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# Induction of Defense Related Enzymes against Crown Rot in Banana Fruits Treated with Plant Oils and Plant Extracts

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## ABSTRACT

In present study, peroxidase activity was found to be increased when fruits are treated with thyme oil @ 0.05% (52 µg changes in absorbance min-1g-1) which was followed by propiconazole @ 0.1% (55.81 µg changes in absorbance min-1g-1) which decreased in all other treatments. In polyphenol oxidase activity was found to be increased when fruits are treated with thyme oil @ 0.05% (4.27 µg changes in absorbance min-1g-1) which was followed by propicanazole @ 0.1% (4.0 µg changes in absorbance min-1g-1) which decreased in all other treatments. In the case of phenylalanine ammonia lyase activity was found to be increased when fruits are treated with thyme oil @ 0.05% (6.20 µg changes in absorbance (min-1g-1) which was followed by propicanazole @ 0.1% (6.0 µg changes in absorbance min1g-1) which decreased in all other treatments. In the case of β- 1, 3- glucanase activity was found to be increased when fruits are treated with thyme oil @ 0.05% (217.4 µg changes in absorbance min-1g-1) which was followed by propicanazole @ 0.1% (205.81 µg changes in absorbance min-1g-1) which decreased in all other treatments.

**Keywords:** Crown rot, Peroxidase, polyphenol oxidase, phenylalanine ammonia lyase

## Introduction:

Banana is a popular fruit worldwide due to its flavor, texture, nutritional value and convenience, being easy to peel and eat (Robinson 1996). However, banana is susceptible to several diseases resulting in massive and extensive postharvest losses during transportation and storage (Basel et al. 2002). Laseodiplodia theobromae is a well known pathogen to cause post harvest disease in fruits, especially crown rot disease in banana. Fungicides are used as the primary means of controlling post harvest diseases (Eckert and Ogava 1988). Chemical control using fungicides such as carbendazim, methyl thiophanate, Imazalil and bitertanol is still the most common practice in controlling crown rot disease of banana (Siriwardana et al. 2017). The use of synthetic fungicides has been a major cause for the development of resistant fungal strains and increased amount of toxic

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residues in food products (Divya jagana et al. 2018). Among the safer alternatives to synthetics, use of plant oils has attracted researchers for the management of several diseases of fruits (Smid et al.1994; Dixit et al. 1995). A wide range of secondary metabolites such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins and sterols are present in the higher plants. Such plant derived chemicals may be exploited for their different biological properties (Tripathi et al. 2004). Various plant extracts are used as the control for the fungal pathogens because of their antifungal properties and antibacterial properties (Parveen et al. 2013; Koka JA et al. 2017). The antifungal components present in the plant extracts are phenols, flavonoids, isoflavonoids, coumarins, pyrones and aldehydes which effect the growth of pathogenic fungi (Jantasorn et al. 2016). Therefore plant oils and plant extracts used to manage plant diseases. This study aims to investigate the induction of defense re in banana fruits treated with some plant oils and plant extracts.

**Materials and Methods:**

**Sample Collection**

The following eight treatments were used to treat the samples.

T1 : Basil oil (0.07%)

T2 : Lemon grass oil (0.09%)

T3 : Citrodara oil (0.09%)

T4 : Thyme oil (0.05%)

T5 : Lavender oil (0.1%)

T6 : Neem extract (60%) + Tulasi extract (50%)

T7 : Propicanazole (0.025%)

T8 : Control

The above treated samples were collected at different time intervals (1, 3, 5, 7 and 9 days) after infection. Three replications were maintained in each treatment.

**Enzyme extraction**

The plant tissues collected from plants were immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2/ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4oC. The homogenate was centrifuged for 20 min at 10,000 rpm. Plighting extract prepared from leaves was used for the estimation of peroxidase (PO), polyphenol oxidase (PPO), L-phenylalanine ammonia-lyase (PAL).

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**Spectrophotometric assay**

**Peroxidase (PO)** (Hammerschmidt et al. 1982)

Peroxidase activity was assayed spectrophotometrically (Hartee 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H2O2 which was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 nm was recorded at 30 sec. interval for 3 min and the boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture min-1 g-1 on fresh tissue.

**Polyphenol oxidase (PPO)** (Mayer et al. 1965)

The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 200 ml of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 µm min-1 g-1 fresh weight of tissue.

**Phenylalanine ammonia-lyase (PAL)** (Ross and Sederoff 1992)

The assay mixture containing 100 µl of enzyme, 500 µl of 50 mM Tris HCl (pH 8.8) and 600 µl of 1mML-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later, 1.5 ml of toluene was added and vortexed for 30 sec. The centrifuged (1000 rpm, 5 min) toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was
expressed as moles of cinnamic acid min-1 g fresh tissue-1.

**Results and Discussion**:

**Changes in peroxidase (PO) activity**

In present study, the results revealed that peroxidase activity was found to be increased when fruits are treated with plant oils and extracts when compared with the inoculated control. Two to four fold increase in the activity was observed in the treatment T4- Thyme oil @ 0.05% (52 µg changes in absorbance min-1g-1) in the fifth day which was followed by propicanazole @ 0.1% (55.81 µg changes in absorbance min-1g-1) which then starts to decrease after five days where other treatments showed lesser activity(Figure 1).

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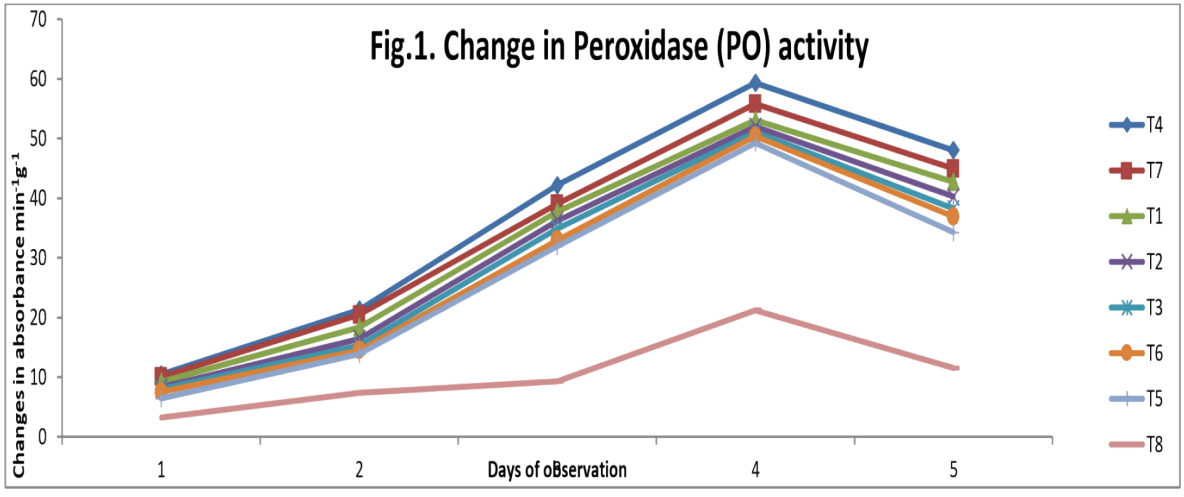
**Changes in polyphenol oxidase (PPO) activity**

In present study, polyphenol oxidase activity was found to be increased gradually when fruits are treated with thyme oil @ 0.05%. The observed value in fifth day was 4.27 µg changes in absorbance min-1g-1 which was followed by propicanazole @ 0.1% which was 4.0 µg changes in absorbance min-1g-1 in fifth day. The activity was found to be decreased in all other treatments (Figure 2).

**Changes in phenylalanine ammonia lyase (PAL) activity**

In present study, phenylalanine ammonia lyase activity was found to be increased in treatement T4-Thyme oil @ 0.05% (6.20 µg changes in absorbance min-1g-1) which was followed by the treatment T7-Propicanazole @ 0.1% (6.0 µg changes in absorbance min-1g-1) in the fifth days. It increased five times in the treatment T4 in the fifth day which then decreased. The PAL activity was reduced and seems to be lesser in all other treatments (Figure 3).

Sangeetha et al. (2013) also reported that banana fruit treated with zimmu leaf extract effectively increased the PAL, β1,3- glucanase and chitinase. The results suggested that enhancing the activity of defence enzyme increases the resistance of the banana fruit. Similarly, Jadesha et al. (2012) reported that the peroxidase activity was significantly increased both in peel and pulp of inoculated fruits dipped in leaf extracts. The increase in peroxidase activity was 1.4, 38.8, 36.26 and 9.4 per cent in peel and 5.0, 31.6, 60.0 and 62.5 per cent in pulp. Similarly it enhanced the activity of polyphenol oxidase and phenylalanine ammonia-lyase activity. The results reveal that S. torvum was found to induce more defence enzyme activity. These earlier reports relies with the present work.



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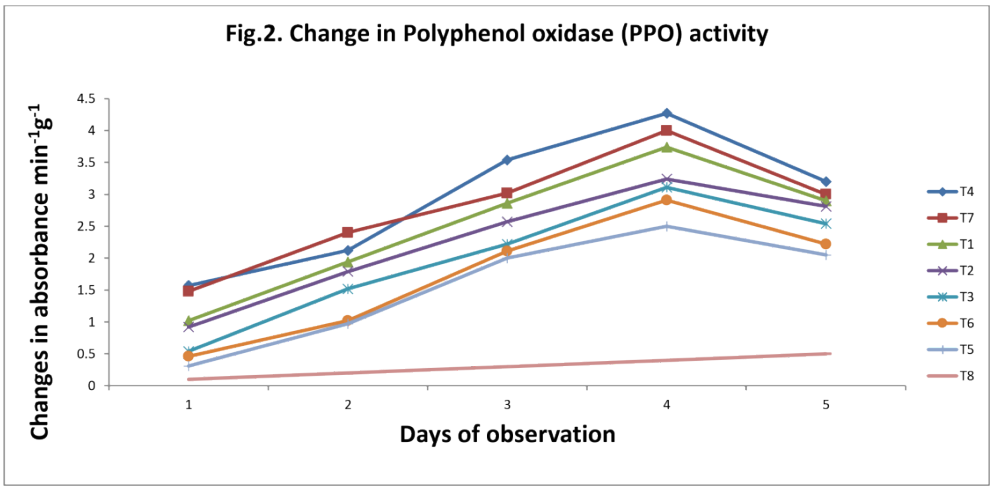
T5 - Lavender oil (0.1%) T6 - Neem extract (60%) + tulasi extract (50%)

T7 - Propicanazole (0.1%) T8 - Control

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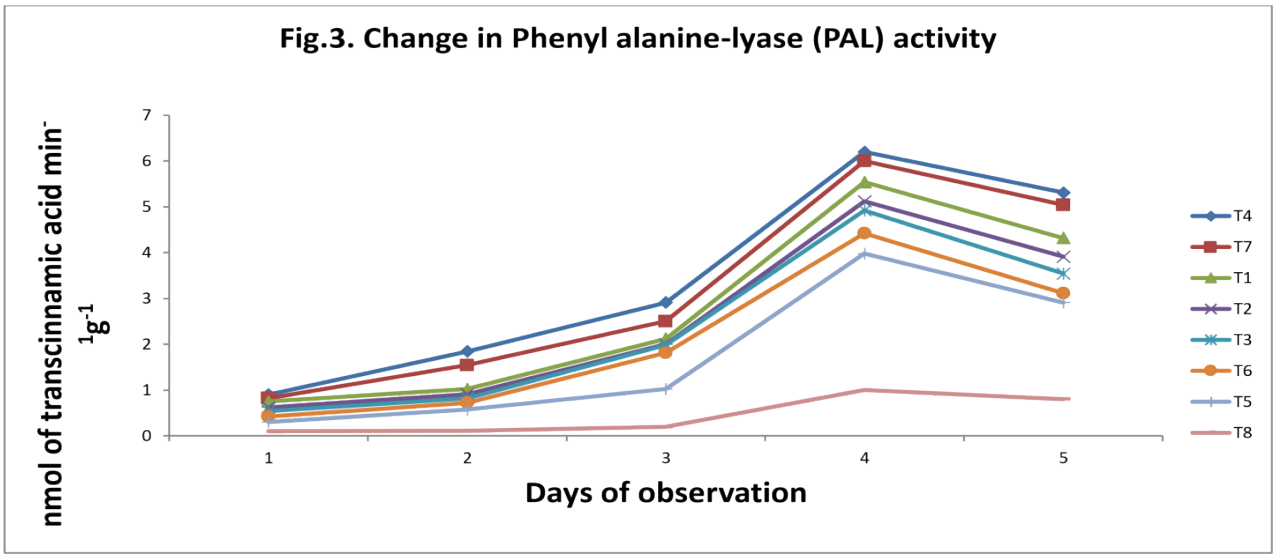
T7 - Propicanazole (0.1%) T8-Contro

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