate 2). The study showed that at 2.5 % concentration maximum inhibition of 36.45 % was seen by the Aloe vera extract followed by *L.inermis* with 16.93% and *Vitex nigundo* at 10.58%. At 5% concentration of the botanicals, maximum inhibition was seen with *Geranium sanguineum* at 27.69% followed by *Allium sativum* with 26.96% and *L.inermis* with 26.67%. At 7.5% concentration, *Moringa oleifera* showed a maximum inhibition of 40.815 followed by *S. glauca* with 37.64% and 32.25% with *Geranium sanguineum*. The results showed that extracts of the different plant species are substantially varied in their antifungal potentials. These differences are to be expected since plants vary in their chemical constituents, habitats and stages at which they were collected. Differences in the nature and concentration of inhibitory material even between different plants parts have been reported elsewhere (Ogbebor and Adekunle, 2008). Many plant and plant products have been reported as having antimicrobial activities against plant pathogenic fungi (Sokovicet *et al.*, 2009).

**Table.1** List of botanicals used to study the antifungal effect on *C. gloeosporioides*

Sl. No.	Common name	Scientific name	Plant part used
1	Neem	<i>Azadirachta indica</i>	Leaves
2	Vitex	<i>Vitex negundo</i>	Leaves
3	Aswagandha	<i>Ashwagandha somnifera</i>	Leaves
4	Aloevera	<i>Aloe vera</i>	Leaves
5	Moringa	<i>Moringa oleifera</i>	Leaves
6	Henna	<i>Lawsonia inermis</i>	Leaves
7	Thyme	<i>Thymus vulgaris</i>	Leaves
8	Simarouba	<i>Simaroubaglauca</i>	Leaves
9	Geranium	<i>Geranium sanguineum</i>	Leaves
10	Lantana	<i>Lantana camara</i>	Leaves
11	Onion	<i>Allium cepa</i>	Bulb
12	Garlic	<i>Allium sativum</i>	Bulb

**Table.2** *In vitro* evaluation of fresh botanicals against mycelial growth of *Colletotrichum gloeosporioides* of mango

Sl. No.	Botanicals		Inhibition (%)			Mean Inhibition (%)
	Common name	Scientific name	Concentration			
			2.5	5.0	7.5	
1	Neem	<i>Azadirachta indica</i>	23.94 (29.29)	42.01 (40.40)	58.84 (50.09)	<b>41.59</b> <b>(40.16)</b>
2	Vitex	<i>Vitex negundo</i>	23.29 (28.86)	42.35 (40.60)	44.93 (42.09)	<b>36.85</b> <b>(37.38)</b>
3	Onion	<i>Allium cepa</i>	16.47 (23.94)	28.55 (32.30)	32.91 (35.01)	<b>25.97</b> <b>(30.64)</b>
4	Aswagandha	<i>Ashwagandha somnifera</i>	2.46 (9.02)	5.76 (13.89)	25.92 (30.61)	<b>11.38</b> <b>(19.72)</b>
5	Garlic	<i>Allium sativum</i>	16.01 (23.59)	26.33 (30.87)	29.54 (32.61)	<b>23.96</b> <b>(29.31)</b>
6	Aloevera	<i>Aloe vera</i>	6.58 (14.86)	24.71 (29.81)	32.48 (34.74)	<b>21.25</b> <b>(27.45)</b>
7	Henna	<i>Lawsonia inermis</i>	30.32 (33.41)	48.55 (44.17)	59.03 (50.20)	<b>45.96</b> <b>(42.68)</b>
8	Moringa	<i>Moringa oleifera</i>	3.16 (10.24)	9.72 (18.17)	26.73 (31.13)	<b>13.20</b> <b>(21.30)</b>
9	Thyme	<i>Thymus vulgaris</i>	16.08 (23.64)	52.84 (46.63)	27.10 (31.37)	<b>32.00</b> <b>(34.45)</b>
10	Simarouba	<i>Simarouba glauca</i>	30.28 (33.39)	49.33 (44.62)	59.41 (50.42)	<b>46.34</b> <b>(42.90)</b>
11	Geranium	<i>Geranium sanguineum</i>	18.51 (25.48)	22.50 (28.32)	30.22 (33.35)	<b>23.74</b> <b>(29.16)</b>
12	Lantana	<i>Lantana camera</i>	28.37 (32.18)	31.68 (34.25)	44.85 (42.04)	<b>34.96</b> <b>(36.25)</b>
13	Control	-	0.00	0.00	0.00	<b>0.00</b>
		<b>Mean</b>	<b>17.96</b>	<b>32.03</b>	<b>39.33</b>	-
		<b>S. Em±</b>	<b>(23.10)</b>	<b>(31.28)</b>	<b>(38.64)</b>	-
		<b>CD @ 1%</b>	<b>2.13</b>	<b>3.91</b>	<b>5.82</b>	-
			<b>6.17</b>	<b>11.32</b>	<b>16.85</b>	-

**Table.3** *In vitro* evaluation of dry botanicals against mycelia growth of *Colletotrichum gloeosporioides* of mango

Sl. No.	Botanicals		Inhibition (%)			Mean Inhibition (%)
	Common name	Scientific name	Concentration			
			2.5	5.0	7.5	
1	Neem	<i>Azadirachta indica</i>	7.18 (15.54)	21.17 (27.39)	33.60 (35.43)	<b>20.65</b> <b>(27.03)</b>
2	Vitex	<i>Vitex nigundo</i>	10.58 (18.98)	20.53 (26.94)	24.68 (29.79)	<b>18.59</b> <b>(25.54)</b>
3	Onion	<i>Allium cepa</i>	6.76 (15.07)	13.43 (21.50)	25.36 (30.24)	<b>15.10</b> <b>(22.87)</b>
4	Aswagandha	<i>Ashwagandha somnifera</i>	8.88 (17.34)	11.88 (20.16)	23.26 (28.83)	<b>14.67</b> <b>(22.52)</b>
5	Garlic	<i>Allium sativum</i>	5.92 (14.08)	26.96 (31.28)	31.47 (34.12)	<b>21.45</b> <b>(27.49)</b>
6	Aloevera	<i>Aloe vera</i>	36.45 (37.14)	16.36 (23.86)	21.98 (27.96)	<b>24.93</b> <b>(29.95)</b>
7	Moringa	<i>Moringa oleifera</i>	5.09 (13.04)	24.10 (29.40)	40.81 (39.70)	<b>23.33</b> <b>(28.88)</b>
8	Henna	<i>Lawsonia inermis</i>	16.93 (24.30)	26.67 (31.09)	27.80 (31.82)	<b>23.80</b> <b>(29.20)</b>
9	Thyme	<i>Thymus vulgaris</i>	8.89 (17.35)	22.50 (28.32)	24.47 (29.65)	<b>18.62</b> <b>(25.56)</b>
10	Simarouba	<i>Simarouba glauca</i>	8.48 (16.93)	23.23 (28.81)	37.64 (37.84)	<b>23.31</b> <b>(28.87)</b>
11	Geranium	<i>Geranium sanguineum</i>	5.06 (13.00)	27.69 (31.75)	32.25 (34.60)	<b>21.66</b> <b>(27.74)</b>
12	Lantana	<i>Lantana camera</i>	11.88 (20.16)	15.45 (23.16)	25.74 (29.65)	<b>17.69</b> <b>(24.87)</b>
13	Control	-	0.00	0.00	0.00	<b>0.00</b>
	<b>Mean</b>		<b>11.01</b> <b>(18.58)</b>	<b>20.83</b> <b>(23.16)</b>	<b>29.09</b> <b>(32.71)</b>	-
	<b>S. Em±</b>		<b>2.59</b>	<b>1.81</b>	<b>2.08</b>	-
	<b>CD @ 1%</b>		<b>7.51</b>	<b>5.24</b>	<b>6.04</b>	-

**Plate.1** *In vitro* evaluation of fresh plant extracts on mycelial growth of *Colletotrichum gloeosporioides*



2.5 %



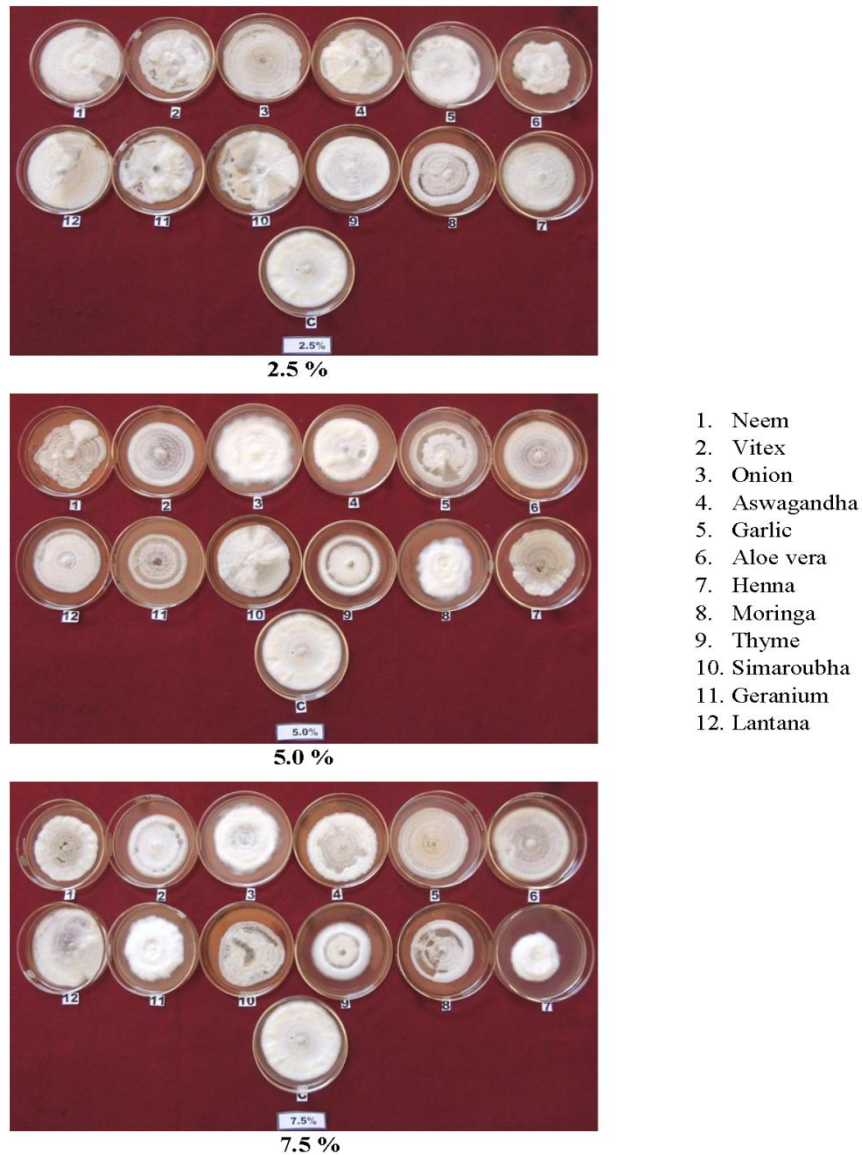
5.0 %



7.5 %

1. Neem
2. Vitex
3. Onion
4. Aswagandha
5. Garlic
6. Aloe vera
7. Henna
8. Moringa
9. Thyme
10. Simaroubha
11. Geranium
12. Lantana

**Plate.2** In vitro evaluation of dry plant extracts on mycelial growth of *Colletotrichum gloeosporioides*



In conclusion, the inhibitory effects of crude extracts indicate that they can be selected for better understanding of the effect of these extracts on the pathogen as well as take up field and postharvest studies so that we have environmental friendly and safe options of managing the disease.

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