



Lemon grass oil: effect on physico-chemical properties and postharvest life of banana cv. Amritasagor

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ABSTRACT

Banana undergoes various diseases at the entire stages of its life. The most important post-harvest disease of Banana is Anthracnose which causal organism is *Colletotrichum gloeosporioides*. The objective of the current research work was to test the effectiveness of the use of lemon grass extract oils (*Citrus medica*) to minimize the postharvest losses commenced by anthracnose (*Colletotrichum gloeosporioides*) disease in banana fruits. In this experiment, the antifungal activity of essential oils were assayed under in vitro and in vivo condition by post-harvest application of lemon grass oil (20%) on banana fruits with fungicidal treatment (diphenochonazol) and sterilized water treatment as a control. Single-factor experiment was carried out in a completely randomized design (CRD) with five replications at postharvest analysis laboratory, Department of Horticulture, Patuakhali Science and Technology University, Bangladesh. Among the physico-chemical parameters; pH, TSS and sugar contents increased significantly whereas titratable acidity and ascorbic acid decreased during post-harvest storage period in all treated and untreated fruits. There was significant increasing tendency was obtained in relation to disease incidence and disease severity in control fruits compared to other lemon grass extract treated fruits and fungicide treated fruits. Among the physico-chemical parameters, lemon grass oil treated fruits produced significantly the highest pH (5.99), lowest total soluble solids (19.24%), total sugar (9.04%), reducing sugar (5.48%), titratable acidity (0.52) and ascorbic acid content (9.04 mg/100g) compared to control. Postharvest disease was severe at 12 days of storage; it was maximum (100%) in distilled water treated fruits and minimum in lemon grass oil treated fruits (42.20%). Based on storage performance, lemon grass extract treated fruits resulted in the longest shelf life (12.33 days) and shortest (6.33 days) was recorded in control fruits. This experiment's findings recommend the application of lemon grass essential oils @20% on 'Amritasagor' Banana prior to storage to improve and extend the postharvest storage.

Key words: Banana, Lemon grass extract, Microbial analysis, Nutritional quality and Shelf-life

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I. Introduction

In the world, Banana is one of the top five fruit crops which hold an emergent position among the fruits of Bangladesh not only for its highest production but also for its accrescent popularity to a large number of producers as an economic crop. Among the fruit crops grown in Bangladesh, banana occupies the first position in terms of production comprising nearly 42% of the total (Akter et al., 2013). From wet tropical to dry subtropical, it is adaptable to a vast area of climates. From the nutritional point of view, a medium sized banana per 100g carries energy (89 Kcal), carbohydrates (22.84 g), protein (1.09 g), fat (0.33 g), vitamin-A (64.29 IU), vitamin-C (8.7 mg), vitamin B complex and many other essential minerals in a notable amount (Singh, 1998).

Banana fruits are highly perishable and affected by different microbial contaminates because ripe bananas are very perishable. Due to the enhancement of respiration in storage conditions, a histrionic change in physic-chemical characteristics in banana has occurred while peel color changes and pulp texture decreases due to conversion of starch into sugar (Kajuna et al., 1997 and Prabha and Bhagyalakhmi, 1998). Significant increase in weight loss, TSS content along with microbial activity also occurs during ripening (Misir et al., 2012). An appropriate postharvest treatment without any negative impact on human health is an urgent necessity to decrease these unwanted changes in banana fruits. Due to shorter shelf life of banana, Bangladesh has lost a vast amount of money every year (Almamun, 2014). Proper storage practices are pre-requisite for banana fruits to avoid quality deterioration which occurs generally due to the incidence of various post-harvest diseases and accelerated softening of fruit pulp. While maintaining the quality it is essential to control the diseases and to delay the onset of the ripening processes for the fruits to be competitive in the market.

Postharvest decays of fruits and vegetables account for significant amounts of postharvest losses. Among the various constraints, the utmost emergent is anthracnose caused by *Colletotrichum musae*. Flower blight, fruit rot, and leaf spots are among the symptoms of this disease (Ahmed and Rahman 1974). Young infected fruits exhibit black spots, shrivel and drop off. Fruits infected at mature stage convey the fungus into post-harvest storage and cause notably large amounts of loss during storage, transit and marketing. It is important to note that chemical fungicides are the primary means supposed to hinge the post-harvest diseases of fruits; however, environmental and human health risks are high (Janisiewicz and Korsten, 2002). Controlled atmosphere (CA) techniques are costly, while modified atmosphere packaging (MAP) has been demonstrated to ameliorate chilling injury and fungal decay in several crops (Yahia and Paull, 1997). Thus, there is a necessity to have alternative technology to lessen the disease incidence and enhance the post-harvest life of banana without undesirable physico-chemical changes taking place during the storage period. Agricultural commodities which are free from synthetic pesticide residues, consumer preference is high however, synthetic pesticides may also kill various beneficial micro-organisms and their toxic forms may persist in soil and enhance the incidence of resistance among pathogens towards synthetic chemicals (Ramezani et al, 2002). Thus, new preservation techniques are needed, which have to be considered as human-safe and eco-friendly. Natural plant products including essential oils which are biodegradable and eco-friendly are catching the attention of biological researchers worldwide from several alternatives to synthetic chemical preservation techniques. These products from higher plants are bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals. Numerous reports showed that oils from some plant species are harmful to fungal pathogens (Macias et al, 1997). Lemon grass extracts i.e. essential oils were effective in the control of *Colletotrichum musae* (Wilson et al., 1987). This experiment was designed to evaluate the influences of lemon grass extract against growth and spore germination of *Colletotrichum musae* under in vitro condition and its effect on the physico-chemical parameters, disease incidence, and disease severity and post-harvest life of banana under in vivo condition.

II. Materials and Methods

The present experiment was carried out at Postharvest Processing Lab of the Department of Horticulture, Patuakhali Science and Technology University during the period from 01 July to 15 August, 2016 to study the post-harvest storage life and quality changes of Banana cv. Amritisagor as influenced by promising postharvest treatments of lemongrass extract.

Experimental material

Hands of mature green banana color index 1 (green) were brought from a commercial orchard located at Dumki. Banana fruit which is free from physical injury, insect or pathogen infection as well as more or less similar in size (200-250 g) were selected for the experiment.

Design of experiment

The experiment was organized in a completely randomized design (CRD) with five replications including five bananas in each replication at ambient condition. Three treatments were imposed on banana fruits under the experiment where fruits treated with distilled water were indicated as control groups (T1), fruits treated with fungicide dipheniconazol® 0.5ml/litre considered as positive control (T2) and fruits immersed with 20% lemon grass extract oil (T3) were considered as post-harvest treatment.

Preparation of lemon grass extracts (Hydrodistillation)

The lemon grass extracts were prepared and applied on the fruit samples at postharvest analysis laboratory, Department of Horticulture, Patuakhali Science and Technology University. Two hundred grams of freeze dried samples were subjected to hydrodistillation. The hydrodistillation was carried out by using the Clevenger equipment at 100°C for 6 hours in glass Dean and Stark apparatus modified to allow the lowest phase return. The oil with water mix was added, to trap the condenser, through the top of the condenser. Later oil with water mix was collected every hour. Then, a few amounts of hexane was added through the condenser. The mixtures were combined and dried over anhydrous Na₂SO₄ for 24 hours and then filtered (it was filtered with 0.001 millimeter micropore filter paper). Finally, yellowish essential oil extracts were found which were stored at 4°C for further use.

In vitro screening of plant essential extract against *C. musae* mycelium linear growth and spore germination

The inhibitory effects of lemongrass (*Cymbopogon citrates*) essential oils were tested *in vitro* on mycelial growth of *C. musae*. Preliminary screening was carried out according to [Arrebol et al. \(2010\)](#). The Petri dish with 9 cm diameter was poured with potato dextrose agar (PDA) media. The lemon grass extracts were dispersed to PDA media immediately before it was filled into the Petri dish at a temperature of 25-30°C. The concentrations of lemon grass extract were tested and 20% extracts were optimized to mix up with the media. The controls included the same quantity of distilled water was added with PDA media. The test fungus (anthracnose of banana) was inoculated immediately after preparation of the petri dishes by placing it in the centre of each plate. A 5 mm diameter disk of the test fungus cut with a sterile cork borer from the periphery of actively growing cultures on PDA plates. The petri dishes were incubated in the dark chamber at a temperature of 25°C. Mean growth rates fungal mycelia were observed. Fruit samples were placed on a sterilized surface in three groups having five replications for each treatment and the extracts were sprayed over the fruit according to the treatments *in vivo* condition.

Methods of treatment application

Randomly selected twenty five fruits were dipped into the lemon grass extract (20%) solution for three minutes to ensure that enough quantity of extract being absorbed by the fruit surface. The treated fruits were subjected to air dry at room temperature (26±2°C). In case of fungicidal treatment, five litres of distilled water was taken in a bowl and 2.5 ml of Dipheniconazol® solution (Trade name: Score, Synzenta Bangladesh Limited) was added. The selected fruits were then individually dipped in this prepared fungicidal solution for three minutes to ensure that enough quantity of solution being absorbed. In case of distilled water treatment, each fruit was then individually dipped into the sterilized distilled water for three minutes and allowed to dry for ten minutes. Each of the treated fruit was wrapped with 70 g offset paper and held at 26±2°C and 80±5% RH for 15 days. Every three days, five fruits represented five replications for each treatment were used for the determination of physico-chemical properties of the treated fruits. Data were documented on 0, 3, 6, 9, 12 and 15 days of storage (26±2°C and 80±5% RH).

Parameters studied

The physic-chemical characteristics of treated fruits such as color changes, pulp pH, Total Soluble Solids (TSS), Titratable Acidity (TA), total sugar, reducing sugar, non-reducing sugar, ascorbic acid content,

disease incidence (percentage of fruits infected) and disease severity were studied in the present experiment.

Methods of studying physico-chemical parameters

The changes in peel color of fruits were determined by visual observation changes in peel of the treated fruits were determined. The pH of fruit juice was recorded by using an electric pH meter. The pH meter was standardized with the help of a buffer solution as described by Ranganna (1994). TSS content of banana pulp was measured by using Abbe's Refractometer. A drop of banana juice squeezed from the fruit pulp was dropped on the prism of the Refractometer. Temperature corrections were made by using the methods described by Ranganna (1994). The sugar content of treated fruits of each treatment was estimated by the procedures, described by the Ranganna (1979) with using following formula:

$$\text{Total sugar (\%)} = \text{Reducing sugar (\%)} + \text{Non-reducing sugar (\%)}$$

Ascorbic acid content was determined through the method of Ranganna, (1994) by using 2,6-Dichlorophenol Indophenols by visual titration method. Percentage of fruits infected with disease at different days after storage is considered as disease incidence. The diseased fruits were identified as symptomatically and separated immediately. The disease incidence was calculated as follow:

$$\text{Disease incidence (DI)(\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

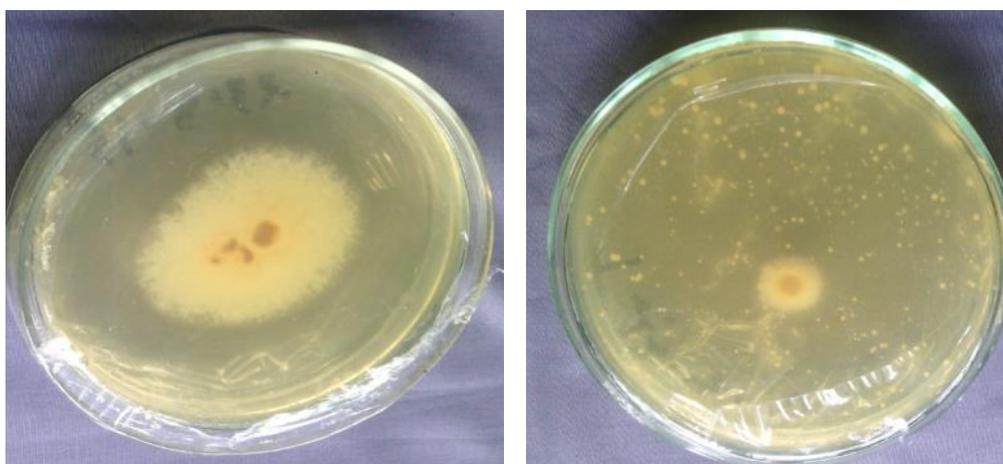
The percent diseased portion of the infected banana fruit is representing as disease severity. The percentage of diseased fruit area was measured based on eye estimation. Post-harvest storage life of banana as subjected by different postharvest treatments was calculated by counting the number of days required to ripen fully with retained optimum marketing and eating qualities.

Statistical analysis

The data which was taken on different days after storage on various parameters were statistically analyzed using MSTATc statistical package. The means for all the treatments were calculated and analyses of variances (ANOVA) for all the parameters were performed by F-test. The significance of difference between the pairs of means was compared by the least significant difference (LSD) test at 1% and 5% levels of probability (Gomez and Gomez, 1984).

III. Results and Discussion

In vitro screening of lemon grass extracts against *C. musae* mycelia growth and spore germination



Control plate of *C. musae*

20% lemon grass extract treated plate

Figure 01. Effect of lemon grass extracts on the mycelia radial growth and spore germination of *C. musae* after four days of incubation at $28 \pm 2^\circ\text{C}$

Visual observation showed that overall spore germination was significantly lower on PDA plates amended with higher concentrations of lemon grass extract than control (Figure 01). Less spore germination was observed on PDA plates treated with concentrations of 20% lemon grass extract after four days of incubation whereas profuse mycelia growth was found on the control plates. This showed that lemon grass extract inhibits the mycelia growth and spore germination of *C. musae*.

Changes in color

Various characters of banana such as shape, size and base of fruits, apex and texture of pulp, color and thickness of peel were observed for “amritosagor” banana after the harvest. Initially, the peel color was green after that it was changing to different color at different days after storage. Storage banana were changing the color from green to pale green, greenish yellow, light yellow and yellow at 3rd, 6th, 9th and 12th day of storage among all postharvest treatments (Table 01). After 12 days of storage, it fully ripened and tends to disintegrate.

Table 01. Effect of different postharvest treatments on peel color changes of banana

Postharvest interventions	Color changes at different days after storage (DAS)				
	Initial color	3 DAS	6 DAS	9 DAS	12 DAS
Distilled water (T ₁)	Mature green	Pale green	Greenish yellow	Light yellow	Dark yellow
Dipheniconazol @ 0.5mL ⁻¹ (T ₂)	Mature green	Pale green	Greenish yellow	Light yellow	Yellow
Lemon grass extracts @20% (T ₃)	Mature green	Pale green	Greenish yellow	Light yellow	Yellow

Pulp pH

The postharvest treatments of lemon grass extract, synthetic fungicide and distilled water showed significant variation in respect of pH content on 6 and 9 DAS but at 3 and 12 DAS did not varied significantly. Results showed that pulp pH was grown up with the increasing storage duration. The maximum pulp pH was recorded in lemon grass extract (5.30, 5.60, 5.81 and 5.99) at 3, 6, 9 and 12 days after storage, respectively. In contrast, the lowest pulp pH was observed in distilled water treated control fruits (5.15, 5.45, 5.50 and 5.90) at similar DAS, respectively (Figure 02). Joshi and Roy (1988) reported that there was a steady rise in pH of the fruits of banana during storage.

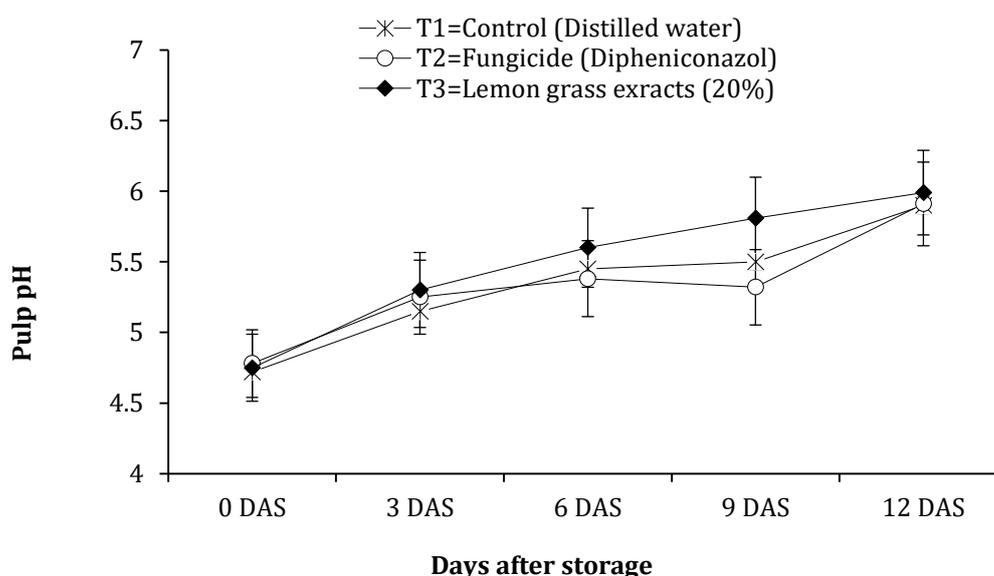


Figure 02. Effect of different postharvest treatments on pulp pH of Banana fruits, the linear line represents LSD at 5% level of probability

Total soluble solids (TSS)

Total soluble solid increased dramatically from 2.94 % to 24.48% during the entire storage period (Figure 04). Barakat et al. (2012), reported that the TSS content of fruits gradually increased with the advancement of storage period that supports the findings of the present experiment. A similar result of

total soluble solid was reported by [Alique and Oliveira \(1994\)](#) in cherimoya. Decrease in acidity and increase in sugar content during fruit ripening was also reported by [Illeperuma and Jayasuriya \(2002\)](#). Among the postharvest treatments, highest TSS content (7.18 % Brix) was recorded in distilled water treated fruits while the lowest (6.13 % Brix) was noticed in lemon grass extract treated fruits of banana at 3 days after storage. Similarly, the highest (13.30, 19.28 and 24.48% Brix) TSS content was found by the control fruit and the lowest (11.12, 15.30 and 19.24 % Brix) TSS content was recorded from the lemon grass extract treated fruit at 6, 9 and 12 days after storage, respectively ([Table 02](#)). This finding resulted that treatment with higher concentration of lemon grass extract enhances the reduction of TSS when applying different treatments after harvest. This might be due to the acidic nature of lemon juice which hindered the physiological changes and delayed the ripening process in treated fruits as reported earlier by [Wills et al. \(1998\)](#). According to them, acid acts as a reserve source of energy to the fruit which tends to decline by the enhanced metabolic activities during fruit ripening.

Table 02. Effect of different postharvest treatments on Total Soluble Solid (TSS) content of Banana

Postharvest treatment	Pulp TSS content at different days after storage				
	Initial stage	3 DAS	6 DAS	9 DAS	12 DAS
T ₁ =Control (Distilled water)	2.94	7.18	13.30	19.98	24.48
T ₂ =Fungicide @ 0.5mL ⁻¹ (Dipheniconazol)	2.87	6.40	11.38	15.76	19.89
T ₃ =Lemon grass extract @20%	2.91	6.13	11.12	15.30	19.24
LSD(0.05)	-	0.063	0.252	0.773	0.720
LSD(0.01)	-	0.095	0.382	1.184	1.091
CV (%)	-	0.52%	1.02%	2.26%	1.37%
Level of significance	-	**	**	**	**

**= Significant at 1% level of probability

Reducing sugar

The postharvest treatments of lemon grass extract exhibited significant variation in respect of reducing sugar content at different storage periods. Among the postharvest treatments of lemon grass extract, the maximum (2.98%) reducing sugar content was observed in control fruits followed by the fruits kept in fungicidal treatment. On the other hand, the minimum (2.18%) was recorded in lemon grass extract treated fruits at 3 DAS. Correspondingly, at 6, 9 and 12 DAS (4.1, 5.58 and 7.12% respectively) was recorded from control fruits while 2.96, 4.06 and 5.48% was calculated from lemon grass extract treated fruits at 6, 9 and 12 DAS respectively ([Figure 03](#)). It was noticed that gradually increased the reducing sugar content with the storage period extension. These increment results were agreed with [Rangavalli et al. \(1993\)](#) who studied the postharvest changes in banana and found a gradual advancement in reducing sugar content. In control guava fruit, lower level of reducing sugar was also reported by [Kaur \(2016\)](#).

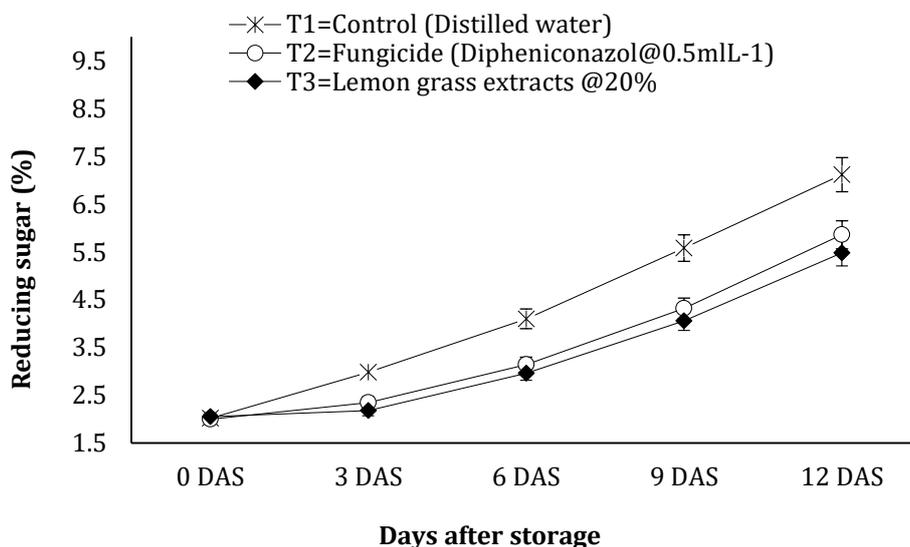


Figure 03. Effect of different postharvest treatments on pulp reducing sugar of Banana fruits, the linear line indicates LSD at 5% level of probability

Total sugar content

Significant difference was found due to the effect of lemon grass extract in relation to total sugar content at all stages during storage interval. The highest total sugar content was observed in untreated fruits (3.99%) where it was minimum (2.6%) in the fruits of lemon grass extract treated at 3 DAS. Similarly, at 12 DAS the highest total sugar (13.49%) was observed in control fruits (Figure 04). The total sugar content increased from 3 DAS to ripe stage (12 DAS), thus it was marked that percent total sugar content gradually increased with the advancement of ripening period. The finding of the current study was agreed with Habiba (2012) who reported that a banana fruit treated with neem extract in comparison to control was increased the level of sugar during postharvest period.

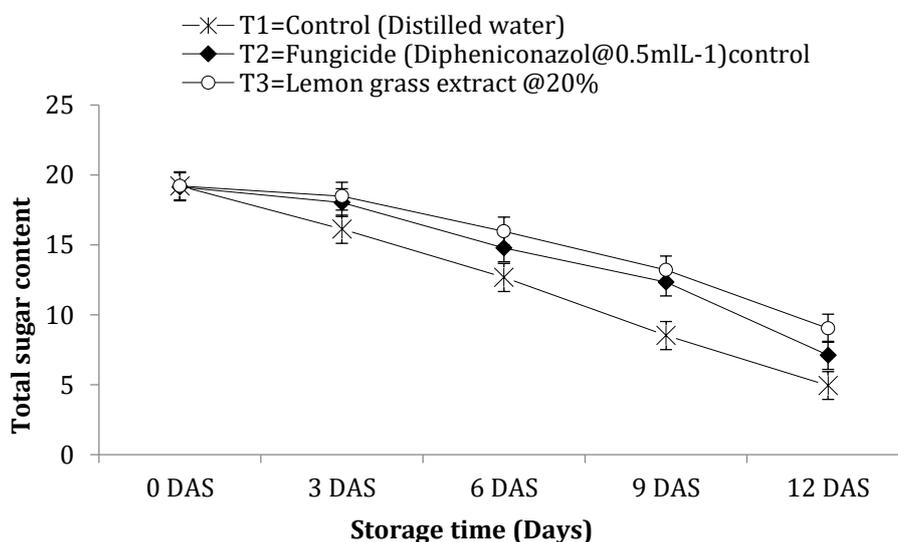


Figure 04. Effect of different postharvest treatments on pulp reducing sugar of Banana fruits, the linear line represents LSD at 5% level of probability

Titrateable acidity

After the application of postharvest treatment, titrateable acidity was decreased with the advancement of storage period. However, the postharvest treatments of lemon grass extract showed statistically more or less similar results at storage period data recording with fungicidal treatment. Comparatively higher reduction trend of TA was seen in control treated fruits (0.88, 0.75, 0.54, 0.38 and 0.21%) at different DAS and comparatively lower reduction trend of titrateable acidity was recorded in the fungicidal treatment (0.90, 0.82, 0.70, 0.54 and 0.43%) and lemon grass extracts (0.92, 0.85, 0.78, 0.64 and 0.52)

(Table 03). The titratable acidity is decreased with the increasing extension period of fruit storage. Similarly, Rashid (2013) found minimum titratable acidity in case of neem extract + perforated polythene treated banana fruits at 12 days of storage. Kaur (2016) also reported that higher results of acid content in postharvest treatment applied guava fruits (0.41%) than control (0.35%).

Table 03. Effect of different postharvest treatments on Titratable Acidity (TA) content of Banana

Postharvest treatment	Pulp Titratable Acidity (TA) content at different days after storage				
	Initial stage	3 DAS	6 DAS	9 DAS	12 DAS
T ₁ =Control (Distilled water)	0.88	0.75	0.54	0.38	0.21
T ₂ =Fungicide @ 0.5mlL ⁻¹ (Dipheniconazol)	0.90	0.82	0.70	0.54	0.43
T ₃ =Lemon grass extract @20%	0.92	0.85	0.78	0.64	0.52
LSD _(0.05)	-	0.063	0.089	0.109	0.101
LSD _(0.01)	-	0.165	0.135	0.165	0.191
CV (%)	-	3.22%	4.08%	6.34%	7.55%
Level of significance	-	*	*	*	*

*= Significant at 5% level of probability

Ascorbic acid content (mg/100g)

Ascorbic acid content was significantly influenced by the application of different postharvest treatments. Significant reduction (16.12 mg/100g to 4.94 mg/100g) of ascorbic content was recorded during the storage period from 3 DAS to 12 DAS at distilled water treated fruits. On the other hand, a fruit treated with lemon grass extract comparatively less reduction pattern of ascorbic acid content was observed (19.22 mg/100g to 9.04 mg/100g) during the storage period from 3 DAS to 12 DAS while statistically similar was found when fruits treated with fungicides which is a synthetic chemical and detrimental to human health. The value of ascorbic acid content was declined with the progress of storage time (Figure 05). In hot water treated lime fruits, vitamin C content was significantly decreased with increasing the storage duration as reported by (Obeed and Harhash, 2006) which supports the findings from the current study.

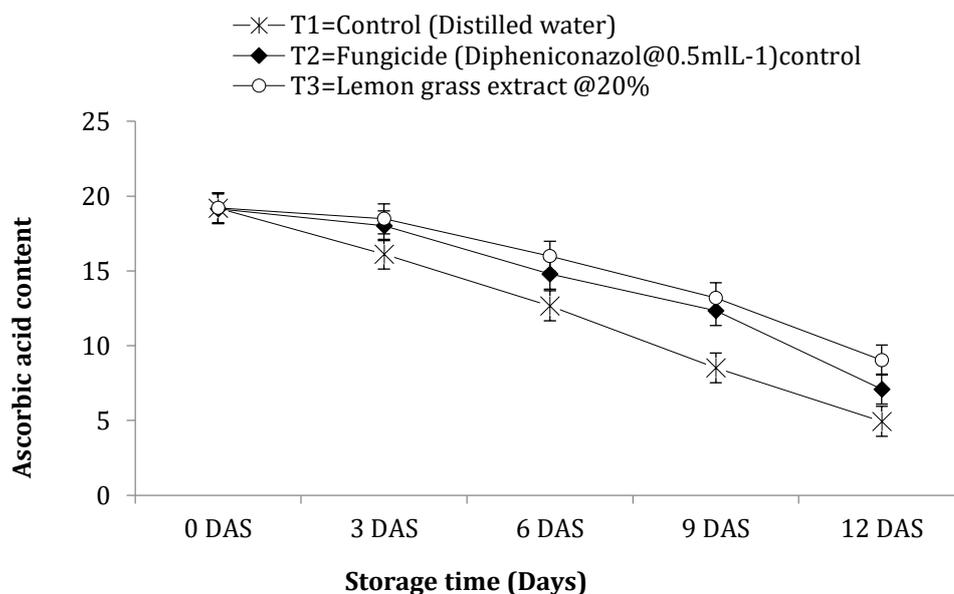


Figure 05. Effect of different postharvest treatments on pulp reducing sugar of Banana fruits, the linear line represents LSD at 5% level of probability

Disease incidence

The postharvest treatments in this experiment showed significant variations in respect of percent disease incidence at advancement of storage period of banana (Figure 06). At 6 DAS, the highest percent disease incidence was identified in control fruits (66.67%) whereas slight infection was found lemon grass extract treated fruits (16.25%). The maximum 100.0% disease incidence was recorded at 12th days after storage in control fruits whereas the minimum (16.25%) infection was identified in lemon grass extract at 6th day of storage.

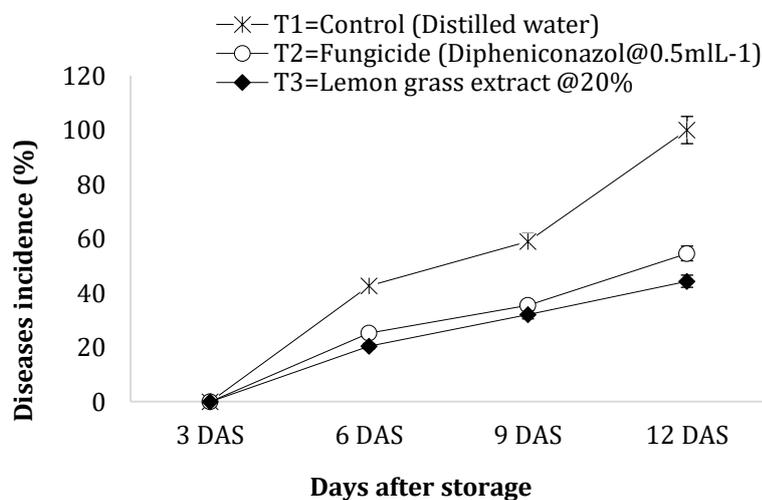


Figure 06. Effect of different postharvest treatments on disease incidence of Banana fruits, the linear line represents LSD at 5% level of probability

Disease severity

The postharvest treatments of lemon grass extract were used as storage treatments of Amritosagor banana for the investigation of percent disease severity where they showed highly significant effects on storage period interval. The highest disease severity was investigated without treated fruits. At 6th, 9th to 12th day of storage the maximum (38.00, 53.3 and 100%, respectively) disease severity was noted in control and the minimum (22.25, 33.33 and 42.2% respectively) was observed in lemon grass extract treated fruits (Figure 07).

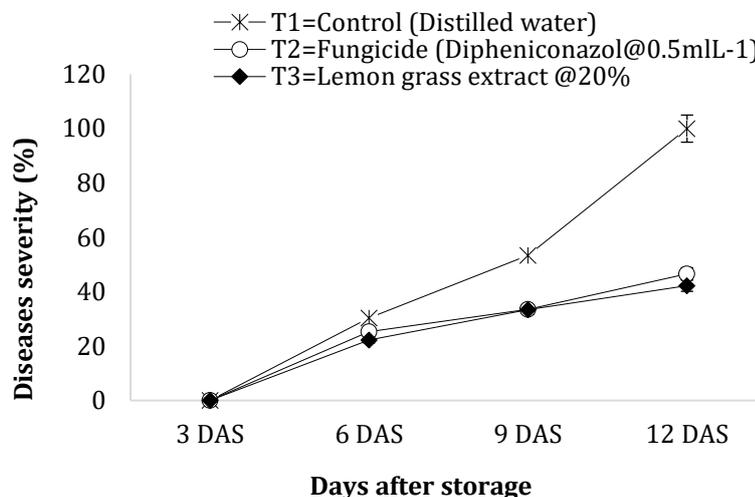


Figure 07. Effect of different postharvest treatments on disease severity (%) of Banana fruits, the linear line represents LSD at 5% level of probability

Shelf life

The enhancement of postharvest life of banana fruits has been one of the most important concerns of the researchers. Significantly longest storage life was showed by lemon grass extract treated fruits (12.33 days) and lowest storage duration in control fruits (6.33 days) (Figure 08). Stephen (2014) reported that the combined effect of guava leaf extract (20%) and lemon extract (15%) has increased the post-harvest life of fruits and vegetables.

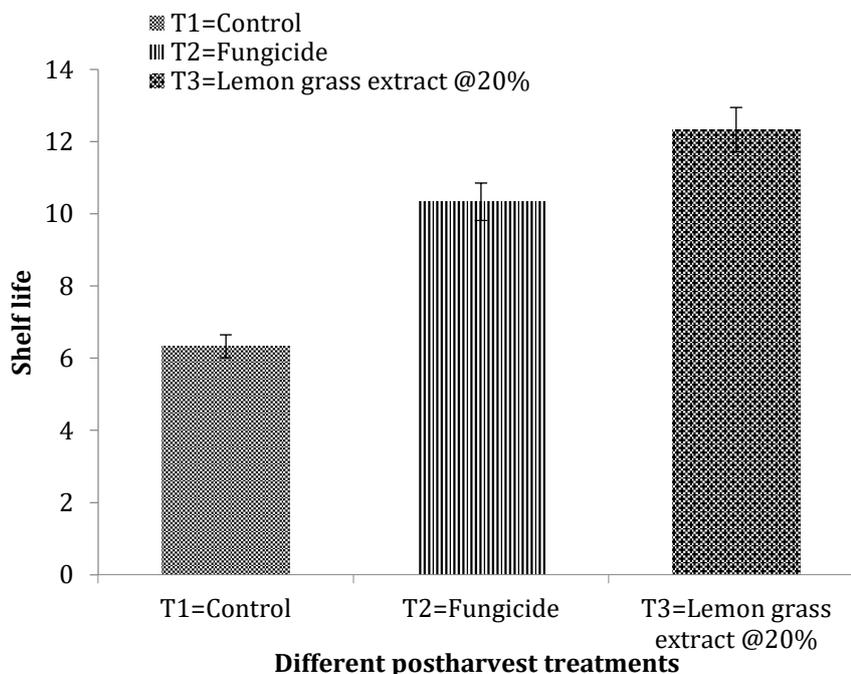


Figure 08. Effect of different postharvest treatments on disease incidence of Banana fruits, the linear line represents LSD at 5% level of probability

IV. Conclusion

The results of the current experiment showed the possibility of use of lemon grass extract to gradually minimize the postharvest losses caused by anthracnose disease in banana fruits. From this experiment, it could be inferred that the lemon grass extracts @20% have significantly influenced the physico-chemical properties, disease incidence, disease severity and shelf life of banana in ambient conditions. So, lemon grass extracts could be used as an effective source of sustainable eco-friendly botanicals, after successful completion of large scale various trials.

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