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Microbial, enzymatic and physico-chemical quality changes during storage of tomatoes (*Solanum lycopersicum*) harvested from polyhouse vs. open conditions

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ABSTRACT

Protected cultivation of vegetables is gaining importance owing to its perpetual demand throughout the year. Effect of cultivating tomato (*Solanum lycopersicum* L.) cultivar Himsona under polyhouse conditions on the microbial and physico-chemical quality and enzymatic behavuior was studied and compared with open conditions at the time of harvesting and during storage under ambient conditions. Study on microflora of tomato fruits grown under open and polyhouse conditions revealed presence of fungi, viz. *Acremonium* sp., *Rhizopus* sp. and *Aspergillus* sp. in fruits harvested from open while fruits under polyhouse condition contained fungus *Acremonium* sp. only. Spoilage started in open fruits from 6th day of storage under ambient conditions due to dominance of *Rhizopus* sp. The bacterial counts on polyhouse grown fruits were about 100 times less than open cultivated fruits. Polygalacturonase activity was initially higher (0.139 U/ml) in open conditions compared to polyhouse condition (0.074 U/ml). It increased sharply to 1.50 U/ml on 4th day of storage in open conditions compared to slow increase under polyhouse condition (0.140 U/ml). On 6th day senescence started as evidenced by decrease in polygalacturaonase activity and increase in surface fungal counts in fruits from open conditions, which finally spoiled on 8th day due to dominance of *Rhizopus* sp. The fruits from polyhouse were still marketable at the end of 8th day. The study proves that tomato fruits grown in polyhouse are microbiologically safer and have better shelf life.

Key word: *Acremonium* sp., *Aspergillus* sp., Polygalacturonase, Protected cultivation, *Rhizopus* sp.,

Protected cultivation of fruits, vegetables and cut-flowers is gaining importance in the recent past globally. Polyhouse production has already been proven as profitable due to enhanced production. About 115 countries in the world are into greenhouse vegetable production commercially. The world scenario shows the area under protected cultivation to be nearly 623 302 ha, while total estimated world greenhouse vegetable production area is 402981 ha (Sabir and Singh 2013). Greenhouse technology is more popular in developed countries. It allows precision farming and overcomes limitations of space and disadvantages of climate change. Promotion of protected cultivation has revolutionized vegetable cultivation. Protected cultivation in the form of greenhouses, net houses, low tunnels, mulches etc. offers several advantages to grow crops of high quality and yields, thus using the land and other resources more efficiently.

Tomato is one of the most important vegetable crop, rich source of lycopene and many other neutraceutical compounds, and most widely grown under protected cultivation all over the globe. It is used as a salad, vegetable, fruit and in canning processes. Its shelf life depends upon the surface microflora present on the fruits at the time of harvesting and the storage environment. It is well studied that protected cultivation improves the crop productivity by controlling the wind velocity, moisture, temperature, nutrients, light intensity etc. (Sabir and Singh 2013) but its effect on surface microflora and the hydrolyzing enzyme activity responsible for spoilage has not been established.

In this study, physico-chemical quality, microbial safety and enzymatic behaviour of mature tomato cultivar Himsona grown under polyhouse condition was compared with that of those grown under open conditions at the time of harvesting at colour break stage and during storage under ambient conditions (30$\pm$5°C and 85% RH)

MATERIALS AND METHODS

Mature tomatoes cultivar Himsona (indeterminate habit, cluster bearing and high yielding) having uniform size were harvested hygienically from polyhouse (TPH) and open conditions (TOC) at ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow, collected in separate aseptic bags and brought to the lab. The freshly harvested fruits were packed separately in brown paper bags with 15 fruits per bag and three replicates for daily sampling. Samples were withdrawn from each bag on alternate days up to 8 days at room temperature (30-35 °C) and 85.0% RH until the fruits were unacceptable for marketing. The total soluble solids were recorded by using hand refractometer

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(Erma Japan). Total acidity was measured by the method of AOAC (1975). Physical loss in weight was recorded by weighing the fruits. The lycopene content was estimated as per the method of AOAC (1975) and the Absorbance was measured at 510 and 480 nm using a double beam UV-VIS spectrophotometer. Polygalacturonase enzyme (PG) activity was determined through the methodology described and modified by Garg and Ashfaque (2010). One unit of PG activity was defined as the quantity of enzyme necessary to produce one micromole (µm) of galacturonic acid per minute. The experiment was conducted in completely randomized design and the data represent the mean and standard error mean. The data were further subjected to analysis of variance and means were compared using least significance difference (LSD) at the $P<0.05$ level. The surface microbial counts were taken as per methods detailed by Garg *et al*. (1992). 100 g of randomly selected tomato samples were rinsed thoroughly with sterile distilled water in sterile bags and further serially diluted 10 fold. One ml of each decimal dilution was pour plated using plate count agar (PCA) for bacteria and Rose Bengal Chloramphenicol agar for yeast and mould. Bacterial colonies were further maintained on Nutrient Agar plates, while yeast and mould on Yeast Extract Potato Dextrose Agar and Potato Dextrose Agar, respectively. The plates were incubated for 24-48 hr in 30°C, and then the microbial colonies were counted. The microbial identification was carried out based on colony characters and microscopic observations as described by Pitt and Hocking (2009).

RESULTS AND DISCUSSION

The quality of fruits at zero day was better in tomatoes from polyhouse (TPH) than tomatoes from open condition (TOC) as reflected by higher TSS (3.6°B) and lower acidity (4.0%) compared to corresponding values under open conditions, i.e. 3.2°B and 5.6%, respectively. Rana *et al.* (2014) reported higher acidity in tomato fruits grown in open conditions. Loures (2001) found a TSS content of 4.77°B and 4.95°B for 'Carmem', grown in the field and in a protected environment, respectively. Changes in PLW, TSS, acidity and lycopene of freshly harvested and stored tomatoes have been depicted in Table 1. No physical loss in weight was observed in TPH, upto 6 days, while in TOC weight loss was observed in 2 days old samples. TSS was initially low in TOC but it increased faster than TPH. Faster decrease in acidity was observed in TOC compared to TPH. Higher lycopene content and uniform red colour and better appearance was observed in TPH than TOC. Lekshmi and Celine (2015) reported that polyhouse tomato fruits exhibited a higher content of lycopene. Similar results were reported by Singh *et al.* (2005), Suchindra *et al.* (2012) and Cheema *et al.* (2013).

Table 1 Changes in PLW, TSS, acidity and lycopene content of freshly harvested and stored tomatoes grown under open vs. protected conditions

Day	PLW (%)		TSS (°B)		Acidity (%)		Lycopene (mg/100g)	
	TOC	TPH	TOC	TPH	TOC	TPH	TOC	TPH
0	-	-	3.2	3.6	0.56	0.40	4.74	5.50
2	0.52	0	4.0	4.0	0.55	0.40	5.40	5.73
4	1.85	0	4.0	4.0	0.41	0.39	4.90	5.44
6	4.13	0	4.4	4.0	0.39	0.37	4.45	5.42
8	5.17	0.47	4.2	4.0	0.31	0.32	4.00	5.20

TPH, Tomatoes from polyhouse; TOC, Tomatoes from open condition

Fruits and vegetables possess nearly ideal conditions for the survival and growth of many types of microorganisms. Surface microbial analysis indicated higher microbial load in TOC than TPH. The bacterial counts on polyhouse fruits were about 100 times less than on open cultivated fruits (Table 2). Rana *et al.* (2014) also reported reduced disease incidence on tomatoes grown under poly house conditions. Tomato contains large amount of water which makes them more susceptible to microbial spoilage which affects its storage life. Ghosh (2009) reported that fungi were the source of spoilage in tomato samples rather than bacteria, with *Aspergillus* and *Fusarium* and *Penicillium* as the dominant fungal genera. Oyemaechi *et al.* (2014) analyzed the microbial agents responsible for spoilage of market sold tomatoes. It was reported that among the fungal isolates *Candida tropicalis* had the highest rate of occurrence (34.5%), followed by *Aspergillus niger* (31.9%), *Penicillium notatum* (17.2%), *Fusarium oxysporum* (12.9%), *Absidia corynbifera* (26%) and *Rhizopus stolonifer* (0.9%). Our results are in concurrence with the above reports. Three types of fungi, viz. *Acremonium* sp., *Rhizopus* sp. and *Aspergillus* sp. were observed on TOC surface compared to one (*Acremonium* sp.) on TPH (Table 2).

Fungi in particular produce an abundance of extracellular pectinases and hemicellulases that are important factors for fungal spoilage. Since the internal tissues are

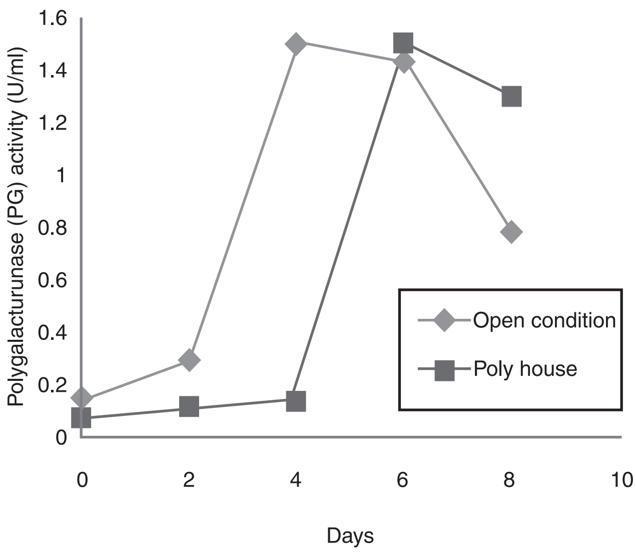
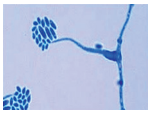
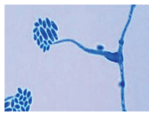
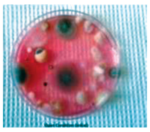
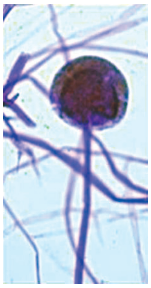
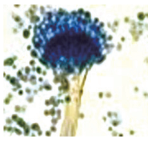


Fig 1 Changes in polygalacturonase activity of tomatoes grown under open and protected conditions during storage

Table 2 Comparison of microbial load of harvested / stored tomato from open and poly house conditions

Day	Open Condition				Poly house Condition			
	Yeast and mould (cfu/g)	Bacteria (cell/g)	Types of fungal colonies	Microscopic view (400×)	Yeast and mould (cfu/g)	Bacteria (cell/g)	Types of fungal colonies	Microscopic view (400×)
0	2×10^2	4×10^2		 *Acremonium* sp.,	2×10^1	4×10^2		 *Acremonium* sp.
2	4×10^3	6×10^4		 *Rhizopus* sp.,  *Aspergillus* sp.	3×10^2	5×10^4		
4	4×10^4	4×10^6			5×10^3	7×10^4		
6	8×10^4	6×10^6			5×10^3	7×10^6		
8	9×10^6	8×10^8			7×10^4	8×10^6		

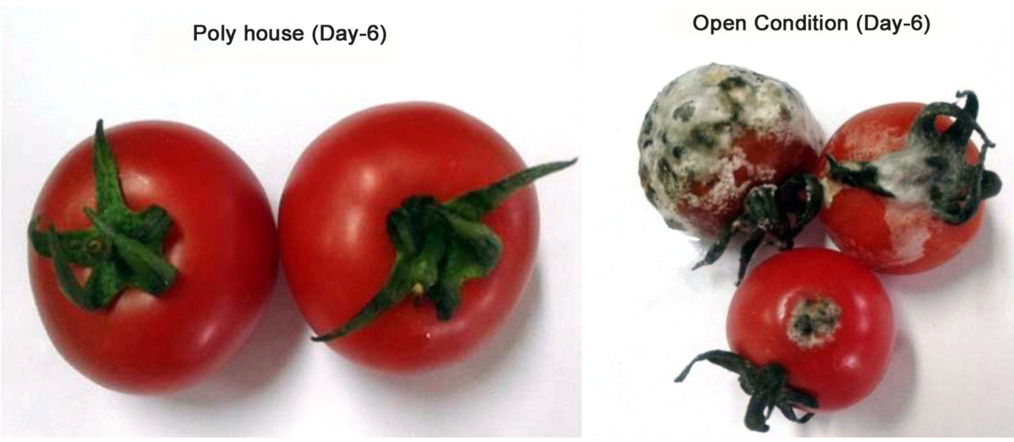


Fig 2 Visual observation of tomatoes on day – 6

nutrient rich comprised mainly of the polysaccharides, cellulose, hemicellulose, and pectin and starch. Spoilage microorganisms exploit the host by producing extracellular lytic enzymes that degrade these polymers. *Rhizopus* sp. and *Aspergillus* sp. are fungi having strong hydrolytic enzyme (Garg and Ashfaque 2010) which might be responsible for the faster decay of TOC. Our results indicated that polygalacturonase activity was initially higher (0.139 U/ml) in fruits grown under open conditions compared to polyhouse condition (0.074 U/ml). It increased sharply to 1.50 U/ml on 4th day of storage in open conditions compared to slow increase in polyhouse condition (0.140 U/ml) (Fig.1). On 6th day senescence started as evidenced by decrease in polygalacturaonase activity and increase in surface fungal counts in fruits from open conditions which finally spoiled on 8th day due to dominance of *Rhizopus* sp. (Fig 2). The fruits from poly house were still marketable at the end of 8th day. Similar results were reported by Ajayi *et al.* (2007), wherein production of polygalacturonase was observed by *Rhizopus arrhizus* during the deterioration of tomato.

The study indicated that tomato fruits grown in poly house have lesser surface microbial growth and better shelf stability due to delayed enzyme production. The results support the use of protected structures for getting tomato fruits with better quality and higher shelf life.

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