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Performance of *Bacillus Cereus* strain PSTU HORT-10 to Increase Self Life of Banana

Md. Mahadi Hasan[1], Md. Rasal-Monir[2*], Meer Rifath Jahan Usha[2], Manas Kirtania[3], Md. Fakhrul Hasan[1]

[1]*Department of Horticulture, Patuakhali Science and Technology University, Dumki, Patuakhali*
[2]*Department of Horticulture, Sher-e-Bangla Agricultural University, Sher-e-Bangla nagar , Dhaka*
[2]*Department of Agrochemistry, Sher-e-Bangla Agricultural University, Sher-e-Bangla nagar , Dhaka*
Corresponding Author: Md. Rasal-Monir

Abstract: *This study was conducted to increase the shelf life of banana by controlling the anthracnose of banana. The pathogenic fungi was collected and confirmed as Colletotrichum musae. Bacillus cereus strain PSTU HORT-11 are used as antagonistic bacteria. To evaluate the postharvest efficacy of antagonistic bacteria, an experiment was laid out in completely randomized design with four replications. Three treatments viz. T_1: Positive control (Diphenoconazol@ 0.5 ml/L), T_2: Bacterial treatment (suspension of Bacillus cereus strain PSTU HORT-10) and T_3: Negative control (sterilized water) were used in this study. The bacterial treatment markedly reduced the weight loss (11.570%) compared to the negative control and slowed down the ripening of banana fruits. Moreover, a delayed change in total anthocyanin and carotenoid contents, external peel color, titratable acidity, ascorbic acid content, sugar content and pH was also observed in fruits treated with antagonistic bacterial suspension without compromising the fruit quality. It was more effective in reducing disease incidence and disease severity in naturally infected fruits at the end of 12 days storage at 24 ± 20 C and 60 ± 10% RH, which was alike the results found with Diphenoconazol @ 0.5 ml/L, Thus, the shelf life was extended up to 3 days compared to negative control. This study provided an eco-friendly method which is alternative to fungicidal treatment for postharvest storage of banana.*

Keywords: *Bacillus cereus strain PSTU HORT-1, Colletotrichum musae, Banana, self life*

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I. Introduction

Banana (*Musa spp.)*belongs to the family Musaceae is the most popular fresh fruit all over the world. It was first domesticated in the tropical regions of South East Asia. It is a perennial herbaceous plant that grows from the underground rhizome. It flourishes well under tropical, moisture-rich, humid, low-lying farmlands. Banana is a nutritious gold mine. Fresh bananas provide adequate levels of minerals like copper, magnesium, and manganese. Magnesium is essential for bone strengthening and has a cardiac-protective role as well. Manganese utilized as a co-factor for the antioxidant enzyme, superoxide dismutase. Copper is an essential trace element in the production of red blood cells. Bananas are rich source carbohydrates and potassium. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure, countering harmful effects of sodium.Banana is a good source of vitamin-B6 (pyridoxine); provides about 28% of daily-recommended allowance.

Banana grows very quickly and can be harvested all year round. During 2012-13, the world acreage of banana was 5007520 hectares, while the world production 103632349 Metric Tons and productivity was 20.7 Metric Ton/Hectare. The major banana producing countries around the world are china, Philippines, Ecuador, Brazil, Indonesia, Angola, Guatemala, Tanzania, and Mexico. Owing to favorable climate, huge quantities of bananas are also produced in Bangladesh each year though the quality fruits are concentrated solely in Narsingdhi, Tangail, Jessore, Barisal, Kustia and Dhaka regions. Districts of wild grown Banana are Sylhet, Moulvibazar, Netrokona, Rangamati, Khagrachhari, Bandarban (M. F. Hossain, 2014). Banana ranks first in terms of area and fourth in terms of production in Bangladesh. It occupies 121718 ha of land and total production is 774,000 tons (BBS, 2013).

Postharvest losses of banana are reported to be 45-48% in India, 25% (by weight) in Srilanka, 37-43% in Pakistan and 25-27% in Bangladesh (M.M. Molla et al., 2010) quantitavely. Being a climacteric fruit, Banana is susceptible to diseases. The important cause of the postharvest loss is the pathogenic microorganisms that infect fruits through wounds or latent infections during the preharvest period (Arras and Maltoni, 2004). Precisely, fungal diseases are one of the major causes of fruit decay as they account for 80-90% of all losses in postharvest industry and to the consumer (Sommer, 1985). There are a number of fungi that attacks banana

fruits after harvest which causes heavy fungal infections during storage and transit. The major postharvest fungal diseasesare Anthracnose, Stem end rot, Fingertip rot and*Fusarium* rot. Among them, anthracnose which is caused by Colletotrichum musae is widely distributed all banana growing regions of the world and remain one of the biggest concern for banana producers due to huge postharvest losses (Fivaz, 2009).

Presently, Fungicidal treatment is considered to be the most effective method, but it often gives rise to resistant pathogens (Reimann and Deising, 2000) and also associated with environmental health risks (Janisiewicz and Korsten, 2002; Conway et al., 2004). Besides, non-chemically treated bananas are more appealing to the consumers. Therefore, all of these issues impose a necessity to develop new sustainable alternatives to synthetic fungicides for eco-friendly management of postharvest diseases (Droby et al. 2009).

During the last few decades, there have been increased efforts to develop a number of alternatives to chemical fungicides (Smilanick et al. 1999; Droby et al. 2009). Meanwhile, biological control using microbial antagonistic has emerged as a desirable and rapidly developing alternatives due to increasing global chemo-phobia and environmental social awareness (Emmert and Handelsman, 1999). Among the bio-control agents, antagonistic bacteria are attracting research focus, with their wide antimicrobial spectrum, good antagonistic effects, genetic stability, low nutrition requirements, and high security (Zhou et al., 2007).

Unfortunately, there was little information concerning bio-control of postharvest diseases of fruit. As far as knowledge goes, controlling of anthracnose using biocontrol agent is nearly absent in our country. Therefore, this research has been taken to evaluate the best antagonistic bacteria to prolong the shelf life of banana.

II. Materials And Methods

Postharvest application of antagonistic bacteria (***Bacillus cereus*** strain PSTU HORT-10) on the storability and quality of banana. The postharvest application of antagonistic bacteria on banana fruit was conducted to investigate the significance differences among the treatments on the storability and quality of banana fruits.

Experimental site

The experiment was conducted at the "Postharvest processing and analysis unit of Postharvest laboratory", Department of Horticulture, PSTU.

Experimental materials

Matured, green, approximately uniform size banana fruits without visible defects were brought from local market. The fruits were then carefully handled to avoid mechanical injury. The fruits were then carefully transported to post-harvest laboratory. 24 fruits were selected in each treatment and total no. of fruits was 72. 8 fruits represented each replication for each was used for determination of physio-chemical characteristics.

Design and Layout of the experiment

Bacillus cereus strain PSTU HORT-10 (antagonistic bacterium) was selected as treatment for this study compared with other treatments, which were:

1) **Treatment (antagonistic bacterial suspension):** Cell suspension of the best antagonistic bacterium
2) **Positive control:** Diphenoconazol solution at the rate of 0.5 ml/L of water (Trade name: Score, Syngenta Bangladesh Limited)
3) **Negative control:** Sterilized distilled water

Methods of treatment application

Fruits were randomly selected from the experimental fruit lot. Each of the fruits was washed in running tap water for removing any surface contamination followed by distilled water. The fruits were placed on the table of the laboratory at ambient condition to allow air dry at room temperature (26±2C). The postharvest treatments were applied to the fruits as per the following methods.

Preparation of bacterial suspension (Treatments)

For bacterial suspension treatment, the banana hands were immersed into prepared aqueous bacterial suspension which bacterial colony showed antagonism performance in this experiment and kept for 10 minutes before placing on the paper placed on the table in the laboratory at ambient condition.

Preparation of Diphenoconazol solution (Positive control)

Five litres of distilled water was taken in a bowl and 2.5 ml of Diphenoconazol'P solution (Trade name: Score, Synzenta Bangladesh Limited) was added. Twenty four fruits were randomly selected from the experimental fruit lot. The selected fruits were then individually dipped in this prepared fungicidal solution for three minutes to ensure that enough quantity of solution being absorbed. The treated fruits were allowed to air dry for a period of ten minutes.

Immersed in Sterilized distilled water (Negative control)

Twenty four fruits were randomly selected from experimental fruit lot. Each fruits was then individually dipped into the sterilized distilled water for ten minutes and allowed to dry for a period of ten minutes.

The banana fruits were randomly selected for the post-harvest treatments. After the applications of treatments, the fruits were wrapped by 70gm offset paper and stored at 28+2c and 66 to 74% relative humidity. During the entire storage period, the fruits, used for the experiments, were keenly observed but data were collected until the disease severity become 30%. Data were recorded at 3days interval.

Atmospheric condition of storage room

The temperature and relative humidity of the storage room were recorded daily during the study period with a digital thermo hygrometer (Wet and dry bulb hygrometer, Zeal, G.H. ZEALTA). The minimum and maximum temperatures during the study period of the storage room were 26 c to 30 c, respectively. The minimum and maximum relative humidity was 80% and 90%, respectively

Physical characteristics of treated fruits

The physical characteristics of treated fruits such as total weight loss, peel firmness, external peel color, pulp to peel ratio, sensory evaluation of ripe fruits were studied in the present experiment.

Determination of weight loss

The banana hands used in this study were weighed using a top balance and kept for storage. Percent total weight loss was calculated based on the following formula

$$\text{Percent weight loss (WL)} = \frac{W1-W2}{W1} \times 100$$

Where, W_1 = initial weight of fruit (days); W_2 = weight of fruits at various storage periods.

Determination of color

The peel color of the leather was determined using an Android Application Software namely "On Color Measure" (developed by PotatotreeSoft, Version 3.0) equipped with an aim pointer. It provides the easiest way to store the information of each color detection. Color measurements were done at each face of leather and a mean value was obtained. The leather color determination was expressed in chromaticity values of Red (R*), Green (G*) and Blue (B*). For measuring the color the camera was aimed at the target color point and clicked on crosshair pointer and moved it to any place on the screen.

The dashboard displays the information of the color detected. Grab button was clicked to capture the screen image and saved all the detailed color information including RGB, HSV, color names, hex code and screen images.

Chemical characteristics of treated fruits

The Chemical characteristics of treated fruits such as total anthocyanin content of peel and the carotenoid content, soluble acid concentration (SSC), pH, Reducing sugar content, Non-reducing sugar content, ascorbic acid content of fruit pulp were studied ion the present experiment.

Anthocyanin and carotenoid measurement

The total anthocyanin content of peel and the carotenoid content of pulp were determined by the method of sims and gamon. For chlorophyll and carotenoid measurement,5g tissue samples were properly homogenized and 10 ml (1:2) 80% cold acetone (pH = 7.8) and centrifuged for 4 min at 800 rpm at 4ºC. The clear supernatant diluted to a final volume of 5 ml with additional acetone and used for the estimation of total anthocyanin content, carotenoids content and evaluated for antioxidant activity. The absorbance of the extract solutions at 665nm, 646nm, 663nm, 470 nm, 529 nm and 650nm wave length was measured with a double beam spectrophotometer). Content of chlorophyll-a and chlorophyll-b as well as anthocyanin content and carotenoid content were calculated by using the following formula:

$$\text{Chlorophyll-a (mg\ml)} = 12.21\ A_{665} - 6.88\ A_{649}$$

$$\text{Chlorophyll-b (mg\ml)} = 20.13\ A_{646} - 5.03\ A_{663}$$

$$\text{Anthocyanin (µmol/ml)} = A_{529} - 0.288\ A_{650}$$

$$\text{Anthocyanin (µmol/g} \times 207.247 = \text{µg/g)} = A_{529} - 0.288\ A_{650}$$

$$\text{Carotenoid (µg/ml - µg/g)} = \frac{1000\ A\ 470 - 3.27\ Ca - 104\ Cb}{229}$$

Where, A_X is the absorbance of the extract solution in a 1-cm path length cuvette at wavelength X.

Determination of pH

The remainder of the filtrated juice from TA determination was used to measure the pH of the fruit pulp. The pH was determined by using a glass electrode pH meter. Beforebeing used, the pH meter was

calibrated with buffers at pH 4.0 followed by pH 7.0. After that, the glass electrode was placed into the filtrate to measure the pH and stabilized reading was recorded. For accuracy of the reading, a glass electrode was hide after each reading with distilled water and wiped to dry with soft tissue paper.

Determination of soluble solid concentration (SSC)

The soluble solids concentration of banana will be determined by using a digital refractometer. The remaining of the filtrated juice from TA determination was used to measure the SSC of the fruit pulp. Before measurement, the refractometer will be calibrated with distilled water to give a 0% reading. About 1-2 drops of the filtrate was placed on the prism glass of the refractometer to obtain the % SSC reading. The readings were multiplied by dilution factor to obtain an original % SSC of the pulp tissues. Since differences in sample temperature could affect the measurement of SSC (Boourne, 1992), each of the reading was standardized to a temperature of 20℃ by adding 0.28% to obtain % SSC at 27℃.

Determination of ascorbic acid

Ascorbic acid was determined according to the dye method by Ranganna (1977). Ten gram of pulp tissue was homogenized with 40 ml of 3% cold metaphosphoric acid (HPO3) using a blender for two minutes and filtrated through Whatman filter paper no.2. Five millimeters of aliquot was titrated with 2, 6-dichlorophenol-indophenol dye until the filtrate changed to pink color that persisted for at least 15 seconds. The titrated volume of dye solution used will be recorded and ascorbic acid content will be calculated using the formula:

Ascorbic acid (mg 100 g -1)

$$=\frac{Titre\ (mL)\times dye\ factor\ (0.125)\times vol.made\ up\ (50\ mL)\times 100}{Aliquot\ used\ for\ estimation\ (5\ mL)\times sample\ weight\ (10g)}$$

To standardize the dye, 5 ml of standard ascorbic acid solution was added to 5 ml of 3% cold HPO_3. The mixture will be titrated with the dye solution to a pink color, which persisted for 15 seconds.

The dye factor was calculated as follows:

$$\text{Dye factor} = \frac{0.5}{Titre\ (ml)}$$

Determination of total sugar

Sugar content of fruit was estimated by the following procedures described by the Lane and Eynon (1923).

Standardization of fehling's solution

50 of both Fehling's solution A and Fehling's solution B were mixed together in a beaker. 10 ml of mixed solution was pipetted into a conical flask and 25 ml distilled water added to it. Standard sugar solution was taken in a burette. The conical flask containing mixed solution begin to boil, 3 drops of methylene blue indicator solution were added without removing the flask from the hot plate. Mixed solution was titrated by standard sugar solution. The end point was indicated decolorization of the indicator. Fehling's factor was calculated by using formula:

$$\text{Fehling's factor (g of invert sugar)} = \frac{Titre\ \times 2.5}{100}$$

Preparation of sample

50 ml fruit juice mixed with 100ml of distilled water and 5ml of neutral led acetate solution and then kept for ten minutes and the mixture was homogenized. Then the blender material was transferred to a 250ml volumetric flask. The volume made up to the mark with distilled water. The solution was then filtered.

Determination of reducing sugar

10ml of mixed Fehling's solution was taken in a 250ml conical flask and made 250ml with distilled water. Purified juice solution was taken in a burette. Conical flask containing mixed Fehling's solution was heated on a plate. 3 to 5 drops of methylene blue indicator was added into the flask when boiling started and titrated with solution taken in burette. The end point was indicated by decolorization of indicator. Percent reducing sugar will be calculated according to the following formula:

$$\text{Reducing sugar (\%)} = \frac{F\times D\times 100}{T\times W\times 1000}$$

Where,

F=Fehling's solution

D=Dilution

T=Titre, and

W=Weight of sample

Determination of total invert sugar

50ml of purified solution (filtered) was taken in a 250ml conical flask. 5ml citric acid and 50ml distilled water added to it. The conical flask containing sugar solution boiled for inversion of sucrose and cooled. The solution was transferred to a 250ml volumetric flask and neutralized by 1N NaOH using phenolphthalein indicator. The volume was made up to the mark with distilled water. Then the mixed Fehling's solution was titrated using similar procedure followed as in case of invert sugar (reducing sugar) mentioned earlier. The percentage of total invert sugar was calculated by using the formula used in incase of reducing sugar.

Estimation of non-reducing sugar

Non-reducing sugar was estimated by using the following formula:
Non-reducing sugar (%)=Total invert sugar (%) - Reducing sugar (%)
Estimation of total sugar=Reducing sugar(%)+Non-reducing sugar(%)

Bio-control activity on natural infected fruits

Bio-control agents have own mechanism to maintaining the quality of host. They have antimicrobial properties that help to controlling the mechanism of diseases.

Disease incidence

Data on DI was recorded as the percentage of fruits showing post-harvest diseases out of the total no. of fruits in each treatment. Disease incidence was calculated using the formula:

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

Disease Severity

Data on disease severity was indexed on a 0-4 scales, where, 0= no disease symptom on the fruit surface area, 1= 1-10% diseased area, 2= 11-20% diseased area, 3= 21-30% diseased area and 4 = 31% and over diseased area. Disease severity was recorded according to the following formula described by Singh (1984):

$$\text{Disease Severity (DS) (\%)} = \frac{\sum(\ Severity\ \ Rating\ \times number\ \ of\ fruits\ \ in\ that\ rating\ )}{Total\ \ number\ \ of\ fruit\ \ assessed} \times highest\ scale\ \times 100$$

Shelf life of banana

Shelf life of banana fruits as influenced by different post-harvest treatments was calculated by counting the number of days required to ripen fully with retained optimum marketing and eating qualities.

Experimental design and statistical analysis

Data were analyzed using one way ANOVA with the Web Agri Stat Package 2.0 (WASP). Means compared by the Duncan's multiple tests and statistical significance was determined at 5% level using WASP

III. Results And Discussions

Application of *Bacillus cereus*strain PSTU HORT-10 on the storability and quality of banana

Changes in physical characteristics during ripening

Various parameters regarding the physical changes of banana were observed and results are presented in the following sub-headings:

External peel color

External color is one of the important visual characteristics of banana fruits for its quality evaluation. The changes of outer color of banana fruit cv. Sagor was monitored by measuring the value of Red (R), Green (G) and Blue (B) during the storage of 12 days at 25±2ºc. The intensity of green color of fruit skin in negative and positive control decreased gradually with advancing storage period and turned to light yellow as evidenced by increasing values of red and blue. However, the changes in colour slowed down with the bacterial treatment. The initial values of R, G and B were 95, 124and 45 respectively in bacterial suspension treated fruits. The water treated negative control fruits demonstrated the maximum colour change at each storage interval as showed by rapid increase in R values with ranged from 95 to 204 compared to treatment (95 to 139) (Figure 1). On the other hand, the fruits under the bacterial treatment exhibited a slower change in peel colour as indicated by lesser decreased in G and B values which ranged from 124 to 157 (Figure 3) and 45to 92 (Figure 4.1.2),respectively after 3 to 12 days of storage.

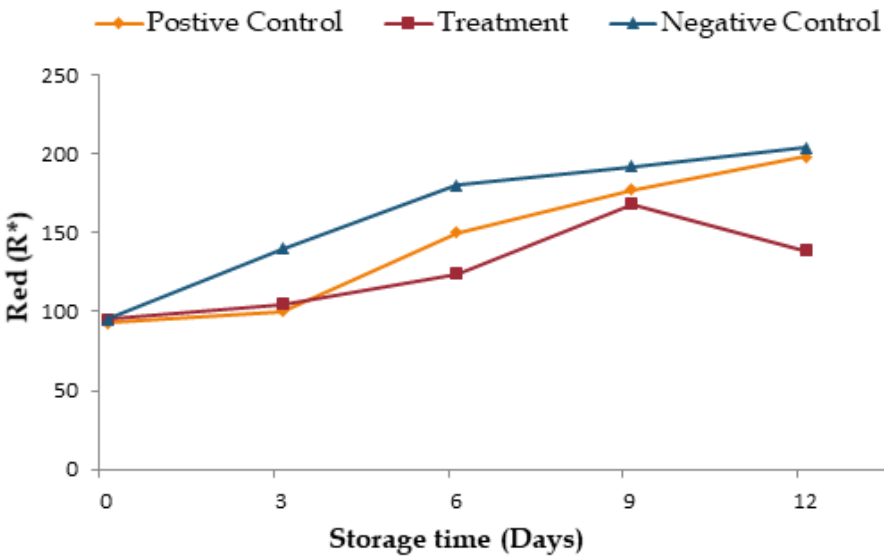

Figure 1: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5 ml/L) and negative control (sterilized distilled water) on peel color (Red) of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days

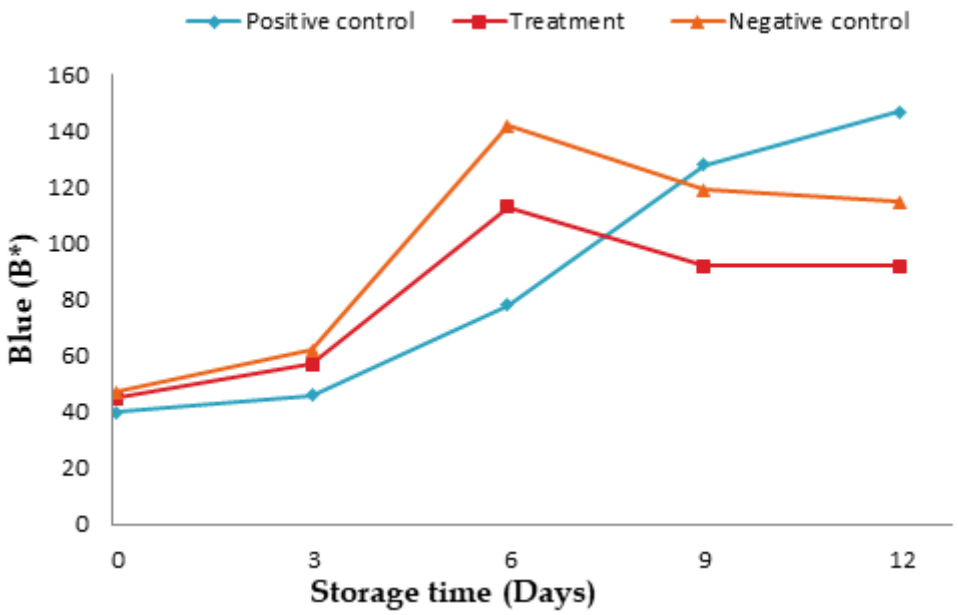

Figure 2: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5 ml/L) and negative control (sterilized distilled water) on peel color (Blue) of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days

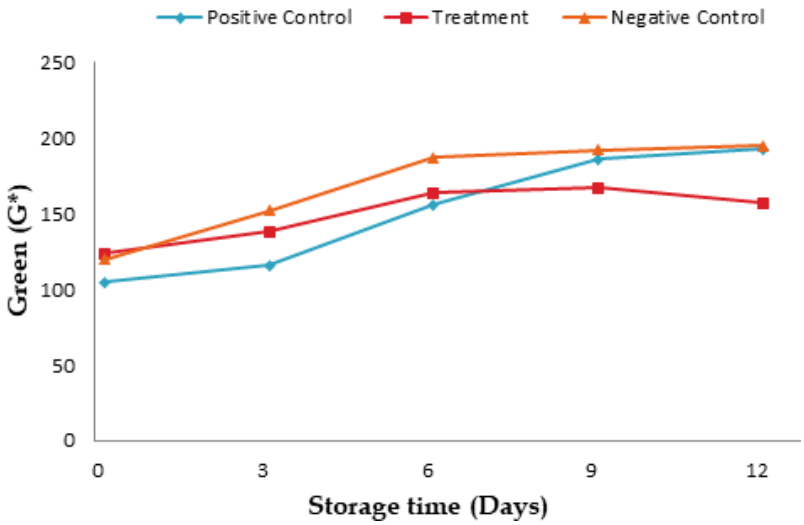

Figure3: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5 ml/L) and negative control (sterilized distilled water) on peel color (Green) of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days

Positive control (3 day) | Bacterial treatment (3 day) | Negative control (3 day)

Positive control (6 day) | Bacterial treatment (6 day) | Negative control (6 day)

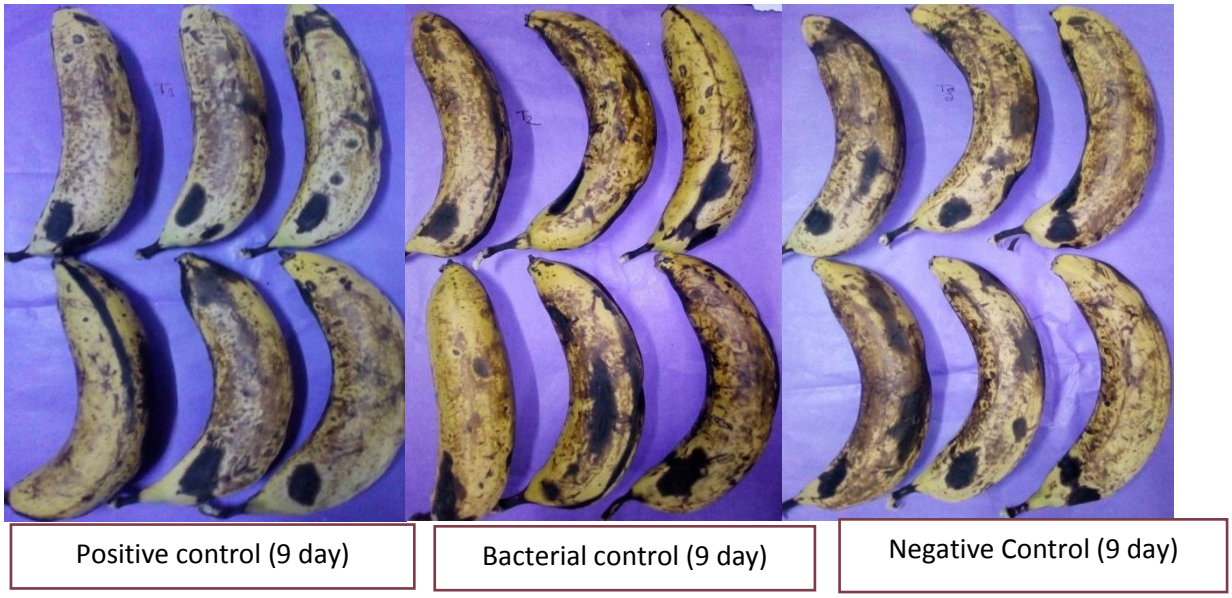

Plate 1. Photographs taken at different days after storage (DAS)

Weight loss

The results revealed that all fruits showed a progressive loss of weight during storage period at (Table 1). However, the minimum weight loss of banana fruits was recorded in bacterial suspension T_2 (3.37 %) and diepheniconazol®, positive control (5.53%) compared to water treated negative control T_3. Bacterial suspension treated Fruits weight loss were ranged between 3.37 to 11.570 % (Table 1) after 3 to 12 days of storage.

The water treated control fruit, on the other hand, exhibited the maximum weight loss at each storage interval with the values ranged between 7.03 to 17.455%. No significant differences in weight loss between positive treatment (T_1) and bacterial suspension (T_2) fruits were observed at 6 days of storage; however, control (T_3) fruits showed higher weight loss than those of other treatments by the end of 12 days storage.

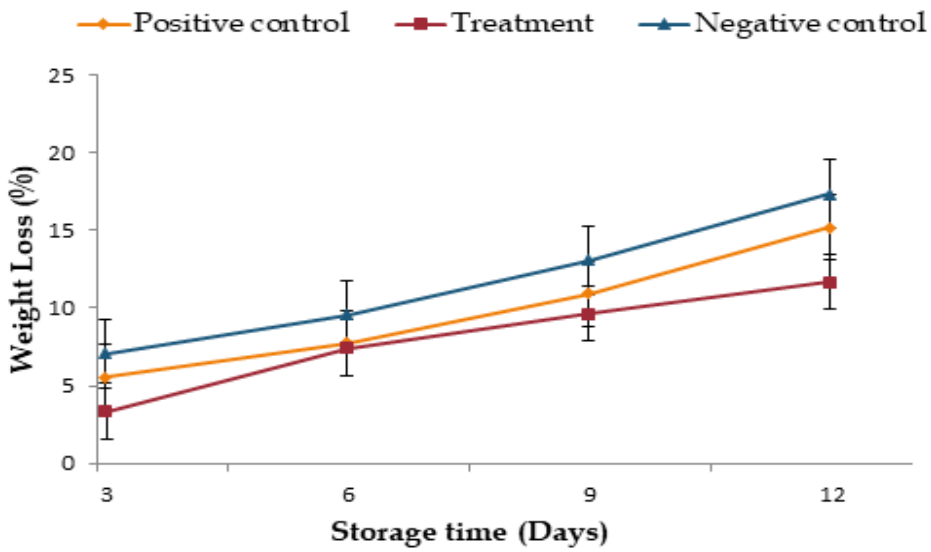

Figure 4: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5 ml/L) and negative control (sterilized distilled water) on Percent weight loss of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days, vertical bar represents standard error.

Table 1: Effects of postharvest treatments on weight loss of banana during storage

Postharvest Treatments	Weight loss (%) at different days after storage (DAS)			
	3	6	9	12
Positive control	5.53 b	7.75 b	10.38 b	15.30 b
Treatment	3.37 c	7.43 b	8.87 c	11.57 c
Negative control	7.03 a	9.56 a	13.01 a	17.46 a
CD at 0.05	0.353	0.341	0.658	0.907
% CV	4.161	2.587	3.826	3.836
Level of sig.	*	*	*	*

CD = Critical difference, CV= Coefficient of variation, * = Significant at 5% level

Changes in chemical characters during ripening

pH

In banana fruits, the pH is significantly changed for all treatments but, the increasing rate is slower in positive control compared to treatments and negative controls (Figure 4). At the 12th days of storage pH value of negative control was significantly higher (5.96) than the treatment control (5.44) and the positive control (5.23) at the same time of storage.

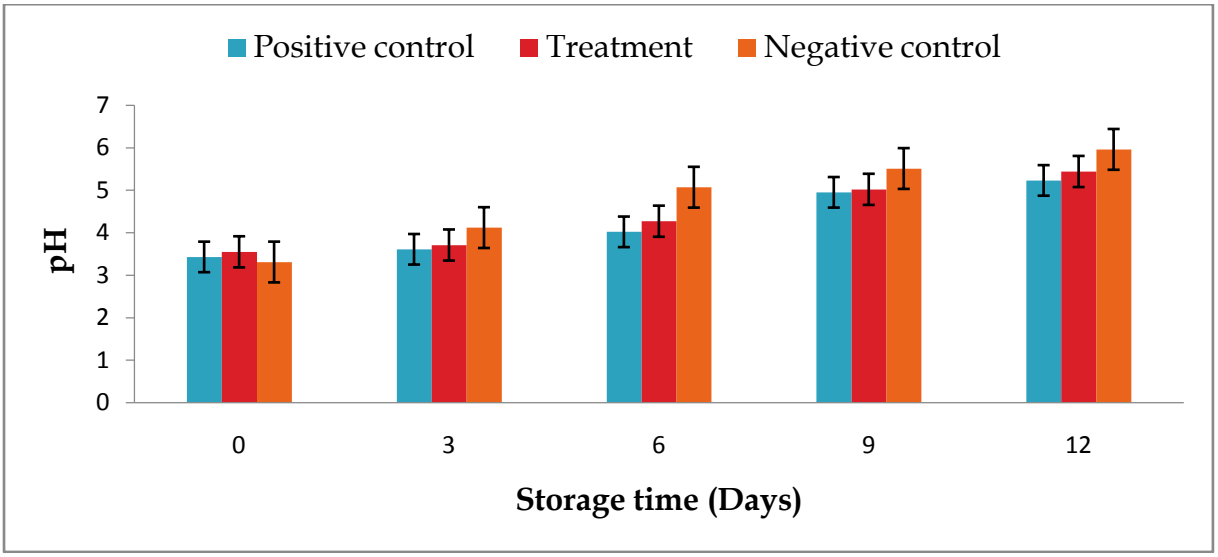

Figure 5. Relationship between pH and storage duration of banana fruits treated with treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water), vertical bar represents standard error.

Total anthocyanin content

Anthocyanins are well known because of their antioxidant properties and their pigmenting power that make them attractive to be used as food colorants (Ajila and Bhat, 2007). It has been reported that anthocyanins are comparatively higher in ripe banana peel than raw peel (Ajila and Bhatt 2007). Anthocyanins are a group of phenolic compounds in the plant kingdom and they exhibit good antioxidant properties. Anthocyanins below pH 2 exist primarily in the form of flavyllium cation and at this low pH, they absorb light around 510 nm, while degraded anthocyanins in the polymeric form absorb light below 2 pH and pH 4.5.

Application of different treatments, significant difference was found in anthocyanin concentration during the storage period of banana fruits; while the difference of anthocyanin content in the diphenicona zol 5 mI/L treatment (T1) and bacterial suspension are significant (Figure 4). Considering the anthocyanin concentration the bacterial suspension and diphenicona zol treatments are significantly different from the negative control. The maximum anthocyanin was found in distill water treatment (1.334 mg/ g) at 12 days while diphenicona zol® and bacterial suspension treatments were (1.226 and 1.118 mg/ g) respectively.

In banana peel colour changes from green to yellow during ripening is mainly due to the loss of total chlorophyll and synthesis of total carotenoids. In the present study the green color of negative control and positive control treated fruits decreased rapidly during storage compared to bacterial control which might be due to the synthesis of anthocyanins, contributing to the development of red-yellow colour in the flesh of banana.

Table 2. Comparison of Treatment Means of anthocyanin content with Critical Difference (0.05) at different days of storage

Postharvest Treatments	Anthocyanin content at different days after storage			
	3	6	9	12
Positive control	0.531 b	0.785 a	0.937 b	1.226 b
Treatment	0.438 c	0.652 c	0.875 c	1.118 c
Negative control	0.562 a	0.728 b	0.979 a	1.334 a
CD at 0.05	0.020	0.018	0.013	0.041
% CV	2.494	1.573	0.857	2.101
Level of sig.	*	*	*	*

CD = Critical difference, CV= Coefficient of variation, * = Significant at 5% level

Total Carotenoids

The results revealed that all fruits showed progressive increases of carotenoid concentration during storage period. However, the maximum carotenoid present of banana fruits was recorded in water treated T_3 (0.961 mg/g) and following bacterial suspension treatment (0.879). However, negative control (T_3) fruits showed higher carotenoid concentration (0.961) than those of other treatments by the end of 12 days storage.
Hornero-Mendez and Minguez-Mosquera (2000) stated that fruit ripening is the change in color; it is a consequence of Chlorophyll disappearance, when the reddish/ yellowish coloration due to carotenoids become perceptible.

Table 3: Comparison of different Treatment Means of carotenoids content of banana with Critical Difference (0.05) at different days of storage

Treatments/Days	Total carotenoids content at different days after storage				
	0	3	6	9	12
Positive control	0.158 b	0.325 c	0.427 c	0.561 c	0.738 c
Treatment	0.171 a	0.338 b	0.457 b	0.765 b	0.879 b
Negative control	0.135 c	0.451 a	0.561 a	0.889 a	0.961 a
CD at 0.05	**0.010**	**0.009**	**0.013**	**0.009**	**0.010**
% CV	**3.891**	**1.599**	**1.629**	**0.781**	**0.699**
Level of sig.	*	*	*	*	*

CD = Critical difference, CV= Coefficient of variation, * = Significant at 5% level.

Titratable acid content of banana pulp

Titratable acidity (TA) of banana fruits gradually decreased with time during storage (Figure 4.4). Data showed that treatments and storage periods had significant effect on the concentration of TA. Significantly the highest TA (0.76%) was recorded under bacterial suspension treatment in fruits at the beginning of storage (3 days) and lowest in water treated control (0.64%) (Figure 6). There is little bit difference between bacterial suspension and diphenicona zol® treatment during 9th day of storage.
The decrease titratable acidity during storage may be attributed to the utilization of organic acids in respiration process and other bio-degradable reactions (Ulrich, 1974)

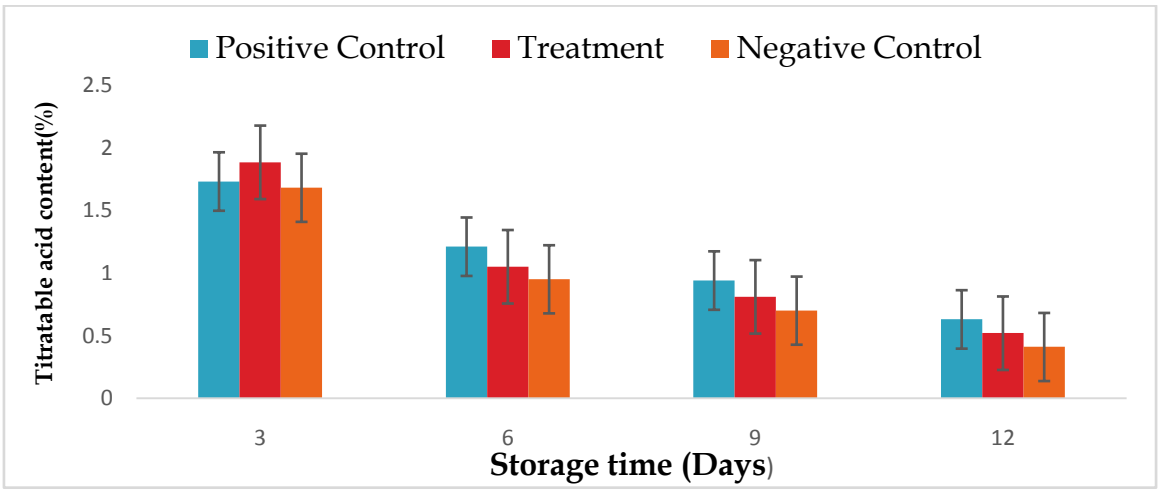

Figure 6: Relationship between titratable acid content and storage duration of banana fruits treated with treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water), Vertical bar represents standard error.

Ascorbic acid content

The different treatments used in the investigation showed statistically significant variation in relation to ascorbic acid at different days of storage . Maximum ascorbic acid content (17.25%, 12.37%) were recorded in T_1 treatment while the minimum (15.98%, 10.31%) were recorded in bacterial suspension treated fruits at 3rd and 6th days of storage respectively (Figure 7) .But in 12th day the highest ascorbic acid content (4.28%) in bacterial suspension treatment and lowest in T3(2.56%) due to the maximum fruits were rotten.

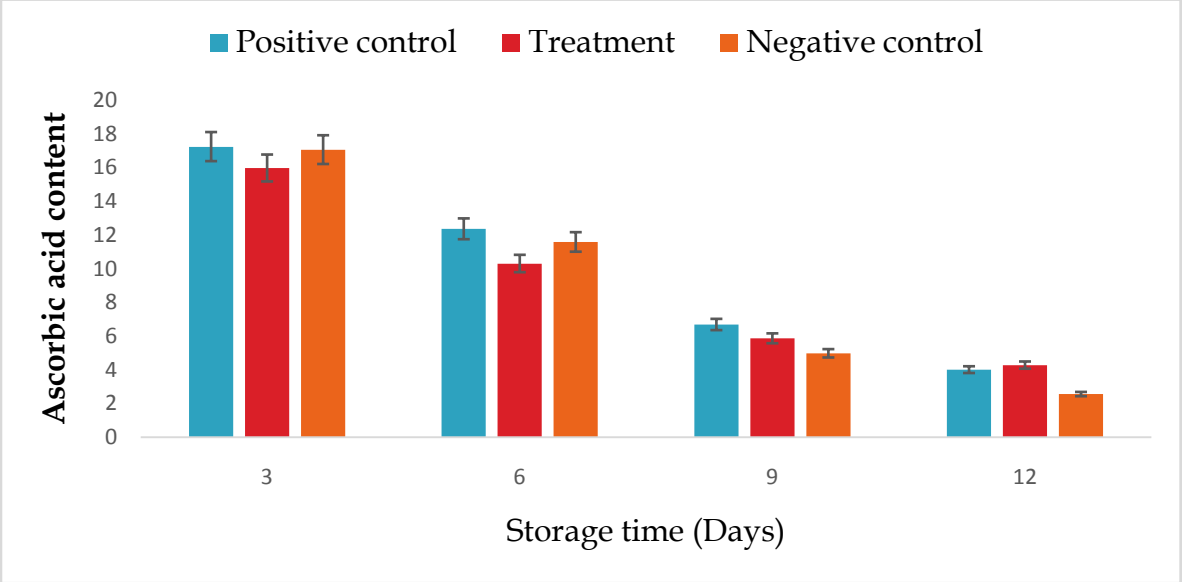

Figure 7: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water) on ascorbic acid content of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days, Vertical bar represents standard error.

Total Soluble solids

Changes in the TSS of banana fruits with different treatments during the storage period are presented in (Table 4.).The maximum TSS content (4.83%, 13.17%, 17.09%) were recorded in water treated while the minimum (4.06%,9.68%,15.89%) were recorded in bacterial suspension treated fruits at 3rd, 6th and 9th days of storage respectively . But in 12th day the highest TSS content (24.01 %) in bacterial suspension treatment and lowest in distill water treatment (18.13 %). The sharp decline in TSS values indicated faster metabolic rates of the water treated fruits. However, the bacterial suspension treated fruits were attributed to higher TSS, which was significantly similar to dipheniconazol® O.5ml/L treated fruit twelve day of storage.

The increasing trend of percent total soluble solids contents of fruit during storage could be attributed mainly to the breakdown of starch into simple Sugars. This observation is somewhat similar to Pinakiet *al. (1997).*

Table 4: Effects of postharvest treatments on total soluble solids content of banana during storage

Postharvest Treatments	Total soluble solids (%) at different days after storage (DAS)			
	3	6	9	12
Positive control	4.58 b	10.07 b	16.67 b	22.27 b
Treatment	4.06 c	9.68 c	15.89 c	24.01 a
Negative control	4.83 a	11.03 a	17.09 a	18.13 c
CD at 0.05	0.113	0.107	0.120	0.128
% CV	1.575	0.652	0.454	0.372
Level of sig.	*	*	*	*

CD = Critical difference, CV= Coefficient of variation, * = Significant at 5% leve

Reducing sugar

Postharvest storage treatments used in the study showed a slightly effect on reducing sugar content of banana. Variation among the treatment in relation to reducing sugar was statistically significant at different days of storage. Maximum reducing sugar content (5.50%) was found in T1 treatment where the $T_3$was the lowest (4.76%) at 12 days.The reducing sugar content minimum (2.25%) in positive control at 3rd days of storage.
All treatments reducing sugar were increased gradually.

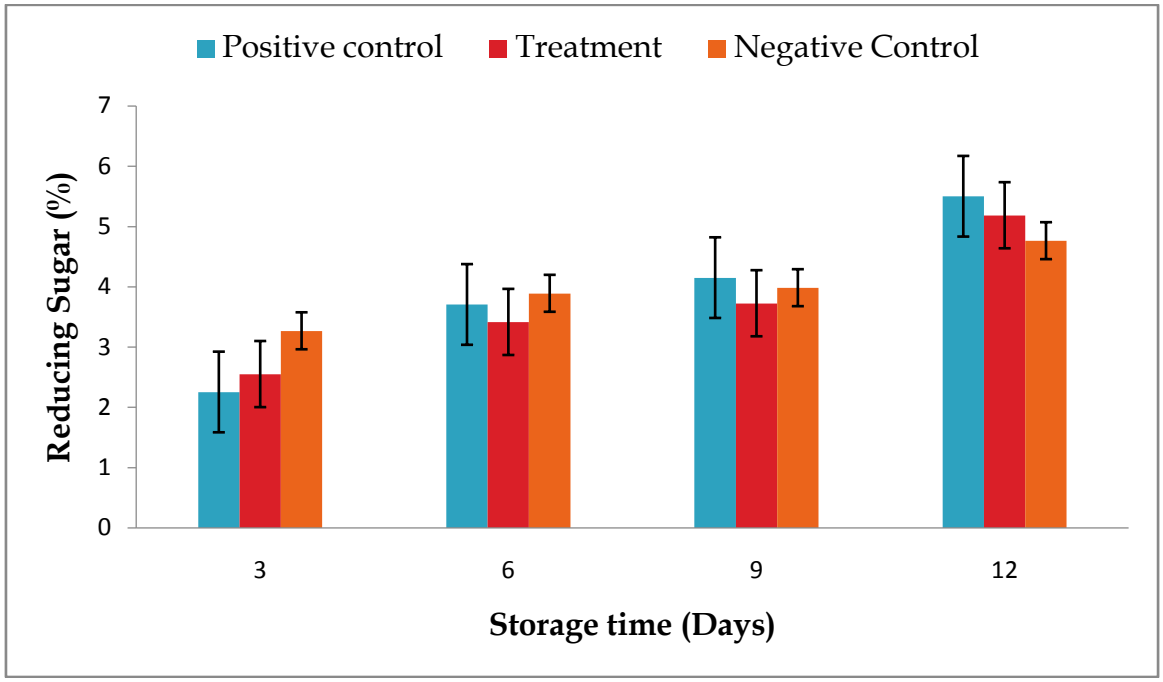

Figure 8: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water) on reducing sugar contentof banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days, vertical bar represents standard error.

Non reducing Sugar

The effect of postharvest treatments used in the study showed a noticeable effect on non-reducing sugar content during the storage of 3 day interval of banana. Variation among the treatment in relation to non-reducing sugar was statistically significant at different days of storage. Maximum non-reducing sugar content (8.420%) was found in bacterial treatment and minimum (5.203%) in distilled water treated fruit at 12th days of storage (Figure 8).

Non-reducing sugar content was increased with the increasing storage period and postharvest treatments of bacterial treatment showed significant variation.

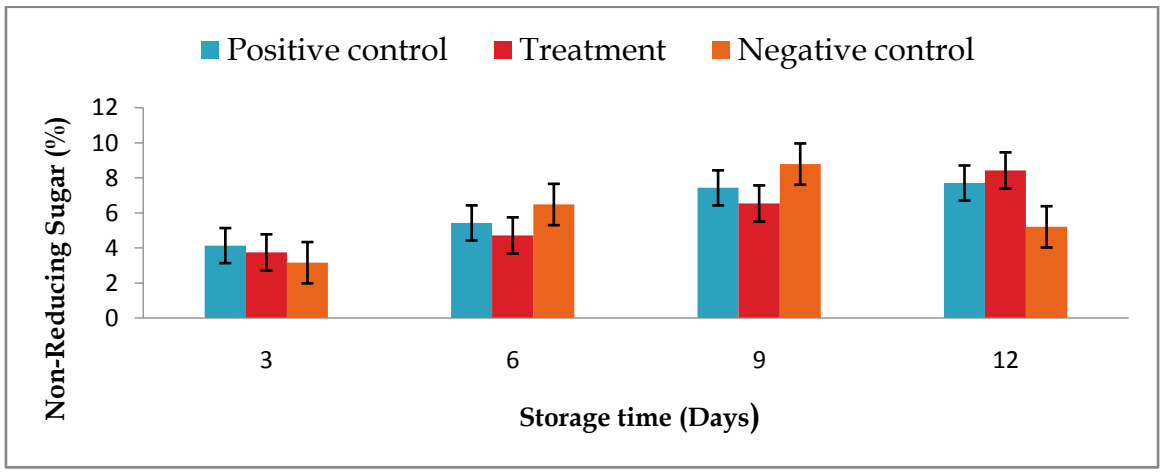

Figure 9: Effect of treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water) on non-reducing sugar content of banana fruits during storage at 26±2ºC and 55±10 % RH for 12 days, vertical bar represents standard error.

Changes in microbial characteristics

Disease incidence

The naturally infected fruits subjected to treatments with antagonistic bacteria and dipheniconazol®(0.5mI/L) showed significantly lowest anthracnose incidence than in fruits dipped in distil water.

The incidence of the disease gradually increased with ripening period. Among the postharvest treatments, positive treatment T_1-dipheniconazol(0.5ml/L) produced the minimum disease incidence (29.27%) at 6 days of storage of where water treated (T_3) treatment was maximum (47.77%) and it was rapidly increased where it showed 100% disease incidence at 12 days of storage. The treatment of bacterial suspension (T2), the highest disease incidence (72.15%) was recorded at 12 days of storage which was nearly about the positive treatment (T_1) gave the highest disease incidence (65.25%) at 12 days of storage.

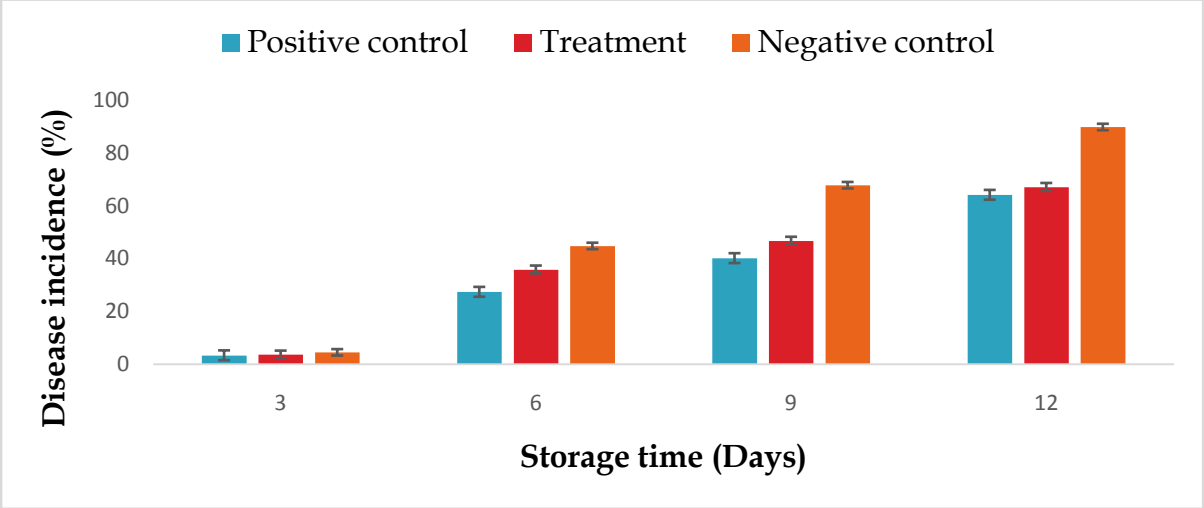

Figure 10: Relationship between disease incidence and storage duration of banana fruits treated with treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water) , Vertical bar represents error.

Disease severity

Anthracnose severity on naturally infected banana fruits subjected to different treatments is presented in (Figure 10). The postharvest treatments of dipheniconazol treatment and bacterial suspension showed highly significant compared to distill water treatment on disease severity effects on storage period interval. The dipheniconazol® treatment (T_1) and bacterial suspension (T_2) were the most effective treatment, which showed significantly lower anthracnose severity on naturally infected fruits than in fruits dipped distilled water (T_3). The maximum disease severity(53.25%) was found in water treated fruit while others were 29.25 and 31.35 % respectively.

Table 5: Effects of postharvest treatments on disease severity of banana during storage

Postharvest Treatments	Disease severity (%) at different days after storage (DAS)			
	3	6	9	12
Positive control	0 b	20.19 b	24.33 c	29.82 b
Treatment	0 b	21.07 b	26.18 b	31.35 b
Negative control	5.33 a	27.38 a	35.79 a	53.25 a
CD at 0.05	0.136	1.150	1.339	1.980
% CV	4.776	3.130	2.909	3.253
Level of sig.	*	*	*	*

CD = Critical difference, CV= Coefficient of variation, * = Significant at 5% level

Isolation and Identification of pathogen from infected test fruits

Infected test fruits were picked up and studied in the applied microbiology lab, Department of Horticulture of PSTU for identifying the disease causing organism. In anthracnose, initially tiny brown spots developed which enlarge as the ripening progressed. The sunken spots appeared as tear-shaped patterns anywhere on the peel. Eventually, the whole fruits rot and fungal bodies were formed on the rotten surfaces.

Shelf life

Shelf life of banana fruits was significantly influenced by postharvest treatments of bacterial suspension. The extension of shelf life of fruits has been one of the most important concerns of the researchers. Results revealed that the longest shelf life (15 days)of banana fruits were recorded in those fruits treated with dipheniconazol® (positive treatment), following bacterial suspension (14 days) and shortest shelf life(12.5 days) was recorded in control precede by T_3 fruits in (Figure 4.10). Shelf life extension with bacterial suspension treatment was possibly due to the suppression of microbial growth. Girdhay and AnuragPayasi, (2007) also

reported that the ripe banana fruits were subjected to dip treatment for 4 h using chemicals, bio control agent or their combinations, and shelf life of treated fruits stored at room temperature was monitored by assaying fruit firmness. The treatment of fruit with 1% garlic clove extract or 10% onion bulb extract and adjusting the pH to 4.5 extended the shelf life by 4-5 days when compared with untreated fruit Treatment with a combination of 1 % garlic extract and 1 rom sodium metabisulphite and adjusting the pH to 4.5 prolonged the shelf life of ripe banana to 13-14 days when compared with 6-7 days for the untreated fruit The treatment delayed the fruit softening by diminishing the rate of starch and pectin degradation. The rate increase in b-amylase and polygalacturonase activities along with softness was also retarded in treated fruits when compared with control fruits.

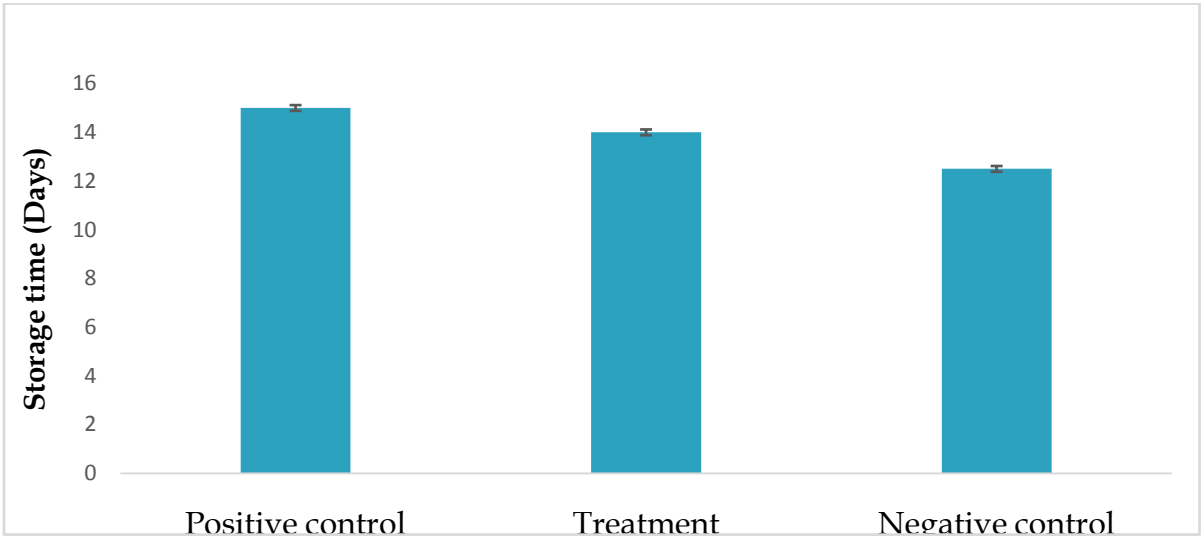

Figure 11: Relationship between shelf life and storage duration of banana fruits treated with treatment (bacterial suspension), positive control (Diphenoconazol® @ 0.5m1/L) and negative control (sterilized distilled water), Vertical bar represents error percentage.

IV. Conclusions

Apparently, the present study found that, fruits treated with antagonistic bacterial suspension delayed several physio-chemical changes and thus extended shelf life of banana. The bacterial treatment significantly retained fruit firmness, decreased weight *loss* and delayed the changes in external color, soluble solids concentration, titratable acidity, ascorbic acid contents and pH of the fruits during storage. It is suggested that the composite mixture of bacterial suspension with other microbial antagonists or safe additives like different salts (NaCI, $MgCl_2$ etc.), coating materials (aloe gel coating, chitosan coating etc.) at suitable concentrations could be commercially used as promising natural, harmless and eco-friendly technology for prolonging the shelf life of banana.

However, postharvest use of fungicides has been increasingly curtailed by the development of pathogen resistance to many key fungicides, lack of replacement fungicides and public perception that fungicides are harmful to human health and the environment. This negative perception has promoted governmental policies restricting use of fungicides. Thus, *Bacillus cereus* strain PSTU HORT-10 antagonistic bacteria may be the promising one in the field of postharvest.

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