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Role of plant extracts in inducing the systemic acquired resistance in harvested banana against anthracnose disease

[*]G. Jadesha[1], Hemachandra Haller[2], Manish K Mondhe[3], Manjunath Hubballi[4], K. Prabakar[4] V. Prakasam [4]

[1]Department of Plant Pathology, Acharya N G Ranga Agricultural University, Hyderabad
[2]Department of Plant Pathology, University of Agricultural Sciences, Dharwad
[3]Department of Plant Pathology, Dr. P D K V Akola University, Akola
[4]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore

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ABSTRACT

Mechanism of disease suppression by plant products have proved that, the active principles present in them and/or induce systemic resistance in host plants. In the present investigation we studied the expression of induced systemic resistance in banana fruits upon treatment with plant extracts. Banana fruits are dipped in 10 per cent of leaf extracts of Solanum torvum, Zimmu and Allium alliaceum for five minutes and inoculated with conidial suspensions (10^6/ml) of Colletotrichum musae by pin prick method. The changes in the induction of defence mechanism of the inoculated fruits, inoculated fruits dipped in leaf extracts and uninoculated control are analyzed separately both in peel and pulp 2, 4, 6, 8 and 10 days after treatment. The Peroxidase(PO), Polyphenol oxidase(PPO) and Phenylalanine ammonia-lyase(PAL) activity was significantly increased both in peel and pulp of the inoculated fruits dipped in leaf extracts as compared to inoculated fruits alone. Activity of defense enzymes are highest at six day after inoculation and decline in subsequent days.

Key words: Induced systemic resistance, Plant extracts, Banana, Anthracnose

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INTRODUCTION

Banana (*Musa* spp.) is the most important fruit crop in India and cultivated over an area of 5.29 lakh ha with an annual production of 162.25 lakh tones [8]. It is not only known for its antiquity but also closely interwoven in our national heritage with its multifaceted uses. Hence, it is referred as Kalpatharu.

The crop is affected by several diseases among which the post-harvest diseases are most important as they not only deteriorate the quality and nutritive value of the fruits but render them unfit for consumption and marketing, thereby causing severe losses to farmers and retailers. Important post-harvest diseases of banana are anthracnose, stem end rot and crown rot. Anthracnose disease caused by *Colletotrichum musae* (*Berk. & M. A. Curtis*) *Arx* is occurring in almost all the banana growing countries. Primarily anthracnose is considered as a storage disease but infections of immature fruits do occur in the field itself.

The most commonly used fungicides for the management are benzimidazole fungicides, such as benomyl and thiabendazole [14] and *C. musae* has developed resistance to these fungicides in addition to the residual toxicity of the chemicals on fruits. [6].

It is therefore an utmost urgency to identify alternative control methods, particularly those which are environmentally safe and biodegradable, without sacrifying the productivity. Thus, replacement of synthetic fungicides by natural products (particularly of plant origin) are gaining the importance. Investigations on mechanism of disease suppression by plant products have suggested that the active principles present in them may either act on pathogen directly or induce systemic resistance in host plants resulting in reduction of disease development [27]. The utilization of a plant's own defense mechanism is the subject of current interest in the management of pests and diseases. Induction of defense genes by prior application of inducing agents is termed as "Induced resistance" [17].

MATERIALS AND METHODS

Experimental materials and isolation of pathogen

The healthy unripened banana fruits of Robusta, free from injuries and scars were procured from local market, Coimbatore. The leaves of *S. torvum*, Zimmu and *A. alliaceum* were collected from botanical garden located at Tamil Nadu Agricultural University, Coimbatore.

Isolation of Pathogen was isolated from the fruits exhibiting typical symptom of anthracnose on PDA medium using tissue segment method of [28] and was further purified through single spore isolation method [30].

Preparation of plant extract

The leaves of *Solanum torvum*, Zimmu and *Allium alliaceum* were collected and washed under tap water and then the leaves were rinsed with sterile distilled water. 100 gram of leaf material was weighed and ground using a grinder by adding one liter sterile distilled water.

Pin prick method of inoculation

Banana fruits at stage-1 [19] free from bruish and blemish were selected, washed with running tap water, surface sterilized with 0.1 per cent mercuric chloride and subsequently washed in sterile distilled water. Fruits were dipped in 10 per cent leaf extracts of *S. torvum*, zimmu and *A. alliaceum* for five min and allow to air dry under aseptic condition. Conidial suspension of inoculated the pathogen by pin pricking method, then a circle of about five mm diameter was made with an Indian ink and injuries were made using a sterile needle in the marked area and conidial suspensions (10^6/ml) of *C. musae* were inoculated into the fruits separately. The inoculated area of the fruit was covered with moist cotton and was kept inside sterile, perforated polythene bags (200 gauge) which were sprayed with sterile distilled water so as to provide required humidity.

The roles of plant extracts in inducing the defense mechanism in the banana fruits against the pathogen were estimated. The changes in the induction of some of defense enzymes in the inoculated fruits, inoculated fruits dipped in leaf extracts and uninoculated control are analysed separately both in peel and pulp on 2, 4, 6, 8 and 10 days after treatment.

Assay of defense related enzymes

Peroxidase activity was assayed using a slight modification of the method of [18]. The peroxidase activity was expressed as changes in the absorbance of the reaction mixture/min/g on fresh weight basis [16]. Polyphenol oxidase activity was assayed using the modified method of [26]. Phenylalanine ammonia-lyase activity was determined at 30°C by direct spectrometric measurement of the conversion of L-phenylalanine to transcinnamic acid at 290 nm [7]. An extinction co-efficient of 9630/mole/cm was determined for transcinnamic acid in 0.1M borate buffer (pH 8.8) [36].

Statistical analyses

The data were statistically analyzed [29] using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines [11].

RESULTS

Changes in the activity of Peroxidase

The peroxidase activity was significantly increased both in peel and pulp of the inoculated fruits dipped in leaf extracts as compared to inoculated fruits alone. The increase in peroxidase activity was 1.4, 38.8, 36.26 and 9.4 per cent in peel and 5.00, 31.6, 60.0 and 62.5 per cent in pulp over untreated control on tenth day was recorded in inoculated fruits treated with extracts of *S. torvum,* zimmu and *A. Alliaceum* respectively (Table 1).

Changes in the activity of Polyphenol oxidase

A significant increase in polyphenol oxidase activity was noticed from the second day onwards in the inoculated fruits dipped in leaf extracts over inoculated fruits alone and the trend continued up to six days both in peel and pulp. The increase in polyphenol oxidase activity was 0.00, 64.28, 57.14 and 42.85 per cent in peel and 10.0, 90.0, 77.0 and 17 per cent in pulp over untreated control on tenth day in the inoculated fruits alone and inoculated fruits dipped in leaf extracts of *S. torvum,* zimmu and *A. alliaceum* respectively (Table 2).

Changes in the activity of Phenylalanine ammonia-lyase activity

Phenylalanine ammonia-lyase activity was significantly increased both in peel and pulp of the inoculated fruits dipped in leaf extracts when compared to inoculated fruits alone. In the inoculated fruits alone and inoculated fruits dipped in leaf extracts of *S. torvum,* zimmu and *A. alliaceum* the increase was 3.17, 179.3, 121.7 and 139.8 per cent in peel and 16.6, 175.0, 108.3 and 133.0 per cent in pulp over untreated control on tenth day.

Among the three extracts *S. torvum* was found to induce more defense enzyme activity.

DISCUSSION

ISR mediated by plant extracts

Activation of the plant's own defense system with the aid of biotic and abiotic inducer is a novel technology in the management of plant diseases. Plant products have been considered as one of the major groups of compounds that induce systemic resistance. Biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development. In the present investigation, application of botanical extracts has been studied for their induction of systemic resistance mechanism in terms of induction of defense related enzymes *viz*., peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL).

Peroxidase

Peroxidases are involved in phenyl propanoid metabolism, regulation of plant cell elongation, phenol oxidation, polysaccharide cross-linking, IAA oxidation, cross-linking of extensis monomers, oxidation of hydroxy-cinnamyl alcohols into free radical intermediates and wound healing [35]. Plant peroxidases are heme - proteins that use H_2O_2 to oxidize a large variety of hydrogen donors such as phenolic substances, amines, ascorbic acid, indole and certain inorganic ions [33]. Various experiments suggested the involvement of plant peroxidases not only in biosynthetic processes related to wall development such as lignification [23] , suberification [31] and polymerization of hydroxyproline-rich glycoproteins [3], but also in the regulation of cell wall elongation [13], wound healing [23] and resistance against infection by pathogens [5].

In the present study, a significant increase in peroxidase activity was observed upto six days in the inoculated fruits dipped in leaf extracts and thereafter the activity declined both in peel and pulp (Fig.1). [2] reported that mango fruits inoculated with the spore suspension of *C. gleosporides,* and sprayed with 12.5 per cent leaf extracts of *A. alliaceum* showed a threefold increase in peroxidase ativity. These findings are in similarity with the results of present investigation.

Polyphenol oxidase (PPO)

Polyphenol oxidase is a copper containing enzyme, which oxidises phenolics to highly toxic quinones and involved in the terminal oxidation of diseased plant tissues and is attributed for its role in disease resistance [22]. In the present study, polyphenol oxidase activity was

Table 1. Expression of peroxidase activity of banana fruits after treatment with plant extracts

Treatment	Change in absorbance / min / g of fresh tissue													
	Peel*					Per cent increase over control		Pulp*					Per cent increase over control	
	Days after treatment							Days after treatment						
	2	4	6	8	10	6th day	10th day	2	4	6	8	10	6th day	10th day
T1	0.43^{b}	0.57^{c}	0.913^{c}	0.747^{d}	0.680^{c}	60.17	1.40	0.220^{c}	0.357^{b}	0.74^{c}	0.570^{c}	0.42c	131.2	5.00
T2	0.57^{a}	0.913^{a}	1.823^{a}	1.243^{a}	0.937^{a}	219.6	38.80	0.253^{b}	0.537^{a}	1.13^{a}	0.727^{b}	0.72^{a}	253.1	80.0
T3	0.56^{a}	0.827^{b}	1.523^{c}	1.120^{b}	0.913^{a}	166.6	36.26	0.253^{b}	0.540a	0.97^{b}	0.77a	0.64^{b}	203.2	60.0
T4	0.59^{a}	0.793^{b}	1.610^{b}	0.950^{c}	0.733^{b}	180.7	9.40	0.267^{a}	0.387^{b}	0.94^{b}	0.543^{c}	0.65^{b}	193.7	62.5
T5	0.40^{b}	0.513^{d}	0.570^{e}	0.617^{e}	0.670^{c}	-	-	0.210^{d}	0.253^{c}	0.32^{d}	0.380^{d}	0.407^{c}	-	-

T1- Fruits inoculated with pathogen *T4- Fruits inoculated and sprayed with A. alliaceum*
T2- Fruits inoculated and sprayed with S. torvum *T5- Uninoculated control*
T3- Fruits inoculated and sprayed with Zimmu
**Mean of five replications.*

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

The values in parentheses are arcsine transformed.

Table 2. Influence of plant extracts on Polyphenol oxidase activity in harvested banana fruits

Treatment	Change in absorbance / min / g of fresh tissue													
	Peel*					Per cent increase over control		Pulp*					Per cent increase over control	
	Days after treatment							Days after treatment						
	2	4	6	8	10	6th day	10th day	2	4	6	8	10	6th day	10th day
T1	0.107^{b}	0.133^{b}	0.193^{d}	0.177^{c}	0.14^{b}	51.96	0.00	0.060^{b}	0.077^{c}	0.100b	0.103^{b}	0.110^{d}	19.2	10.0
T2	0.140^{b}	0.190^{a}	0.387^{a}	0.247^{a}	0.23^{a}	216.6	64.28	0.070^{a}	0.180^{ab}	0.313^{a}	0.217^{a}	0.19^{a}	259.7	90.0
T3	0.153^{b}	0.187^{a}	0.313^{c}	0.233^{b}	0.22^{a}	158.3	57.14	0.070^{a}	0.170^{b}	0.247^{ab}	0.240^{a}	0.177^{b}	174.4	77.0
T4	0.273^{a}	0.183^{a}	0.350^{b}	0.250^{a}	0.20^{a}	191.6	42.85	0.073^{a}	0.187^{a}	0.397^{a}	0.233^{a}	0.167^{c}	333.3	67.0
T5	0.100^{b}	0.117^{c}	0.127^{e}	0.133^{d}	0.14^{b}	-	-	0.063^{b}	0.080^{c}	0.087^{b}	0.093^{b}	0.10^{d}	-	-

T1- Fruits inoculated with pathogen *T4- Fruits inoculated and sprayed with A. alliaceum*
T2- Fruits inoculated and sprayed with S. torvum *T5- Uninoculated control*
T3- Fruits inoculated and sprayed with Zimmu
**Mean of five replications.*

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.
The values in parentheses are arcsine transformed.

Significantly increased in the inoculated fruits treated with leaf extracts upto six days and thereafter declined both in peel and pulp. (Fig. 2).

This was contrary to the finding of [21] that the polyphenol oxidase activity decreased in the apple fruits inoculated with *G. cingulata* and *Monilinia laxa.* [4] reported that polyphenol oxidase activity was higher in the mango fruits inoculated with *B. theobromae* and *C. gleosporioides* than the uninoculated control. The increase in polyphenol oxidase activity might be due to activation of latent host enzyme, solubilisation of host polyphenol which was normally particulate or even due to *de novo* synthesis [25]. [1], [2] have reported similar results in chilli and mango fruits after treatment with plant extracts.

Phenylalanine ammonia-lyase (PAL)

Phenylalanine ammonia lyase is the first key enzyme in the phenylpropanoid metabolism and plays a significant role in the regulation of biosynthesis of phenols in plants [24]; [20]. PAL catalyzes the conversion of phenylalanine to trans-cinnamic acid which supplies the precursors for flavonoid pigments, lignins and phytoalexins [15]. Several studies indicated that the activation of PAL and subsequent increase in phenolic content in plants is a general response associated with disease resistance [34]. Inhibition of PAL affects subsequent pathways of phenolic compound synthesis. The stimulation of phenyl propanoid metabolism by challenge inoculation of pathogen was reported by [9], PAL is involved in the biosynthesis of phytoalexins, lignins, polyphenols and salicylic acid associated with disease resistance [10]. Inhibition of PAL affects subsequent pathways of phenolic compound synthesis. The

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Fig. 1. Changes in peroxidase activity of *C. musae* inoculated banana fruits as influenced by plant extracts

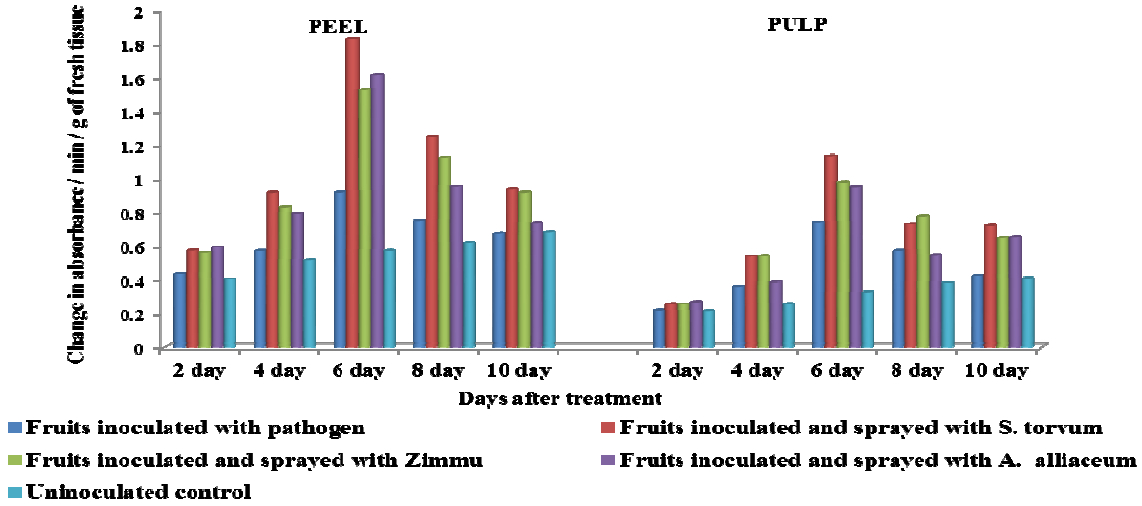
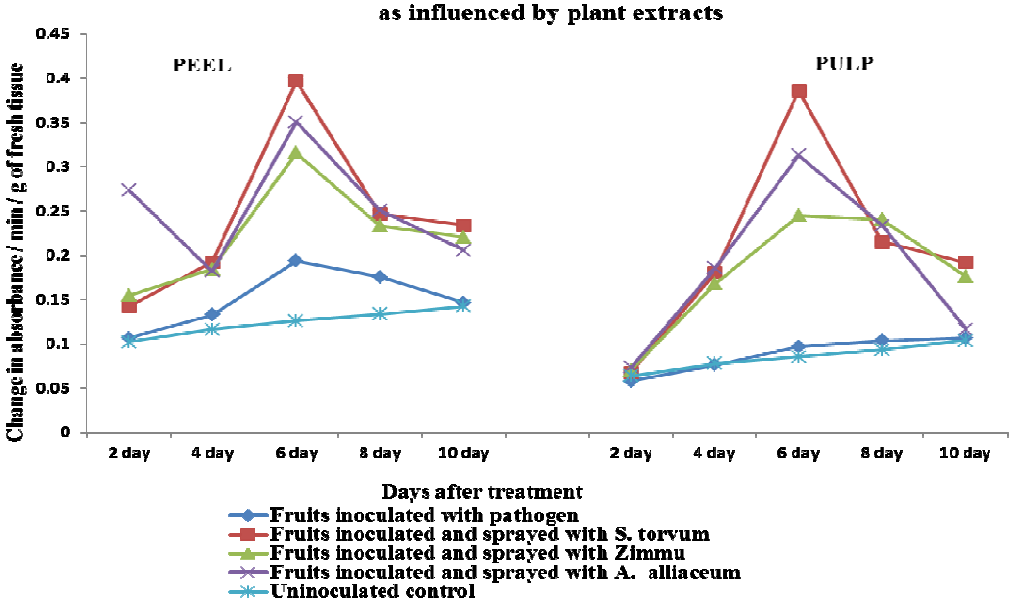


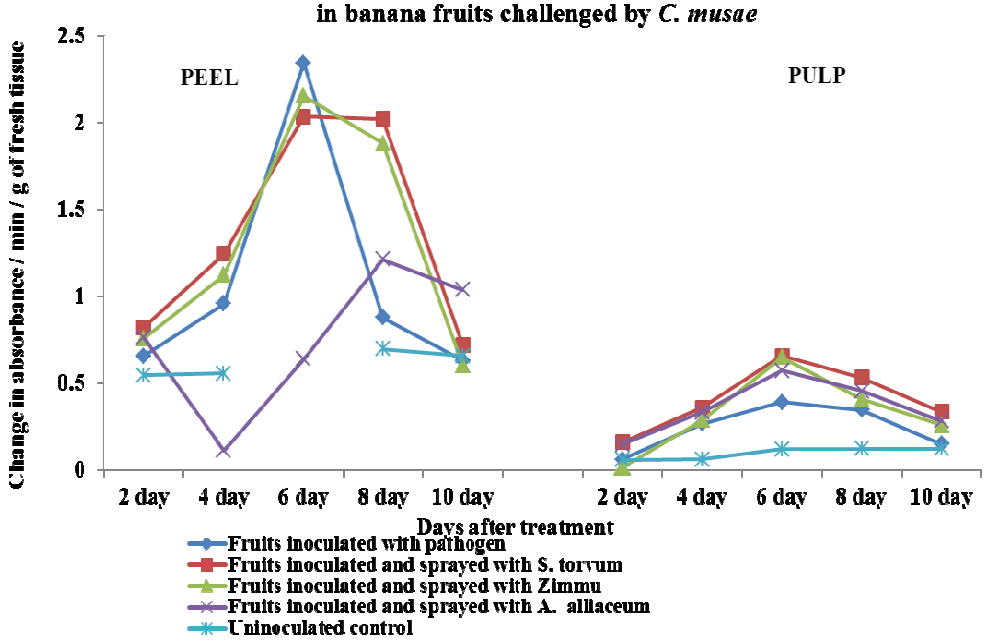
Fig. 2. Induction of Polyphenol oxidase in *C. musae* inoculated banana fruits as influenced by plant extracts



In the present study, the activity of phenylalanine ammonia-lyase increased in the inoculated fruits dipped in leaf extracts of *S. torvum* , zimmu and *A. alliaceum* upto sixth day after treatment both in peel and pulp of banana fruits (Fig. 3). Since the production of phenolic compounds depend on PAL activity [12], increased phenolic synthesis in the treated banana plants might be due to increased activity of PAL. [1] found that *C. capsici* inoculated chilli fruits sprayed with leaf extract of *Abrus precatorius* recorded two fold to three fold increased activity of phenylalanine ammonia lyase. [32] reported that induction of Phenylalanine ammonia lyase in *X. axonopodis* pv *malvacearum* inoculated cotton plant when sprayed with zimmu leaf extract. [2] reported a threefold increasing in Phenylalanine

ammonia lyase activity in the mango fruits inoculated with the spore suspension of *C. gleosporides*, and sprayed with 12.5 per cent leaf extracts of *A. alliaceum*.

Fig. 3. Influence of plant extracts in inducing the phenylalanine ammonia-lyase in banana fruits challenged by *C. musae*



CONCLUSION

Management of plant disease by application of plant extracts has became one component in integrated disease management. Mechanism of disease suppression by plant products have proved that, the active principles present in them and/or induce systemic resistance in host plants. Induction of defense enzymes *viz*, Peroxidase(PO), Polyphenol oxidase(PPO) and Phenylalanine ammonia-lyase(PAL) in banana is one of key factor in suppression of pathogen and disease development.

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