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Effects of Foliar Application of Melatonin on Head Yield and Quality of Broccoli cv. Palam Samridhi

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ABSTRACT

Background: Broccoli is a cruciferous vegetable with green flower buds. It has a reputation as a superfood as it is low in calories but contains a wealth of nutrients and antioxidants that support many aspects of human health. Broccoli is a good source of fibre and protein and contains iron, potassium, calcium, selenium and magnesium as well as the vitamins A, C, E, K and a good array of B vitamins including folic acid.

Methods: Thirty days old and uniform seedlings of broccoli cv. Palam Samridhi were transplanted in the field at a spacing of 45 × 45 cm. Different concentrations of Mel, *viz* 0, 20, 40, 60 and 80 ppm with three replications were sprayed at 15 days after transplanting (DAT). Head diameter, weight, chlorophyll and carotenoids, sugar, proteins and antioxidant were analyzed at harvest.

Result: Results showed that the maximum head diameter (10.7 cm), weight (233.8 g), chlorophyll (0.79 mg/g.FW), carotenoid (0.028 mg/g.FW), phenol (178.1 mg/100 g DW), total antioxidant (362.3 mg GAE 100 g DW) and MSI (45.38 %) levels were recorded with Mel 60 ppm. However, the highest levels of sugar (129.0 µg/g) and protein (88.0 µg/g) were recorded with Mel 80ppm followed by Mel 60 ppm.

Key words: Antioxidants, Broccoli sprout, Melatonin, Protein, Sugar.

INTRODUCTION

Broccoli [*Brassica oleracea* (L.) var. italica Plenk] is a relatively new vegetable for many Indians. It belongs to family Brassicaceae. Broccoli is an Italian word from the Latin brachium, meaning an arm or branch. The term sprouting as used in sprouting broccoli refers to the branching habit of this type, the young edible inflorescences often being referred to as sprouts. Broccoli has gained attention because of the struggles on patenting genotypes with high concentrations of glucosinolates showing positive effects in cancer treatment (Wolf *et al*., 2014). The sprouting broccolis are thought to have originated from the eastern Mediterranean then introduced into different parts of the world (Owis, 2015). The United States is the world's largest producer of broccoli. The flowers of broccoli are borne on a faceted floral shoot so that the inflorescence terminates the axis of the plant. The inflorescence, which has been described as a corymb, a corymbose panicle or a modified racemose panicle, consists of functional floral buds, perfect flowers, stem and bracts. At the time of harvesting, the inflorescence is a growing, faceted axis bearing a large number of immature, stalked flowers, floral buds and varied bracts which are smaller and simpler in form than the vegetative leaves. The bracts are absent from the terminal portion of the inflorescence (Buck, 1956; Gray, 1982).

Melatonin (C13H16N2O2) is a low molecular weight organic compound ubiquitously available in almost all living organisms from bacteria to mammals and considered as the nature's most versatile biological signal (Pandi-Perumal *et al*., 2006). Chemically, melatonin is N-acetyl-5-methoxytryptamine, an indolic compound derived from serotonin (5-hydroxytryptamine). Both biogenic amines are synthesized from the amino acid tryptophan through a well characterized biosynthetic pathway (Arnao and Hernández-Ruiz, 2014; Lee *et al*., 2015). Phytohormone IAA (indolyl-3-acetic acid) bears some resemblance to melatonin since both are indole compounds and have a common biosynthetic pathway through the tryptamine in the tryptophan dependent IAA biosynthetic pathway.Melatonin has been reported to involve in regulating plant growth and development and especially play critical role (s) in plant defense systems (Weeda *et al*., 2014). The present investigation was carried out toinvestigatethe effectof foliar application of melatonin on improving head yield andquality attributes of broccoli.

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MATERIALS AND METHODS

The investigation was carried out at the Division of Basic Sciences and Humanities, Faculty of Horticulture, SKUAST-Kashmir during the year 2017 and 18. One month old uniform

and healthy seedling of broccoli cv. Palam Samridhi was collected from the nursery, Division of Vegetable Science (SKUAST-K) and transplanted at a spacing of 45 × 45 cm in a well prepared field. Different concentrations of melatonin (Mel) *viz* 0, 20, 40, 60 and 80 ppm were sprayed through hand spray on the foliage of plants at 15 days after transplanting (DAT). One plot constituted one sample unit and each treatment consisted of four plots with 6 plants per plot.

Head diameter (at spherical) was measured by measuring tape then average of five plants was computed to get the mean head polar diameter. Head diameter (at spherical) was measured by measuring tape then average of five plants was computed to get the head equatorial diameter. After harvesting, five broccoli head were weighed at pan balance and average individual head weight was computed in terms of grams. Total yield of broccoli head sprouts from each plot was taken in kilogram and then, it was also converted to yield in quintal per hectare.

For total soluble sugar 100 mg of dried sample was hydrolysed with 5.0 ml of 2.5N HCl by keeping it in a boiling water bath for 3 hours. After cooling at room temperature the content was neutralized with solid sodium bicarbonate until effervescence ceases and volume was to 100 ml. Centrifuged the content at 500 g for 10 minutes and supernatant was collected. The amount of total soluble sugars was estimated by phenol sulphuric acid method (Dubois *et al*., 1956) colorimetrically. Extraction of protein was done by grinding 0.2 g of sample with 0.1 M phosphate buffer (ph 7.5) in a mortar and pestle. Centrifuge it and use the supernatant for protein estimation (Bradford, 1976). Bovine serum albumin (BSA) was used as protein standard. Total phenol was determined by spectrophotometeric measurement of blue colured complex by the reaction of phenols with phosphomolybdic acid in Folion Ciocalteur in alkaline medium (Bray and Thorpe, 1954). The total anti-oxidant capacity was measured by the method described by Prieto *et al*. (1999). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. Malondialdehyde (MDA) content generated as product of lipid peroxidation in the leaves of broccoli was estimated in different treatments according to Cakmak and Horst (1991). Leaf sample of 0.12 g were ground in 1.2 ml 0.1% (w/v) trichloroacetic acid (TCA), then centrifuged at 12000 g for 10 min. Then 0.3 ml 0.5% (w/v) thiobarbituric acid (TBA) was added to 0.3 ml of the supernatant. The resulting mixture was boiled at 100°C for 20 min. The reaction was stopped by placing the reaction tubes in an ice bucket. The absorbance values were measured at 532, 600 and 450 nm. The interference of soluble sugars in the samples at A_{532} and A_{450} was corrected by subtraction. The MDA content (µmol g^{-1} FW) was calculated according to the formula:

$$MDA\ (\mu\ mol/g\ FW) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

Where:

A_{532}, A_{600} and A_{450} represent the absorbance of the mixture at 532, 600 and 450 nm, respectively.

The leaf membrane stability index (MSI) was determined according to the method of Premachandra *et al*. (1990) as modified by Sairam (1994). Leaf discs (100 mg) were thoroughly washed in running tap water followed by washing with double distilled water. Thereafter, the discs were heated in 10 ml of double distilled water at 40°C for 30 minutes. Then electrical conductivity (C_1) was recorded by EC (Electrical Conductivity) meter. Subsequently, the same samples were placed in a boiling water bath (100°C) for 10 minutes and their electrical conductivity was also recorded (C_2). The MSI was calculated as:

$$\text{Membrane stability index (MSI)} = [1- (C_1/C_2)] \times 100$$

The data recorded on various variables were statistically analysed by using technique of analysis of variance and significance was determined as given by Panse and Sukhatme (1967).

Results and Discussion

Physical characters of broccoli head

Perusal of the data (Table 1) indicated that broccoli plant sprayed with 0 ppm melatonin (control) showed the minimum polar as well as equatorial diameter of 7.1 cm and 6.5 cm, respectively. However, there was significant improvement in polar as well as equatorial diameter of broccoli when sprayed with different doses of melatonin and Mel 60 ppm resulted in maximum diameter of 11.6 cm and 10.4 cm, respectively. The polar and equatorial diameter recorded with Mel 80 ppm (10.7 cm and 9.5 cm) was found at par with Mel 60ppm. Mel 40 ppm resulted in the second highest head diameter (10.1 cm and 8.1 cm) followed by 9.7 cm and 6.9 cm polar and equatorial diameter in Mel 20 ppm, respectively. Results further indicated that maximum individual head weight and yield (240 g and 1.9 kg/plot) was recorded with Mel 60 ppm which was at par with Mel 80 ppm (233.8 g and 1.8 kh/plot). The next highest head weight and yield (221.5 g and 1.7 kg/plot) was recorded with Mel 40 ppm followed by Mel 20 ppm (208.1 g and 1.6 kg/plot) against the minimum head weight and yield (199.9 g and 1.5 kh/plot) in Mel 0 ppm (control), respectively.

Enhanced head size, weight and yield in Mel treated broccoli may be attributed to improved photosynthesis, plant growth and vigour (Mansha Gul *et al*., 2018, 2021) that might have resulted in a better development of source leaves which synthesized and supplied more photosynthates to the sink for the formation of broccoli heads. Similar trend has also been noticed by Sermenli *et al.* (2011) for main head size in broccoli.

Food reserves and pigmentation

Findings of the study (Table 2) showed that application of Mel 80 ppm resulted in maximum levels of sugar and protein contents (129.0 and 88.0 µg/ml), respectively of broccoli

sprouts. Mel 60 ppm was established as the second best treatment in terms of sugar and protein content (125.4 and 81.1 µg/ml) followed by 40 ppm (118.2 and 79.5 µg/ml) and 20 ppm (111.3 and 73.8 µg/ml) of Mel compared to minimum sugar and protein contents (105.0 and 68.0) in control. Perusal of the data again pointed out that amount of both chlorophyll and carotenoid present in head of control plants (0.31 and 0.012 mg/gFW) was better when sprayed with different concentrations of melatonin wherein Mel 60 ppm was established as the most superior treatment and produced the highest amount of chlorophyll and carotenoid content (0.79 and 0.028 mg/gFW). Mel 80 ppm was proved as the next superior treatment in producing these compounds (0.64 and 0.021 mg/gFW). Mel 40 ppm and Mel 20 ppm followed the above treatments in order and resulted in chlorophyll and carotenoid content of 0.55 and 0.017 and 0.42 and 0.014 mg/g.FW, respectively.

An elevated level of fruit sugar and protein contents has been reported by Debnath *et al*. (2018) in tomato. Yang *et al*. (2019) stated that melatonin induced significant accumulations of fructose, glucose and sucrose in apple through the down regulation of *FRK2* expression, a gene encoding fructokinase (FRK).

Increased amount of chlorophyll due to foliar application of Mel may be attributed to its role in delaying Chl breakdown and simultaneously accelerating its *de novo* synthesis (Szafranska *et al*., 2017). Tal *et al*. (2011) stated that melatonin can also preserve chlorophyll content in plants by the radical rummaging activity of indoleamine of melatonin. Melatonin-induced increase in carotenoid content has been attributed to the expression of genes related to carotene biosynthesis (Sun *et al*., 2020). Results of the present study that increasing chlorophyll contents also showed a similar trend with respect to carotenoid also conforms the findings of earlier research (Ranmalbhai, 2014).

Antioxidant and membrane characters

Analysis of different antioxidant and membrane characters (phenol, total antioxidant, MDA and MSI) of broccoli sprouts under the influence of foliar spray of Mel (Table 3) indicated that the Mel 60 ppm resulted in highest value of phenol content in broccoli sprouts (178.2 mg/100 g DW) compared to the lowest value (151.9 mg/100 g DW) in control. The highest value of phenol in Mel 60 ppm was followed by Mel 80 ppm (170.1 mg/100 g DW), Mel 40 ppm (160.9 mg/100 g DW) and Mel 20 ppm (160.9 mg/100g DW). Data regarding total antioxidant showed that Mel 80ppm resulted in highest value of antioxidant (365.4 mg GAE 100 g DW). However, the values of antioxidant recorded with Mel 40 ppm (358.2 mg GAE 100g DW) as well with Mel 60 ppm (362.3 mg GAE

Table 1: Effect of foliar application of melatonin (Mel) onagronomic characters of broccoli head

Treatments	Polar diameter (cm)	Equatorial diameter (cm)	Head weight (g)	Yield (kg/plot)
Mel 0 ppm	7.1	6.5	199.9	1.5
Mel 20 ppm	9.7	6.9	208.1	1.6
Mel 40 ppm	10.1	8.1	221.5	1.7
Mel 60 ppm	11.6	10.4	240.4	1.9
Mel 80 ppm	10.7	9.5	233.8	1.8
CD (p≤0.05)	1.0	1.1	10.5	0.1

Table 2: Effect of foliar application of melatonin (Mel) onhead chlorophyll and carotenoid content of broccoli.

Treatments	Total sugars (µg/ml)	Soluble proteins (µg/ml)	Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Mel 0 ppm	105.0	68.0	0.31	0.012
Mel 20 ppm	111.3	73.8	0.42	0.014
Mel 40 ppm	118.2	79.5	0.55	0.017
Mel 60 ppm	125.4	81.1	0.79	0.028
Mel 80 ppm	129.0	88.0	0.64	0.021
CD (p≤0.05)	3.1	6.01	0.10	0.001

Figures in parenthesis are squire root transformed data for the purpose of statistical analysis.

Table 3: Effect of foliar application of melatonin (Mel) on antioxidant and membrane characters of broccoli sprouts.

Treatments	Phenols (mg/100 g DW)	Total antioxidant (mg GAE 100 g DW)	MDA (µ mol MDA/g FW)	MSI (%)
Mel 0 ppm	151.9	319.7	0.21	27.16 (5.21)
Mel 20 ppm	160.9	336.8	0.18	32.22 (5.68)
Mel 40 ppm	166.4	358.2	0.16	39.56 (6.29)
Mel 60 ppm	178.2	362.3	0.12	45.38 (6.96)
Mel 80 ppm	170.1	365.4	0.13	46.22 (6.57)
CD (p≤0.05)	5.8	7.9	0.01	0.25

100 g DW) were found at par with Mel 80 ppm. These values were significantly followed by Mel 20 ppm (336.8 mg GAE 100 g DW) while as untreated plants showed the minimum antioxidant value (319.7 mg GAE 100 g DW) than all other treatments. Perusal of the data further revealed that the amount of malondialdehyde (MDA) measured in the broccoli sprouts was found minimum (0.12 µ mol MDA/g FW) with Mel 60ppm which was also at par (0.13 µ mol MDA/g FW) with the plants treated with Mel 80 ppm. The minimum MDA value obtained was followed significantly in ascending order by Mel 40 ppm (0.16 µ mol MDA/g FW), Mel 20 ppm (0.18 µ mol MDA/g FW) and maximum (0.21 µ mol MDA/g FW) in control. Measurement of antioxidant potential in terms of membrane stability index (MSI) revealed that Mel 80 ppm exhibited highest MSI (46.22%) which was found at par with MSI value of 45.38% under Mel 60 ppm treated plants. However, these values were significantly followed by Mel 40 ppm (39.56%) and Mel 20 ppm (32.22%) while the least value of MSI (27.16%) was recorded with control.

The application of exogenous melatonin alleviated reactive oxygen species and cell damage induced by means of repairing mitochondria (Sharif *et al*., 2018). Melatonin is known to play an important role in the detoxification of reactive oxygen and free radicals and functions as an antioxidant in living organisms. As a broad-spectrum antioxidant, melatonin can directly eliminated ROS and the subsequent products, its derivatives, AFMK (N1-acetyl-N2-formyl-5-methoxyknuramine) and AMK (N1-acetyl-5-methoxykynuramine), can also scavenge ROS and further terminate the cascade reaction of lipid peroxidation. Thus, one molecule of melatonin may eventually scavenge ten molecules of radicals at least. Melatonin treatment can markedly decreased the content of ROS and thus alleviate oxidative damages induced by the excessive ROS accumulation (Huang *et al*., 2019).

Conclusion

Foliar application of Mel (@ 60 ppm) at 30 days after transplanting resulted in significant improvement in head diameter and head weight. This treatment also resulted in highest chlorophyll and carotenoid contents in broccoli sprouts. However, total sugar and protein contents of broccoli sprouts were significantly highest with Mel 80 ppm. A significantly highest level of phenols and MSI were evident with Mel 60 ppm while as the level of total antioxidants was at par with Mel 60 and 80 ppm. An inverse trend was recorded in case of MDA level of broccoli sprouts.

Conflict of interest

All authors decclare that they no conflict of interest.

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