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Effect of pre and post-harvest application of chitosan on sensory quality and microbial count parameters of grape Cv. Manik Chaman during storage at 0 °C temperature

VB Yadav, Dr. KH Pujari and VR Kekan

Abstract
An experiment entitled, "Effect of pre and post-harvest application of chitosan on Sensory quality and microbial count parameters of grape Cv. Manik Chaman during storage at 0 °C temperature" was conducted in the Department of Post-Harvest Management of Fruit, Vegetable and Flower Crops, P.G. Institute of P.H.M., Killa-Roha during the year 2017-2018. The study aimed at minimizing post-harvest handling losses in table grapes by using following experiment. The experiment was conducted in Factorial Completely Randomized Design (FCRD) for different parameters with six main treatments *viz*. untreated fruits (control), 0.1% pre-harvest spray and 0.5 to 2% post-harvest dipping of chitosan, with 0, 15, 30 and 45 days storage period at 0 °C temperature and the grape berries were analyzed for the changes in sensory qualities and microbial count parameters. It was observed that the pre-harvest spray and post-harvest dipping of chitosan treatments recorded delay in increase in microbial count and delay in decreasing sensory qualities of grape Cv. Manik Chaman irrespective of treatments.
As regards the organoleptic evaluation, the grape clusters with 0.1% pre-harvest spray and 1.0% post-harvest dipping of chitosan treatment obtained highest sensory score at 45 days of storage at 0 °C temperature condition as compare to other treatments. Thus, it is suggested that 0.1% pre-harvest spray and 1.0% post-harvest dipping of chitosan is optimum for grape.

Keywords: Grape, chitosan, dipping, storage, sensory qualities and microbial count

Introduction
Grape (*Vitis vinifera* L.) is one of the most consumed fruit crops grown worldwide. Grape is the third most widely cultivated fruit after citrus and banana (Anon., 2015) [7]. India ranks 7th position in grape production (Shikamany, 2001; Gade *et al.*, 2014) [50, 16]. It is one of the most important crops in India, generally grown in the subtropical regions of India (Shinde, 2016) [52]. Grape is believed to have originated in Armenia near the Black and Caspian seas in Russia, and belong to the *Vitaceae* family.
India ranks 7th in the world with total production of 2,922 thousand metric tonnes from about 137 thousand ha area and productivity is 14.9 tonnes/ha. Maharashtra is leading state in area under cultivation (90.91 thousand ha) and total production (2048.11 thousand metric tonnes); followed by Karnataka (23.35 thousand ha; 429.78 thousand metric tonnes), Tamilnadu (2.44 thousand ha; 34.10 thousand metric tonnes), Mizoram (4.47 thousand ha; 22.55 thousand metric tonnes) and Kerala (1 thousand ha; 15.50 thousand metric tonnes) (Anon., 2017a) [8]. Maharashtra is the biggest producer of grapes in the nation and holds the 1st position. Over 80% of the total grapes exported past years were from Maharashtra. Nasik, Satara, Solapur, Sangli, Pune and Ahmednagar are major grape growing belts in the state (Anon., 2018) [8].
The quality of grapes in market not only depends on various activities carried out in the vineyard, but the operations and handling during and after harvesting also play important role. The post-harvest practices are influenced by various factors like variety, market, market requirement, packaging material, handling practices etc. Now, post-harvest practices are becoming more important as quality and cost factors are making market more competitive. Involvement of labor issues, unavailability of skilled labour as per requirements etc. are creating problem and increasing cost of produces in the market (Sharma, 2016) [48].
Manik Chaman variety is a mutant of Thompson seedless variety of grape. This variety is grown in Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka. It has wide adaptability with seedless, ellipsoidal-elongated, golden-yellow berries with medium-thin skin.

The juice is straw coloured, sweet with a TSS of 20-22 °B. This variety has a good keeping quality and is used for table purpose and raisin making. Average yield is 20-25 t/ha. Manik Chaman is also reported to respond better to G. A. application than Thompson Seedless (Anon, 2017e) [8]. As per the Vitis International Variety Catalogue, the details the variety are; Prime name- Manik Chaman, Color of berry skin- BLANC, Variety number- VIVC 16872 (Erika., 2014) [15].

Table grape is a highly perishable, non-climacteric fruit. Its shelf life is usually shortened by firmness loss, berry drop, discoloration of the stem, desiccation and fungal rots. The most common commercial method to control decay of the table grape fruit is the use of SO_2 during cold storage, either by fumigation or generators (Crisosto, *et al*., 2002; Smilanick *et al*., 1990) [11, 53]. As chitosan can form a semi-permeable film, a chitosan coating might be expected to modify the internal atmosphere, as well as to decrease transpiration losses and regulate the quality of the fruits (El Ghaouth, Arul and Ponnampalam, 1991; Olivas and Barbosa-Canovas, 2005) [28]. Meanwhile, chitosan has broad-spectrum antimicrobial activity, which has been well documented (Ait Barka, *et al.*, 2004; Plascencia-Jatomea *et al*., 2003; Reddy *et al*., 1998; Sathiyabama and Balasubramanian, 1998) [3, 32, 34, 46] and *in vivo* studies showed that chitosan treatment could control or delay postharvest decay of fruits and vegetables (Bautista-Ban˜os *et al*., 2006) [9].

Chitosan is a linear polysaccharide consisting of β-(1→4)-linked 2-amino-2- deoxy-D-glucose residues, originating from de-acetylated derivative of chitin, which is the second most abundant polysaccharide in nature after cellulose. It is non-toxic, biodegradable, bio-functional, and biocompatible. Chitosan has strong anti-microbial, anti-cracking, anti-browning, anti-stress, and anti-fungal activities that could effectively control fruit decay. It could easily form coating on fruit and vegetable, and the respiration rate of fruit and vegetable was reduced by adjusting the permeability of carbon dioxide and oxygen (Bautista-Ban˜os *et al.*, 2006) [9]. It is regarded as a promising material for an edible coating on fruit (Olivas and Barbosa-Canovas, 2005) [28].

However, the previous researchers mainly focused on the control effect by treatment with chitosan inoculation and on the physiological and pathological regulation of the fruit by chitosan coating. There are a few reports on the increase of postharvest disease resistance, by preharvest chitosan spray (Reddy *et al*., 2000; Romanazzi *et al*., 2006) [35, 37]. There are no reports about the effect of the combination of pre-harvest and postharvest treatments of chitosan on the Sensory quality and microbial count parameters of grapes during storage.

Keeping this in view, the present investigation entitled, "Effect of pre and post-harvest application of chitosan on Sensory quality and microbial count parameters of grape Cv. Manik Chaman during storage at 0 °C temperature", was carried out with the following objective.

To study the Effect of pre and post-harvest application of chitosan on Sensory quality and microbial count parameters of grape Cv. Manik Chaman during storage at 0 °C temperature.

Materials and Methods

The present investigation was undertaken in the Department of Post-Harvest Management of Fruit, Vegetable and Flower Crops, Post Graduate Institute of Post-Harvest Management, Killa-Roha. Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (M.S.) during the winter season of 2017. The material used and the methods adopted during the investigation are as given below.

The Department laboratory of Post-Harvest Management of Fruit, Vegetable and Flower Crops, Post Graduate Institute of Post-Harvest Management (PGI-PHM), Killa-Roha is located at 18°25'35.54'', North latitude and 73°10'45.01'', East longitude and at an elevation of 17.50 meters above MSL. The climate of Killa-Roha is warm and humid with the mean annual rainfall 2000-3000 mm, mostly received from 1st June to 15th October.

Experimental details

Table 1: Experimental details

Experimental Design	Factorial Completely Randomized Design (FCRD)
No. of Treatments	Six
No. of Replications	Four
No. of Treatments combination	6×4=24
No. of plants sprayed with 0.1% chitosan	2000
No. of grape clusters per treatment	Thirty six

Treatments details

Factor A

Different levels of chitosan concentration used for pre-harvest spraying and post-harvest dipping of grape

Table 2: Treatments details

Sr. No.	Treatments	Concentrations of chitosan used for	
		Pre-harvest spraying (%)	Post-harvest Dipping (%)
1.	T_1 (control)	NIL	NIL
2.	T_2	0.1%	NIL
3.	T_3	0.1%	0.5%
4.	T_4	0.1%	1.0%
5.	T_5	0.1%	1.5%
6.	T_6	0.1%	2.0%

Factor B: Storage period

S-1: 0 day

S-2: 15 days

S-3: 30 days

S-4: 45 days

Plant materials and treatments

Table grapes (*Vitis vinifera*) of the cultivar Manik chaman were harvested at the ripe stage from a commercial vineyard from Yadav grape farm, At- Palsawade, Post- Devapur, Tal- Man, Dist- Satara, (M.S.) with 2.5-4.5 cm stalk from grape orchard (Plot No.- 27) located at 17.57', North latitude and 74.86', East longitude and elevation of 473 meters above MSL. The grapes were harvested at minimum T.S.S of 16^0B and sugar acid ratio of 20:1.

Pre-harvest preparation and application of chitosan

For experimental purpose, 2000 vines were selected (0.80 Ha areas), the 0.1% chitosan solution was prepared by dissolving the purified Emulsifier chitosan which having brand name RESCUE-D (Omega Fine Chemicals, Dombivali (E). in 400 litres of de-mineralized water, with continuous stirring, When dissolved, the pH value of the chitosan solution was adjusted to 5.6 using pH balancer "Decorus" (Poorva Chem tech Pvt Ltd, Nashik.) to increase spray elements absorption. At 10 days before harvest, the chitosan solution was sprayed on grape clusters once by using a tractor mounted "Cima Low Volume Venturi Air Sprayer" until clusters were wet to runoff. The spraying of dissolved 0.1% chitosan solution was done at 4.30 pm. during evening time. After application of chitosan on clusters whole plant was allowed for full rest up to harvesting.

Maturity indices for harvesting

As grape is a non-climacteric fruit, it was harvested at minimum TSS of 16 ^{0}B and sugar acid ratio of 20:1.

Method of harvesting

Only attractive bunches fulfilling minimum quality requirement were harvested. A day prior to picking, the broken, along with decayed, deformed, undersized, and dis coloured berries were removed by cutting their pedicels from the selected bunch, using a long nosed scissors. One care was taken not to injure other sound berries by the scissor. The grape bunches were harvested during the early morning hours before the berry temperature rises above 25 °C.

Pre-cooling

The grapes were pre-cooled at 2-4 °C for 4 hours in visi cooler before post-harvest treatment of chitosan.

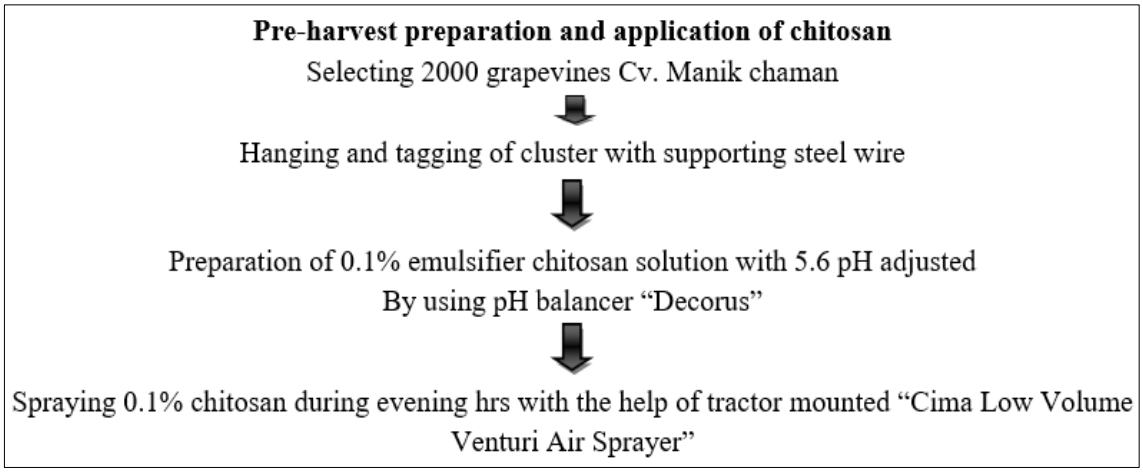
Post-harvest preparation and dipping of Chitosan

Clusters were selected for size and colour uniformity. Blemished, damaged, or diseased berries were discarded carefully. Immediately after harvest, the fruits were brought to the laboratory for preliminary tests. The grape berries were surface-sterilized with 2% sodium hypochlorite for 2 minutes at room temperature rinsed with tap water in order to remove the heavy dirt, pesticides and fungal spores covering the fresh harvested clusters and allowed to dry them at room temperature. After preparation, the fruits were weighed to about 400 g. and then randomly distributed into 6 groups before treatment.

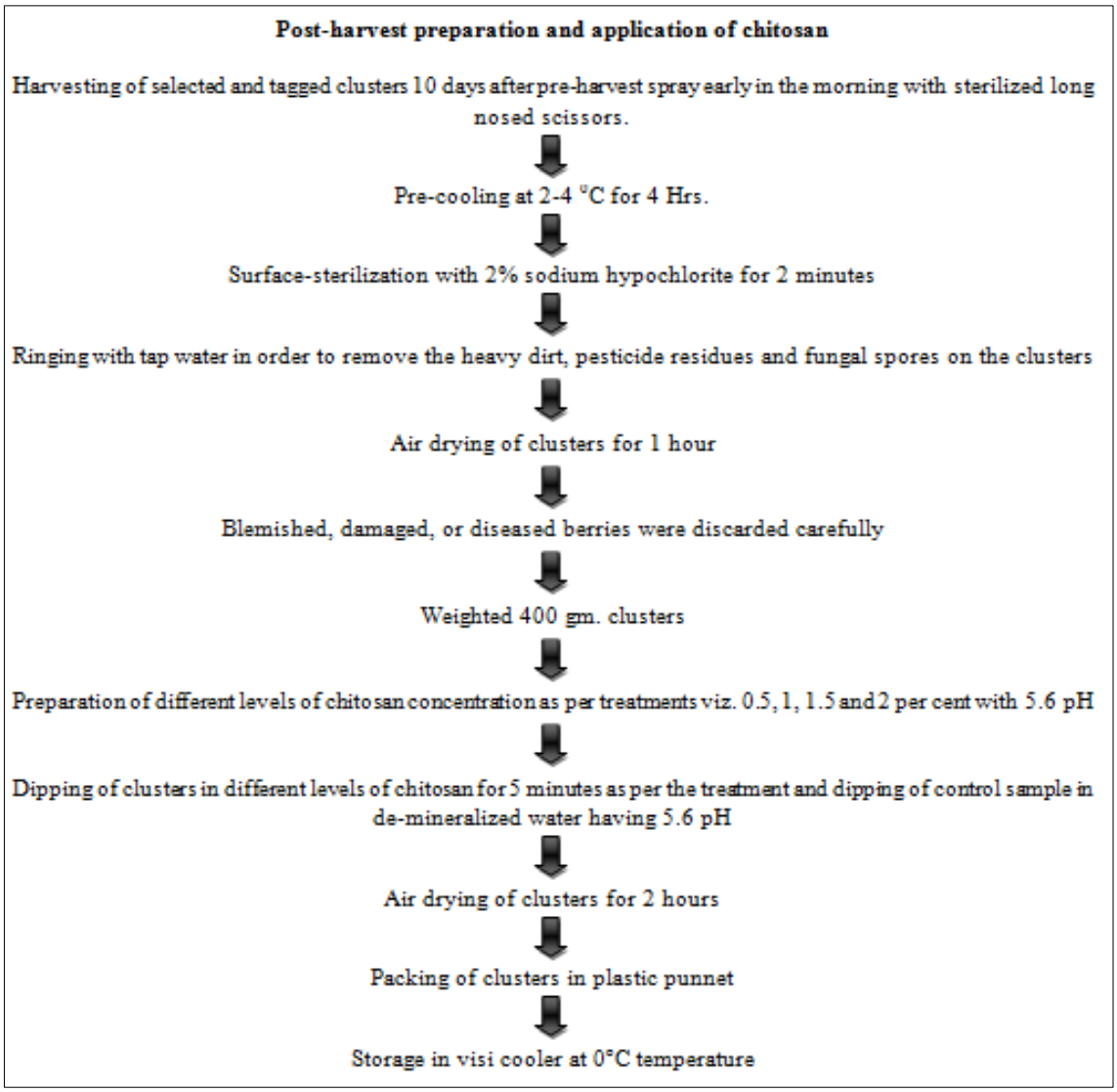
The emulsifier chitosan which having brand name RESCUE-D (Omega Fine Chemicals, Dombivali (E) was dissolved in de-mineralized water to prepare 0.5, 1, 1.5 and 2% chitosan solution respectively under continuous stirring. The grape bunches were dipped in the solutions for 5 min and then left for 2 hrs. at room temperature for drying. The control samples were dipped in the de-mineralized water with 5.6 pH.

Packaging and storage of treated clusters

The treated grapes were packed in plastic pun net and stored in the visi cooler (Manufactured by Fri go glass India Pvt. Ltd., Marketed by Blue star Ltd.) at a temperature of 0 °C and 85-95% relative humidity for 45 days. The qualitative traits were evaluated at 0, 15, 30, and days of storage.



Flow Sheet 1: Pre-harvest preparation and application of chitosan



Flow Sheet 2: Post-harvest preparation and application of chitosan

Effect of pre and post-harvest application of chitosan on sensory score of grape Cv. Manik Chaman during storage at 0 °C temperature

- **Colour, flavour, texture and overall acceptability**

The sensory qualities with respect to the colour, flavour, texture and overall acceptability were evaluated by a panel of judges using 9 points hedonic scale (Amerine *et al.*, 1965) [1] as given below. The overall rating was obtained by averaging score of evaluation. The fruits with sensory score of 5.5 and above were rated as acceptable.

Table 3: Sensory score for evaluation of product under 9 point hedonic scale

Sensory score	Rating
1	Like extremely
2	Like very much
3	Like moderately
4	Like slightly
5	Neither liked nor disliked
6	Dislike slightly
7	Dislike moderately
8	Dislike very much
9	Dislike extremely

- **Rachis appearance**

The rachis appearance quality of the grapes was assessed on inoculated bunches at an interval of 45 days to end of storage based on the clusters Visual index. (Karabulut *et al.*, 2004; Luo, 2007 and Papachatzis *et al.*, 2013) [21, 23, 30].

Table 4: Rachis appearance

Visual index score	Visual index rating
1	Fresh and green
2	Green
3	Semidry
4	50% dry
5	Completely dry

- **Berry appearance**

The quality related to the berry appearance of the grapes was assessed on inoculated bunches at an interval of 45 days to end of storage based on the clusters Visual index. (Karabulut *et al.*, 2004; Luo, 2007 and Papachatzis *et al.*, 2013) [21, 23, 30].

Table 5: Berry appearance

Visual index score	Visual index rating
1	Excellent
2	Good
3	Slightly dull
4	<50% brownish and soft berries
5	>50% brownish and soft berries

- **Fruit shriveling**

Fruit shrivelling was evaluated based on a 4-score scale rating. (Karabulut *et al.*, 2004; Luo, 2007; and Hosseini-Farahi *et al.*, 2015) [21, 23, 19].

Table 6: Fruit shriveling

Scale score	Rating
1	Very shrivelling
2	Low shrivelling
3	Normal
4	Very smooth

- **Decay**

During storage, the natural decay incidence was evaluated in terms of a decay index. The disease severity of each grape berry in each bunch was assessed according to the following empirical scale. (Karabulut *et al.*, 2004); (Romanazzi *et al.*, 2006; Luo, 2007; and Meng and Tian, 2009) [21, 37, 23, 26].

Table 7: Decay

Empirical scale score	Empirical scale rating
0	Healthy berry
1	One lesion less than 2 mm in diameter
2	One lesion less than 5 mm in diameter
3	Several lesions or less than 25% of berry surface infected
4	More than 25% of berry surface infected, sporulation present

Effect of pre and post-harvest application of chitosan on Microbial count of grape Cv. Manik Chaman during storage at 0 °C temperature

The microbial analysis of the grapes with pre-harvest and post-harvest chitosan treatment was carried out at 0, 15, 30 and 45 days of storage as per the method described by Kiiyukia (2003) [22].

- **Bacteria**

Nutrient Agar media was prepared by weighing required quantity of nutrient agar and diluted with double distilled water to a known volume. The media was then autoclaved at 121 °C for 20 min. When the temperature of media reached to 40 °C, it was used for plating.

The plating was carried out with 0.1 ml sample in sterile petri plates under the Laminar Air Flow. The sample of each treatment was taken on a separate petri plate, followed by pouring of approximately 20 ml of media (35-40 °C) on the sample and mixing was done by tilting plate properly. Plates were sealed with para film and incubated at 37 °C for 48 hrs to check bacterial count. The total microbial plate count was measured in colony forming unit/gram (cfu/g).

- **Fungi**

Potato dextrose agar media was prepared for the colony count of fungi. Plates were sealed with para film and incubated at 37 °C for 5-6 days for fungal count. The total microbial plate count was measured in colony forming unit/g initially and at the end of storage of period of 90 days.

- **Statistical analysis**

The data collected on the changes in sensory qualities and microbial count analysis of grapes berries were statistically analyzed by the standard procedure given by Panase and Sukhatme (1985) [29] and Amdekar (2014) [6] using Factorial Completely Randomized Design and valid conclusions were drawn only on significant differences between treatment mean at 5% level of significance.

Result and Discussion

Effect of pre and post-harvest application of chitosan on sensory quality of grape Cv. Manik Chaman during storage at 0 °C temperature

- **Colour**

The data on effect of pre and post-harvest application of chitosan on the changes in sensory score for colour of grape Cv. Manik Chaman are presented in Table-8 and graphically depicted in Figure-1.

The data indicate that the maximum (7.95) mean sensory score for colour of grape berry was observed in the treatment T_4, which was significantly superior to rest of the treatments. It was followed by the treatments T_5 and T_6 in that orders. However, minimum (5.66) mean score for colour of grape was recorded in the treatment T_1 followed by the treatment T_2 and T_3.

Thus, it is clear from the data that the sensory score for colour of grape berry increased with increase in the level of post-harvest chitosan concentration that imparted attractive fresh green colour to the berry and rachis.

Table 8: Effect of pre and post-harvest application of chitosan on sensory score for colour of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Sensory score for colour				Mean
	Storage period (Days)				
	0	15	30	45	
T1	7.65	6.5	5.00	3.50	5.66
T2	7.87	7.25	6.00	4.75	6.47
T3	7.95	7.50	6.5	5.50	6.86
T4	8.38	8.03	7.80	7.60	7.95
T5	7.95	7.77	7.30	7.05	7.52
T6	7.92	7.64	6.88	6.09	7.13
Mean	7.95	7.45	6.58	5.75	
		S.Em ±		CD at 5%	
Treatments (T)		0.12		0.33	
Storage (S)		0.10		0.30	
Interaction (T×S)		0.20		0.56	

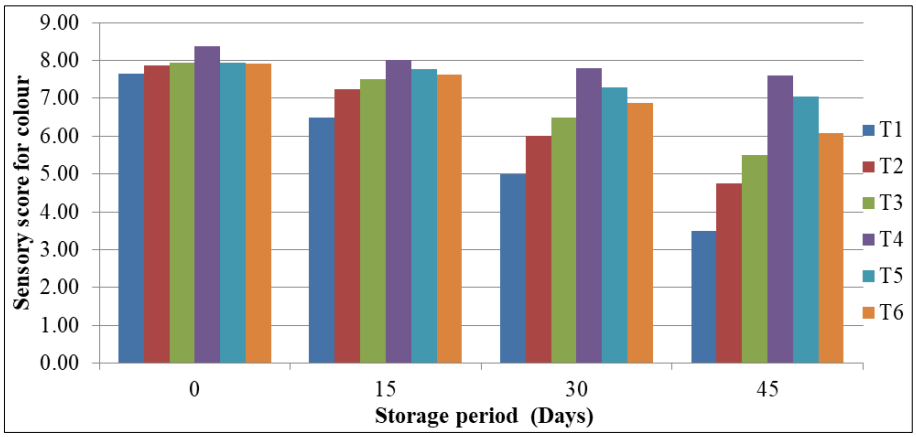


Fig. 1: Effect of pre and post-harvest application of chitosan on sensory score for colour of grape Cv. Manik Chaman during storage at 0 °C temperature

The mean sensory score for colour varied significantly during storage period of 45 day. It was highest mean (7.95) at the time of initial stage i.e. 0 day and lowest mean (5.75) at 45th day of storage. It is evident from the data that the likeness for colour of grape decreased during storage period of 45 days. It might be due to the change in fresh green colour to pale green and brown during storage. Chitosan coating slowed down the respiration rate, reduced the changes in colour of skin and flesh and increased the shelf life of fruits (Maftoonazad and Ramaswamy 2005) [24].

The similar decreasing trend in sensory score for colour was observed by Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14] in grape. The result in similar were also observed by Dang *et al.*, (2010) [12] in sweet cherries. Das *et al.*, (2013) [13] in tomatoes. Salunkhe (2015) [45] and Venkateswerlu *et al.*, (2017) [54], in banana. Patil (2016) [31] in pomegranate, Sethi (1987) [47], Abbasi *et al.*, (2009) [2], Shinde (2014) [51], Purohit (2015) [33] and Mansute (2016) [25] in mango fruit.

Interaction effect between treatments and storage period was found to be statistically significant for mean sensory colour for score of the grape berry and rachis at 5% level of significance. As per result, the highest (8.38) sensory score for colour was recorded in treatment T_4 but at par with the treatment T_3, T_4 and T_6 at initial day and the lowest (3.50) was observed in treatment T_1 at end of 45 days of storage.

- **Flavour**

The data pertaining to the changes in sensory score for flavour of grape Cv. Manik Chaman influenced by pre and post-harvest chitosan application are presented in Table-9 and graphically depicted in Figure-2.

Maximum (7.93) mean score for flavour of grape berry Cv. Manik Chaman was observed in the treatment T_4 and was significantly superior to rest of the treatments. It was followed by the treatments T_5 and T_6. The minimum (5.78) mean score for flavour of grape berry Cv. Manik Chaman was obtained by the treatment T_1 at the end of storage. Thus, it is clear from the data that the sensory score for flavour of grape berry Cv. Manik Chaman increased with increase in the level of chitosan for post-harvest coating. This may be due to chitosan coating slowed down the respiration rate, reduced the colour changes of skin and flesh and increased the shelf life of fruits (Maftoonazad and Ramaswamy 2005) [24].

Table 9: Effect of pre and post-harvest application of chitosan on sensory score for flavour of grape Cv. Manik Chaman during storage at 0°C temperature

Treatments	Sensory score for flavour				Mean
	Storage period (Days)				
	0	15	30	45	
T1	7.50	6.7	5.2	3.70	5.78
T2	7.7	7.25	6.00	4.75	6.43
T3	7.90	7.40	6.3	5.20	6.70
T4	8.20	8.00	7.80	7.70	7.93
T5	7.9	7.70	7.50	7.30	7.60
T6	7.8	7.30	7.00	7.50	7.15
Mean	7.83	7.39	6.63	5.86	
		S.Em ±		CD at 5%	
Treatments (T)		0.10		0.29	
Storage (S)		0.09		0.26	
Interaction (T×S)		0.18		0.50	

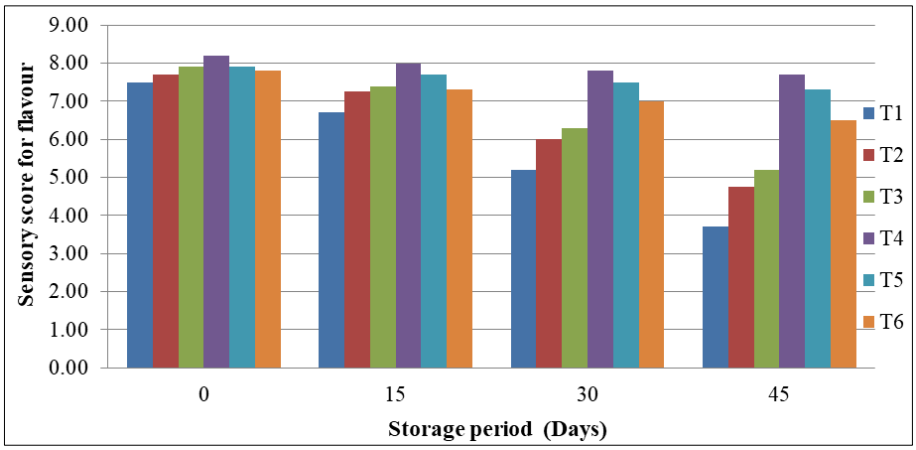


Fig 2: Effect of pre and post-harvest application of chitosan on sensory score for flavour of grape Cv. Manik Chaman during storage at 0 °C temperature

The mean sensory score for flavour varied significantly during storage period of 45 days. It was highest (7.83) at the time of initial stage and lowest (5.86) at 45th day of storage. It was revealed from the data that the likeness for flavour of grape Cv. Manik Chaman decreased during storage period of 45 days. It might be due to the loss of fresh flavour during storage.

Similar decreasing trend in sensory score for flavour with decrease in the levels of chitosan concentration was observed in grapes by Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14].

Identical results related to the present investigation were reported by Dang *et al.*, (2010) [12] in sweet cherries. Das *et al.*, (2013) [13] in tomatoes. Salunkhe (2015) [45], Venkateswerlu *et al.*, (2017) [54], in banana. Patil (2016) [31] in pomegranate. Sethi (1987) [47], Abbasi *et al.*, (2009) [2], Shinde (2014) [51], Purohit (2015) [33] and Mansute (2016) [25] in mango.

Interaction effect between different levels of pre and post-harvest application of chitosan to grape Cv. Manik Chaman and storage period was found to be statistically significant for mean sensory score for flavour of the grape berry and rachis at 5% level of significance. As per result, the highest (8.20) sensory score for flavour was recorded in the treatment T_4 but at par with the treatments T_6, T_5 and T_3, at initial day and the lowest (3.70) was observed in the treatment T_1 at end of 45 days of storage.

- **Texture**

The data on the effect of pre and post-harvest application of chitosan on changes in sensory score for texture of grape berry Cv. Manik Chaman are presented in Table-10 and graphically depicted in Figure-3.

Maximum (7.88) mean sensory score for texture of grape berry was observed in the treatment T_4, which was significantly superior to rest of the treatments, followed by the treatments T_5 and T_6, while minimum (5.78) mean sensory score for flavour of grape berry was recorded by the treatment T_1, followed by the treatment T_2 and T_3. Thus, it is clear from the data that the sensory score for texture of grape berry increased with increase in the level of chitosan concentration for post-harvest coating of grape berries. Chitosan imparted superior texture to the berry and rachis as it slowed down the respiration rate and reduced the changes in colour of skin and flesh fruits (Maftoonazad and Ramaswamy 2005) [24].

The mean sensory score for texture of grape berry varied significantly during storage period of 45 days. It was highest (7.96) at the time of initial stage i.e. 0 day and lowest (5.83) at 45th day of storage. It is revealed from the data that the likeness for texture of grape berry decreased during storage period of 45 days. It might be due to the loss of moisture during storage.

The similar results were observed by Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14] in grape.

Identical observations were was also reported by Dang *et al.*, (2010) [12] in sweet cherries. Das *et al.*, (2013) [13] in tomatoes. Salunkhe (2015) [45] and Venkateswerlu *et al.*, (2017) [54] in banana. Patil (2016) [31] in pomegranate, Sethi (1987) [47], Abbasi *et al.*, (2009) [2], Shinde (2014) [51], Purohit (2015) [33] and Mansute (2016) [25] in mango

Table 10: Effect of pre and post-harvest application of chitosan on sensory score for texture of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Sensory score for texture				Mean
	Storage period (Days)				
	0	15	30	45	
T1	7.80	6.60	5.10	3.60	5.78
T2	7.90	7.28	6.06	4.84	6.52
T3	7.90	7.48	6.36	5.24	6.75
T4	8.20	8.00	7.80	7.50	7.88
T5	8.00	7.70	7.50	7.30	7.63
T6	7.95	7.50	7.00	6.50	7.24
Mean	7.96	7.43	6.64	5.83	
		S.Em ±		CD at 5%	
Treatments (T)		0.08		0.24	
Storage (S)		0.07		0.21	
Interaction (T×S)		0.14		0.40	

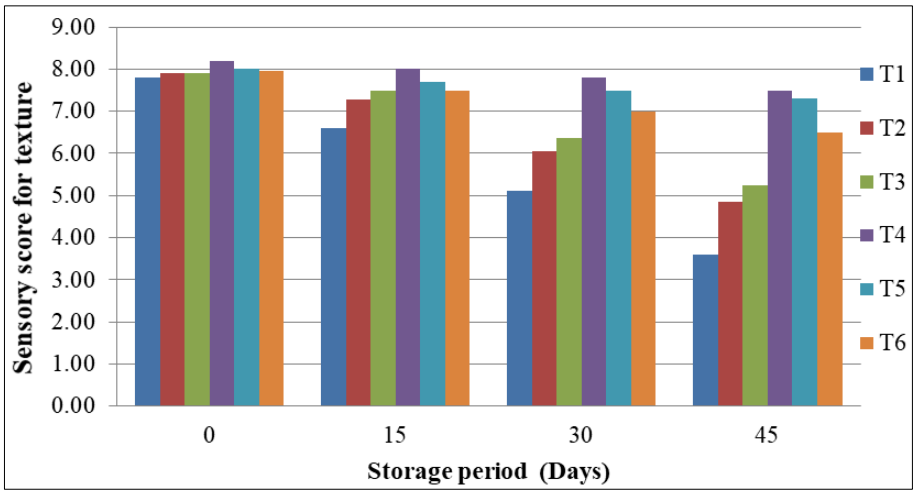


Fig 3: Effect of pre and post-harvest application of chitosan on sensory score for texture of grape Cv. Manik Chaman during storage at 0 °C temperature

Interaction effect between treatment and storage period was found to be statistically significant for mean sensory texture of the grape berry and rachis at 5% level of significance. As per result, the highest (8.20) sensory score for texture was recorded in treatment T_4 but at par with treatments T_5, T_6, T_2 and T_3 at initial day and the lowest (3.60) was observed in the treatment T_1 at end of 45 days of storage.

- **Overall acceptability**

The data pertaining to the changes in sensory score for overall acceptability of grape berry Cv. Manik Chaman influenced by pre and post-harvest application of chitosan treatment are presented in Table-11 and graphically depicted in Fig.-4.

Maximum (7.92) mean sensory score for overall acceptability of grape berry was observed in the treatment T_4, which was significantly superior to rest of the T_5 which was followed by the treatment T_6 and T_5. Minimum (5.74) mean score for overall acceptability grape berry was recorded by the treatment T_1 at the end of storage, followed by the treatments T_2 and T_3.

Thus, it is clear from the data that the sensory score for overall acceptability of grape berry Cv. Manik Chaman increased with increase in the level of chitosan concentration for treatment which imparted attractive fresh green colour to the berry and rachis.

However, the fruit treated with 2% chitosan did not ripen fully during cold storage. The thick film layer of 2.0% chitosan coating over grape berries, attracted the respiration processes affecting the organoleptic quality of the grape berries. Therefore, the fruits obtained lower sensory score with for colour, flavour and texture of the grape berries, thereby resulting poor sensory score for overall acceptability as compared to 1.0 or 1.5% post-harvest chitosan treatment. The identical results were also reported by Ali *et al.*, 2010; 2011.

The mean sensory score for overall acceptability varied significantly during storage period of 45 days. It was highest (7.92) at the time of initial stage i.e. 0 day and lowest (5.81) at 45th day of storage. It is revealed from the data that the likeness for overall acceptability of grape decreased during storage period of 45 days. It might be due to the loss of fresh green colour to pale green, brown, texture, flavour, reach is and berry appearance, influenced by physiological processes respiration and transpiration during storage.

The similar decreasing trend in overall acceptability with decrease in the levels of chitosan concentration post-harvest dipping treatments for grape observed by Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14].

Table 11: Effect of pre and post-harvest application of chitosan on sensory score for overall acceptability of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Sensory score for overall acceptability				Mean
	Storage period (Days)				
	0	15	30	45	
T1	7.65	6.60	5.10	3.60	5.74
T2	7.82	7.26	6.02	4.78	6.47
T3	7.92	7.46	6.38	5.31	6.77
T4	8.26	8.01	7.80	7.60	7.92
T5	7.95	7.72	7.43	7.22	7.58
T6	7.89	7.48	6.96	6.36	7.17
Mean	7.92	7.42	6.62	5.81	
		S.Em ±		CD at 5%	
Treatments (T)		0.07		0.19	
Storage (S)		0.06		0.17	
Interaction (T×S)		0.12		0.33	

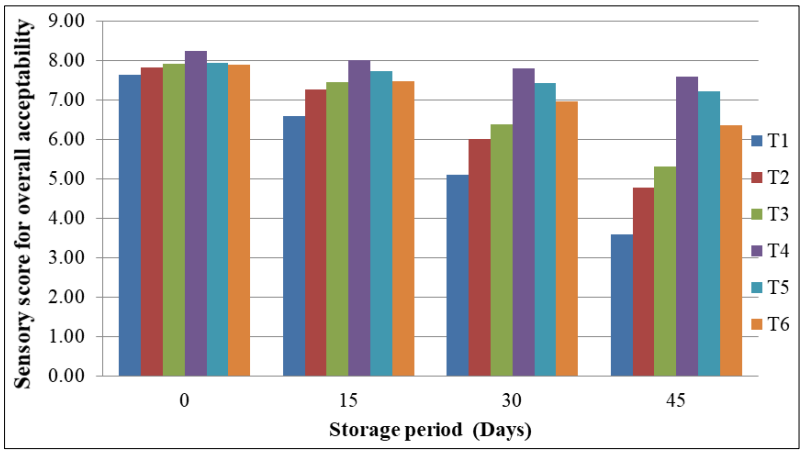


Fig.4: Effect of pre and post-harvest application of chitosan on sensory score for overall acceptability of grape Cv. Manik Chaman during storage at 0 °C temperature

The results related to the present investigation were also reported by Dang *et al.*, (2010) [12] in sweet cherries. Das *et al.*, (2013) [13] in tomatoes. Salunkhe (2015) [45] and Venkateswerlu *et al.*, (2017) [54] in banana. Patil (2016) [31] in pomegranate. Sethi (1987) [47], Abbasi *et al.*, (2009) [2], Shinde (2014) [51], Purohit (2015) [33] and Mansute (2016) [25] in mango.

Interaction effect between treatments and storage period was found to be statistically significant for mean overall acceptability of the at 5% level of significance. As per result, the highest (8.26) sensory score for overall acceptability was recorded in the treatment T_4 at initial day and the lowest (3.60) was observed in the treatment T_1 at the end of 45 days of storage.

- **Rachis appearance**

The rachis appearance quality of the grapes was assessed on inoculated bunches at an interval of 15 days to the end of storage based on clusters Visual index (Karabulut *et al.*, 2004; Luo 2007 and Papachatzis *et al.*, 2013) [21, 23, 30].

Table 12: Effect of pre and post-harvest application of chitosan on sensory visual index rating for rachis appearance of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Sensory visual index rating for rachis appearance				Mean
	Storage period (Days)				
	0	15	30	45	
T1	2.00	3.00	5.00	5.00	3.75
T2	1.00	2.00	4.00	5.00	3.00
T3	1.00	1.50	2.50	4.00	2.25
T4	1.00	1.00	1.00	1.20	1.05
T5	1.00	1.00	1.50	1.80	1.33
T6	1.00	1.20	2.00	2.40	1.65
Mean	1.17	1.62	2.67	3.23	
		S.Em ±		CD at 5%	
Treatments (T)		0.08		0.24	
Storage (S)		0.07		0.21	
Interaction (T×S)		0.14		0.40	

*The lowest score indicate the highest cluster visual index rating

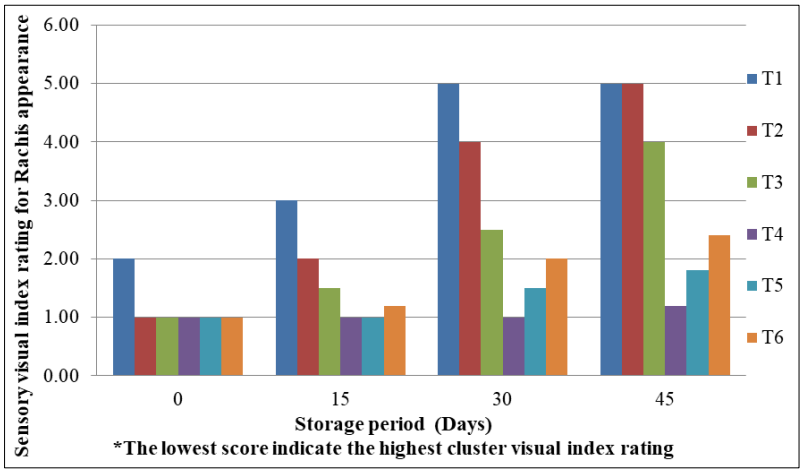


Fig.5: Effect of pre and post-harvest application of chitosan on sensory visual index rating for rachis appearance of grape Cv. Manik Chaman during storage at 0 °C temperature

The data on the effect of pre and post-harvest application of chitosan on changes in sensory visual index rating for rachis appearance of grape Cv. Manik Chaman are presented in Table-12 and graphically depicted in Figure-5.

Maximum (1.05) mean visual index rating for rachis appearance of grape Cv. Manik Chaman was observed in the treatment T_4, significantly superior to rest of the treatments, which was followed by treatment T_5 and T_6. However Minimum (3.75) mean visual index rating for rachis appearance of grape Cv. Manik Chaman was obtained in the treatment T_1 followed by the treatment T_2 and T_4. Thus, it is clear from the data that the sensory rating for rachis appearance of grape increased with increase in the level of and post-harvest application of chitosan that checked the drying of rachis of the grape clusters.

Chitosan coating delayed the rachis dehydration and browning, which is associated with decayed berries. These symptoms first appeared on pedicels, followed by lateral branches and finally on the central axis, due to increased polyphenol oxidase activity (Carvajal-Millan *et al.*, 2001). It is worth noting that the stem is a physiologically active part with greater respiration intensity than the berry and it is a key issue in grape storage (Crisosto *et al.*, 2002) [11].

The mean sensory visual index rating for rachis appearance varied significantly during storage period of 45 days. As per the result, it was highest (1.17) mean at the time of initial stage i.e. 0 day and lowest (3.23) mean found at 45th days of storage. It is revealed from the data that the likeness for rachis appearance of grape decreased during storage period of 45 days. It might be due to decreased freshness and green appearance during storage.

The similar decreasing trend in the rating of rachis appearance in grape was observed by Karabulut *et al.*, (2004) [21], Luo (2007) [23], Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14].

Interaction effect between the treatments and storage period was found to be statistically significant for mean sensory visual index rating for rachis appearance of the grape rachis at 5% level of significance. As per the result, the highest (1.00) sensory visual index rating for rachis appearance was recorded in treatment T_2, T_3, T_4, T_4 and T_6 at initial day and the lowest (5.00) was observed in the treatment T_1 and T_2 at end of 30 and 45 days of storage at 0 °C temperature.

Berry appearance

The berry appearance quality of the grapes was assessed at an interval of 15 days to end of storage using Visual index (Karabulut *et al.*, 2004; Luo, 2007 and Papachatzis *et al.*, 2013) [21, 23, 30].

The data pertaining to the changes in sensory visual index rating for berry appearance of grape Cv. Manik Chaman influenced by pre and post-harvest chitosan treatment are presented in Table-13 and graphically depicted in Figure-6.

Maximum (1.00) mean visual index rating for berry appearance of grape berry Cv. Manik Chaman was observed in the treatment T_4 but at par with the treatment T_5 and significantly superior to rest of the treatments. However, minimum (3.50) mean visual index rating for rachis appearance of grape berry Cv. Manik Chaman was obtained by the treatment T_1 at the end of storage, followed by the treatment T_2, T_3 and T_6.

Thus, it is clear from the data that the sensory score for berry appearance of grape increased with increase in the level of chitosan concentration for post-harvest coating as chitosan prevented moisture loss and influenced respiratory exchange. In general, this positive effect of edible coatings is based on their hygroscopic properties, which enables formation of a water barrier between the fruit and the environment, thus reducing external transfer and appearance (Morillon *et al.*, 2002) [27].

The mean sensory score for berry appearance varied significantly during storage period of 45 days. As per the result the berries appearance rating was highest (1.17) at initial stage i.e. 0 day and lowest (2.87) mean found at 45th day of storage. It is revealed from the data that the likeness for berry appearance of grape decreased during storage period of 45 days. It might be due to the loss of freshness and green appearance of berries during storage.

Table 13: Effect of pre and post-harvest application of chitosan on sensory visual index rating for Berry appearance of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Sensory visual index rating for Berry appearance				Mean
	Storage period (Days)				
	0	15	30	45	
T1	2.00	3.00	4.00	5.00	3.50
T2	1.00	2.00	3.00	4.00	2.50
T3	1.00	1.50	2.00	3.00	1.88
T4	1.00	1.00	1.00	1.00	1.00
T5	1.00	1.00	1.20	1.50	1.18
T6	1.00	1.20	1.50	2.50	1.55
Mean	1.17	1.62	2.13	2.87	
		S.Em ±		CD at 5%	
Treatments (T)		0.08		0.24	
Storage (S)		0.07		0.21	
Interaction (T×S)		0.14		0.40	

*The lowest score indicate the highest cluster visual index rating

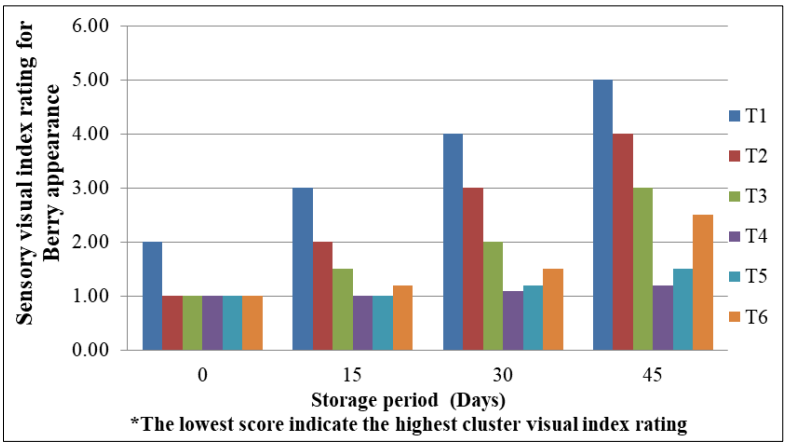


Fig.6: Effect of pre and post-harvest application of chitosan on sensory visual index rating for Berry appearance of grape Cv. Manik Chaman during storage at 0 °C temperature

The similar decreasing trend in sensory ratings of berry appearance was noticed by Karabulut *et al.*, (2004) [21], Luo (2007) [23], Xu *et al.*, (2007) [23], Papachatzis *et al.*, (2013) [30] and Elwahab *et al.*, (2014) [14].

Interaction effect between the treatments and storage period was found to be statistically significant for mean sensory rating for berry appearance of the grape at 5% level of significance. As per result, the highest (1.00) sensory visual index rating for berry appearance was recorded in the treatment T_2, T_3, T_4, T_5 and T_6 at initial and the lowest (5.00) was observed in the treatment T_1 at end of 45 days of storage.

Fruit shriveling

Fruit shriveling was evaluated based on a 4-score scale rating (Karabulut *et al.*, 2004; Luo, 2007 and Hosseini-Farahi *et al.*, 2015) [21, 23, 19].

The data on effect of pre and post-harvest application of chitosan on changes in the sensory rating for fruit shriveling of grape berry Cv. Manik Chaman are presented in Table-14 and graphically depicted in Figure-7.

Table 14: Effect of pre and post-harvest application of chitosan on sensory scale rating for fruit shrivelling of grape Cv. Manik Chaman during storage at 0°C temperature

Treatments	Sensory scale rating for fruit shrivelling				Mean
	Storage period (Days)				
	0	15	30	45	
T1	3.50	3.00	2.00	1.00	2.38
T2	4.00	3.00	2.40	2.00	2.85
T3	4.00	3.50	2.50	2.40	3.10
T4	4.00	4.00	3.80	3.60	3.85
T5	4.00	3.80	3.40	3.20	3.60
T6	4.00	3.60	3.20	2.80	3.40
Mean	3.92	3.48	2.88	2.50	
		S.Em ±		CD at 5%	
Treatments (T)		0.08		0.24	
Storage (S)		0.07		0.21	
Interaction (T×S)		0.14		0.40	
*The highest score indicate the highest scale rating					

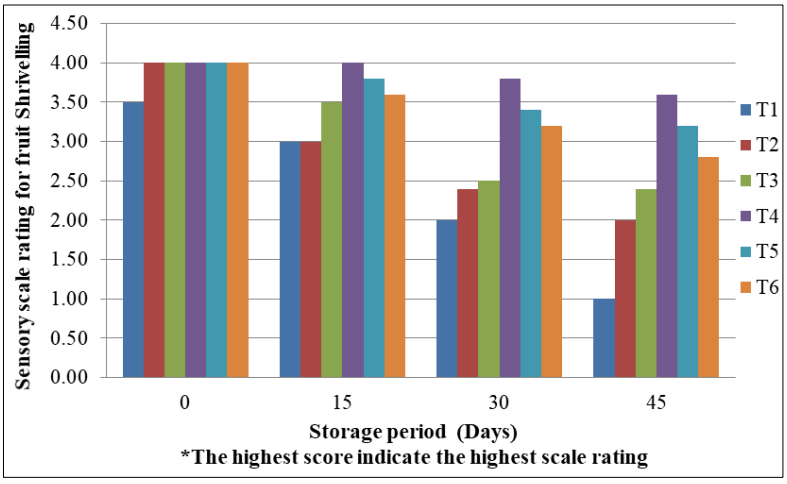


Fig 7: Effect of pre and post-harvest application of chitosan on sensory scale rating for fruit shrivelling of grape Cv. Manik Chaman during storage at 0 °C temperature

Maximum (3.85) mean sensory scale rating for fruit shriveling of grape Cv. Manik Chaman was observed in the treatment T_4, which were significantly superior to rest of the treatments. It was followed by the treatments T_5 and T_6. However, minimum (2.38) mean visual index rating for fruit shriveling of grape berry Cv. Manik Chaman was obtained by the treatment T_1 at the end of storage, followed by the treatments T_2 and T_3.

Thus, it is clear from the data that the sensory rating for fruit shriveling of grape berry decreased with increase in the level of chitosan concentration for post-harvest coating of grape. As the chitosan coating acts as a gas barrier, it down the loss of the respiration processes and moisture loss and allows retention of the firmness of fruits during storage as also reported Yaman and Bayoundurh (2002) [56].

The mean sensory scale rating for fruit shriveling varied significantly during storage period of 45 days. It was highest (3.92) at initial stage i.e. 0 day and highest (2.50) at 45th day of storage. It is revealed from the data that the rating for fruit shriveling of grape berry decreased during storage period of 45 days. It might be due to loss of moisture affecting the firmness of the berries during storage.

Identical decreasing in trend sensory rating for fruit shriveling in grape was observed by Karabulut *et al.,* (2004) [21], Luo (2007) [23], Xu *et al.,* (2007) [23], Papachatzis *et al*., (2013) [30] and Elwahab *et al.,* (2014) [14].

Interaction effect between treatments and storage period was found to be statistically significant for mean sensory scale rating for fruit shriveling of the grape berry at 5% level of significance. As per result, the highest (4.00) sensory scale rating for fruit shriveling was recorded in treatment T_2, T_3, T_4, T_5 and T_6 at initially and the lowest (1.00) was observed in the treatment T_1 at end of 45 days of storage.

Fruit decay

During storage, natural decay incidence was evaluated in terms of a decay index. The disease severity of each grape berry in every bunch was assessed by using the empirical scale (Karabulut *et al.,* 2004; Romanazzi *et al.,* 2006; Luo, 2007 and Meng and Tian, 2009) [21, 37, 23, 26].

The data on effect of pre and post-harvest application of chitosan on changes in sensory empirical scale for fruit decay index of grape berry Cv. Manik Chaman are presented in Table-15 and graphically depicted in Figure-8.

Maximum (0.13) mean empirical scale score for fruit decay index of grape was observed in the treatment T_4 which was at par with the treatment T_5 and significantly superior to rest of the treatments, it was followed by the treatment followed by the treatment T_6. The minimum (2.63) mean empirical scale score for decay was found in the Treatment T_1 followed by the treatment T_2 and T_3 at 45 days storage at 0 °C temperature.

Thus, it is evident from the data that the sensory empirical scale score for fruit decay of grape berry increased with increase in the level of chitosan concentration for post-harvest coating of grape. The chitosan biopolymer has a dual mechanism of action: it inhibits the growth of decay causing fungi (Allan and Hadwiger, 1979) [5] and induces defence response in host tissues (Shibuya and Minami, 2001) [49]. This response elicits phytoalexin formation (Hadwiger and Beckman, 1980) [17] and induces the production of antifungal hydrolases (Zhang and Quantick, 1998) [58].

As per result the treatment T_2 (0.1% Pre-harvest spray of chitosan) also recorded lower (1.88) empirical scale for decay as compared to control i.e. T_1 (2.63) during 45 days storage at 0 °C temperature. Chitosan, a mostly deacetylated β-(1-4) - linked D-glucosamine polymer, is a structural component of fungal cell walls. Chitosan has been reported to enhance resistance against many fungal diseases when applied as either a pre or postharvest treatment (Reglinski *et al.,* 2005) [36].

Table 15: Effect of pre and post-harvest application of chitosan on sensory empirical scale rating for fruit Decay of grape Cv. Manik Chaman during storage at 0°C temperature

Treatments	Sensory empirical scale rating for fruit Decay				Mean
	Storage period (Days)				
	0	15	30	45	
T1	0.00	2.00	3.50	5.00	2.63
T2	0.00	1.00	2.50	4.00	1.88
T3	0.00	0.60	1.50	3.00	1.28
T4	0.00	0.00	0.00	0.50	0.13
T5	0.00	0.00	0.00	1.00	0.25
T6	0.00	0.00	0.75	1.31	0.52
Mean	0.00	0.60	1.38	2.47	
		S.Em ±		CD at 5%	
Treatments (T)		0.07		0.21	
Storage (S)		0.06		0.18	
Interaction (T×S)		0.12		0.35	

*The lowest score indicate the highest empirical scale rating

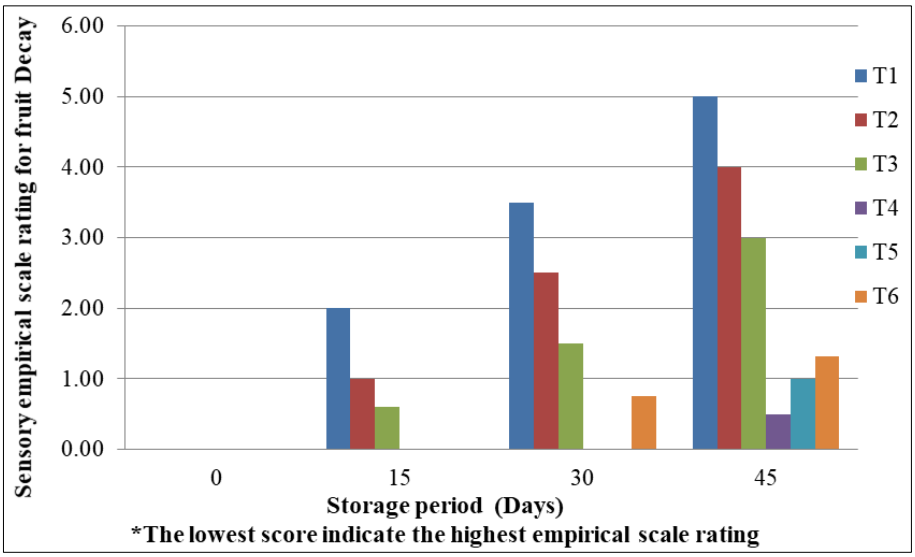


Fig. 8: Effect of pre and post-harvest application of chitosan on sensory empirical scale rating for fruit Decay of grape Cv. Manik Chaman during storage at 0 °C temperature

The mean sensory empirical scale score for fruit decay index varied significantly during storage period of 45 days. It was maximum (0.00) mean at initial stage i.e. 0 day and lowest (2.47) at 45th day of storage. It is revealed from the data that the sensory scale for fruit decay of grape berry decreased during storage period of 45 days. It might be due to the more number of infected berries during storage.

Similar result related to the present investigation in grape was observed by Romanazzi *et al.*, (2002) [38], Karabulut *et al.*, (2004) [21], Romanazzi *et al.*, (2005) [43], Romanazzi *et al.*, (2006) [37], Luo (2007) [23], Xu *et al.*, (2007) [23], Meng and Tian (2009) [26], Romanazzi (2010), Papachatzis *et al.*, (2013) [30]. Youwei and Yinzhe (2013) and Elwahab *et al.*, (2014) [14].

Interaction effect between treatments and storage period was found to be statistically significant for mean sensory empirical scale fruit decay index of the grape berry at 5% level of significance. As per the result, the highest (0.00) sensory empirical scale for fruit decay index was recorded all treatments at initial day and at 15th and 30th day of storage by the treatment T_4 and T_6 at 15th and the lowest (5.00) was observed in the Treatment T_1 at end of 45 days of storage.

Effect of pre and post-harvest application of chitosan on microbial activity of grape Cv. Manik Chaman

1. Microbial count for fungi (cfu/1g)

The data on the effect of pre and post-harvest application of chitosan on changes in microbial count for fungi (cfu/1g) of grape berry Cv. Manik Chaman are presented in Table-16 and graphically depicted in Figure-9.

Table 16: Effect of pre and post-harvest application of chitosan on Microbial count for fungi (cfu/g) of grape Cv. Manik Chaman during storage at 0 °C temperature

Treatments	Microbial count for fungi (cfu/g) Storage period (Days)				Mean
	0	15	30	45	
T1	0.00	2.12	6.64	10.36	4.68
T2	0.00	1.03	3.58	6.13	2.69
T3	0.00	0.00	2.44	3.38	1.46
T4	0.00	0.00	0.00	1.42	0.36
T5	0.00	0.00	0.00	1.14	0.29
T6	0.00	0.00	0.00	1.02	0.26
Mean	0.00	0.53	2.04	3.91	
		S.Em ±		CD at 5%	
Treatments (T)		0.027		0.078	
Storage (S)		0.024		0.070	
Interaction (T×S)		0.047		0.134	

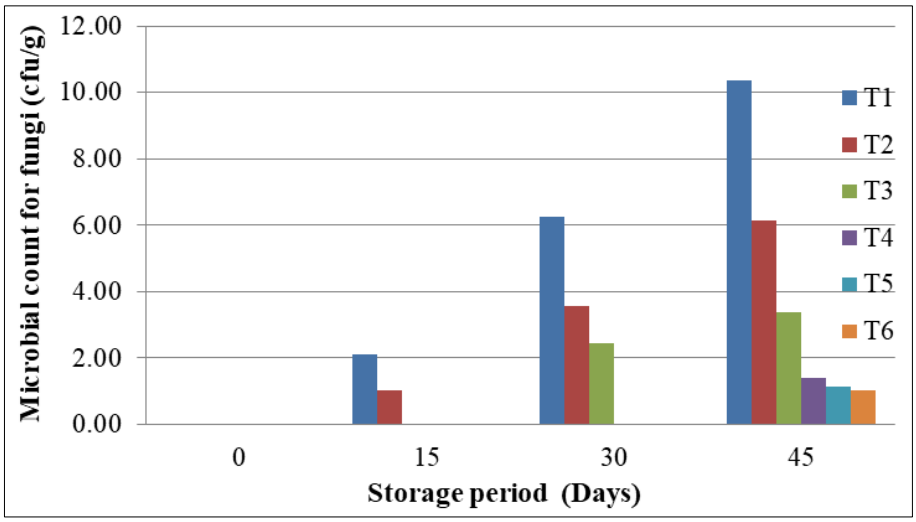


Fig 9: Effect of pre and post-harvest application of chitosan on Microbial count for fungi (cfu/g) of Grape Cv. Manik Chaman during storage at 0 °C temperature

The highest (4.68 cfu/g) count was recorded in the treatment T_1 at 45 days storage at 0 °C temperature. Minimum (0.26 cfu/1g) mean microbial count for fungi of grape berry was observed in the treatment T_6. However, it was at par with the treatments T_5. Thus, it is clear from the data that microbial count for fungi on grape berry decreased with increase in the level of chitosan concentration for post-harvest treatment of grape.

The antimicrobial activities of chitosan appear to rely on electrostatic interactions between positive chitosan charges and the negatively charged phospholipids in the fungal plasma membrane. Chitosan first binds to the target membrane surface and covers it, and in a second step, after a threshold concentration is has been reached, chitosan causes membrane permeabilization and the release of the cell contents.

The mean microbial count for fungi varied significantly during storage period of 45 days. It was lowest (0.00 cfu/1gm) in at initially and the highest (3.91 cfu/1g) at 45th days of storage. It was revealed from the data that microbial count for fungi increased during storage period of 45 days.

The antifungal property of chitosan might be related to its forming a physical barrier against infection, reducing the conidial germination and mycelial growth of *B. cinerea* and resulting in the long lasting protection of grape berries against gray mold (Romanazzi *et al.*, 2002) [38].

The similar decreasing trend in microbial count for fungi in grape was observed by Romanazzi *et al.*, (2002) [38], Karabulut *et al.*, (2004) [21], Romanazzi *et al.*, (2005) [43], Romanazzi *et al.*, (2006) [37], Luo (2007) [23], Xu *et al.*, (2007) [23], Meng and Tian (2009) [26], Romanazzi (2010), Papachatzis *et al.*, (2013) [30], Youwei and Yinzhe (2013) and Elwahab *et al.*, (2014) [14].

Interaction effect between treatments and storage period was found to be statistically significant for mean microbial count for fungi of the grape berry and at 5% level of significance. As per result, the lowest (0.00 cfu/1gm) microbial count for fungi was recorded in all treatments 0 day and 15th and 30th day of storage in the treatment T_4, T_5 and T_6 and the highest (10.36 cfu/1gm) was observed in the treatment T_1 at end of 45 days of storage.

2. Microbial count for bacteria (cfu/1g)

The data related to the microbial count of bacteria on grape during storage period are presented. As regards microbial analysis for bacteria of the grape, it is observed that there was no microbial growth of bacteria observed in pre and post-harvest of chitosan treated grapes at initial as well as at 0, 15, 30, 45 days of storage at 0°C temperature.

Conclusion and future scope

From the present investigation, it could be concluded that the admirable effect of 0.1% pre-harvest spray and 0.5 to 2% post-harvest dipping of chitosan on Sensory quality and microbial count parameters of Grape Cv. Manik Chaman during 45 days of storage period at 0°C temperature. The pre and post-harvest application in chitosan in Grapes can modify the internal atmosphere (by altering the permeability to water, oxygen and carbon dioxide), thereby decreasing the transpiration loss, reducing respiration rate, reducing microbial growth and delay's in senescence process of clusters an compared to untreated Grape Cv. Manik Chaman.

As regards the organoleptic evaluation, the Grape clusters treated with 0.1% pre-harvest spray and 1.0% post-harvest dipping of chitosan got maximum sensory score for colour, flavour, texture, overall acceptability, rachis appearance, berry appearance, fruit shrivelling and fruit decay as compared to control treatment. Thus, it is suggested that 0.1% pre-harvest spray and 1.0% post-harvest dipping of chitosan is optimum for grape.

Future scope also that chitosan define not only maintains firmness but also improves the postharvest quality during cold storage and also suggests that chitosan is promising as an eco-friendly edible coating to be used in commercial postharvest applications for prolonging the storage life of grapes.

Acknowledgement

I extend my sincere thanks to Dr. K. H. Pujari (Guide/Chairman and Associate Dean, PGI-PHM, Killa-Roha) and to my advisory committee members for giving me proper guidance throughout the course of study. I also sincerely thank Post Graduate Institute of Post-Harvest Management, Killa-Roha (Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli) for supporting the research financially.

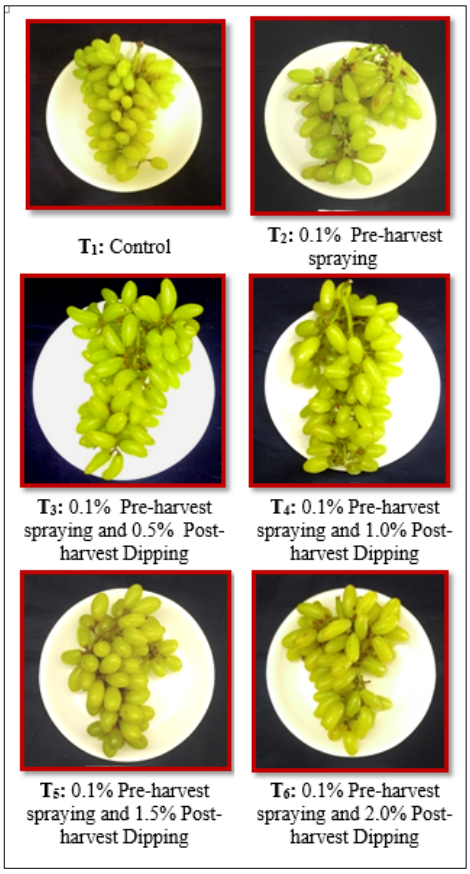


Plate 1: Effect of pre and post-harvest application of chitosan on grape Cv. Manik Chaman during 0 day storage at 0 °C temperature

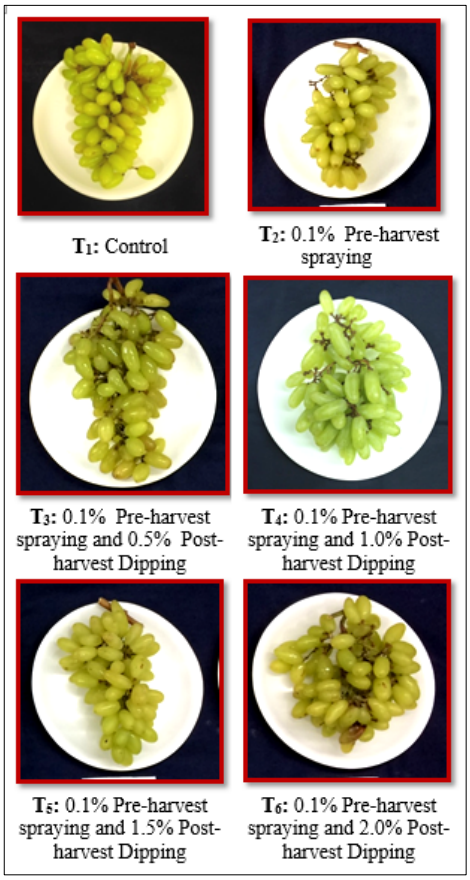


Plate 2: Effect of pre and post-harvest application of chitosan on grape Cv. Manik Chaman after 15 days of storage at 0 °C temperature

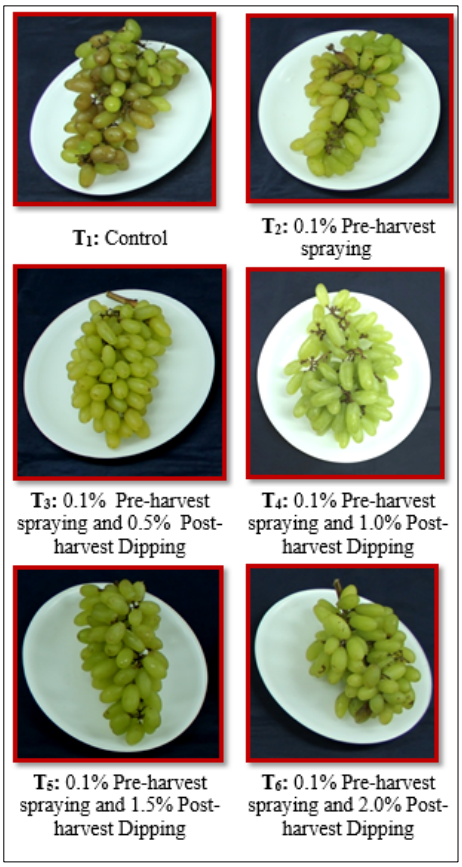


Plate 3: Effect of pre and post-harvest application of chitosan on grape Cv. Manik Chaman after 30 days of storage at 0 °C temperature

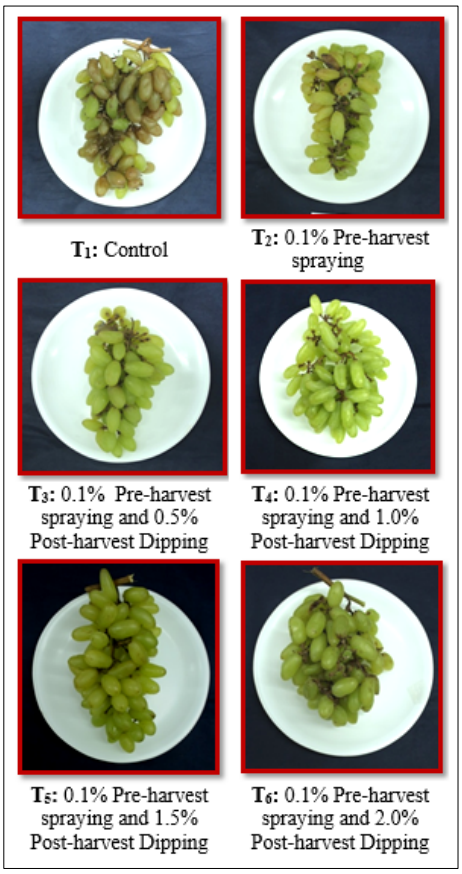


Plate 4: Effect of pre and post-harvest application of chitosan on grape Cv. Manik Chaman after 45 days of storage at 0 °C temperatur

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