



RESEARCH ARTICLE

Effect of exogenous elicitors in enhancing the postharvest quality and antioxidant potential of *Spinacia oleracea* cv. Pusa Jyothi

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ABSTRACT

The effect of exogenous elicitors such as methionine and salicylic acid on spinach were investigated in the current study. Leaves were treated with 100, 200 μ M of salicylic acid and 10 and 50mM of methionine as elicitors. Complete retention of chlorophylls was possible in the spinach treated with 200 μ M of salicylic acid. The shelf life of spinach was increased up to 9 days in comparison with the control which deteriorated in 5 days of storage at 15°C. Both the treatments were effective in improving the phenolic and antioxidant potential of spinach during the storage. The results from the present study highlights the synergetic effect of elicitors in extension of shelf life and quality of the spinach. In future, strategies can be developed based on the effect of these elicitors, especially salicylic acid on improvement of postharvest quality of fruits and vegetables.

Keywords: Antioxidant, ascorbic acid, elicitors, methionine, postharvest losses, salicylic acid, spinach, storage

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INTRODUCTION

The most preferred and major source of essential vitamins and minerals are fresh fruits and vegetables. They contain the nutrients which are needed for the wellbeing for human beings. The quality of fresh vegetables has been checked on the basis of their appearances, texture, flavor, nutritional value etc. (Mahajan et al., 2013). Due to their concentrations of vitamins (vitamins C and A), minerals (electrolytes), recently photochemical (antioxidants), vegetables have historically held a place in dietary guidance (Joanne and Llyod, 2012). However, fresh vegetables are nothingbut perishable living products which requires certain activities to maintain the quality of the vegetables for certain period of time. The food production relied on locally and seasonally available crops can reduce the production, storage and transport cost, thereby reducing the ecological footprint considerably (Singh, 2018).

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Spinach (*Spinacia oleracea*) is one of the seasonal plants that are widely grown throughout India. Spinach is a green leafy vegetable with a broad, crisp, dark green leaves. Botanically Spinach is called as, *Spinacia oleracea* which belongs to the Amaranthaceae family. It is an annual (mostly winter) crop which has a quick maturing period (Ramesh et al., 2016). Spinach is being called by different names, Palak (Sanskrit), Palak-ki-sag (Hindi), Basalay-Soppu (Kannada), Vasolekiray (Tamil), Visalacheera (Malayalam), Palakura (Telugu), Phalanga-saga (Bengali) etc. Spinach contains various bioactive compounds such amino acids, vitamins, carbohydrates, fats and fatty acids, macro and micro nutrients etc. are known for its medicinal properties (Anil et al., 2017; Sowparthani and Radhika, 2020). Other than its nutritional value spinach also has many therapeutic uses such as gastrointestinal disorder, appetite stimulation, growth stimulation in children, constipation, anemia (due to its high iron content), high Blood pressure, bronchitis etc. (Tahseen, 2016; Ramesh et al., 2016).

Spinach-derived phytochemicals and bioactive are able to perform certain biological functions such as scavenge reactive oxygen species and prevent macromolecular oxidative damage, modulate expression and activity of genes involved in metabolism, proliferation, inflammation, and antioxidant defense, and curb food intake by inducing secretion of satiety hormones. These biological activities contribute to the anti-cancer, anti-obesity, hypoglycemic, and hypolipidemic properties of spinach (Roberts and Moreau, 2016). Freshly harvested spinach is highly perishable similar to any fruits and vegetables. This problem may be due to their high rates of respiration and transpiration and also the possibility of enzymatic and microbiological deterioration. Therefore, spoilage of fresh-cut vegetables may result from degradation of physiological and sensory characteristics (color, texture, and odor) as well as from microbiological degradation (Supriya et al., 2017; Kakade et al., 2015). Normally, spinach has a shelf life period of 3-4 days under refrigeration. Generally various post-harvest (physical, chemical or gaseous) treatments may be applied to maintain the quality with high nutritional value. These treatments are combined with appropriate management of the temperatures (Mahajan et al., 2013). Post-harvest quality of the spinach is maintained by using certain elicitors. These elicitor treatments enhance the phytochemical content and quality composition in spinach under post-harvest practices (Baenas et al., 2014). Post-harvest elicitor treatments may stimulate the production of various enzymes which produce the secondary metabolites that induce resistance along with the formation of different phenolic compound that are toxic to microbes (Demartelaere et al., 2017).

Elicitors such as Salicylic acid and L- Methionine were used to enhance the post-harvest quality of Spinach. Certain method was used for the analysis of the color and quality, free phenols, Total Phenols, Ascorbic acid and the Chlorophyll contents in the Spinach leaves. The postharvest quality of spinach was enhanced and the shelf life of the plants was increased to 9 days postharvest. Salicylic acid and L- Methionine acted as a defensive mechanism to increase the post-harvest quality of spinach. The major goal of post-harvest treatments is to increase the shelf life period of fruits and vegetables. According to Demartelaere et al. (2017), elicitors have been highly efficient due to their ability to increase the shelf life of fruits and vegetables by decreasing the decay percentage and improving the nutritional value. Elicitors stimulate the production of peroxides, polyphenol oxidase, ammonia-lyase enzymes etc, which helps in the production of secondary metabolites which in turn initiates resistance from certain organisms. These secondary metabolites which are induced production of various phenolic components that reduced the microbial contamination and post-harvest losses. Elicitors are classified into various types such as, Biotic, Abiotic (physical and chemical) elicitors, and also some of the plant hormones (Baenas et al., 2014). Plant defense mechanisms can be enhanced by inducing either of these elicitations (Baenas et al., 2013). Initially, the term elicitor was used for molecules capable of inducing the production of phytoalexins, but it is now commonly used for compounds stimulating any type of plant defense. Since then, elicitors are being used as a many compound for various pre-harvest and post-harvest treatments.

Depending upon the pathogen the plant has encountered, it can activate the innate defense mechanisms. These mechanisms are responsible for this differential recognition and response that involve crosstalk among different signal transduction pathways. The understanding of these pathways has led to the discovery of compounds which helps in the activation of plant defense mechanism (Garcia-Brugger et al., 2006).

In the current study, salicylic acid and L- Methionine were used as elicitors to increase the post-harvest quality of spinach. Salicylic acid is a signaling molecule that respond to the biotic and abiotic stress of the plants. This molecule can be used to induce catalytic reactions by specific enzymes involved in the biosynthesis of Plant cell (Mendozaa et al., 2018). Salicylic acid has a great role providing resistance to plants due to its exogenous application against many fungal pathogens (Subramani et al., 2019). Natural phytochemicals considered antioxidant compounds which have ability to damage free radical in the organisms related to their bioactivity to inhibit lipoxygenase, scavenge free radicals and to chelate metals (Maria et al., 2012; Jaradat et al., 2015). Phenolic compounds help in stimulating the quality and nutritional value of fruits and vegetables by certain modification in the color, flavor and also the aroma (Papoulias et al., 2009).

MATERIALS AND METHODS

Material collection

Fresh Spinach (*Spinacia oleracea*) plants were obtained from a local farm in Hoskote (Bangalore). The Plant belongs to the Pusa Jyothi Variety. It was immediately transferred to the lab and placed in a refrigerator.



Fig 1: Farm where Spinach (*Spinacia oleracea*) Pusa Jyothi was collected



Fig 2: Samples divided and labeled as T1, T2, T3, T4 and T5 before treating with 100 μ Molar Salicylic acid, 200 μ Molar Salicylic acid, 10m Mole L-Methionine, 50m Mole L- Methionine and control respectively

Treatments and storage

Spinach leaves were handpicked after washing with sterile water. The leaves were removed from the plant and were divided into 5 parts being 50gms each. The weights were weight using the electronic weighing machine to get the correct weight of the leaves. The leaves were placed in different trays which were prewashed with distilled water. The trays were labeled T1, T2, T3, T4 and T5 and the first Four sets of leaves were treated with 100 μ M (micromolar) Salicylic acid, 200 μ M Molar Salicylic acid, 10 mM (milli molar) L-Methionine and 50 mM L- Methionine respectively. The last set (T5) was kept for control. After the treatment leaves were kept for drying under room temperature. Once the leaves were dried the leaves were placed in sealed air tight plastic zipper bags. Minute holes were made at various regions of the zipper bag for the air flow. The zipper bags were placed in refrigerator at 15°C. Every 3rd day, the samples were taken out of the refrigerator and analyzed for weight loss, Chlorophyll 'a' & 'b', Total phenol content, Free phenol content and Ascorbic acid contents. 3rd day, 6th day and 9th day after the post-harvest treatment were used for analysis. Samples were analyzed for the post-harvest quality changes as given in Fig. (2)

Color change and visual quality

The Surface color of the samples was analyzed visually and no particular methods were used for the analysis of color change. Initially the leaves were bright green color (as shown in Fig. 1 and 2). And the color change was noted on every alternative 3rd day after the treatment until the 9th day of the post-harvest treatment.

Weight loss

The samples were weighted after the post-harvest treatment and during the storage at 3rd, 6th and 9th day. Results were seen in the percentage of the weight loss compared to the initial weight (50gm/set).

Chlorophyll

The chlorophyll content of the leaves was determined by hexane acetone method. Equal volume of Hexane and Acetone were measured and a Hexane: Acetone Solution was prepared. 1 gm of the sample was weighed and taken in a mortar to which 10ml of Hexane: Acetone Solution was added. The sample was later homogenized using pestle. Chlorophyll

extracted into Hexane: Acetone solution was collected from mortar by filtering the homogenate using a muslin cloth. The homogenate was washed with hexane acetone. The final content was made up to 15ml. Concentration of chlorophylls 'a', 'b' was quantified in samples by reading the optical density at 640 and 700 nm.

Total phenol

The Total phenol content of the sample (spinach leaves) were determined by Folin–Ciocalteu (F–C) reagent. 1gm of the sample was weighed and taken in a mortar to which 10ml of 1.2M HCl in 50% Methanol was added. The sample was homogenized using pestle. The homogenate was heated at 90°C for 3hrs with vortexing every 30 min. The samples were cooled at room temperature diluted with methanol. The sample was centrifuged at 5,000 rpm for 10 minutes. The supernatant was transferred into a new test tube and the pellet was discarded. For the assay, 0.2ml of the extract was taken in a new test tube to which 1ml of distilled water was added. 0.5ml of 1:1 FC reagent was added to the solution and was kept for incubation in a dark room for 30 minutes. The color of the solution was changed from its initial color to a blue colored solution. Concentration of Total phenol was quantified in samples by reading the optical density at 650nm.

Free phenol

Similar to Total Phenol, Free Phenol was also determined by Folin–Ciocalteu (F–C) reagent. 1gm of the sample was weighed and taken in a mortar to which 10ml of 50% Methanol was added and homogenized using a pestle. The solution was vortexed for about a minute. The homogenate was heated at 90°C for 3hrs with vortexing every 30 min. The samples were cooled at room temperature diluted with methanol. The sample was centrifuged at 5,000 rpm for 10 minutes. The supernatant was transferred into a new test tube and the pellet was discarded. 0.2ml of the extract was taken into which 2ml of distilled water was added. 0.5ml of 1:1 FC reagent was added to the solution along with 2ml of 20% sodium carbonate. The solution was kept for incubation in a dark room for 30 minutes. Concentration of Free phenol was quantified in samples by reading the optical density at 650nm.

Ascorbic acid

1gm of the sample was weighed and taken in a mortar to which 10ml of 5% Metaphosphoric acid and glacial acetic acid was added and homogenized using a pestle. The homogenate was centrifuged at 5000rpm for 10 minutes. The pellets were discarded and the supernatant was collected into a new test tube. 0.3 - 0.4 ml of aliquot is taken a test tube to which 1ml of 2% 2,4-Dinitrophenylhydrazine (DNPH) and 2 drops of 10% Thiourea is added. The tubes were kept for incubation at 37°C (RT) for 3 hours. After incubation, the reaction was terminated by adding 5ml of 85% Sulphuric acid. Concentration of Ascorbic acid was quantified in samples by reading the optical density at 540nm.

RESULTS

Color change

Gradual change in color and visual quality of spinach were observed in all the samples during the storage. Change was checked every 3rd day over the span of 9 days post treatment. Control had lost its visual quality and degradation of color was high than treated samples (T1 to T4). Texture, leaf color was maintained comparatively well in treated samples than

in control. On the 9th day post treatment, the samples were completely different than they were before stored at 15°C on the 1st day.

Weight loss

Weight loss of the spinach was observed by weighing the samples in a weighing machine every 3rd day post treatment (Fig. 3). The initial weight of the samples was reduced gradually during the storage post treatment. Weight loss of control and the samples treated (T1 to T4) increased and by the end of 9th day control had lost 63.7% weight whereas treated samples had lost 38.4% - 56.91% Control has highest weight loss and treated samples had significantly low weight loss.

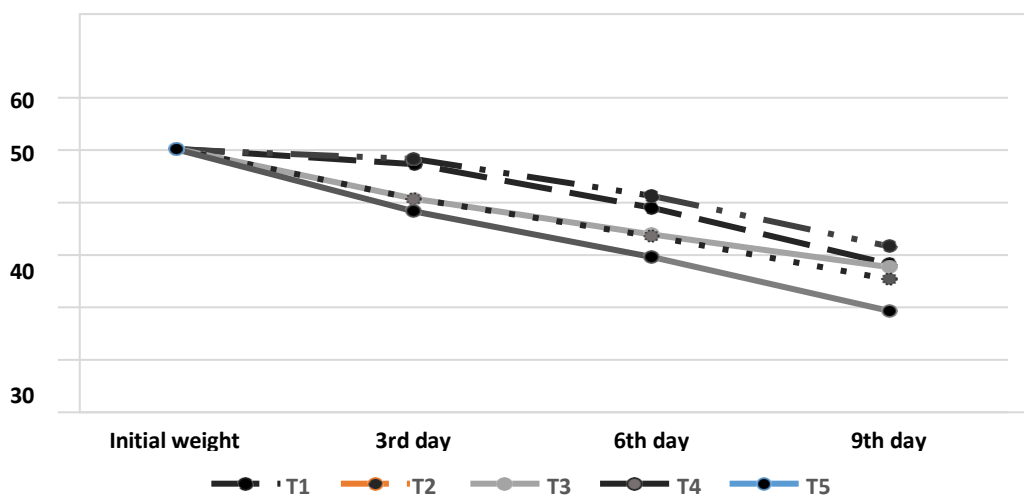


Fig 3: Effect on weight loss on the sample during different postharvest treatments of Spinach during cold storage at 15°C. Each data point represents the mean of replicate samples.

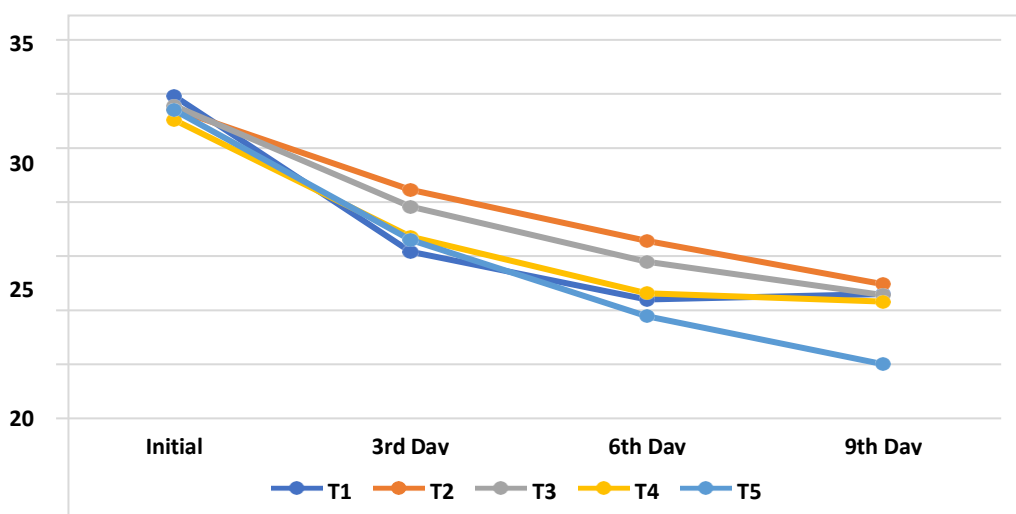


Fig 4: Effect on Total Chlorophyll on the sample during different postharvest treatments of Spinach during cold storage at 15°C for a period of 9 days. Each data point represents the mean of replicate samples.

Total chlorophyll

Total chlorophyll content of the sample was seen to be decreased throughout the storage at 15°C for a period of 9 days in all the treatments (Fig. 4). Total chlorophyll amount in spinach at harvest was 30.16 mg 100 g⁻¹ and at the end of the storage (9th day), the highest total chlorophyll content was determined to be in between 10.34 mg 100 g⁻¹ to 13.67 mg 100 g⁻¹ the samples treated (T1 to T4) while the control (T5) was 5.80 mg 100 g⁻¹. Total chlorophyll amount in samples treated were seen to be 34.28% to 45.32% in T1 to T4, but 19.23% in control (T5). Treatment was more effective compared to control with respect to chlorophyll content.

Total phenol

Total phenolic content of the sample (T1-T5) was seen to be decreased initially (Fig. 5). During storage, the highest total phenolic content was determined in T4 on day one which on comparison to other treated samples showed most phenolic content throughout the storage. Sudden increase was seen in T2 on day six and T3 on day nine. At the end of the storage, the least total phenol content was obtained from T2 (0.28 mg gallic acid kg⁻¹) while the rest of the treatments T1, T3, T4 and T5 (control) were 0.35 mg gallic acid kg⁻¹, 0.55 mg gallic acid kg⁻¹, 0.57 mg gallic acid kg⁻¹ and 0.36 mg gallic acid kg⁻¹ (control), respectively.

Free phenol

Free phenolic content from the treated samples (T1 – T5) was determined at 3 days interval for a period of 9 days (Fig. 6). During the post-harvest treatment, the free phenol content was seen to be decreased over the storage period. During storage, the highest free phenolic content in the treated samples was determined in T2 and the lowest was seen in control (T5) with 0.43 mg gallic acid kg⁻¹ and 0.59 mg gallic acid kg⁻¹, respectively.

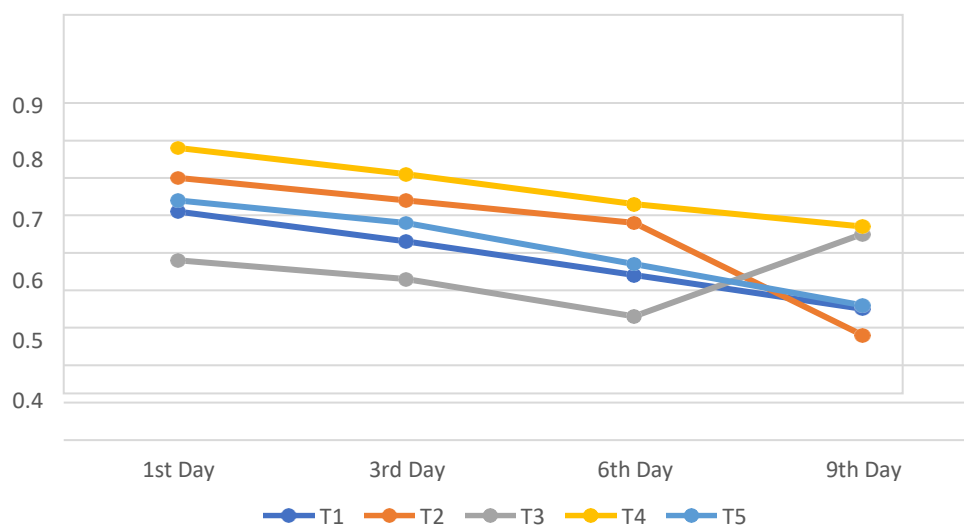


Fig 5: Effect on Total Phenol on the sample during different postharvest treatments of Spinach during cold storage at 15°C for a period of 9 days. Each data point represents the mean of replicate samples .

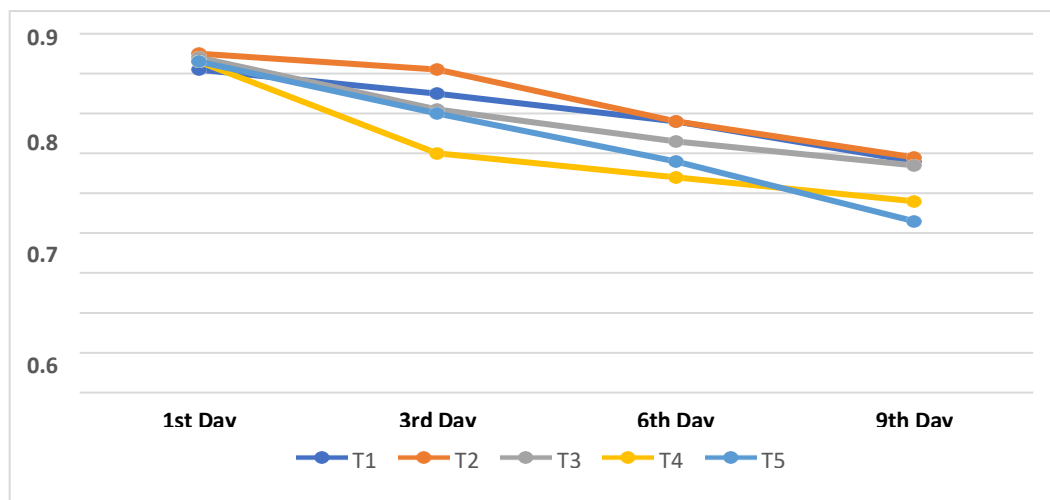


Fig 6: Effect on Free Phenol Concentration on sample during different postharvest treatments of Spinach during cold storage at 15°C for a period of 9 days. Each data point represents the mean of replicate samples.

Ascorbic acid

Ascorbic Acid content in spinach at harvest in T1 to T4 (samples treated) was observed as 0.19, 0.32, 0.15, 0.18 mg 100 g⁻¹ respectively and in Control it was observed to be 0.22 mg 100 g⁻¹ (Fig. 7). At the end of the storage (9th day), the highest Ascorbic acid content was determined to be 0.13 mg 100 g⁻¹ in T2. Control and other storage treated spinach underwent a significant loss in ascorbic acid with control showing 0.03 mg 100 g⁻¹ at the end of the storage.

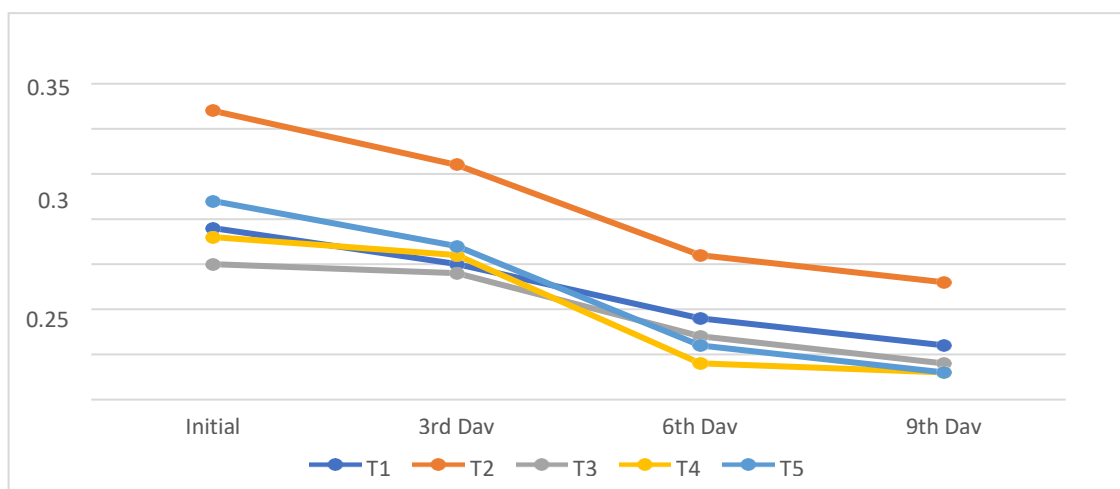


Fig 7: Effect on Ascorbic Acid Concentration on sample during different postharvest treatments of Spinach during cold storage at 15°C for a period of 9 days. Each data point represents the mean of replicate samples.

DISCUSSION

The results of this study show the effects of 100µ Molar Salicylic acid, 200µ Molar Salicylic acid, 10m Mole L-Methionine and 50m Mole L- Methionine on the post - harvest quality of Spinach.

Salicylic acid is proven to be the best elicitor molecule identified in controlling postharvest decay and retaining the quality of fruits and vegetables (Wang et al., 2022). Postharvest quality of any green leafy vegetables depends on maintaining the nutritional values, Visual quality, weight, Ascorbic acid and the Phenolic compounds that helps in stimulating the quality of the samples for a longer storage period. Spinach is one of the easily perishable green leafy vegetables, which has a low shelf life and poor visual quality under unfavorable post – harvest conditions. In another related study performed in red-fleshed kiwi fruit, treatment of fruits with 1-methyl cyclopropane (MCP) improved the antioxidant capacity and increased levels of phenols were also observed (Xia et al., 2020).

Phenolics and ascorbic acid are the compounds which are involved in the maintenance of antioxidant status. During storage, the highest total phenolic content was determined in T4 on day one which on comparison to other treated samples showed most phenolic content throughout the storage. Sudden increase was seen in T2 on day six and T3 on day nine and the highest ascorbic acid content was determined in T2 but Control and other treated spinach underwent a significant loss in ascorbic acid content by end of the post treatment storage that is on the 9th day. Phenolics are reported to be the best antioxidant against oxidative stress and in combating the ROS based on their accumulation in plants especially leaf tissues (Ramakrishna and Ravishankar, 2011).

Chlorophylls were retained in the treated spinach leaves compared with control till the 9th day of treatment and study. Similar kind of data was reported in spinach by Gorelick et al. (2020). Elicitation is a process of induction of both physiological and biochemical changes in the plants with the help of chemicals or molecules generally known as elicitors (Angelova et al., 2006). These molecules are mainly involved in the endogenous defense mechanism in plant and even exogenous spraying of these elicitors have proven to protect the plant and help them to develop resistance against environmental stress (Shachi, 2023). A positive correlation was observed with the elicitors and elevated amount of all secondary metabolites in the plant selected under current study.

CONCLUSION

Plants are rich in numerous potential bioactive compounds and consumption of these vegetables are found to be good for health and wellbeing. One such interesting source of nutritional compounds is spinach. Even though spinach is known for its antioxidant potential, perishability of the crop is very high due to lesser shelf life. Shelf retention is achieved in the current study upto 9 to 10 days by the treatment of exogenous elicitors. Salicylic acid could retain the chlorophylls content in the treated leaves in comparison with the control. Moreover, the higher levels of phenolics, ascorbic acids were also reported which indicated the involvement of these metabolites in the defensive mechanism in spinach leaves. Hence the study focuses on the green approach in postharvest physiology and technology and even in area of nutraceuticals. Future strategies can be developed and implemented based on the efficient role of elicitor molecules. Individual endogenous elicitors can be isolated and identified and based on the advanced technology in postharvest biology can retain and improve shelf life and quality of various susceptible and perishable crops like spinach.

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