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IN VITRO EVALUATION OF DIFFERENT PLANT EXTRACTS, PLANT OILS, ORGANIC AMENDMENTS AND ANTAGONIST AGAINST ANTHRACNOSE DISEASE OF PAPAYA CAUSED BY *GLOEOSPORIUM PAPAYII*

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Abstract

The present investigation was carried to study the different plant extracts,plant oils, organic amendments and bacterial antagonists against anthracnose disease of papaya. Five isolates of *G. papayii* were isolated from the diseased fruits,I4 was the most virulent. Anthracnose caused by *Gloeosporium papayii* is considered to the important disease in papaya growing regions. Among the treatments of plant extracts, plant oils and organic amendments, neem Leaf extract, neem oil cake and cinnamon oil recorded the least mycelial growth.

Keywords: Amendments, *Gleosporium papayii*, Papaya, Plant oils

I. Introduction

Papaya (*Carica papaya* L.) is an important fruit crop, belonging to family Caricaceae. Papaya is to native on Southern Mexico and neighbouring Central America and grown in all tropical countries and many subtropical countries between 32◦ North and South latitudes but the high commercial production is found between 23◦ N and S latitudes (Nakasone and Paul, 1998). Papaya is known as "common man's fruit". It is rich sources of vitamin A, C and calcium. The amount of vitamin A in papaya is the second highest among fruits and comes next to mango (Aykroyed, 1951). It contains fair quantities of vitamin C, some riboflavin and niacin and a good source of calcium, phosphorus and iron. Papaya has more carotene compare to other fruits such as apples, guavas, sitaphal and plaintains.

Papaya is an economically important fruit crop in Hawaii, Australia, India, Srilanka, Philliphines and South-east Asia including Thailand. The major papaya producing regions are Asia, South America, North Central America and Africa. About 65 per cent of the world's production is from South America. Another 35 per cent is from North Central America and Africa. In India, the major growing states in the country are Madhya Pradesh, Tamil Nadu, Kerala, Gujarat, Karnataka, Bihar, Uttar Pradesh, West Bengal, Orissa, Jammu and Kashmir, Himachal Pradesh. Approximately 3800 ha area is covered under Papaya with a production of 2, 61,900 tons with an average productivity of 68.921 tonnes/ hectare and total production and area of India during 2003-2004 was 18,50,000 tonnes and 18,000 hectares with average productivity of 23.130 tonnes/hectare (FAOSTAT, 2012).The Food and Agriculture Organization (FAOSTAT) reported annual papaya production in India was 5636,000 Metric Tonnes from 133,000 hectares of land during the year 2013-2014.

Papaya fruits are very susceptible to diseases caused by many microorganisms especially fungi, as papaya fruit is high in moisture and nutrients (Sankat and Maharaj, 1997). *Colletotrichum gloeosporioides*, *Rhizopus stolonifier*, *Botryodiopodia theobromae* were the major post harvest pathogens in Coimbatore markets (Pramod *et al*., 2007).Among the postharvest pathogens, anthracnose is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is the serious one causing heavy loss to farmers (10 to 80%) of the crop production in some developing countries (Poonpolgul and Kumphai, 2007). Anthracnose disease of papaya is an economical disease that occurs during transit, storage and market. Disease symptoms are normally not apparent at the time of harvest, but appear when the fruits are ripening or ripened (Ilag *et al*., 1994).

India is the second largest producer of fruits and vegetables in the world. Fruits contain high levels of sugars and nutrients and their low pH values makes them vulnerable to decay (Singh and Sharma, 2007). It has been estimated that about 20 to 25 % of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006). A considerable amount of fruits and vegetables produced in India is lost due to improper post-harvest operations; as a result there is a considerable gap between the gross production and net availability. Postharvest losses of approximately 40 to 100% have been generally reported in papaya in developing countries (Rahman *et al*., 2011). Postharvest loss reduction is essential for increasing food availability and cost reduction.

In India, the disease is prevalent throughout the country and has been reported to occur in Uttar Pradesh, Madhya Pradesh, Bihar, Karnataka, Kerala and Tamil Nadu. Post harvest losses are high in India because of lack of cold chain facilities, poor logistics connectivity in the hilly areas etc. Various types of physical damage such as surface injuries, impact bruishing and vibration brushing are the major contributors to deterioration.

Post harvest diseases of fruits are traditionally managed by chemical fungicides. However, residues play a major role in the application of fungicides during harvest. Biological control is one of the option to overcome these problems in the management of postharvest disease. Biological control is effective, eco-friendly and alternative approach for any disease management practice.

Modern agriculture is highly dependent on chemical pesticides. However, the repeated use of such chemicals may result in the environmental pollution and may lead to development of resistance in target organism. With due to the concentration of the above fact, the present study is formulated with the following objectives to manage the anthracnose disease of Papaya (*G. papayii*) by eco-friendly methods.

II. Materials and methods:

2.1.Isolation of the pathogen

Fruits of papaya showing characteristic symptoms of anthracnose were collected separately from different papaya growing areas of Tamil Nadu. The infected portions were cut into small pieces along with some healthy portion of diseased fruits and used for isolation of the pathogen using sterilized scalpel separately and these were surface sterilized with 0.1 per cent mercuric chloride for thirty seconds. These bits were then washed separately in repeated changes of sterile distilled water. Sterilized PDA medium amended with 100 ppm of streptomycin sulphate (to avoid bacterial contamination) was prepared and poured @ 20 ml per sterile Petri plate and allowed to solidify. The surface sterilized tissue bits were placed separately at equidistance @ 3 tissue bits per plate. All these works carried out under aseptic conditions. The plates were incubated at room temperature (28 ± 2◦C) for five days and observed for the fungal growth. The fungal culture of each isolate was purified by single hypal tip method and maintained on PDA slants.

2.2. Pathogenicity of *G. papayii*

2.2.1. Preparation of spore suspension

Three nine mm disc of seven days culture of each isolate of *G. papayii* was transferred into sterilized test tube using cork borer and the isolates of *G. papayii* (I1, I2, I3, I4 and I5) were inoculated into PDA medium and incubated for seven days

2.2.2. Pathogenicity tests

Pathogenicity tests were performed with a five isolates of *G. papayii,* from all morphological groups and locations, using papaya fruit (*Carica papaya* var. CO8) (Table 1). Fruits were disinfested by immersing them in two per cent sodium hypochlorite solution for 1 min, washed twice with sterile distilled water and dried at room temperature. An aqueous conidial suspension (1 × 106 spores ml-1) was prepared from seven day old cultures of each isolate and then placed on the fruit by the wound/ drop method. This method involved pin – pricking the surface of the fruit to a 1mm depth and placing 20 µl of conidial suspension over the wound. Three fruits were tested per isolate and experiments were conducted twice. The inoculated fruit, along with appropriate controls (fruit inoculated with sterile distilled water) were incubated at room temperature (25◦ C) in humid chamber. Symptoms were recorded six days after inoculation (d.a.i) and re-isolation, according to Koch's postulates, was made from all resulting lesions.

2.2.3. *In vitro* evaluation of plant extracts against *G. papayii*

2.2.3.1.Preparation of extracts of different botanicals (Ansari, 1995)

Fifty grams of either fresh green leaves or flowers or rhizome of different plant extracts having antimicrobial activity were collected separately and washed thoroughly with distilled water and crushed using 50 ml of sterile distilled water in a sterile pestle and mortar. The extracts of each plant parts were collected separately by squeezing the macerate with sterile cotton wool and each was passed through a double layer cheese cloth and then finally the filtrate was passed through Whatman No.1 filter paper separately. Each filtrate was centrifuged at 5000 rpm for 20 minutes and then each supernatant was filtered with sterilized sintered funnel separately. Then each filtrate was further diluted to a concentration of 5 per cent and 7.5 per cent using sterile distilled water. The efficacy of the above extracts was tested against the growth of the *G. papayii* under *in vitro* condition .

2.2.3.2. Efficacy of different plant extracts against the growth of *G. papayii in vitro* (I4)

The efficacy of plant extract was tested against G. papayii using poisoned food technique (Table 2).The standard extract solution (100%) of each plant extract was mixed with PDA medium separately at the ratio of 1:4 and 1:6.5 so as to get 5 per cent and 7.5 per cent concentration of each plant extract and sterilized in an autoclaved at 15 lb for 20 minutes and allowed to solidify. A nine mm actively growing PDA culture disc of virulent isolate (I4) of *G. papayii* was cut by using sterilized cork borer and placed at the centre of the medium. The plates were incubated at room temperature (28 ± 2 °C) for seven days. PDA medium without any plant extracts served as control. Three replications were maintained for each treatment. The radial growth of mycelium of the pathogen was measured in each plate separately when the fungus fully grown in the control plate or whichever was earlier. The results were expressed as per cent growth inhibition over control (Schmitz, 1930)

2.2.3.3. Efficacy of different plant oils against the growth of *G.papayii in vitro* (Poisoned food technique)

The efficacy of four different plant oils *viz.*, cinnamon oil (0.05% and 0.1 %), clove oil (0.05 % and 0.1 %), neem oil (1 % and 2 %) and mahua oil (1 % and 2 %) were tested against virulent isolate (I4) of *G. papayii in vitro* (Table 3). The required quantity of each plant oils was mixed with the PDA medium separately, sterilized and autoclaved at 15 lb for 20 minutes and cooled. Twenty ml of each plant oils medium was immediately poured into each sterilized Petri plates separately. The PDA medium without any oil was served as control. Three replications were maintained for each treatment. All the operations were carried out under aseptic conditions. The plates were incubated at room temperature (28 ± 2 ° C) for seven days. When the control plate showed the full growth of the pathogen, the diameter of the mycelial growth was measured in all the other treatments and the results are expressed in per cent inhibition over control (Schmitz, 1930).

2.2.4. Preparation of aqueous extract of oil cakes

Different oil cakes (100 g each) were taken and made into powder separately. These were soaked @ 1g/1.25 ml of sterile distilled water separately and kept at overnight. Each this was ground using a pestle and motar separately and filtered through a two layer of muslin cloth and each filtrate was collected separately and centrifuged at 10,000 rpm for 15 minutes. The supernatants of extract of each oil cake obtained were served as the standard solution (100%) (Dubey, 2002).

2.2.4.1.Efficacy of oilcake extracts against the growth of *G. papayii in vitro*

The efficacy of each oil cakes extract was tested against *G. papayii* using posoined food technique (Schmitz, 1930). The required quantity of standard extract solution (100 %) each oil cake was mixed thoroughly with the sterilized freshly prepared PDA medium so as to get 5 per cent and 10 per cent concentration of each oil cake. The fifteen ml of sterilized PDA medium containing extract of each oil cake was poured into each sterilized Petri plate separately and then allowed to solidify. A nine mm mycelial disc of *G. papayii* was placed at the centre of each Petri plate and incubated at room temperature for seven days. The PDA medium without the extract of any oil cakes served as control. Three replications were maintained for each treatment. The diameter of mycelial growth (cm) of *G. papayii* in each treatment was recorded separately when the pathogen reached the full growth in the control plate and the result was expressed in per cent inhibition of mycelial growth over control.

Table 1: **Pathogenicity of *G. papayii* (I_4) on papaya fruits**

S. No.	Inoculum	Fruit rot incidence (PDI)			
		Days after inoculation (DAI)*			
		2	4	6	8
1.	Spore suspension of *G.papayii* (2 drops)	31.92 (34.06)**	45.23 (40.53)	64.48 (46.86)	72.20 (58.20)
2.	Spore suspension of *G.papayii*(4 drops)	40.39 (39.45)	65.17 (53.82)	78.23 (62.20)	83.09 (65.74)
3.	Control I (Sterile distilled water - 2 drops)	NO SYMPTOM			
4.	Control II (Sterile distilled water - 4 drops)	NO SYMPTOM			
CD (P=0.05)		**1.09**			

Table 2: Efficacy of different plant extracts against *G. papayii* under *in vitro* condition

S .No	Treatments	Mycelial growth (cm)*			
		Concentrations			
		5%	Percent reduction over control	7.5%	Per cent reduction over control
1.	Neem (*Azadirachta indica*)	3.23	64.11	2.9	67.87
2.	Lantana (*Lantana camera*)	4.03	55.2	3.8	57.78
3.	Tulsi (*Ocimum sanctum* L.)	4.2	53.33	4	55.55
4.	Notchi (*Vitex negundo* L.)	4.36	51.55	4.2	53.33
5.	Kuppaimeni (*Acalypha indica*)	4.7	47.78	4.5	50
6.	Garlic (*Allium sativum*)	4.5	50.00	3.06	66.00
7.	Ginger (*Zinger officinale*)	6.4	28.89	4.10	54.44
8.	Turmeric (*Curcuma longa*)	4.5	50.00	3.07	65.89
9.	Onion (*Allium cepa*)	6.5	27.77	6	33.33
10.	Oomathai (*Datura stramonium* L.)	8.0	11.11	6.4	28.89
11.	Control	9	-	9	-
	CD (P = 0.05)	**0.24**		**0.25**	

Table 3: Effect of organic amendments on the mycelial growth of *G. papayii* under *in vitro* condition.

S. No.	Treatments	Mycelial growth (cm)*			
		Concentrations			
		5%	Percent reduction over control	10%	Per cent reduction over control
1.	Neem oil cake	3.6	60.00	2.00	77.77
2.	Pungam oil cake	4.00	55.00	2.8	68.88
3.	Groundnut oil cake	4.2	53.33	3.0	66.66
4.	Gingelly oil cake	4.6	48.88	4.00	55.55
5.	Cotton oil cake	5.5	38.88	4.90	45.55
6.	Control	9	-	9	-
	C.D (P=0.05)	**0.17**		**0.15**	

Table 4: Efficacy of different plant oils on the mycelial growth of *G. papayii* under *in vitro* condition.

S. No	Treatments	Conc. (%)	Mycelial growth (cm)*	Percent reduction over control	Conc. (%)	Mycelial growth (cm)*	Per cent reduction over control
1.	Neem oil	1	3.8	57.78	2	1.06	78.89
2.	Mahua oil	1	3.9	56.67	2	2.70	70.00
3.	Clove oil	0.05	2.8	68.89	0.1	1.90	88.20
4.	Cinnamon oil	0.05	1.2	86.67	0.1	0.00	100
5.	Control	-	9	-	-	9	-
CD (P= 0.05)		-	**0.28**	-	-	**0.08**	-

III. Results discussion:

The papaya fruits inoculated with 4 drops of spore suspension of *G. papayii* shown 78.23 PDI at 6 DAI and the uninoculated control fruits exhibited no symptoms and then reisolation, according to Koch's postulates, was made from the resulting lesion(Table 1). The results of *in vitro* screening of plant extracts (5% and 7.5%) against *G. papayii* revealed that the leaf extract of neem (*Azadirachta indica*) was more effective against the growth of *G. papayii* by recording the maximum reduction of mycelial growth (64.11 and 67.87 per cent) (Table 2). The effect of four different plant oils were tested on the mycelial growth of *G. papayii in vitro*. Among these, the cinnamon oil (0.05 and 0.1 %) recorded the maximum mycelial growth reduction of 86.67 % and 100 % over control The least mycelial growth reduction (56.67 % and 70 %) was observed in mahua oil (1 and 2 %) (Table 3). The extract of cotton cake (5 %) was found to record the minimum reduction of mycelial growth (38.88 per cent) of *G. papayii* over control. The extract of cotton cake (10 %) was found to record the minimum reduction of mycelial growth (45.55 %) of *G. papayii* over control (Table 4).

IV. Discussion

Five pathogenic isolates of *G. papayii* were established by the standard isolation procedure and all these five isolates possessed the morphological characters similar to *G. papayii* as described by Prasanna kumar (2001). The pathogen was identified as *Colletotrichum gloeosporioides (*Penz.) Penz. and Sacc. based on its mycelial, condial characteristics through standard mycological keys (Barnett *et al.*, 1972). Kota (2003) proved the pathogenicity of *C. gloeosporioides* on mango and banana. The pinprick and spore suspension method employed for pathogenicity test was similar to those described by Stanghellini and Aragaki (1966).

Ashoka (2005) reported that neem (50.45 %) was found effective in inhibiting mycelial growth of *C. gloeosporioides* under *in vitro*. Kumar and Yadav (2007) found that the leaf extracts of *Azadirachta indica, Datura stramonium* and clove extract of *Allium sativum* reduced spore germination of *C. capsici* and *C. gloeosporioides*. Kannan (2010) reported that among the oil cake extract tested, mahua cake at 10 per cent level, recorded minimum mycelial growth and maximum percentage of inhibition (68.74%) followed by neem seed kernel extract (3.33 cm and 61.59%), pungam cake (3.43cm and 60.43%) and neem cake (4.27 cm and 50.74%) extracts in *A. alternata* causing leaf blight of *Aloe vera*.

V. Conclusion

Data recorded for per cent disease incidence were calculated for each treatment. Later data were subjected to statistical analysis following the method of variance described by Gomez and Gomez (1984). Least significant difference (LSD) at 5 % level was calculated to determine significant difference between treatments.

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