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Application of plant extracts induced the changes in biochemical composition of Banana fruits

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ABSTRACT

Banana fruits were dipped in 10 per cent leaf extracts of Soanum torvum, Zimmu and Allium alliaceum for five min and inoculated the pathogen with conidial suspensions (10^6/ml) of Colletotrichum musae by pin prick method of inoculation, The changes in the biochemical constituents of the inoculated fruits, inoculated fruits dipped in leaf extracts and uninoculated control are analyzed separately both in peel and pulp at 2, 4, 6, 8 and 10 days after treatment. The rate of reduction in starch content was higher in the fruits inoculated with C. musae than inoculated fruits dipped in leaf extract and there was a progressive reduction in its content as the storage period increased. Sugar content is increased both in peel and pulp of banana as over period. The extent of sugar content increase was much higher in the fruits inoculated with C. musae alone than C. musae inoculated fruits dipped in leaf extracts over untreated control. The reduction of phenolic content was more pronounced in the C. musae inoculated fruits alone as compared with C. musae inoculated fruits dipped in leaf extracts. In addition, ascorbic acid content was increased in the inoculated fruits dipped in leaf extracts when compared to the fruits inoculated with pathogen alone.

Key words: *Soanum torvum*, Zimmu, *Allium alliaceum*, and biochemical changes

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INTRODUCTION

Banana (*Musa* spp.) the most important fruit crop grown in tropical and subtropical regions of India is having a great socio-economic significance. Based on gross value, it is considered to be the fourth most important food crop in the world after rice, wheat and milk/milk products. Apart from the antiquity this has closely interwoven in our national heritage with its multifaceted uses and hence, referred as Kalpatharu (a plant with virtues). In India it is cultivated over an area of 5.29 lakh ha with an annual production of 162.25 lakh tones [4].

Crop is known to be debilitated by an array of fungal, bacterial and viral disease, of which anthracnose incited by *Colletotrichum musae* [*Berk. & M. A. Curtis] Arx* is a wide spread, destructive disease affecting quality and nutritive value of the fruits. Primarily anthracnose is considered as a storage disease but infections of immature fruits do occur in the field. The widely practiced existing management practices for this diseases mainly relied on the

chemical fungicides especially benomyl and thiabendazole [11]. Though a good control has been achieved with chemical their residual impact on biological entity as whole is quiet evident. Apart from this appearance of new fungicide resistant strains of pathogen coupled with high cost involved in the management through chemicals has forced to find an alternative arsenal. In this context, biological control especially with plant extracts seems to be the possible option. Many people have reported the usage of plant extracts for managing this disease [1]. The efficacy of *S. torvum*, zimmu and *A. alliaceum* against *C. musae* has been demonstrated by many workers [27], [1].

The usage plant derived fungicides have undoubtfully given good control of disease in many cases. However, their impact on quality of the fruits has been ignored greatly. Understanding the biochemical nature of fruits sprayed with effective plant-derived fungicides will aid in safety usage of these fungicides for management of disease under storage conditions. A thorough survey has clearly depicted scarcity of information in this direction. The present study reports the influence of plant extracts on the biochemical parameters of banana.

MATERIALS AND METHODS

Experimental materials and isolation of pathogen

The healthy banana fruits of robusta with no injuries and scars was obtained from local market. The leaves *S. torvum*, zimmu and *A. alliaceum* were obtained from medicinal plants orchard located at Tamil Nadu Agricultural University, Coimbatore. Pathogen was isolated from the fruits exhibiting typical symptom of anthracnose on PDA medium using tissue segment method of [21] and was further purified through single spore isolation method [22].

Preparation of plant extract

The leaves of *S. torvum*, zimmu and *A. alliaceum* were collected and washed under tap water and then the leaves were rinsed with sterile distilled water. Ten per cent of leaf extracts were prepared by grinding 100 gram of leaf material in one-liter sterile distilled water.

Application of plant extracts and challenge inoculation of pathogen

Banana fruits at stage-1 [8] free from bruish and blemish were selected, washed with running tap water, surface sterilized with sodium hypochlorite (For fruits or any soft tissue mercuric chloride should not be used) 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 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 to provide required humidity.

The samples of fruits viz., pulp and peel were collected at pulp 2, 4, 6, 8 and 10 days after treatment and these samples were subjected for biochemical analysis. Five replications were maintained for each treatment.

Estimation of quality parameters

Various quality parameters like starch, total sugar, reducing sugar, non-reducing sugar, phenols, and ascorbic acid were estimated at different days after harvest. Total sugar content in fruits at different days after harvest was estimated by Anthrone method [7] and reducing sugar was estimated through method suggested by [31]. The difference between total sugar and reducing sugar corresponds to the non-reducing sugar**.**

Total phenol was estimated through Folin- Ciocalteau reagent as per procedure of [2] and Ortho dihydroxy phenol content of the ethanolic extract was estimated by the method described by [12] using Arnow's reagent. In addition [28] was adopted for Ascorbic acid content by use of estimated by 2-6-dichlorophenol indophenol titration method.

Statistical analyses

The data were statistically analyzed [21] using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines.

RESULTS AND DISCUSSION

Starch content

The most striking chemical changes which occur during ripening of banana are hydrolyses of starch and accumulation of sugars [11]. It was observed that there was an appreciable reduction in the starch content in the inoculated fruits compared to inoculated fruits dipped in leaf extracts. The reduction in starch content was noticed from the second day of treatment and reached a maximum on the tenth day. On tenth day after treatment the per cent reduction of starch in inoculated fruits and treated with leaf extract of *S. torvum*, zimmu and *A. alliaceum* were 43.47, 25.62, 31.73 and 28.26 per cent in peel and 20.23, 8.6, 12.9 and 18.11 per cent in pulp over untreated control, respectively (Table 1). Degradation of starch and conversion into sugars was less in inoculated fruits dipped in *S. torvum*, zimmu and *A. alliaceum.*

[29] reported that pulp of the fresh green fruit had more starch content, but during ripening it was completely hydrolyzed and only one to two per cent was noticed in fully ripened fruits. Drastic reduction in starch content of banana fruits was noticed starting from the first day upto five days in the inoculated fruits treated with paraquat when compared to untreated fruits [18].

Table 1. Influence of plant extracts on starch content of banana fruits

Per cent starch content												
Treatment	Peel*						Pulp*					
	Days after treatment					Per cent decrease over control	Days after treatment					Per cent decrease over control
	2	4	6	8	10		2	4	6	8	10	
T1	3.57 ^{d} (11.14)	3.30 ^{b} (10.46)	2.30 ^{d} (8.72)	1.70^{d} (7.48)	1.30d (6.54)	43.47	14.39^{d} (22.42)	10.23^{e} (18.65)	7.48^{d} (15.87)	6.24^{c} (14.46)	3.83^{e} (11.29)	26.20
T2	3.94^{b} (11.45)	3.67 ^{ab} (10.93)	3.16 ^{b} (9.27)	2.60^{b} (8.78)	2.00^{b} (8.52)	13.04	14.74^{b} (22.58)	11.17^{b} (19.52)	8.304^{b} (16.74)	6.59^{b} (14.87)	4.74^{b} (12.57)	8.6
T3	3.82 ^{c} (11.61)	3.54 ^{a} (11.18)	2.81^{c} (9.66)	2.32^{c} (8.76)	1.76^{c} (7.46)	23.47	14.62^{c} (22.74)	10.83^{c} (19.21)	8.33^{b} (16.78)	6.56^{b} (14.84)	4.52^{c} (12.27)	12.9
T4	3.83^{c} (11.29)	3.54 ^{a} (11.18)	2.83^{c} (9.68)	2.33^{c} (8.78)	1.70^{bc} (7.42)	35.29	14.62^{c} (22.74)	10.53^{d} (18.91)	8.13^{c} (16.57)	6.60^{b} (14.88)	4.52^{d} (12.27)	12.9
T5	4.12 ^{a} (11.71)	3.76 ^{a} (11.10)	3.40 ^{a} (10.6)	3.00^{c} (9.97)	2.30^{a} (8.72)	-	15.08^{a} (22.85)	11.80^{a} (20.09)	9.23^{a} (17.69)	7.60^{a} (16.00)	5.19^{a} (13.17)	-

T1- Fruits inoculated with pathogen, T2- Fruits inoculated and sprayed with S. torvum, T3- Fruits inoculated and sprayed with Zimmu, T4- Fruits inoculated and sprayed with A. alliaceum, T5- Uninoculated control

**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.

Sugar content

The increase in the total sugar was more pronounced from the second day and the trend continued up to ten days after treatment. There was a significant increase in the total sugar content in both peel and pulp of the inoculated fruits when compared to inoculated fruits dipped in leaf extracts. The increase was found to be 66.34, 6.20, 18.67 and 9.7 per cent in peel and 60.56, 9.70, 23.19 and 25.00 per cent in pulp at tenth day in the inoculated fruits and treated with leaf extract of *S. torvum*, zimmu and *A. alliaceum* respectively when compared to untreated control (Table 2).

The reducing sugar content significantly increased both in peel and pulp of the inoculated fruits dipped in leaf extracts when compared to untreated control. The increase was comparatively lesser in the fruits inoculated and dipped in leaf extracts than the fruits inoculated alone. The present increase in inoculated fruits alone and dipped in leaf extracts of *S. torvum*, zimmu and *A. Alliaceum* were 8.22, 5.02, 6.42 and 6.80 per cent in peel and, 12.24, 8.14, 9.50 and 9.68 per cent in pulp and on tenth day, respectively (Fig. 1).

The non-reducing sugar content also increased significantly in peel and pulp of the inoculated fruits as well as in inoculated fruits dipped in leaf extracts when compared to untreated control. The increase in the infected fruits alone, and inoculated fruits dipped in leaf extracts of *S. torvum*, zimmu and *A. Alliaceum* were found to be 0.54, 0.36, 0.37 and 0.36 per cent in peel and 0.45, 0.26, 0.27 and 0.28 per cent in pulp and on tenth day respectively (Fig 2).

Increased level of reducing sugar content [20] and total sugar content [2] were reported in ripening banana fruits. [25] reported that the reducing sugar content increased in the healthy and diseased skin portions of the diseased banana fruits as compared to the skin of the healthy fruits. [17] reported that the sugar contents were increased in the banana and mango fruits inoculated with *B. theobromae* over healthy control up to six days, followed by a decline.

[24] reported that the biochemical changes taking place during ripening phase includes reduction in the content of starch, increase in soluble sugars and reduction in titrable acidity. In lemon fruits, the content of reducing sugars decreased upto 8 days and afterwards it increased due to inoculation with *C. gloeosporioides* [6].

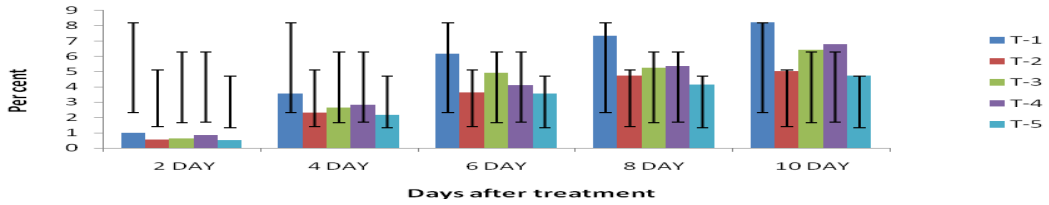
Table 2. Total sugar content of banana fruits as influenced by plant extracts

Per cent total sugar												
Treatment	Peel*						Pulp*					
	Days after treatment					Per cent increase over control	Days after treatment					Per cent increase over control
	2	4	6	8	10		2	4	6	8	10	
T1	2.66^{c} (9.35)	4.02^{d} (11.57)	6.46^{e} (14.73)	7.65^{e} (16.06)	8.55^{d} (17.00)	66.34	3.91^{d} (11.40)	4.63^{c} (12.43)	6.73^{e} (15.03)	10.28^{d} (18.70)	12.46^{a} (20.67)	60.56
T2	2.11^{a} (8.35)	3.35^{b} (10.54)	4.22^{b} (11.86)	5.08^{b} (13.03)	5.46^{a} (13.51)	6.20	3.31^{a} (10.49)	3.96^{a} (11.48)	4.72^{b} (12.45)	7.04^{b} (15.39)	8.52^{c} (16.97)	9.70
T3	2.12^{a} (8.37)	3.57^{c} (10.90)	5.15^{d} (13.12)	5.64^{d} (13.73)	6.10^{c} (14.12)	18.67	3.46^{b} (10.73)	4.27^{b} (11.92)	5.62^{d} (13.71)	8.02^{c} (16.45)	9.56^{b} (18.15)	23.19
T4	2.25^{cb} (8.63)	3.63^{c} (10.99)	4.74^{c} (12.57)	5.39^{c} (13.43)	5.64^{b} (13.73)	9.7	3.56^{c} (10.83)	3.95^{a} 11.46	5.45^{c} (13.50)	8.05^{c} (16.45)	9.70^{b} (18.27)	25.0
T5	2.10^{a} (8.33)	3.24^{a} (10.38)	4.12^{a} (11.72)	4.82^{a} (12.68)	5.14^{a} (13.10)	-	3.33^{a} (10.52)	3.88^{a} (11.36)	4.59^{a} (12.37)	6.43^{a} (14.69)	7.76^{d} (16.18)	-

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

Fig.1. Changes in reducing sugar content of banana fruits treated with plant extracts

A. Peel



B. Pulp

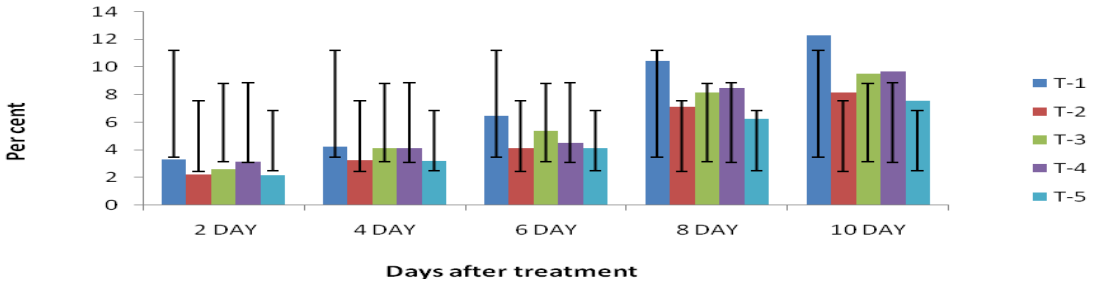
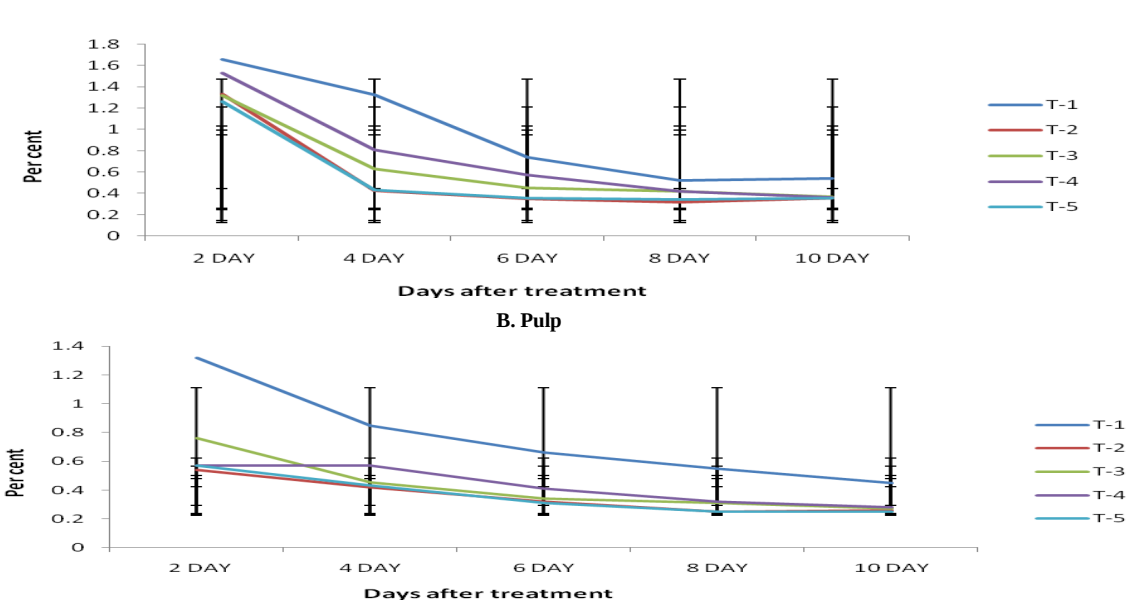


Fig. 2. Changes in non-reducing sugar content of banana fruits after treated with plant extracts



Vertical bar indicates standard deviation of five replication
T1- Fruits inoculated with pathogen
T2- Fruits inoculated and sprayed with S. torvum
T3- Fruits inoculated and sprayed with Zimmu
T4- Fruits inoculated and sprayed with A. alliaceum
T5- Uninoculated control

[19] Reported that the sugar contents increased in the banana fruits treated with paraquat than in the healthy fruits. [18] Reported that the total and reducing sugar contents increased significantly both in peel and pulp of the *C. gloeosporioides* inoculated fruits treated with paraquat when compared to control

Phenolic content

Phenolics are fungitoxic in nature and the induction of phenolics accumulation is due to the activation of the shikimic acid pathway which comes from phenylalanine and tyrosine. Phenols were reported to be fungistatic in action conceivably because of inactivation of enzymes required for pathogenesis [13]. The failure of the pathogen to infect unripe fruit is attributed to the presence of phenolics [28]. It was observed that there was an appreciable reduction in both total and OD phenol contents as the period of storage prolonged up to 10 days in the inoculated fruits when compared to inoculated fruits dipped in leaf extracts. The reduction in total phenol content was noticed from the second day of treatment and reached a maximum reduction on the tenth day. On tenth day after treatment the per cent reduction of total phenol in inoculated fruits and inoculated fruits treated with leaf extract of *S. torvum*, zimmu and *A. alliaceum* was found to be 32.45, 0.87, 8.70 and 7.80 per cent in peel and 62.79, 18.60, 16.27 and 23.25 per cent in pulp over untreated control respectively (Table.3). With respect to OD phenol the per cent reduction was found to be 0.12, 0.23, 0.31 and .031 per cent in peel and 0.08, 0.13, 0.14 and 0.14 per cent in pulp respectively. (Fig.3). Thus in the present study, the reduction of phenolic content was more pronounced in the *C. musae* inoculated fruits alone as compared with *C. musae* inoculated fruits dipped in leaf extracts of *S. torvum*, zimmu and *A. alliaceum*. It indicated that reduction in phenol content of *C. musae* inoculated fruits was due to pathogen attack at the same time leaf extracts resisted the phenol degradation in fruits where it has great role in disease resistance in fruits.

Similarly, the reduction in phenolic content during ripening was found to be responsible for making the fruit susceptible to pathological breakdown [16]. Peel in the unripe banana fruit contains three times more phenol than pulp [17]. The reduction in total soluble solids, total phenol and OD phenol in the pear fruits inoculated with *G. cingulata* was reported by [26]. [19] reported that the total phenol content was found to be reduced in the banana fruits treated with different concentrations of paraquat. [18] Reported that there was a significant reduction in phenolic content in inoculated fruits treated with paraquat as compared to control. The reduction in the content has

started even from first day and increased upto five days. [5] Reported the reduction in total and OD phenol content due to the infection of *C. gloeosporioides* in mandarin orange, sweet orange and lemon. These reports are in accordance with the results of present investigation.

Ascorbic acid content

Ascorbic acid functions as one of the biological oxidation-reduction substrate. It is easily oxidised to dehydro-L-ascorbic acid by ascorbic acid oxidase or by certain other oxidative enzymes to diketo form (2, 3 diketogluconic acid). Increase in ascorbic acid oxidase during pathogenesis was reported by [10]. Ascorbic acid content of fruits was found to be decreased with increase in the storage period. The reduction in ascorbic acid content was gradual with increase in storage period in the mango fruits treated with 2,4-D [19]. In the present study, the ascorbic acid content was significantly reduced both in peel and pulp of the inoculated fruits alone when compared to inoculated fruits pretreated with leaf extracts.. Generally ascorbic acid content in peel was higher than the pulp. On tenth day after treatment the per cent reduction of ascorbic acid in inoculated fruits alone as well as in inoculated fruits treated with leaf extract of *S. torvum*, zimmu and *A. alliaceum* was found to be 1.64, 3.94, 3.20 and 3.26 per cent in peel and 3.15, 6.02, 5.64 and 5.17 per cent in pulp, respectively. (Fig 4). Similar results were also reported by [12] in the postharvest disease of guava. Decrease in ascorbic acid content during ripening of fruits was reported by [15] and [3]. [19] reported that banana fruits treated with paraquat (500 ppm) recorded a considerable reduction in ascorbic acid content compared to untreated one.

Table 3. Total phenolics of banana fruits treated with plant extracts

**mg of catechol /g of tissue /min*												
Treatment	**Peel***						**Pulp***					
	Days after treatment					**Per cent decrease over control**	**Days after treatment**					**Per cent decrease over control**
	2	**4**	**6**	**8**	**10**		**2**	**4**	**6**	**8**	**10**	
T1	1.44^{b}	1.27^{d}	1.09^{c}	0.94^{d}	0.77^{c}	32.45	0.73^{b}	0.52^{c}	0.36^{c}	0.26^{c}	0.16^{c}	62.79
T2	1.55^{a}	1.48^{b}	1.33^{a}	1.23^{a}	1.13^{a}	0.87	0.80^{a}	0.64^{a}	0.56^{a}	0.45^{a}	0.35^{b}	18.60
T3	1.56^{a}	1.44^{c}	1.27^{b}	1.11^{b}	1.04^{b}	8.70	0.78^{a}	0.57^{b}	0.48^{b}	0.31^{b}	0.36^{b}	16.27
T4	1.58^{a}	1.41^{c}	1.25^{b}	1.02^{c}	1.05^{b}	7.80	0.79a	0.58^{b}	0.48^{b}	0.31^{b}	0.33^{b}	23.25
T5	1.58^{a}	1.52^{a}	1.36^{a}	1.25^{a}	1.14^{a}	-	0.81^{a}	0.65^{a}	0.57^{a}	0.45^{a}	0.43^{a}	-

T1- Fruits inoculated with pathogen, T2- Fruits inoculated and sprayed with S. torvum, T3- Fruits inoculated and sprayed with Zimmu, T4- Fruits inoculated and sprayed with A. alliaceum, T5- Uninoculated control

**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

Fig. 3. Changes in Ortho-dihydroxy phenol content of banana fruits treated with plant extracts

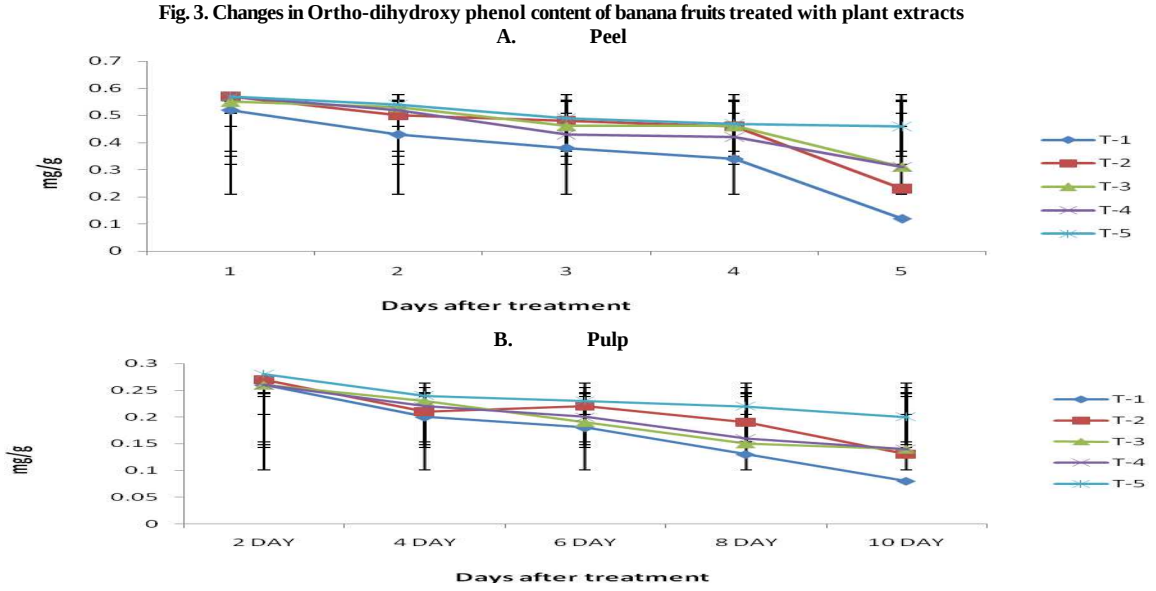
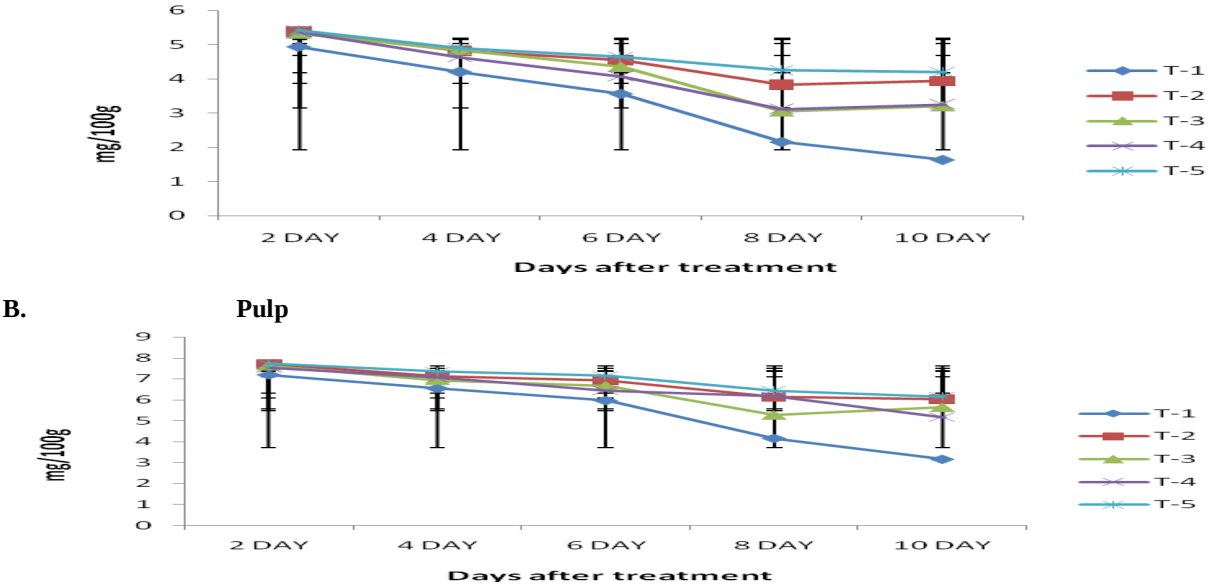


Fig. 4. Changes in ascorbic acid content of banana fruits after treatment with plant extracts



Vertical bar indicates standard deviation of five replication
T1- Fruits inoculated with pathogen
T2- Fruits inoculated and sprayed with S. torvum
T3- Fruits inoculated and sprayed with Zimmu
T4- Fruits inoculated and sprayed with A. alliaceum
T5- Uninoculated control

CONCLUSION

Plant extracts have a potential role in inducing the biochemical changes in fruits where it can manage the disease effectively. Rate of reduction of phenolic and starch content is low in banana fruit treatment of plant extracts compare to inoculated fruits alone, and rate of increase of sugar content is high in banana fruits inoculated alone compare to treated with plant extracts. Total phenolic, starch and sugar content in banana will decide the pathogen infection, and plant extracts will influence the biochemical changes in banana fruits manage the disease effectively.

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