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Research Article

Quality of Tomatoes as Influenced by Bio-Chemicals and Controlled Atmosphere during Storage

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Abstract: A study was conducted to assess the potential of neem leaf extract, *Aloe vera* gel and Chitosan on the quality of two tomato cultivars (Sahil& Rio Grande) during storage in comparison with traditional post-harvest fungicide thiophanate methyl. Total soluble solids, acidity, color, respiration rate and ethylene production of bio-pesticides, treated tomatoes were compared (at 7 day interval, till 35 days) with those of treated tomatoes (thiophanate methyl) and control sample. Treated tomatoes (both varieties) were stored at 10^{o}C and 75% humidity without CO_2 and with 3% CO_2 for 35 days in two different environment chambers. The selected parameters which affect the post-harvest quality loss were significantly controlled in the treated tomatoes (neem leaf extract and stored with 3% CO_2) as compared to control and all other treatments except thiophanate methyl that also showed a significant effect.

Keywords: Bio-chemicals, Controlled atmosphere, Storage life, Tomatoes

1 INTRODUCTION

Maintaining food quality and ensuring the food safety are two major objectives of storages and packing houses. Selecting the best options and integrating the various components in a safe and cost-effective management scheme has added complexity to storage and packing operations. In addition, there is increasing scrutiny of postharvest practices for all fresh produce due to the hazardous pesticide residual effects. Socioeconomic and environmental damage due to application of hazardous pesticides on crop plants is estimated about 8.1billlions dollars annually [1].

Biopesticides are important areas to fulfill the challenges in a sustainable way. Biopesticides are derived from natural materials such as animals, plants, bacteria, and certain minerals widely used for controlling insects and disease causing pathogens by non-toxic mechanisms. Demand for chemicals free crop products is expected to enhance the demand for biopesticides in near future. Organic food market are other driving factors for increasing trend in biopesticides market, since future organic industry is solely dependent upon the chemical free crop protection products to safeguard the environment. The global biopesticide market was valued at 1.3 billion dollars in 2011 and is expected to expand much more in nearby future.

Europe is expected to be the fastest growing market in the near future owing to the strict laws for pesticides. Biopesticides are usually inherently less toxic; generally affect only the selective pest, effective in very small quantities, easily biodegradable, thereby resulting in lower exposures and largely avoiding the environmental pollution. When used as a component of Integrated Pest Management programs, it can greatly control major pest threat without affecting the crop yield [2]. As of early 2013 there were approximately 400

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registered active ingredients of biopesticides and more than 1250 actively registered biopesticide products [3].

Herbal extracts have gained a lot of attention because of numerous potential applications and properties. Antibacterial, antifungal and film forming properties of plant extracts has made their use an ideal to improve the stability of perishable fruits. A large number of chemicals have been developed for the control of postharvest diseases of crop plants. But due to hazardous side effects of these chemicals, more and more emphasis is being given to the use of bio control agents. Now major challenge is felt in the field of plant pathology to introduce some eco-friendly and safe alternative control strategies for agricultural commodities, which led researchers to turn their attention to herbs and plants as sources of bio agents to control postharvest decay of farm products during storage. Recently, our team has been published research on the potential of biopesticides to enhance the shelf life of tomatoes [4] and the present study was the further extension of our research work on different quality parameters.

2. MATERIALS AND METHODS

2.1. Chemicals

Sodium hydroxide was purchased from Fisher Scientific, UK. Methanol was procured from VWR International, Geldenaaksebaan 464, 3001, Belgium. Sodium hypochlorite, glacial acetic acid, phenolphthalein, citric, chitosan, pectin and ascorbic acid were purchased from Sigma Aldrich Chemical Co. St. Louis, MO, USA. Topsin-M 70 WP (Thiophanate Methyl) was procured from Nippon Soda Co., Ltd. Japan.

2.2. Plant Material

Two tomato varieties (Sahil & Rio Grande) were purchased from Faisalabad (Agricultural Farm), Pakistan, according to the USDA standard tomato color classification chart [5].The mature and healthy fruits were selected. The solution of sodium hypochlorite (0.05g/100 mL) was used to wash the tomatoes and then air-dried at ambient temperature before further treatment.

2.3. *Aloe vera* Gel Preparation

Aloe vera plant (matured leaves) was obtained from Faisalabad, (Agricultural Farm) Pakistan. The sodium hypochlorite solution (0.05g/100 mL) was used to wash *Aloe vera* leaves. *Aloe vera* gel was collected from the leaves (outer cortex) and blended in a high speed blender. To remove the fibers, the resulting mixture was filtered. The obtained gel matrix after filtration was then pasteurized for 45 minutes at 70°C. The cooling of gel was carried out immediately at ambient temperature. To maintain the pH at 4, the citric acid (4.5-4.6 g/L) and ascorbic acid (1.9-2.0 g/L) were added. The pectin (1g/100 mL) was used to improve the viscosity and coating efficiency of the stabilized *Aloe vera* gel. To prevent oxidation of the gel, the glass bottle (brown color) was used to store the gel [4, 6].

2.4. Neem Leaf Extract Preparation

Neem leaf extract preparation was done by the described methodof Masood et al [4]; Subapriya and Nagini [7] with slight modification. Mature fresh leaves *of Azadirachta indica* were harvested from Faisalabad (University of Agriculture), Pakistan. After washing with sterilized distilled water, the leaves (500g) were soaked in 1000 mL absolute methanol in the flask and left for 24 hours to allow for extraction. And then coarse residues were filtered through muslin cloth. Coarse residues were extracted repeatedly with 500 mL methanol. All extracts were mixed together and coarse filter paper was used to filter them. Rotary evaporator was used to remove the methanol from the extract till the volume was about 500 mL. The air tight amber colored glass bottle was used to store the extract.

2.5. Chitosan Coating Preparation

Chitosan (30g) was dissolved in 3 liters distilled water.The distilled water contained glacial acetic acid (150 mL). The solution of NaOH (1g/L) was used to adjust pH at 5.0 [4].

2.6. Thiophanate Methyl Solution Preparation

The thiophanate methyl solution was prepared by using 1g Topsin-M 70WP (Thiophanate Methyl 70%) into 1 liter of distilled water [4].

2.7. Experiment Plan and Storage

Aloe vera gel (50g/100 mL), Chitosan (3g/100 mL), neem leaf extract (20g/100 mL) and fungicide thiophanate methyl (0.1g/100 mL) coating solutions were used for tomatoes. The fresh fruits were dipped completely into the coating solutions for 15 minutes at 30˚C temperatures. The coated fruits were dried before storage. The untreated and treated tomatoes were keptat 10˚C and 75% relative humidity for 35 days under controlled atmosphere (without CO_2 and with 3% CO_2). Physical and chemical analysis was done after every 7 day interval. The different treatments scheme was as: TC: tomatoes were treated with Chitosan, TN: tomatoes were treated with neem leaf extract, TA: tomatoes were treated with *Aloe Vera* gel, TF: tomatoes were treated with conventional fungicides thiophanate methyl, control: untreated tomatoes.

2.8. Respiration Rate

The rate of CO_2 production was determined by digital CO_2 meter (Inspect Air CO_2 Meter, Model 8560, TSI Incorporated, USA) as determined by Ullah et al [8].

2.9. Ethylene (C_2H_4) Production

Ethylene gas production was determined by digital meter (Drager Safety Pac III, Mexico) as determined by Ullah et al[8].

2.10. Fruit Color

Surface Color was evaluated by a colorimeter (Model Color Tec PCM+ ColorTec Associates, Inc. USA). It was expressed as the ratio between a* and b* parameter which is an indication of color transformation of turning green to red. Ratio $a^*/b^* < 0$ indicates the green color while $a^*/b^* > 0$ indicates red color [9].

2.11. Total Soluble Solid Contents

Total soluble solid contents of freshly extracted juice by using a refractometer (Model RX 5000 Atago, Japan) at 20˚C temperatures were determined as per the method described by Dabeka and McKenzie [10].

2.12. Titratable Acidity

According to the standard technique of Dabeka and McKenzie, [10] acidity of juice was evaluated by titration of juice against NAOH (0.1g/L) using Titratable acidity mini titrator(Model no. HI 84432, HANNA Instruments Inc. Rumania).

2.13. Statistical Analysis

The Complete Randomized Design (CRD) with three-factor factorial was used to analyze data. The level of significance $P < 0.05$ was used. Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA) was used for statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Effects of Post-Harvest Bio-Chemicals on the Total Soluble Solid Contents of Tomatoes during Storage

To evaluate the fruit ripening, the total soluble solid contents are considered basic criteria. The results showed that the total soluble solid contents were very low at the time of harvest, but total sugars increased with the passage of time during ripening (Figure 1). However, during storage of tomatoes, sugar contents significantly changed in neem leaf treated tomatoes with 3% CO_2 as compared to control, Chitosan and *Aloe vera* treated tomatoes except tomatoes treated with thiophanate methyl. Changes in total soluble solid contents of thiophanate treated tomatoes were almost similar with neem leaf treated tomatoes. Effect of biochemical treatment was found non-significant with respect to varieties. Sahil and Rio grande showed the same results regarding change in total soluble solid contents during storage with and without CO_2 storage. Total soluble solid contents of neem leaf were increased from 3.63% to 4.23 with CO_2 while without CO_2 total soluble solid contents changed from 3.64 to 5.1% during 35 days of storage while total soluble solid contents of control at initial was 3.62 % and at the end of storage soluble solid contents were 5.37% with the CO_2 storage condition. While total soluble solid contents of control without CO_2 increased from 3.58 to 5.2%. Results showed that change in the total soluble solid contents of neem leaf treated tomatoes were slow during storage as compared to control and other treatments with CO_2

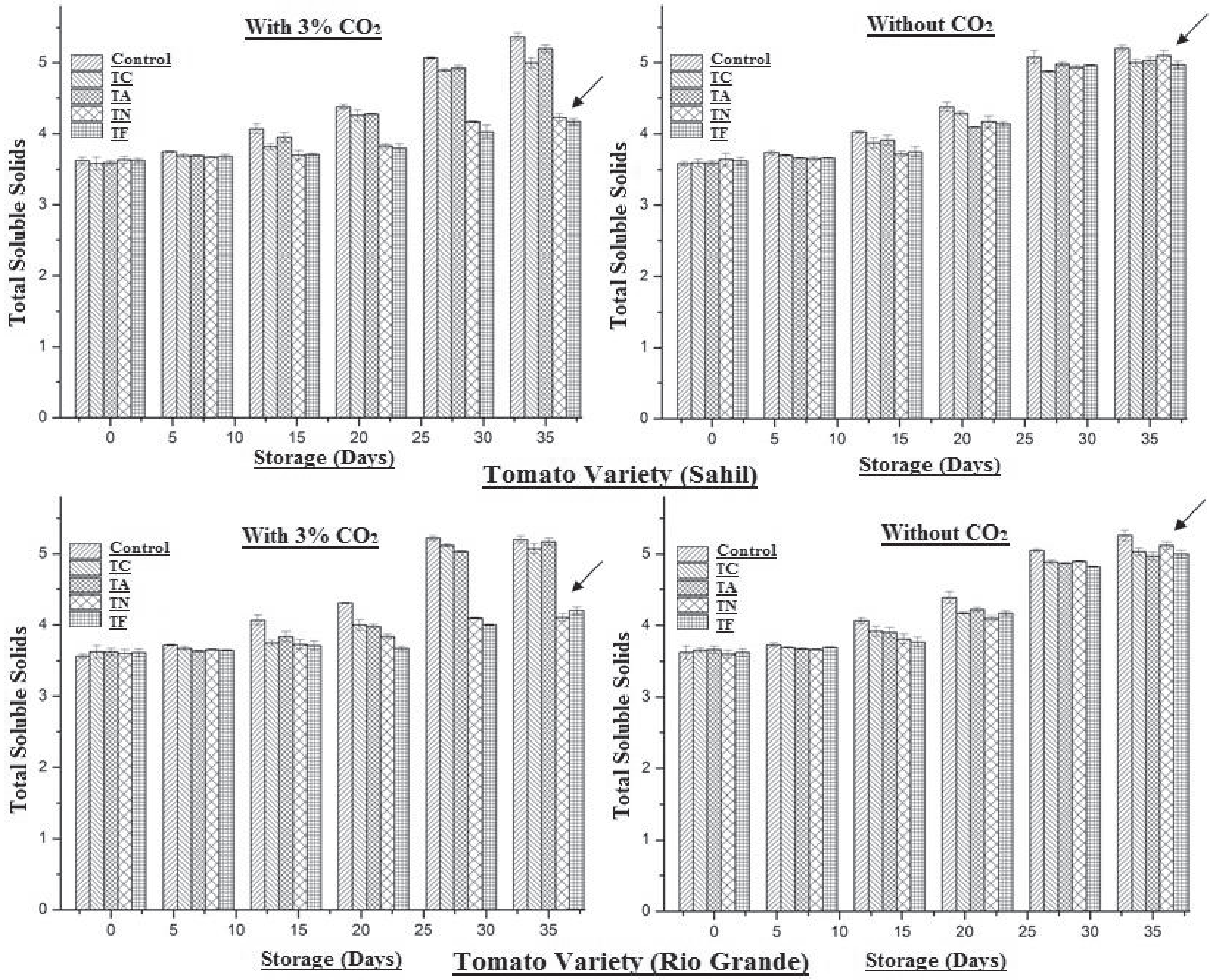

Fig. 1. Effects of post harvest treatments on the total soluble solid contents of the tomatoes during storage. Control: untreated tomatoes, TC: Tomatoes were treated with Chitosan, TA: Tomatoes were treated with Aloevera gel, TN: Tomatoes were treated with Neem leaf extract, TF: Tomatoes were treated with conventional fungicides thiophanate methyl.

storage. Small increase in sugar contents in neem leaf treated tomatoes as compared to control and other treatments (Figure 1) might be due to its slow ripening process and respiration [11]. Neem leaf extract integrated with 3% CO_2 storage slowed down the rate of respiration, transpiration and other metabolic changes due to its natural yield intrinsic activity or incorporation of antimicrobial compounds [12]. The maximum amount of sugars in untreated control might be due to rapid conversion of starch to sugars as a result of moisture loss and decrease in acidity by physiological changes during storage [13]. Our results are also in accordance with Melkamuet al [14]. Similar results were also quoted by Krammeset al [15], Opiyo and Ying [16] using different tomato cultivars.

3.2. Effects of Post-Harvest Bio-Chemicals on the Titratable Acidity of Tomatoes during Storage

In general, fruit titratable acidity tends to decrease and total soluble solid contents increase with maturation. Titratable acidity (TA) values of both the cultivars Sahil and Rio grande tended to reduce during storage under both storage conditions. Effect of interaction between bio-chemicals and CO_2 on the acidity of tomatoes was found highly significant. Acidity of neem leaf treated tomatoes with CO_2 storage decreases slowly as compared with that of control, Chitosan and *Aloe vera* treated tomatoes under same storage conditions (Figure 2). At the first day of storage, TA values of neem

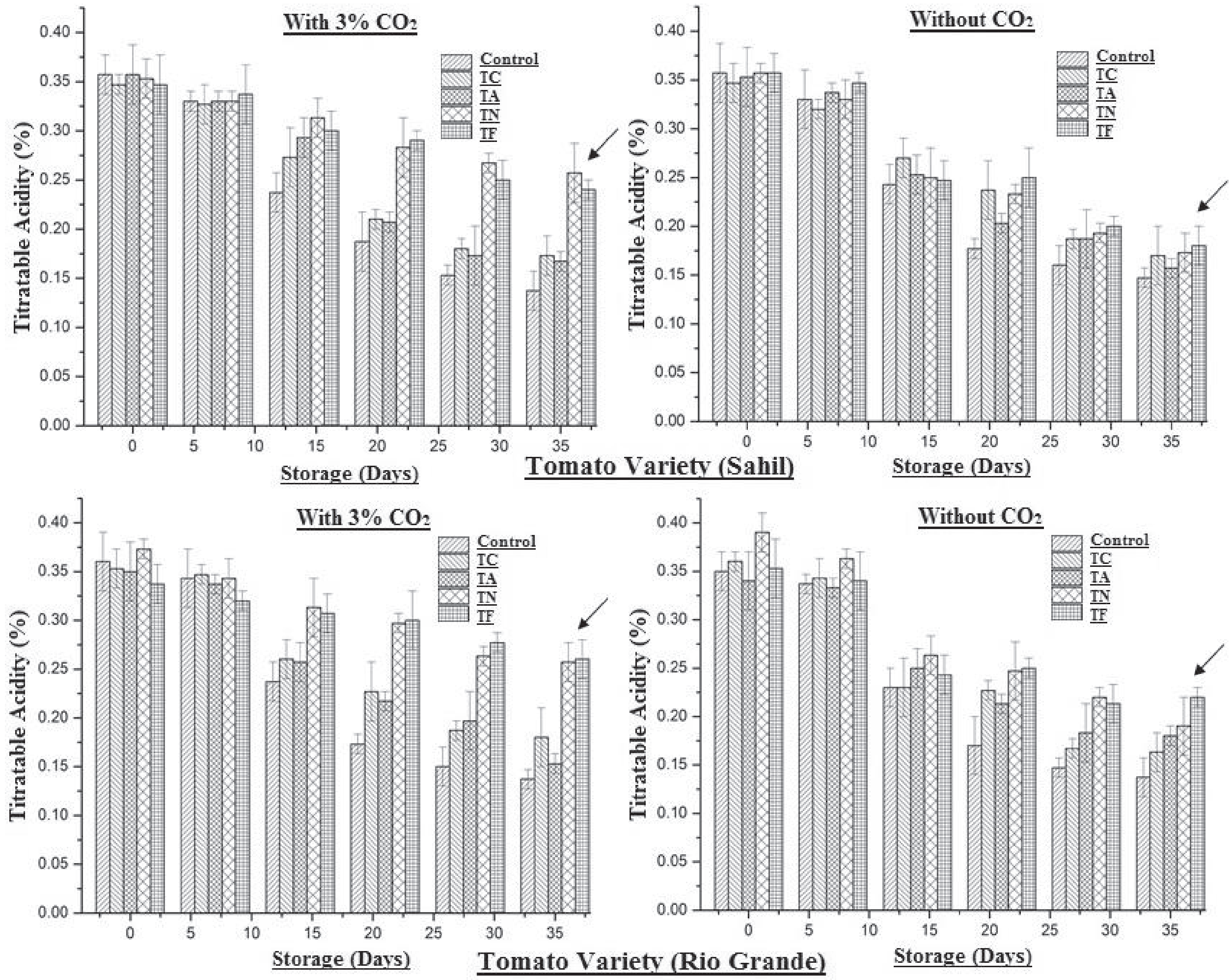

Fig. 2. Effects of post harvest treatments on the titratable acidity of the tomatoes during storage. ▨ Control: untreated tomatoes, ▧ TC: Tomatoes were treated with Chitosan, ▩ TA: Tomatoes were treated with Aloevera gel, ⊠ TN: Tomatoes were treated with Neem leaf extract, ▦ TF: Tomatoes were treated with conventional fungicides thiophanate methyl.

leaf treated tomatoes with CO_2 were 0.35% that a decrease to 0.25% at 35 days while TA of control sample change from 0.35 to 0.13% during storage period of 35 days. The response of both cultivars Sahil and Rio grande in terms of TA change was found not significant. Slow reduction in titratable acidity of neem treated tomatoes stored with CO_2 may be due to the anti-microbial properties of neem leaf extract that slow down the microbial breakdown of organic acids. It is also supported by Castro et al. [17] who observed that the rate of reduction in titratable acidity in coated fruits compared to uncoated fruits is low due to restriction of oxygen availability that leads to reduced respiration rate. Our findings of this study regarding TA change were similar to the findings of Krammeset al [15]; Fernandez-Trujillo and Sanchez [18]; Opiyo and Ying [16] on tomatoes. It is also in agreement with Good enough and Thomas (1981) who found that titratable acidity was affected by CO_2 storage.

3.3. Effects of Post-Harvest Bio-Chemicals on the Color of Tomatoes During Storage

Surface color changes involve loss of chlorophyll, and synthesis of other pigments, such as carotenoids and lycopene, during the ripening period. Thus, color change is often used as an index of the degree of ripeness, and provides primary information about the physiological condition of the fruits [19]. The results of the present study prove that neem leaf extract apparently restrict the change in color from yellow to red and help to retard the senescence. Color a*/b* values increased with increasing USDA color

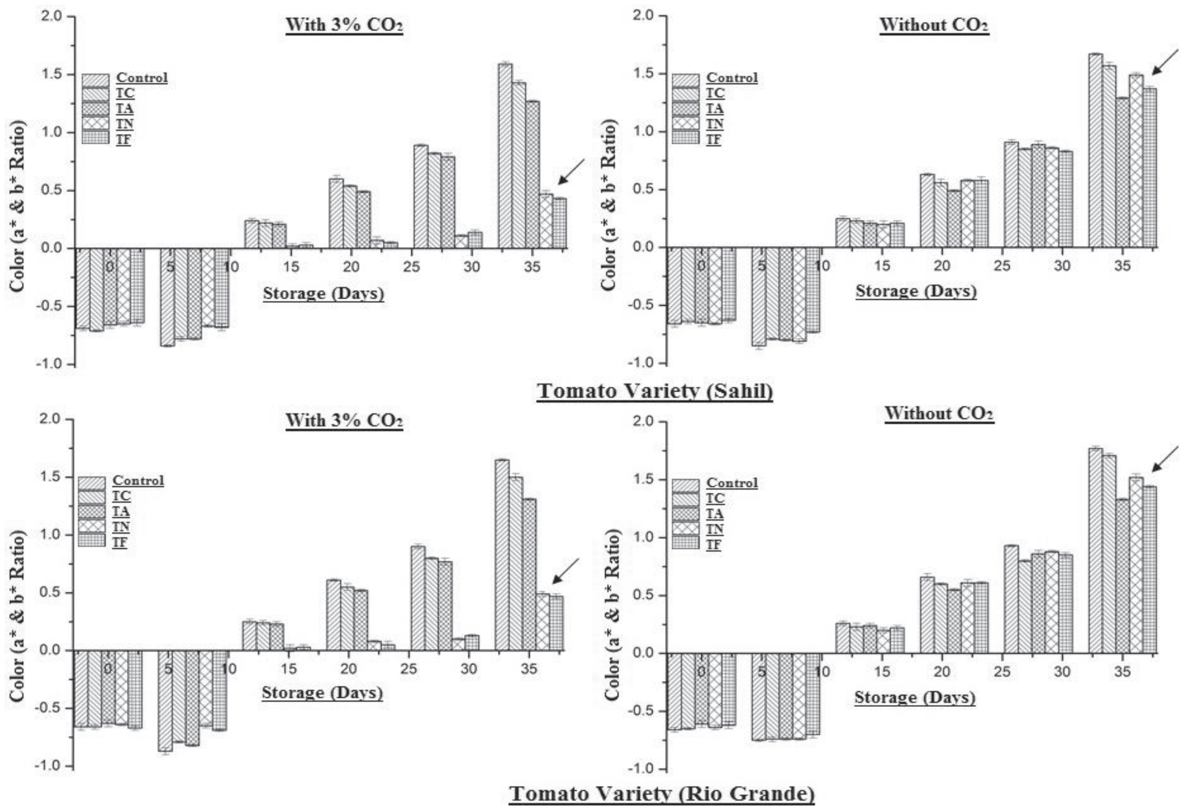

Fig. 3. Effects of post harvest treatments on the color of the tomatoes during storage. Control: untreated tomatoes, TC: Tomatoes were treated with Chitosan, TA: Tomatoes were treated with Aloevera gel, TN: Tomatoes were treated with Neem leaf extract, TF: Tomatoes were treated with conventional fungicides thiophanate methyl.

stages (Figure 3). Color a*/b* values of neem leaf treated tomatoes with 3% CO_2 were significantly differ from control, Chitosan and *Aloe vera* treated tomatoes except thiophanate treatment. Results of thiophanate treatment were also similar with neem leaf treatment at same storage condition. Effect of biochemical was found non-significant with respect to Variety. Effect of bio-chemicals on a*/b* values of Sahil and Rio Grande were almost similar. The color values at the breaker stage of tomatoes were also negative. Variation in color readings between maximum and minimum values increased during ripening of tomatoes. The color development rate of tomatoes increased with increasing maturation [20]. Neem leaf and thiophanate treated tomatoes stored at 3% CO_2 showed less color development as compared to all other treatments at same storage condition. Color a*/b* value of control of Sahil cultivar at the end of storage was 1.59 and 1.67 of Rio grande with 3% CO_2 while a*/b* values of neem leaf treated tomatoes were 0.47 and 0.49 of Sahil and Rio grande respectively. Results showed slow color development of neem leaf treated tomatoes under 3% CO_2 as compared to all other treatments. However thiophanate treated tomatoes showed the same results as compared to neem leaf with respect to color development. Slow color development in neem leaf and thiophanate may be due to the integrated action of the antimicrobial neem leaf extract and modified atmosphere that slow down ripening and respiration processes within the fruit. Color changes are well correlated with chlorophyll breakdown and carotenoid mainly beta-carotene accumulation in the plastids. Carotenes and xanthophylls, especially lycopene, oxidize during the storage and gradually change from bright red to dark brown. Our results are in accordance with the finding of Jiang and Li [21].

3.4. Effects of Post-Harvest Bio-Chemicals on the Respiration Rate of Tomatoes During Storage

Respiration, transpiration and ethylene production are the main factors contributing to the deterioration of fruits and vegetables [22]. Tomatoes treated with

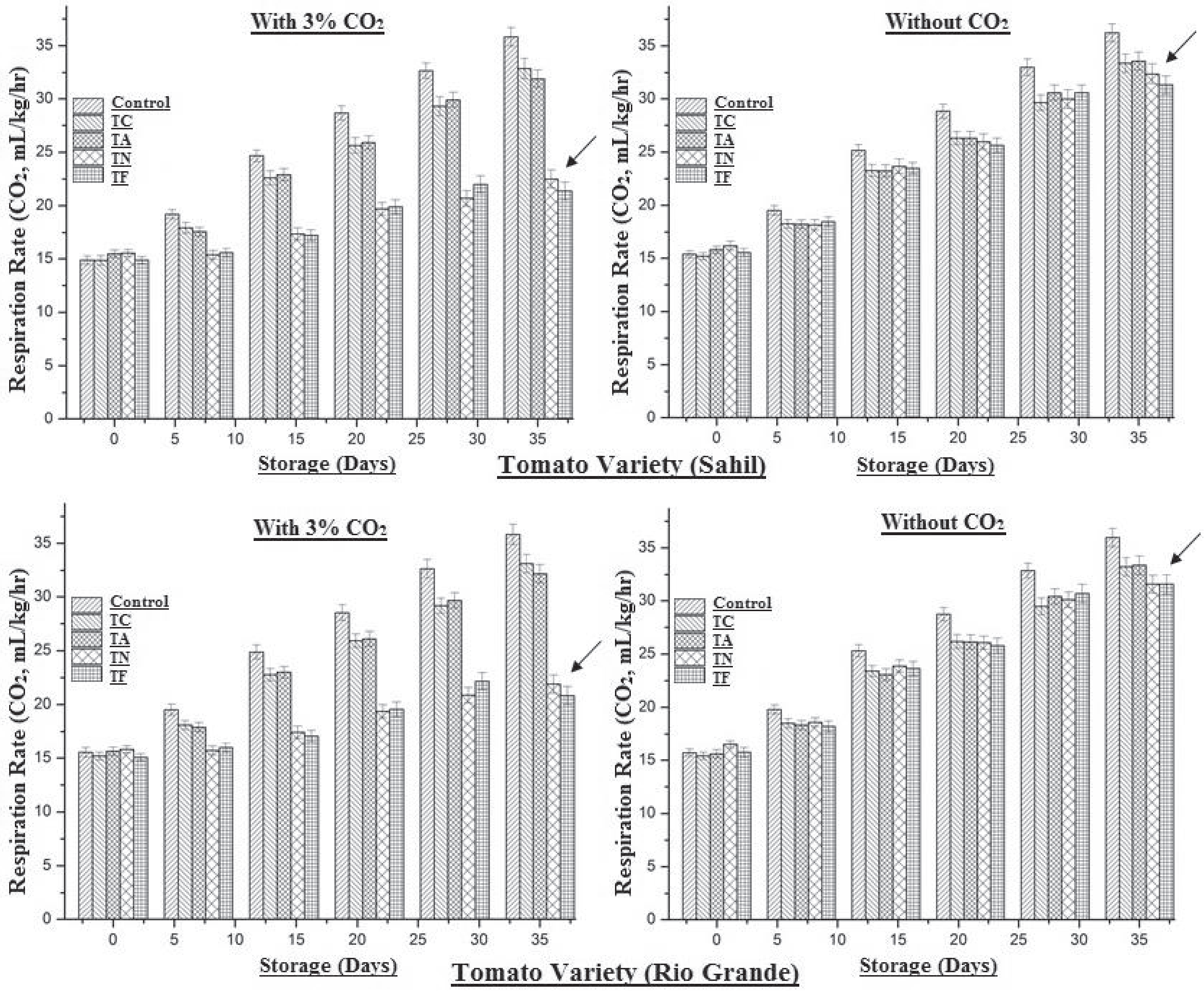

Fig. 4. Effects of post harvest treatments on the respiration rate of the tomatoes during storage. Control: untreated tomatoes, TC: Tomatoes were treated with Chitosan, TA: Tomatoes were treated with Aloevera gel, TN: Tomatoes were treated with Neem leaf extract, TF: Tomatoes were treated with conventional fungicides thiophanate methyl.

neem leaf extract and stored at 3% CO_2 significantly reduced respiration rates during storage as compared to control, Chitosan and *Aloe vera* treatments except thiophanate treated tomatoes at same storage condition (Figure 4). Neem leaf treated tomatoes stored with the CO_2 produced 15.53mL CO_2 $kg^{-1}h^{-1}$ at first day and 22.5mL CO_2 $kg^{-1}h^{-1}$at the last day of storage while control produced 14.87mL CO_2 $kg^{-1}h^{-1}$ at first day and 35.83mL CO_2 $kg^{-1}h^{-1}$at the last day of storage at same storage condition. Effect of biochemical on Sahil and Rio grande cultivars were found non-significant regarding respiration rate. Slow respiration of neem leaf treated tomatoes stored with CO_2 may be due to the anti-microbial properties that resist the senescence of tomatoes during storage. This may also be the result of the fact that reduction of O_2 supply to the fruit surface inhibits respiration rate [21]. The high organoleptic quality of fruit or vegetable products can be obtained by the control of ethylene production and respiration with controlled atmosphere. The respiration rate of fruits and vegetables is reduced by an atmosphere of high carbon dioxide and low oxygen which depress ethylene production, thus stop the ripening process [23]. The shelf life of fruits and vegetables is prolongedby reducing the rate of respiration with limited O_2that ultimately delay the oxidative breakdown of the complex substrates which make up the product. Use of modified or controlled atmospheres should be considered as an interesting alternative to reduce respiration and ethylene production, maintain firmness and delay pathological decay. Knee [24] reported that respiration rate gives an indication ofthe rate of breakdown of respiratory substrates such asstarch, sugars and organic acids. This could have an implication towards better storage qualityof processed fruits as compared to the fresh market tomato varieties.

3.5. Effects of Post-Harvest Bio-Chemicals on the Ethylene Production of Tomatoes During Storage

Ethylene production of tomato fruit was also suppressed by application of neem leaf extract under 3% storage condition. The rate of ethylene release in the control tomatoes initially increased and reached a peak value of 9.87 µL kg-1 h-1 at 35 storage days. Initially the ethylene production was increasing rapidly, but at the end of storage ethylene production was slow down as shown (Figure 5). The ethylene production rate of neem leaf treated tomatoes with CO_2 storage was found significantly slower than control, Chitosan and *Aloe vera* treated tomatoes stored with and without CO_2. Neem leaf treated tomatoes produced 3 µL kg^{-1} h^{-1} at the first day while it reached to maximum 5.83 µL kg^{-1} h^{-1} at 35 days. Ethylene production of thiophanate was very close (5.9 µL kg^{-1} h^{-1})

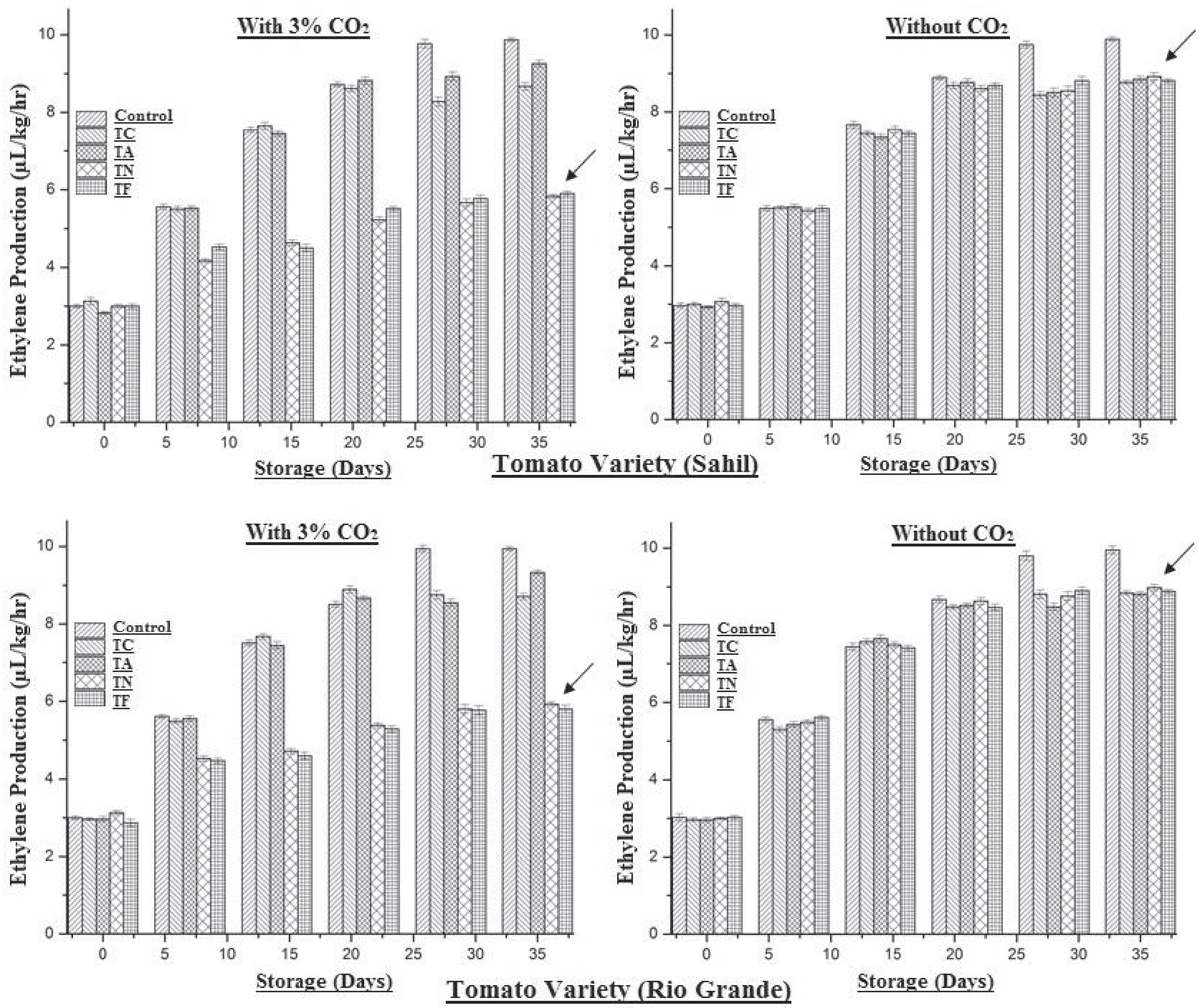

Fig. 5. Effects of post harvest treatments on the ethylene production of the tomatoes during storage. Control: untreated tomatoes, TC: Tomatoes were treated with Chitosan, TA: Tomatoes were treated with Aloevera gel, TN: Tomatoes were treated with Neem leaf extract, TF: Tomatoes were treated with conventional fungicides thiophanate methyl.

with neem leaf treated tomatoes (Figure 5). The results indicate that the interaction between neem leaf treatment and CO_2 storage condition has a significant effect on delay the tomato ripening as compared to all other treatments with and without CO_2. The effects of all bio-chemicals were found non-significant with respect to variety. Both Sahil and Rio grande showed the same results regarding ethylene production with and without CO_2. Limiting pericarp color reddening and ethylene production in tomato fruit lead to a delay in fruit ripening. Neem leaf application integrated with carbon dioxide could effectively retard pericarp color reddening and suppress ethylene production in tomato fruit during storage, which indicates that neem leaf treatment with CO_2 is beneficial in delaying fruit ripening, resulting in higher resistance to the fungal decay as compared to the controls, *Aloe vera* and Chitosan. Low O_2 or elevated CO_2 concentrations may reduce ethylene synthesis directly by affecting the activity of the softening enzymes, particularly 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase by inhibiting ethylene binding to a receptor that triggers autocatalysis [25]. Results are in accordance with the finding of Zapata et al[26], Mejia-Torres et al[27] and Lai et al [28].

4. CONCLUSIONS

Demand for chemical free food products have been well raised and bio-pesticides gradually replacing the highly toxic pesticides in the market. In the present study it was concluded that the neem leaf extract integrated with 3% CO_2 atmosphere can decrease the physiological process during storage and thus reduces the post-harvest losses effectively in tomatoes. Moreover, neem leaf extract coating can be used instead of conventional post-harvest fungicide during storage of tomatoes to control the post-harvest losses.

5. ACKNOWLEDGEMENTS

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