

Original Research Article

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Influence of 1-MCP on Pectin Methylestarase, Peroxidase and Catalase Enzymes in Banana (*Musa paradisiaca* L.) cv. Tella Chakkerakeli during Storage

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ABSTRACT

Keywords

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In order to extend the shelf life of banana cultivar Tella Chakkerakeli was fumigated with 1-methylcyclopropane (1-MCP) at four different concentrations (100, 200, 300 and 400 ppb) @ 24h exposed time and stored at ambient conditions. Among all the treatments, 400 ppb was found to be an effective in extending shelf-life, highest retention of cell wall degrading enzyme such as pectin methylestarase and antioxidant enzymes, peroxidase and catalase. The entire 1-MCP treated banana showed better results over control (kept at open air @ 24h).

Introduction

Banana (*Musa paradisiaca* L.) is one of the major commercial fruit crop grown in tropics and subtropics. It plays a key role in the economy of developing countries. India has vast prospective to cultivate and produce the high quality banana fruits and are exported to the local markets instead of the international markets. Approximately 25 to 30% of the harvested fresh produce is deteriorated in every year due to high perishable nature of the fruits, pitiable handling practices and

inadequate storage facilities. The causes for postharvest losses of fresh produce are an increase in respiration rate, ethylene production, physiological disorders and general senescence. Generally banana is classified as climacteric fruit which is characterized by a low rates of ethylene production and respiration during the pre-climacteric phase, followed by a sudden burst in ethylene production and respiration rate during ripening (Burg and Burg, 1965). The sudden rises in respiration rate and ethylene production during ripening stage are

responsible for major postharvest losses in bananas. Ethylene is a gaseous hormone, it accelerates the ripening in climacteric fruits. The ripening is mainly triggered through the action of ethylene binding to its receptor sites located on cell membrane (Sisler and Serek, 1997).

A novel gaseous anti-ethylene compound 1-Methylcyclopropene (1-MCP) has been reported to have inhibitory effects on ethylene action (Serek *et al.*, 1994, Sisler *et al.*, 1995). It is a stable powder and easily released the ethylene gas when dissolved in water. It acts by binding irreversibly to ethylene-receptors hence, subsequent signal transduction and translation responses are not elicited, causes fruits to be ripen and soften more slowly, thereby maintaining the quality of produce for longer period. 1-MCP treatment extended the green life and/or inhibited the ripening of tomato, banana and plum fruits (Serek *et al.*, 1995, Sisler *et al.*, 1995, Macnish *et al.*, 1997, Abdi *et al.*, 1998, Golding *et al.*, 1998).

In this present paper, we have investigated the effects of 1-MCP on banana fruit ripening by measuring cell wall degrading enzyme i.e pectin methylesterase and antioxidant enzymes such as catalase and peroxidases.

Materials and Methods

Plant material and treatments

The current research work was carried out in the in the Department of Fruit Science, College of Horticulture, V.R.Gudem, Dr.YSRHU during the year 2015-18. Mature green (80-85% Maturity) Tella Chakkerakeli banana bunches were harvested from the experimental farm field of HRS, Kovvur, Dr.YSRHU. Later harvested bunches are dehanded carefully and second hand from each bunch brought to the laboratory. Then the hands were cleaned in tap water, dipped in

0.1% Bavistin for a while and air dried under fan for 10-15 minutes. Later the banana hands were placed in known volume carton boxes (CFB) for imposing 1-MCP. The required quantity of 1-MCP Ansip-F tablets for known volume carton boxes for yielding 100 ppb, 200 ppb, 300 ppb and 400 ppb were calculated (1.1g of tablet in 40L carton box gives 900 ppb concentration) and crushed into powder with mortar and pestle. The 1-MCP powder was placed inside the flask containing a rubber septum. Then warm distilled water (at 40-50⁰C) was added to the flask for dissolving the 1-MCP powder. The flask was then placed inside the container through the top opening and the flask lid is removed immediately before the carton box was completely sealed. This modified method was described by Wongmetha and Lih-Shang Ke (2012) and Alves *et al.*, (2005). After exposure to 24 hrs at room temperature the carton boxes were opened and the banana hands were kept for storage studies at ambient room temperature (32±2°C and 78±2% RH). The banana fruits were sampled for enzyme analysis at 3 day interval. The treatments attempted in this experiment were 1-Methylcyclopropene (100 ppb), 1-Methylcyclopropene (200 ppb), 1-Methylcyclopropene (300 ppb) and 1-Methylcyclopropene (400 ppb) along with control (kept at open air @24h).

Observations recorded

Determination of pectin methylesterase (PME) activity

Pectin methylesterase (PME) activity in banana fruit pulp was measured following the method of Hagerman and Austin (1986) with minor modifications. The method is based on the colour change of a pH indicator during the PME catalysed reaction. In a cuvette, 2.0 mL of pectin (0.5%) is mixed with 0.15 mL of bromothymol blue (0.01%) and 0.83 mL of water. The absorbance of the mixture is read

against water as blank at 620 nm. A constant value of A₆₂₀ at this stage indicates nonexistence of non-enzymatic hydrolysis. The reaction is started by adding 50 µL of enzyme solution and the rate of decrease in A₆₂₀ was recorded. The acid produced by PME action lowers the pH of the medium and thereby cause protonation of the indicator dye to produce a change in absorbance at 620 nm. The change in absorbance is continuously monitored spectrophotometrically and the initial rate of reaction is determined. A standard graph is plotted (OD vs. time) using different known concentrations of glacial acetic acid and the rate of reaction is determined from the linear portion of the graph. The PME activity was expressed as µmol min⁻¹g⁻¹ FW.

Determination of catalase and peroxidase activity

Extract for determination catalase (CAT) and peroxidase (POD) activities were prepared from 0.3 gm of banana pulp, homogenized with a pre-chilled mortar and pestle under ice cold condition in 3 ml of extraction buffer, containing 50 mM sodium phosphate buffer (pH 7.4) with the addition of 1 mM EDTA and 1% (W/V) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for the assay (Costa *et al.*, 2002).

Total catalase (EC 1.11.1.6) activity was determined in the homogenates by measuring the decrease in absorption in 3ml mixture at 240 nm as H₂O₂ ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) was consumed according the method of Aebi (1984) and enzyme activity expressed as µM H₂O₂ oxidized min⁻¹ mg⁻¹ protein. The 3 ml mixture containing 50mM sodium phosphate buffer (2ml) (pH 7.0), 10mM H₂O₂ (950 µl) and 50µl enzyme extract. POD (EC 1.11.1.7) activity was determined in the homogenates by measuring the increase in absorption at

470nm due to the formation of tetraguaiacol ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) in a 3 ml reaction mixture containing 50mM sodium phosphate buffer (2 ml) pH 7.0, 0.1mM EDTA (100 µl), 0.1ml enzyme extract, 10mM guaiacol (400 µl) and 10mM H₂O₂ (400 µl) (Costa *et al.*, 2002) and enzyme activity expressed as µmol guaiacol oxidized min⁻¹ mg⁻¹ protein.

Shelf life (d)

The shelf life of fruits was determined by recording the number of days the fruits remained in good condition during storage. The stage at which more than 50 per cent of the stored fruits became unfit for consumption was considered as end of shelf life in that particular treatment and expressed as mean number of days (Padmalatha, 1993).

Statistics

The experiment was designed in a factorial completely randomized design (FCRD) with three replications. The data was analyzed as per the design and the results were compared from the CD value obtained through ANOVA (Panse and Sukhatme, 1984).

Results and Discussion

Pectin methylesterase (PME) activity

PME activity was followed an increasing trend in all treatment combinations from 1st to 10th day of storage but in untreated mango fruits it reached maximum (0.425 µmol min⁻¹) on the 7th day of storage and declined. However, the rate of increase was significantly high in the untreated banana compared to the treated ones (Table 1 and Fig. 1). Maximum (0.309 µmol min⁻¹g⁻¹ FW) PME activity was noticed in untreated banana and minimum in fruits fumigated with 1-MCP @ 400 ppb (0.159 µmol min⁻¹g⁻¹ FW), followed by 1-MCP @ 300 ppb (0.194 µmol min⁻¹g⁻¹ FW). The

reduced PME activity in the 1-MCP treated banana fruits can be attributed to retarding effects of 1-MCP on the fruit softening. Our results find the support from the reports of Lohani *et al.*, (2004), who found lowered PG and PME enzyme activities in 1-MCP treated banana compared to the untreated fruits. Similar effects of 1-MCP on PME activity of plum (Khan and Singh 2007; Sharma *et al.*, 2012) and papaya (Ahmad *et al.*, 2013) have also been reported.

Peroxidase (POD) activity

Peroxidase activity was followed an increasing trend in all treatment combinations from 1st to 10th day of storage but in untreated

mango fruits it reached maximum (0.943 $\mu\text{mol min}^{-1}$) on the 7th day of storage and declined. However, the rate of increase was significantly high in the untreated banana compared to the treated ones (Table 2 and Fig. 2). Maximum (0.522 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) POD activity was noticed in 1-MCP @ 100 ppb fumigated banana and minimum in fruits fumigated with 1-MCP @ 400 ppb (0.152 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein), followed by 1-MCP @ 300 ppb (0.198 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein). However, the POD activity continually increased but at a slower pace in the banana fruits fumigated with 1-MCP. During ripening process reactive oxygen species were rendered, to scavenge these free radicals, antioxidant enzymes were produced.

Table.1 Effect of postharvest fumigation of 1-MCP on pectin methylestarase ($\mu\text{mol min}^{-1} \text{g}^{-1}$ FW) in Tella Chakkerakeli banana stored at ambient conditions (32±2°C and 78±2% RH)

Treatments (T)	Storage Period(S)				
	1 st Day	4 th Day	7 th Day	10 th Day	Mean
1-MCP @ 100 ppb	0.147	0.211	0.301	0.404	0.266
1-MCP @ 200 ppb	0.129	0.177	0.244	0.323	0.218
1-MCP @ 300 ppb	0.116	0.145	0.208	0.306	0.194
1-MCP @ 400 ppb	0.098	0.126	0.158	0.255	0.159
Control (open air)	0.191	0.337	0.425	0.282	0.309
Mean	0.136	0.199	0.267	0.314	
	SE(m)		C.D.@5%		
Treatment	0.001		0.003		
Storage Period	0.001		0.003		
T×S	0.002		0.006		

Table.2 Influence of postharvest fumigation of 1-MCP on peroxidise ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in Tella Chakkerakeli banana stored at ambient conditions (32±2°C and 78±2% RH)

Treatments (T)	Storage Period(S)				
	1 st Day	4 th Day	7 th Day	10 th Day	Mean
1-MCP @ 100 ppb	0.122	0.254	0.592	1.121	0.522
1-MCP @ 200 ppb	0.107	0.166	0.266	0.472	0.253
1-MCP @ 300 ppb	0.091	0.132	0.215	0.353	0.198
1-MCP @ 400 ppb	0.072	0.113	0.172	0.249	0.152
Control (open air)	0.166	0.554	0.943	0.302	0.491
Mean	0.112	0.244	0.438	0.499	
	SE(m)		C.D.@5%		
Treatment	0.002		0.005		
Storage Period	0.002		0.004		
T×S	0.003		0.010		

Table.3 Effect of postharvest fumigation of 1-MCP on catalase ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in Tella Chakkerakeli stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)

Treatments (T)	Storage Period(S)				
	1 st Day	4 th Day	7 th Day	10 th Day	Mean
1-MCP @ 100 ppb	2.330	2.780	7.567	4.110	4.197
1-MCP @ 200 ppb	1.560	1.930	3.700	6.850	3.510
1-MCP @ 300 ppb	1.320	1.420	3.290	6.020	3.013
1-MCP @ 400 ppb	1.140	1.330	2.620	5.880	2.743
Control (open air)	2.960	4.680	9.167	4.030	5.209
Mean	1.862	2.428	5.269	5.378	
	SE(m)		C.D.@5%		
Treatment	0.018		0.052		
Storage Period	0.016		0.047		
T×S	0.036		0.104		

Table.4 Influence of postharvest fumigation of 1-MCP on shelf life (d) in Tella Chakkerakeli banana stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)

Treatments (T)	Shelf life (d)
1-MCP @ 100 ppb	8.25
1-MCP @ 200 ppb	10.5
1-MCP @ 300 ppb	13.75
1-MCP @ 400 ppb	16.5
Control (open air)	5.5
SE(m)	0.329
C.D.@5%	1.001

Fig.1 Effect of postharvest fumigation of 1-MCP on pectin methylesterase ($\mu\text{mol min}^{-1} \text{g}^{-1}$ FW) in Tella Chakkerakeli banana stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)

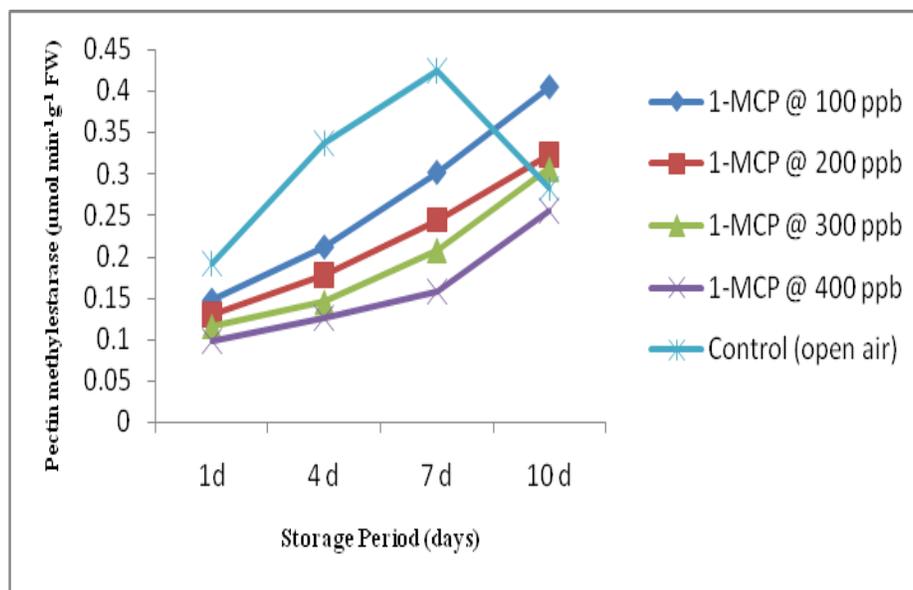


Fig.2 Influence of postharvest fumigation of 1-MCP on peroxidase ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in Tella Chakkerakeli banana stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)

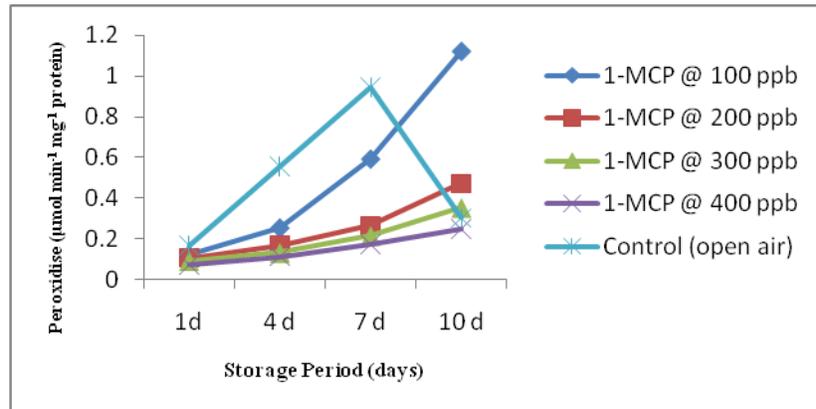


Fig.3 Effect of postharvest fumigation of 1-MCP on catalase ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) in Tella Chakkerakeli stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)

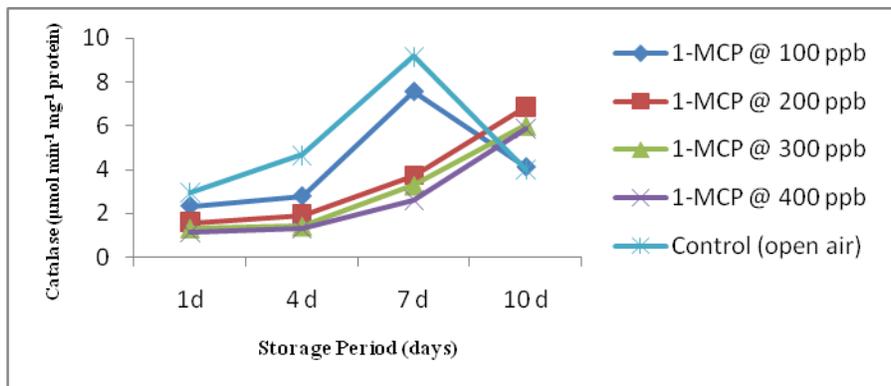
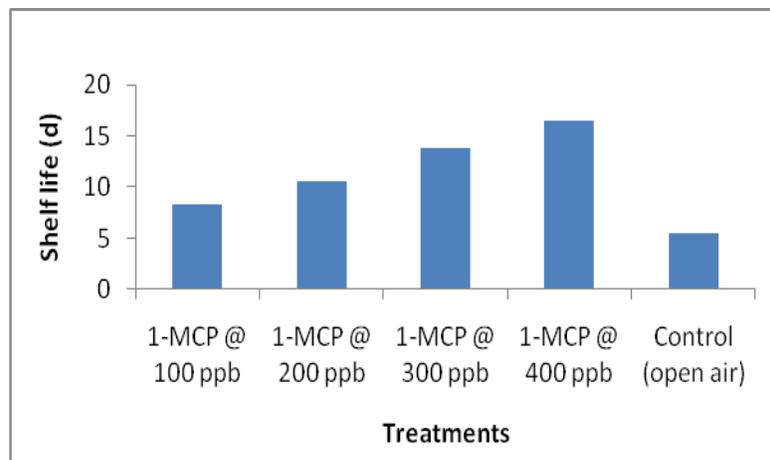


Fig.4 Influence of postharvest fumigation of 1-MCP on shelf life (d) in Tella Chakkerakeli banana stored at ambient conditions ($32\pm 2^\circ\text{C}$ and $78\pm 2\%$ RH)



In this present investigation 1-MCP treated banana fruits recorded less amount of peroxidase (POD) when compared to untreated bananas, this is may be due to delayed ripening process caused by binding of 1-MCP with ethylene receptor that resulted in an apparent delay in the onset of elevated ethylene evolution and respiration rates and also delay in several physiological responses related to ripening. The results obtained under the present investigation are also in agreement with the findings of Dal Cin *et al.*, (2006) and Wang *et al.*, (2009).

Catalase (CAT) activity

Catalase activity was followed an increasing trend in all treatment combinations from 1st to 10th day of storage but in untreated (9.167 $\mu\text{mol min}^{-1}$) and 1-MCP @ 100 ppb (7.567 $\mu\text{mol min}^{-1}$) fumigated banana fruits reached maximum on the 7th day of storage and declined. However, the rate of increase was significantly high in the untreated banana compared to the treated ones (Table 3 and Fig. 3). Maximum (5.209 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) CAT activity was noticed in untreated banana and minimum in fruits fumigated with 1-MCP @ 400 ppb (2.743 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein), followed by 1-MCP @ 300 ppb (3.013 $\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein). However, the catalase activity continually increased but at a slower pace in the banana fruits fumigated with 1-MCP. The reduced CAT content in 1-MCP treated banana fruits can be attributed directly to its ability in inhibiting ethylene action and also to the role of ethylene in triggering free radicals (ROS) production. Such effects of 1-MCP leads to decreased ROS, maintained plasma membrane permeability and decreased the free radical scavenging enzymes production (CAT, POD and SOD). The different effects of 1-MCP on ROS and the antioxidant enzymes may be linked with H_2O_2 and AsA metabolism (production and degradation)

(Wang *et al.*, 2009). This effect was also observed in the previous studies (Larrigaudiere *et al.*, 2004) pear and (Dong *et al.*, 2002) apricot and plum.

Shelf life

Maximum shelf life (16.5 d) was recorded in Tella Chakkerakeli banana treated with 1-MCP @ 400 ppb (d) and lowest shelf life (5.5 d) was recorded in untreated banana. All the 1-MCP treated banana fruits showed improved shelf life compared to control (Table 4 and Fig. 4). The reason for high concentration of 1-MCP was effective in order to suppress the more ethylene binding sites developed in the banana fruit tissues during ripening. These findings are in conformity with, Jiang *et al.*, (1999), Jansasithorn and kanlayanarat, (2006), Moradinezhad *et al.*, (2006, 2010) and Krishna kumar and Thirupathi, (2014) in banana.

It was concluded that exposure of Tella Chakkerakeli banana fruits to 1-methylcyclopropane extended the economic shelf life at ambient conditions ($32 \pm 2^\circ\text{C}$ and $78 \pm 2\%$ RH) compared to the untreated control fruits. 1-MCP (400 ppb) retained cell wall degrading enzyme (PME) and antioxidant enzymes (POD and CAT) and delayed the climacteric ripening process to a greater extent compared to other concentrations.

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