Application methodology and physiological insights of melatonin hormone for water stress alleviation in black pepper (*Piper nigrum* L.)

Abstract

Black pepper is highly sensitive to water deficit stress especially during summer, resulting in significant losses in yield; therefore, strategies aimed at enhancing water stress tolerance are essential. Melatonin improves stress tolerance in plants; however, its method of application and optimum concentration in black pepper under water deficit stress remains unclear. Therefore, we conducted a two pot culture experiment during March and April, 2022 (var. Panniyur-1) to investigate the effects of foliar-sprayed and root-irrigated melatonin (50, 100 and 150 µM L^{-1}) on the recovery per cent and physiological mechanism under water stress. The treatment details were, *WW* - Well-watered; *WS* - Water stressed; *FM50* - Waterstress+50µM Melatonin (Foliar spray); *FRM50* - Water stress + 50µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant); *FM100* - Water stress + 100µM Melatonin (Foliar spray); *FRM100* - Water stress + 100µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant); *FM150* - Water stress + 150µM Melatonin (Foliar spray); *FRM150* - Water stress + 150µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant). The melatonin-induced enhanced stress tolerance could be attributed to improved recovery %, leaf relative water content, photosynthetic pigments, activity of antioxidant enzymes (SOD, POD, and CAT), and ultimately significantly relieved the inhibitory effects of water stress on leaves. After rehydration, melatonin-treated plants recovered more quickly than untreated plants. In addition, melatonin counteracted the water stress induced accumulation in proline content. Overall, the results of this study demonstrated that melatonin at 100 µM L^{-1} (Foliar spray and root irrigation) significantly alleviated the adverse effects of water deficit stress compared untreated plants. In addition, application of exogenous melatonin combined with root and foliar application is superior than foliar spraying alone.

Key words: Black pepper-water stress-melatonin-recovery-physiology

1. Introduction

"Water deficit is one of the major abiotic stress limiting crop productivity" [1]. Severe moisture stress affects the photosynthetic rate of plants by causing damage in the photosynthetic apparatus and reduction in the chlorophyll pigments. Accumulation of proline and maintaining leaf water content are found to be better physiological characters for the characterization of moisture stress tolerance [2]. "Black pepper (*Piper nigrum* L) is basically rainfed crop and requires a well distributed rainfall of 2000-3000mm for better productivity. It is highly sensitive to water deficit stress especially during summer, resulting in significant losses in yield" [3]. Lack of water stress management is a limiting factor to produce black pepper, which is the most important constraint in black pepper productivity.

"Therefore, strategies aimed at enhancing water deficit stress tolerance are essential. Recently, plant growth regulators have been widely used to regulate plant growth and improve plant stress tolerance. Melatonin (N-acetyl-5-methoxytryptamine) is an indole hormone widely present in plants and animals. Many studies reported that exogenous melatonin could enhance plant tolerance to water deficit stress" [4,5]. "Melatonin alleviates oxidative damage during water stress by directly scavenging ROS resulted in decreases in electrolyte leakage and MDA content and by enhancing antioxidant enzyme activities" [6,7]. "Melatonin also has crosstalk with other plant growth regulators such as gibberellin, jasmonic acid, and abscisic acid to regulate various physiological processes in plants under water stress. Application of melatonin altered the metabolic status and delayed protein degradation in the horticultural crops increased the chlorophyll content, the photosynthetic rates, and compatible osmolytes like proline and sugar compared to control plants" [8].

Therefore, it is interesting to investigate the effects of melatonin on black pepper which is the major spices crop of India. This study aimed to investigate the efficacy and appropriate concentration of exogenous melatonin in alleviating the negative effects of water deficit stress in black pepper. Physiological responses and antioxidant enzyme activities were also determined to better understand the mechanism of melatonin in tolerant mechanism.

2. Materials and methods

2.1 Experimental design and treatments

The pot culture experiments were conducted in the ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India. The different concentrations of melatonin with various method of application (foliar and root irrigation) were tested against water stress tolerance in the rooted black pepper cuttings (Variety: Panniyur-1) with six replication (Design: Completely random design) during summer (February to April, 2022).The variety Panniyur-1 is a high yielding black pepper variety which has occupied 70 % of black pepper cultivation area in the Western Ghats of southern India and it is susceptible to water deficit stress during summer (February to May).

The four months old rooted black pepper cuttings are planted in plastic pots containing potting mixture (top soil, sand and FYM 2:1:1, fortified with bio-control agents). The potted plants were placed in a rainproof condition during the entire period of experiment. The standard package of practices was followed in all the treatments as per the recommendations of ICAR-Indian Institute of Spices Research, Kozhikode. The monthly average air temperature of 27.3, 28.8 and 28.9℃, respectively.

After the acclimation period (30 days after planting) out of fifteen plants established (one plant in each pot) eight uniform plants were maintained in each treatment. The sprayed melatonin solution was prepared as follows: 2.3 g melatonin was dissolved in 10 mL ethyl alcohol as a stored solution. 1 mL of this stored solution was diluted to 2 L with deionized water and 0.05 % (V/V) Tween-20 as a surfactant. Every pot was sprayed with 100mL prepared solution. The experiment included eight treatments.

WW	Well-watered
WS	Water stressed
FM50	Waterstress+50µM Melatonin (Foliar spray)
FRM50	Water stress + 50µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant)
FM100	Water stress + 100µM Melatonin (Foliar spray)
FRM100	Water stress + 100µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant)
FM150	Water stress + 150µM Melatonin (Foliar spray)
FRM150	Water stress + 150µM Melatonin (Foliar spray + Soil drenching @ 50ml/plant)

The treatments were imposed when the soil moisture reached 24% (at field capacity) followed by the treated plants were exposed to a gradual, three-week water deficit stress by withholding irrigation until the soil moisture content reached 8-9 % (at stress). Soil moisture content was measured on a dry weight basis using gravimetric method. On day 20 of water stress treatment, the physiological parameters of the marked leaves were measured. Simultaneously, the same leaves were collected, and stored at -80 ^{o}C for the following measurements. The total soluble protein, chlorophyll content, proline content and antioxidant enzyme activity were measured. After completion of the stress period the plants were irrigated and the recovery percentage was measured 10days after irrigation.

2.2 Determination of recovery percent

The total plant recovery was determined by counting the total number of plants survived through the treatment by the actual number of plants per treatment.

$$\text{Plant recovery percentage} = \frac{\text{No. of plants survived}}{\text{Total No. of treated plants}} \times 100$$

2.3 Leaf Relative water content (LRWC)

"LRWC was measured following the method of" [9]. "After plucking leaf samples, their fresh weight (FW) was recorded on an electronic scale. For the measurement of turgid weight (TW), the leaves were submerged in distilled water for 24 h and weighed. Then, the leaf samples were oven dried at 80°C for 72 h to find out their dry weight (DW)". [9] The LRWC was determined as follows:

$$\text{LRWC (\%)} = (FW-DW)/(TW-DW)\times 100$$

2.4 Biochemical assay

All biochemical measurements were carried out on four fresh fully expanded leaves each from the six plants.

"Chlorophyll 'a', chlorophyll 'b, and total chlorophyll content were estimated by adopting the method of [10] and expressed as mg g^{-1} of fresh weight. Soluble protein content was estimated with TCA extract of leaves sample following the method of [11] and expressed in

mg g^{-1} fresh weight. Proline content of the leaf sample was estimated by the method of [12] and expressed as µg g^{-1} of fresh weight".

2.5 Estimation of antioxidant enzyme activity

An aliquot of 200mg of fresh leaves was homogenized in 5 mL grinding buffer i.e. 0.1M phosphate buffer (pH 7) containing 1 mM EDTA,1% PVP under ice cold condition. It was centrifuged at a speed of 15000rpm for 15 minutes at 4°C. The supernatant was collected to separate tubes and used for the enzymatic assays. Catalase assay was determined by [13]. Catalase (CAT) assay was carried out under 25°C with a 1.8mL assay buffer (pH 7), 1 mL $H_2O_2$and 0.2 mL of the enzyme extract. The activity was determined by UVspectrophotometer at 240nm by measuring the time required for the decrease in the activity. For Peroxidase (POD) enzyme assay per formed following [14] with slight modifications, 2.4 mL assay buffer (pH 7), 0.1 mL H_2O_2, 0.02 M guiacol and 0.2mL enzyme extract. The activity was determined at 436nm in UV spectrophotometer.

Superoxide Dismutase (SOD) was carried out by taking 2.3mL 0.1M phosphate buffer (pH7.8), 0.1mL of Na_2Co_3, 0.1 mL EDTA,0.2 mL 200mM methionine,0.1 mL 100µM freshly prepared riboflavin, 0.1 mL Nitro blue tetrazolium (NBT) and enzyme extract of 0.1mL. Two control was kept positive control with no riboflavin and negative control with only one sample kept in dark. The samples and the positive control tubes were exposed to direct sunlight for about a minute until the colour change. The absorbance was noted at 560nm [14,15].

2.6 Soil water content

Soil water content (SWC, ω %) was surveyed by collecting soil from all the pots in the same treatment and taken 100 g from the pooled sample for analysis. Soil sample was dried at 90^{o}C for 24 h. The analysis of soil gravimetric water contents was utilized for the measurements of SWC which was calculated as follows:

$$\omega(\%)=\{(Ww-Dw)/Dw\} \times 100\%$$

Where, Ww and Dw were the wet and dry weight of soil samples.

2.7 Statistical analysis

The experiments were performed in a completely random design with six replication. Differences between the treatments was tested using the WASP-Web Agri Stat Package 2.0 program. Statistical variance analysis was performed using ANOVA (coefficient of variation) and compared with least significant differences (LSD) at 5% level.

3. Results

3.1 Recovery per cent

After rehydration for 10 days, the melatonin treated plants with various concentrations recovered significantly and the plants treated with melatonin (FRM100 and FRM150)

showed faster and 100 % recovery. Water stressed plants without melatonin treatment (WS) showed very less recovery per cent (33%) due to death of plants atthe end of the experiment (Fig. 1).

3.2 Leaf relative water content (LRWC)

In the present study, the results showed that water deficit stress significantly reduced leaf relative water content (27.4 %) in stressed plants compared to control plants. Plants treated with melatonin through foliar and root irrigation showed a significant increase in LRWC (18.3, 24.3 and 24.7 % in FRM50, FRM100 and FRM150, respectively) than the untreated stressed plants. However, the significant difference was observed between the FM and FRM across the melatonin concentrations (Fig.2).

3.3 Chlorophyll pigments

The results of this study indicate that water stress had a significant negative effect on the chlorophyll *a*, chlorophyll *b*, and total chlorophyll levels in black pepper leaves. After 21 days of water deficit stress, chlorophyll concentration significantly decreased, but it was higher in melatonin-treated plants than untreated plants. The treatment FRM100 resulted in significantly higher in chlorophyll *a*, chlorophyll *b*, and total chlorophyll level (163.9 %, 159.1 % and 159.7 %, respectively) compared to untreated stressed plants, whereas other concentrations 50 µM and 150 µM resulted in slight increases in chlorophyll content (Table 1)

3.4 Proline accumulation

Under water stress, proline content in the black pepper leaf increased approximately 2.5-fold compared to the control plant (Fig. 3). Moreover, the proline content was significantly decreased in melatonin-treated plants to a variable degree. Compared with water stressed plants, all the melatonin treated plants showed significant decreases in proline contents. Moreover, the plants treated with FRM150 showed highly significant decreases in proline contents by 51.3 %, compared with water-stressed plants.

3.5 Total soluble protein

In this study, the soluble protein contents in black pepper leaves significantly decreased under water stress. Moreover, the application of melatonin FRM150 caused a significant increase in soluble protein content (39.6 %) compared to water stressed plants (Table 1).These results revealed that the soluble protein and proline in the leaves of black pepper adopt different strategies to avoid water deficit stress, and that exogenous melatonin application may reverse these changes, with the foliar application and root irrigation method especially at the concentration of FRM100 having a better protective effect than the foliar application alone.

3.6 Antioxidant enzyme activities

The experimental results showed that, all measured antioxidant enzymatic activities significantly ($p \leq 0.05$) increased under water deficit stress. The application of melatonin in various concentrations enhanced the activities of enzymatic antioxidants in plants under

water stress. The SOD activity increased slightly after the plant was subjected to water stress. Treating with exogenous melatonin showed significant difference in SOD activity compared to the water stressed plant, while the treatment FRM100, resulted in a significantly highest increase in SOD activity (212.0 U. g FW min^{-1}) and showed on par with FRM150 (Table 2).

CAT activity in the water stressed plant was significantly different compared to the control plant. Treating with exogenous melatonin FRM100 resulting in the significantly highest CAT activity while the treatment FRM150 yield on par with FRM100 (6.85 U. g FW min^{-1}) (Table 2). Similarly, application with FRM100 increased POD activity (9.65 U. g FW min^{-1}) compared to the water stressed plant and showed on par with FRM150 (Table 2). The values of SOD, CAT, and POD activities enhanced to the highest by 75.4 %, 38.9 %, and 81.0 % respectively, in FRM100, as compared to plants under water stress (Table 2).

4. Discussion

4.1 Recovery per cent

Melatonin application decreased leaf osmotic potential, suggesting that melatonin could be involved in regulating plant water status under water stress condition [6], thus improve plant stress tolerance and recovery ability.

4.2 Leaf relative water content

Leaf relative water content is the important factor indicating the water status and ability of plants to survive under water stress conditions. In the current study, the results showed that a significant decrease (26.4 %) in RWC in water stressed plants compared to well-watered plants, which might be due to a blockage of water transport from the roots to the shoots, through mesophyll cell turgidity and low leaf water potential, thicker leaf tissue or reduction of soil moisture [16,17]. “It was documented that the application of melatonin could prevent water loss through/from leaves by increasing the thickness of cuticle and spongy tissues” [18]. According to [16], “exogenous melatonin potentially deals with drought-stressed plants by maintaining the water balance and the turgor of plant cells”.

4.3 Total soluble protein

“Generally, plants produce high levels of osmotic regulating substances like soluble protein to increase cytoplasmic solute concentrations, increase cell osmotic pressure to reduce water potential, and reduce cell water loss” [19]. In this study, the soluble protein contents of black pepper leaves significantly decreased under water stress. High levels of Reactive Oxygen Species affect protein metabolism, causing decrease the protein content. In this present study, the exogenous application of melatonin had a significant effect on protein content of black pepper leaves. This effect is probably because of melatonin promotes the synthesis of heat shock proteins and protect proteins from damage, indicating that melatonin involved in the osmotic adjustment of black pepper plants, consistent with the result of a study on oat [20].

4.4 Chlorophyll pigments

"Water deficit stress directly affects the structure and activity of photosynthetic organs in plant leaves. In present study, application of melatonin effectively inhibited the degradation and increased the chlorophyll content and improved plant photosynthesis" [21]. The results of this study indicate that water stress had a significant effect on the chlorophyll a, chlorophyll b, and total chlorophyll levels in black pepper leaves. The treatment FRM50 resulted in a slight increase in chlorophyll content, whereas the FRM100 treated plants showed significant increase in chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents (102 %, 93.2 %, 99.3 %, respectively) compared with water stressed plants (Table 1).This findings suggests that melatonin under water deficit stress has alleviating effect on chlorophyll metabolism. "Additionally previous research has demonstrated that exogenous melatonin can reduce the drought induced chlorophyll degradation and improve photosynthetic rate in tomato and cucumber" [22,23]. "Melatonin treatment may help to prevent chlorophyll degradation by down-regulating the gene encoding the chlorophyllase enzyme" [24].

4.5 Proline accumulation

Drought stress may cause basic response of damage to the cell membranes, and instability in osmotic regulation [25]. Therefore, the levels of the proline often increase under stress conditions [26,27], as confirmed in the present study, wherein melatonin application significantly reduced the accumulation of proline under the water deficit stress condition (Fig. 3).These results suggest that the proline in the leaves of black pepper plants adopt different strategies to avoid water stress, and that exogenous melatonin application may reverse these changes, with the foliar application and root irrigation method (FRM) having a better protective effect than the foliar application (FM) method alone.

4.6 Antioxidant enzyme activities

Under abiotic stress conditions, more ROS generates by the plants that lead to oxidative damage, and in response to this, plants activate several ROS scavenging enzymes, including SOD, CAT and POD and to protect against oxidative damage [28]. "Melatonin functions as an antioxidant enzyme activator and protects plants from oxidative damage" [29]. Accordingly, the present study showed that water-stressed plants exhibited a decline in the activity of antioxidant enzymes, whereas plants treated with FRM100 showed significant increases in the activity of SOD, CAT and POD. In distinction, control black pepper plants under water deficit stress showed decreased activity of SOD, CAT and POD (Table. 2). Similarly [4] stated that during water stress plants decrease the antioxidant enzyme levels whereas melatonin treated plants showed increased antioxidant enzyme activity. In the present study, 100 μM exogenous melatonin application clearly alleviated oxidative damage in black pepper leaves, especially when used as the foliar application and root irrigation method, which suggests that under water deficit stress, exogenous melatonin treatment effectively protected the cell membrane against oxidative damage.

5. Conclusion

The results revealed that exogenous melatonin could alleviate the oxidative damage caused by water stress. Moreover, exogenous melatonin improved water stress tolerance

by enhancing the antioxidant enzyme activity and reducing ROS production, as well as compatible osmolytes like proline and reducing chlorophyll degradation which leads to maintaining net photosynthetic activity. The data presented in this study demonstrated that melatonin at FRM100 concentrations significantly alleviated the adverse effects of water deficit stress on the black pepper plants compared to other concentrations. Furthermore, the foliar spray and root irrigation of melatonin application resulted in significantly higher physiological regulation than the foliar application alone and reduced the effects induced by water stress in black pepper plants. The findings of this study provide methodology of melatonin application and evidence for the physiological role of melatonin and serve as a platform for its possible application in agricultural/horticultural crops or related fields of research.

References

1. Fedoroff NV, Battisti DS, Beachy RN, Cooper PJ, Fischhoff DA, Hodges CN, Knauf VC, Lobell D, Mazur BJ, Molden D. Radically rethinking agriculture for the 21st century. *Science.* 2010;327:833–834.
2. George KJ, Malik N, Vijesh Kumar IP, Krishnamurthy KS. Gene expression analysis in drought tolerant and susceptible black pepper (*Piper nigrum* L.) in response to water deficit stress. *Acta Physiol Plant.* 2017, 39, 104.
3. Prasada Rao GSLHV.Weather extremes and plantation crops in the humid tropics. Mausam. 2016;67(1):251–258.
4. Wei W, Li QT, Chu YN, Reiter RJ, Yu XM, Zhu DH, Zhang WK, Ma B, Lin Q, Zhang JS, Chen SY. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *Journal of Experimental Botany*. 2015;66:695–707.
5. Zhang N, Sun Q, Zhang H, Cao Y, Weeda S, Ren S, Guo YD. Roles of melatonin in abiotic stress resistance in plants. *Journal of Experimental Botany.* 2015;66(3):647–656.
6. Ye J, Wang S, Deng X, Yin L, Xiong B, Wang X. Melatonin increased maize (*Zea mays* L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. *Acta Physiologiae Plantarum* 2016;38(2):1–13.
7. Cherono S, Ntini C, Wassie M, Mollah MD, Belal MA, Ogutu C, Han Y. Exogenous application of melatonin improves drought tolerance in coffee by regulating photosynthetic efficiency and oxidative damage. *Journal of the American Society for Horticultural Science.* 2021;146(1):24–32.
8. Wang P, Sun X, Li C, Wei Z, Liang D, Ma F. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J. Pineal Res.* 2013;54:292–302.
9. Weatherley PE. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytol.* 1950;49(1):81–97.
10. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiology.* 1949;24:1–15.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951;193:265–275.

Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. *Plant and soil.* 1973;39(1):205–207.
13. Fijałkowski, Krzysztof, Kwarciak-Kozłowska, Anna. Phytotoxicity assay to assess sewage sludge phytoremediation rate using guaiacol peroxidase activity (GPX): A comparison of four growth substrates. *Journal of Environmental Management.* 2020; p.263.
14. Mishra NP, Mishra RK, Singhal GS. Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. *Plant physiology.* 1993;102(3):903–910.
15. Matamoros MA, Loscos J, Dietz KJ, Aparicio-Tejo PM, Becana M. Function of antioxidant enzymes and metabolites during maturation of pea fruits. *Journal of Experimental Botany.* 2010;61(1):87–97.
16. Cui G, Zhao X, Liu S, Sun F, Zhang C, Xi Y. Beneficial effects of melatonin in overcoming drought stress in wheat seedlings. *Plant Physiol. Biochem.* 2017;118:138–149.
17. Rahimi Y, Taleei A, Ranjbar M. Changes in the expression of key genes involved in the biosynthesis of menthol and menthofuran in *Mentha piperita* L. under drought stress. *Acta Physiol. Plant*. 2017;39. s11738-017.
18. Meng JF, Xu TF, Wang ZZ, Fang YL, Xi ZM, Zhang ZW. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: Antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J. Pineal Res.* 2014;57:200–212.
19. Verbruggen N, Hermans C. Proline accumulation in plants: a review. *Amino Acids.* 2008; 35:753–759.
20. Gao WY, Feng Z, Bai QQ, He JJ, Wang YJ. Melatonin-mediated regulation of growth and antioxidant capacity in salt-tolerant Naked Oat under salt Stress. International *Journal of Molecular Sciences.* 2019; 20(5):1176.
21. Shi H, Reiter RJ, Tan DX, Chan Z. INDOLE-3-ACETIC ACID INDUCIBLE 17 positively modulates natural leaf senescence through melatonin mediated pathway in Arabidopsis. *Journal of Pineal Research*. 2015;58:26–33.
22. Liu J, Wang W, Wang L, Sun Y. Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regulation*.2015;77(3):317–326.
23. Wang LY, Liu JL, Wang WX, Sun Y. 2016. Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. *Photosynthetica*. 2016;54:19–27.
24. Sharma A Wang J, Xu D, Tao S, Chong S, Yan D, Zheng B. Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Caryacathayensis* plants. *Science of the Total Environment.* 2020;713:136675.
25. Huang B, Chen YE, Zhao YQ, Ding CB, Liao JQ, Hu C, Zhou LJ, Zhang ZW, Yuan S, Yuan M. Exogenous melatonin alleviates oxidative damages and protects photosystem II in maize seedlings under drought stress. *Front. Plant Sci.* 2019.

26. Ahmad S, Kamran M, Ding R, Meng X, Wang H, Ahmad I, Fahad S, Han Q. Exogenous melatonin confers drought stress by promoting plant growth, photosynthetic capacity and antioxidant defense system of maize seedlings. *PeerJ*, 2019;(10):1–25.
27. Campos CN, Ávila RG, de Souza KRD, Azevedo LM, Alves JD. Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* L. plants. *Agricultural water management.* 2019;211:37–47.
28. Yildiztugay E, Ozfidan-Konakci C, Kucukoduk M, Tekis SA. The impact of selenium application on enzymatic and non-enzymatic antioxidant systems in *Zea mays* roots treated with combined osmotic and heat stress. *Archives of Agronomy and Soil Science.* 2017;63(2):261–275.
29. Imran M, Latif Khan A, Shahzad R, Aaqil Khan M, Bilal S, Khan A, Kang SM, Lee IJ. Exogenous melatonin induces drought stress tolerance by promoting plant growth and antioxidant defence system of soybean plants. *AoB Plants.*2021;13(4):plab026.

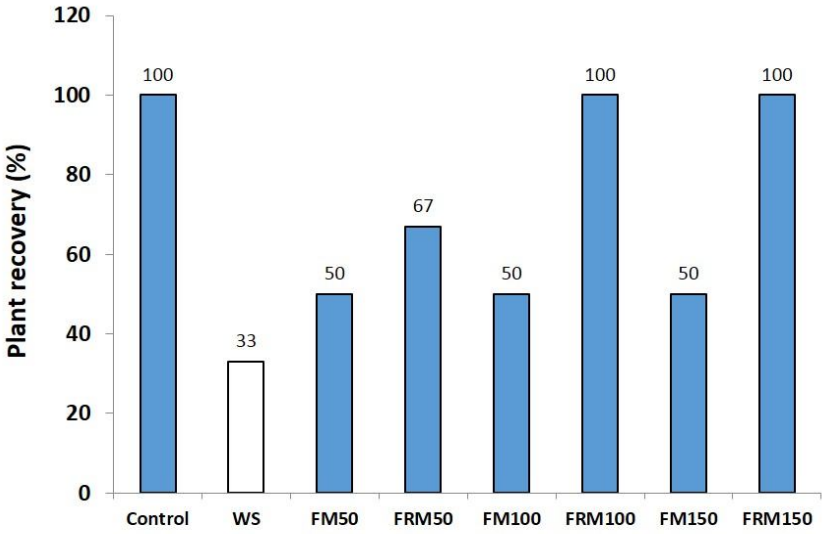

Fig. 1 Effects of melatonin on plant recovery in black pepper under water stress condition

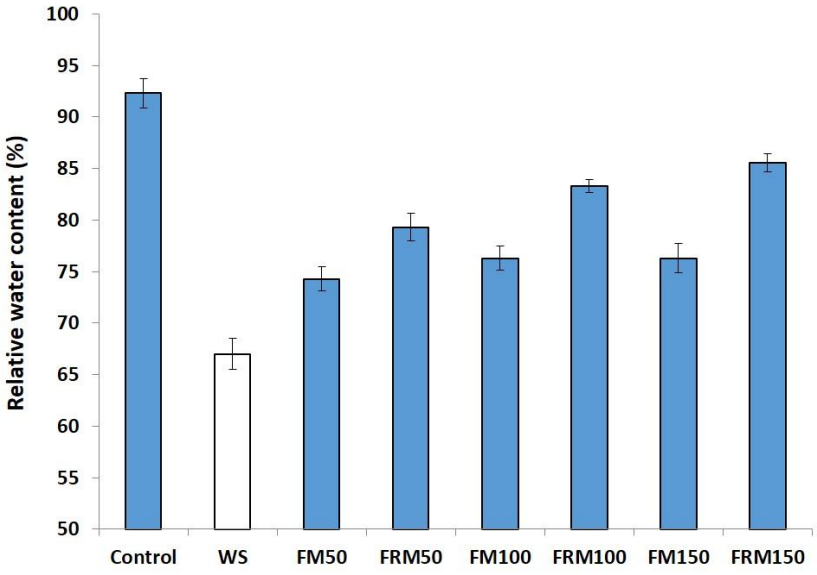

Fig. 2 Effects of melatonin on leaf relative water content (LRWC) in black pepper under water stress condition

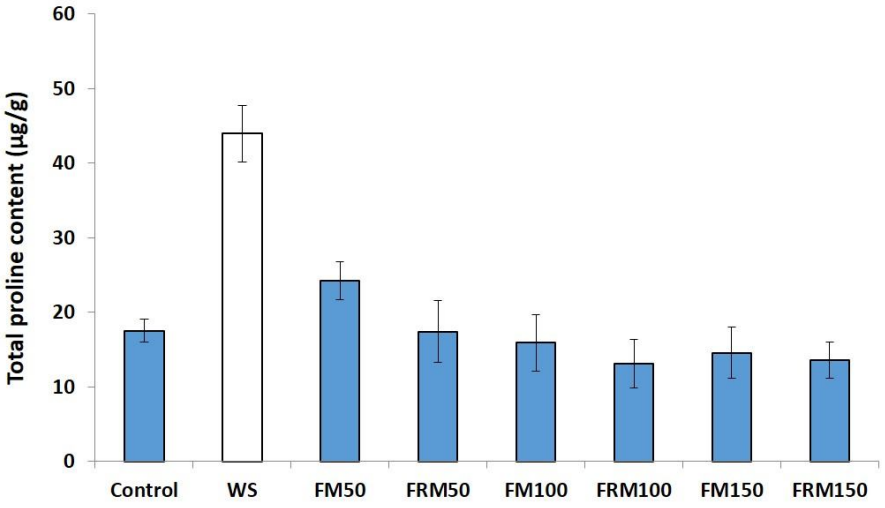

Fig. 3 Effects of melatonin on total proline content in black pepper under water stress condition

Table. 1 Effects of melatonin on Chlorophyll pigments and soluble protein in black pepper leaves under water stress condition

Treatment	Chlorophyll 'a' (mg g^{-1} FW)	Chlorophyll 'b' (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Soluble protein (mg g^{-1})
WW	1.147^{ab}	0.685^{a}	1.985^{ab}	24.46^{a}
WS	0.446^{d}	0.272^{e}	0.787^{e}	13.83^{d}
FM50	0.646^{cd}	0.374^{de}	1.084^{de}	20.04^{b}
FRM50	0.668^{cd}	0.420^{cd}	1.217^{cde}	19.67^{bc}
FM100	0.900^{bc}	0.541^{bc}	1.569^{bc}	15.66^{cd}
FRM100	1.177^{a}	0.705^{a}	2.044^{a}	17.66^{bcd}
FM150	1.010^{ab}	0.518^{bc}	1.436^{cd}	16.99^{bcd}
FRM150	0.977^{ab}	0.642^{ab}	1.536^{bcd}	19.32^{bc}
Mean	0.871	0.519	1.457	18.45
CV%	17.90	13.90	18.4	13.55
CD (0.05)	0.270	0.126	0.466	4.33

Table. 2 Effects of melatonin on antioxidant enzymes activity in black pepper leaves under water stress condition

Treatment	Superoxide dismutase (U. g FW min^{-1})	Catalase (U. g FW min^{-1})	Peroxidase (U. g FW min^{-1})
WW	92.0^{e}	4.20^{d}	4.73^{e}
WS	120.8^{d}	4.93^{c}	5.33^{d}
FM50	150.0^{c}	5.53^{bc}	6.48^{c}
FRM50	162.3^{c}	6.03^{b}	7.10^{b}
FM100	185.0^{b}	5.53^{bc}	6.33^{c}
FRM100	212.0^{a}	6.85^{a}	9.65^{a}
FM150	188.3^{b}	5.43^{bc}	6.10^{c}
FRM150	217.8^{a}	6.88^{a}	9.70^{a}
Mean	166.0	5.67	6.93
CV%	7.20	7.63	5.84
CD (0.05)	17.4	0.632	0.590