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SOME PLANT EXTRACTS AGAINST ANTHRACNOSE INFECTION IN PAPAYA (*Carica papaya*)

Dimpal Kumari
Ex-Research Scholar, JaiPrakash University Chapra (Bihar)
Email ID:dsindhoul@gmail.com

ABSTRACT

Wherever the papaya is grown, the foremost post-harvest disease is Anthracnose through Colletotrichum gloeosporioides infection. The current research was conducted to evaluating plant extracts activity against to manage infection in hold on fruit in each laboratory and field conditions. Plant specimens were collected from native area. The wood alcohal extract of Echinops sp. of 10 ¼L from the concentration of 50 mg/ml resulted within the highest inhibition zone of 13.5 mm against mycelial growth of C. gloeosporioides.

Spore germination of C. gloeosporioides was reduced by 97.6%, 96.8% and 96.2% over the management by extracts of Echinops sp., Thymus serrulatus and Ocimum lamifolium, severally. Among four botanicals evaluated in vivo as 10% and 25% binary compound extracts, Echinops sp. at 25% concentration showed disease severity score at 1.3 out of 5 and maintained quality of papaya fruit throughout 14 days experimental period. Further study is critical on sensory analysis and developing botanicals as natural fungicides.

Key words: Papaya anthracnose; *Colletotrichum gloeosporioides;* Plant extracts; *Echinops* sp.

INTRODUCTION

The most widespread and devastating diseases of papaya, particularly throughout storage is Anthracnose (Tasiwal et al, 2009). It is a serious problem to papaya production and additionally arise constraints to market supply. Its infections area typically predominated within the field at early stages of fruit development; however the infectious

agent remains quiescent til the fruit reaches the ripening stage.

There anti-fungal agent applied typically suppresses disease however additionally toxicant effects area persistent during consumption and then is also neglected due to metabolic disturbances within the human body. Therefore, plant extracts are rising as safer alternatives to traditional fungicides for the management of plant diseases (Tripathi and Shukla, 2007). This natural fungicide has the flexibility to decompose speedily, thereby reducing their risk to human health and also the environment (Fokialakis et al, 2006).

The antifungal activities of various plant species and also the importance of plants as potential sources of natural fungicides are well established. The past researches have demonstrated the anti-fungal potentials of plant extracts against postharvest fungi (Bautista-Banos et al, 2000). The anti-fungal potentials of plant extracts particularly on Anthracnose infection were additionally studied (Peraza-Sanchez et al, 2005). There is a need to future research regarding effective and economical different ways for the management of papaya disease.

This research conducted on some plant species had such secondary substances that ensure their antimicrobial properties and are toxic to phytopathogens (Tripathi and Shukla, 2007: Amare, 2002). Papaya anthracnose is one among the foremost diseases of the crop in India (18). However, restricted studies are accessible regarding papaya, and, thus this paper envisages impact of plant extracts against infection under laboratory and field conditions.

METHODS AND MATERIALS

The fungal agent (*Colletotrichum gloeosporioides*) isolated from papaya fruit lesions and grown in Glucose agar culture tubes at 4°C was used as stock culture throughout the study.

The plant leaves and twigs collected from native area were processed and extracted with wood alcohal. There 50 grams of processed plant specimens were extracted with 250 ml methyl alcohal by stirring for 2 hour on magnetic stirrer. This extract was filtered through filter paper into a 500 ml spherical bottom flask and reduced to dryness at 40°C water bath temperature. Thereforth 50 mg of the methyl alcohol extract of every plant were re-dissolved in 1 ml of the extract solvent and then tested for antifungal activities.

The paper disc assay for anti-fungal activity was additionally performed. For this, 10 ¼L of the extract solution employing a capillary measuring device impregnated to filter paper disc. Then pre-cooled disc was allowed to spore suspension of fungi (105 conida/ml) and when evaporation of carrier solvent incubated to 4 days for reaction. The experiment was arranged in Completely Randomized Design (CRD) with 3 replications. The diameter of inhibition zone was measured in mm, and also the degree of inhibition of fungal growth was recorded on a 0-4 scale (Amare, 2002).

The conidial suspension of target infectious agent was mixed with solvent served as control to check its germination. The experiment was arranged in CRD with 3 replications. A drop of lactophenol was added to the depression slide and also thought of the mount was observed under microscope for spore germination. There germination was thought of once the length of the germ tube exceeded its diameter. The quantity of conidia germinated was counted and expressed as percentage of germination.

The anti-fungal plant extracts impact upon harvested papaya in field condition was additionally evaluated at concentrations of 10 and 25% (w/v) throughout study. The papaya fruits sterilized and then incubated with pathogen spore suspension from 10-day old culture and adjusted upto 105 conidia/ml. These fruits were then treated to plant extracts to 15 hour, whereas the control fruits were dipped into sterile distilled water (Mohmmed et al, 2009). Carbendazim was used as positive control. Five replications (i.e. 5 fruits) were used for each of the treatments. The experiment was laid out in CRD.

The disease severity was rated on 1 to 5 scale, wherever 1=0% infection, 2=1-20%, 3=21-45%, 4=46-70%, and 5=71-100% fruit area affected (Bautista-Banos et al, 2002). Fruit quality

parameters of the fruits were measured following the methods utilized by Mahmud et al. (2008).

There titrated acid quantity in fruit tissues (10 g) were evaluated through homogenisation with water (40 mL) exploiting thinner. Then 5 mL of the filtrate was titrated using 0.1 N NaOH to an endpoint pink through 1 to 2 drops of phenolphthalein (1%) as indicator. The results were expressed as percentage of citric acid per 100 g fresh weight. Ascorbic acid was determined exploiting the dye technique and expressed as mg 100 g-1 of recent fruits (Ranganna, 1977).

Analysis of Variance (ANOVA) was distributed with the minitab software. Least significant difference (LSD) at 5% probability level was used for mean comparison. Disease severity ratings were square root transformed whereas percent spore germination was arcsine transformed prior to statistical analysis.

RESULTS AND OBSERVATIONS

Effect of plant extracts on mycelial growth and spore germination of target pathogen:

The Mycelial growth of *C. gloeosporioides* was considerably ($P<0.05$) inhibited by wood alcohal extracts of tested plant species (Table 1).

The impact of the extracts ranged from weak to strong (shown on 0-4 scale). Strong antifungal activity was exhibited by each leaves and twigs methanol extracts of *Echinops* sp. and *Thymus serrultus*. Growth inhibition score of four was recorded for extracts of these plants, indicating complete inhibition of growth and sporulation of the fungus. *Echinops* sp. had the highest inhibition zone diameter of 13.5 mm, which was then followed by that of *Thymus serrultus*, *Vernonia amygdalina* and *Zingiber officinale* (Table 1). There were significant differences among mycelial growth and spore germination in the presence of anti-microbial plant extracts ($P<0.05$). Among the six methanol extracts, *Echinops* species and *Thymus serrultus* showed strong inhibition with only 1.1% and 2.3% spores germinated, accounting for 98.7 and 97.3% inhibition of spore germination over the control, respectively (Table 1).

Effect of plant extracts on anthracnose development and quality of papaya fruit

All the four liquid extracts tested considerably reduced anthracnose severity on papaya fruit that had been artificially inoculated with *C. gloeosporioides* (Table 2). The severity of anthracnose on a 1-5 scale was 1.3 (e" 1% fruit area infection) in fruits treated with *Echinops* sp. extract at a degree of 25% that was statistically at par with the positive control (carbendazim) after 14 days of incubation.

Fruits treated with 25% liquid extract of *Echinops* sp. had pH and TSS values of 5.57 and 7.8, severally, that are statistically at par with the carbendazim treated fruit. The highest TSS was recorded from the untreated control. There was no distinction in terms of TA among fruits treated with

Table 1: Antifungal activity of Methyl alcohol extracts of some plant species against *C. gloeosporioides* (DI= Differential Inhibition and IE=Inhibition efficiency).

Plant species	Plant family	DI (MM)	IE	SporeGermination (%)
Zingiber officinalis	Zingiberaceae	5.8	2	12.5
Vernonia amygdalina	Asteraceae	6.0	1	32.4
Thymus serrulatus	Lamiaceae	6.7	4	1.9
Ruta chalepensis	Rutaceae	6.7	3	7.2
Ocimum sp.	Lamilaceae	2.1	1	47.3
Ocimum lamifolium	Lamilaceae	3.7	3	2.1
Lantana viloumoides	Verbenaceae	2.1	1	18.7
Ethiops sp.	Asteraceae	13.2	4	1.0
Artemisia afra	Asteraceae	4.2	3	10.4
Control	-NA	0.0	0	85.8
LSD (0.05)	NA	1.26	NA	3.70

Table 2: Impact of plant extracts on Anthracnose disease severity and quality of papaya fruit (TSS=Total soluble solids; TA=Titrable acidity and AA=Ascorbic acid).

Treatments	Disease severity	Quality Parameters			
		pH	TSS	TA	AA
V. amygdalina (10%)	2.4	5.76	9.24	0.156	58.26
V. amygdalina (25%)	3.1	5.56	9.75	0.184	64.61
T. serrulatus (10%)	2.3	5.73	9.54	0.149	52.87
T. serrulatus (25%)	2.5	5.73	9.13	0.145	54.96
R. chalepensis (10%)	2.6	5.73	9.42	0.159	55.42
R. chalepensis (25%)	2.7	5.72	8.73	0.164	58.32
Echinopus sp (10%)	1.3	5.84	9.44	0.144	53.04
Echinopus sp (25%)	2.1	5.72	9.56	0.1500	60.97
Control	4.6	5.89	12.46	0.126	39.72
Carbendazim	-1.2	5.46	7.36	0.18	63.56
LSD (0.05)	0.68	0.19	0.96	0.017	7.12

totally different concentrations of *V. amygdalina*, *R. chalepensis* and *Thymus serrultus*. However, *Echinops* sp. at a degree of 25% resulted in TA value comparable to the fruits treated with carbendazim. In general, fruits treated with liquid extracts of plants had higher titrable acidity and ascorbic acid content than the untreated control (Table 2). Fruits in the untreated control ripened quickly and this led to the reduction of titrable acidity and ascorbic acid content and increase in the pH and total soluble solid contents of papaya fruits.

DISCUSSIONS

The result incontestable that compounds extracted from plants vary in their effictivity in control for *C. gloeosporioides* growth that is probably due to variability on the provision and solubility of active compounds. The findings of this study are in agreement with previous reports on the antifungal activity of *Echinops* sp., *Ruta chalepensis*, *Thymus serrulatus* and *Artemisia* genus (Amare, 2002: Ademe et al, 2013). Previous phytochemical research of *Ruta chalepensis* resulted in isolation of various alkaloids and coumarins and therefore the active ingredients of this plant have antifungal properties that might prove useful to agriculture (Ojala et al, 2002).

Crude extracts of *Vernonia amygdalina* exhibited antifungal activity and therefore the compounds as glycosides, saponins and tannins were identified as responsive to anti-fungal activity (Nduagu et al, 2008). Additionally, extract of *Thymus vulgaris* and *Zingiber officinale* oil are reported to inhibit mycelial growth of phytopathogenic fungi (Lee et al, 2007). Similarly, complete inhibition of *Helminthosporium solani*, *Aspergillu niger*, *Penicillium digitatum* and *Mucor piriformis* was reported by extract Z. *officinale* at 25% concentration. The phytochemical analysis of extracts confirmed the presence of tannins, phlobatannins, steroids, tarpenes, saponins, flavonoids and alkaloids (Cheijina and Ukeh, 2012).

The extracts of tested plants showed high inhibition on spore germination in comparison to papaya. This is in agreement with the report of Barrera-Necha et al. (Barrere-Necha et al, 2008) which reported the inhibition of *C. gloeosporioides* spores with essential oil of *Ruta chalepensis*. Similarly, Anand and Bhaskaran (2009) indicated that in stem ginger extract solely 38.9 and 28.8% of spores of *C. capsici* and *Alternaria alternate* severally germinated. It is noteworthy that inhibition of spore germination by the extracts is fascinating towards the management of papaya anthracnose.

Increased in incidence and severity of the disease resulted in fruit softening and rot that successively results in reduction within the marketability of the fruits (Gamagae et al, 2004). An identical trend was observed in this study. The organic acids in papaya are famous to be largely

are citric and malic acids, and therefore the increase in pH throughout ripening and storage can be due to the metabolic processes of the fruit that lead to decrease of those organic acids (Mahmud et al, 2008).

The reason for increased TSS content during storage is mainly due to conversion of starch into soluble sugar with advances in ripening (Mahmud et al, 2008). Likewise, decrease in acidity throughout storage incontestable fruit ripening (Al Eryani-Raqeeb et al, 2009). Earlier, Selvaraj et al. (1982) and Mahmud et al. (Mahmud et al, 2008) reported that the ascorbic acid and measurable papaya acidity first increases then decrease, whereas pH and therefore the TSS values increase throughout senescence. In last, extract of *Echinops* sp. strangled growth and spore germination of *C. gloeosporioides* likewise anthracnose development by artificially inoculated papaya fruits and will be used for sensible management of papaya infection.

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