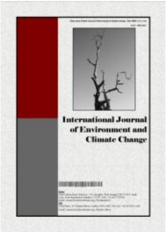

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Exogenous Application of Melatonin-loaded Nanoformulation for Yield Enhancement in Finger Millet (*Eleusine coracana*) under Drought Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the performances of melatonin-loaded nanoformulation on growth, yield and yield attributes of finger millet.

Place and Duration of the Study: The pot culture experiment was carried out under glasshouse conditions at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, during the *rabi* season of 2022.

Methodology: The Experiment was laid out in a completely randomized design (CRD) with four replications, including six treatments: T_1: no spray, T_2: 60 µM CF of MT, T_3: Surfactant alone, T_4: 20 µM NF of MT, T_5: 40 µM NF of MT, T_6: 60 µM NF of MT were used.

Results: Among various melatonin combinations T_6: Foliar spray @ 60 µM NF of MT recorded maximum photosynthetic rate, Fv/Fm ratio, antioxidant enzyme activity, and grain yield.

Conclusion: The present study demonstrated that foliar application of melatonin-loaded nanoformulation 60 µM was found to be an effective concentration for reducing drought effect and improving physiological, biochemical, yield and yield attributes in finger millet under drought conditions.

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Keywords: Finger millet; Melatonin; abiotic stress; photosynthetic activity; antioxidant activity.

1. INTRODUCTION

Agricultural productivity is directly and negatively impacted by drought. In rainfed regions, severe droughts resulted in a 20–40% reduction in agricultural output. Drought stress decreases the leaf water content, cell enlargement, plant growth, and stomatal closure. It leads to reduced photosynthetic rate, stomatal conductance, and photosystem activity, and finally reduces crop productivity. Drought stress causes a major effect on chlorophyll biosynthesis which reduces the intake of CO_2 and ultimately affects photosynthesis and plant biomass. Chlorophyll fluorescence showed the status of plant stress, especially the excitation energy by PSII activity, drought stress causes considerable damage to the PS II reaction center as well as degradation of D1 protein [1]. The changes lead to the production of oxidative molecules which eventually causes oxidative damage to the plant. Plants have developed several defenses against oxidative molecules that caused harm to cellular components, including the release of excess excitation energy and the production of antioxidants like catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD).

Millets are one of the most important agricultural crops. Due to their higher nutritional value millets are referred to as "Nutri-Cereals". These are rich in iron, protein, and antioxidants. Gluten-free status and low glycemic index of millets help prevent diabetes [2]. After sorghum and pearl millet, finger millet (*Eleusine coracana*) is an ancient and important millet crop of India and is served as a major staple food for Africa and Asia. It is widely grown in semi-arid and arid tropics. It has the highest amount of calcium (344 mg) and potassium (408 mg) content. While compare to rice, it has higher dietary fiber, minerals, and sulfur-containing amino acids. Finger millet productivity is low due to several factors viz., marginal and poor soils, inadequate moisture, and poor management techniques, among them deficit moisture stress causes low yield. Drought is the most common environmental stress that affects the growth and development of plants. Even though finger millet is a drought-tolerant crop, the yield potential is reduced when it is grown under rainfed conditions [3]. Among the millets, finger millet is more prone to water deficit [4], as an effect of drought stress causes a reduction in leaf area, radiation use efficiency, dry matter accumulation, and seed weight [5,6] resulting in a reduction of yield up to 50-80% [7] under drought condition. Due to this constraint, the farmers were facing more yield loss. Drought stress ultimately affects the grain filling process in finger millet. In recent years, foliar application of plant growth regulators (PGRs) and nutrients is one of the management options, which have been employed to overcome physiological constraints by rapid change in phenotype of the plant within the season to achieve enhanced production in crops [8]. Recent research has found that using plant growth promoters, antioxidants, and osmoprotectants is a highly effective strategy to encourage plant ability to adapt to drought stress.

Melatonin is emerging and plays a multifaceted role in ameliorating biotic and abiotic stresses of plants. It is a well-known antioxidant in plants and improves the various stress tolerance capacity. Melatonin works primarily as an antioxidant to scavenge free radicals and protect plants from environmental stresses [9]. Melatonin has been reported to alleviate drought stress in many kinds of crops like *Oryza sativa* (rice) [10] *Triticum aestivum* (wheat), *Zea mays* (Maize), *Glycine max* (soybean), *Manihot esculenta* (cassava), *Gossypium hirsutum* (cotton), *Malus domestica* (apple), *Vitis vinifer* (grape) and *Actinidia. chinensis var. deliciosa* (kiwifruit) [11]. Melatonin as seed treatment and foliar spray under drought showed better tolerance in finger millet by recording higher chlorophyll pigments, soluble protein and gas exchange parameters. Chlorophyll fluorescence, relative water content, proline content, osmotic potential and osmotic adjustment and grain yield were improved by melatonin in finger millet under drought [12].

Although there are many reports on the drought tolerance effect of melatonin, whereas, nano-loaded melatonin has not yet been the subject of any investigations. Nanotechnology is a multidisciplinary technology with enormous scope in agricultural research to improve crop production. The use of nanoencapsulation, nanoformulation and nano fertilizer provide site-specific and controlled delivery of active ingredients to increase productivity [13]. Nanoformulated plant growth regulators, such as auxin, have been found to improve plant growth and development [14]. The application of nanotechnology aims to improve farming systems and, enhance crop productivity while encouraging agricultural and environmental sustainability [15]. Therefore, this study proposed to use the nano-loaded formulation of melatonin for reducing the spray volume, and spray fluid concentration to develop cost-effective drought management technology. With this background, the objective was framed to study the effect of melatonin nanoformulation on growth and yield traits in finger millet under drought conditions.

2. MATERIALS AND METHODS

2.1 Synthesis of Melatonin-loaded Nanoformulation

Melatonin and tween 20 were purchased from Sigma-Aldrich, India, the nanoformulation was prepared by using the solvent evaporation method. Melatonin (1.2 mg) was dissolved in 2 5 mL of ethanol (99.9 %) and stirred at the speed of 300 rpm for 1h. About 22.5 mL of tween 20 (8%) was prepared and stirred at 400 rpm for 1h. The solution of melatonin in tween 20 was slowly dropped into an aqueous solution that was stirred (700 rpm) for 1h 30 min. The solution was transferred into a high-pressure homogenizer to reduce particle size (174 nm) by forcing liquid through high pressure and they were mixed again vortex for 15 min. Particle size and distribution pattern of synthesized nanoformulation was determined using Nanopartica SZ-100, Horiba Scientific. The concentration was measured using a UV-Vis Spectrophotometer.

2.2 Planting Materials and Location of the Study

The pot culture experiment was conducted under glasshouse conditions located in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. (11.01 ^{o}N, 76.39 ^{o}E, and 426.7 MSL), during *Rabi* 2022. Finger millet var. CO 15 was used to perform this experiment.

2.3 Research Methodology

In the pot culture experiment, red sandy soil was used. A soil mixture was prepared by combining red soil, sand, and vermicompost in a ratio of 3:1:1, and 20 kg of the soil mixture was filled in each pot. Finger millet seeds were sown directly in the pot at the depth of 2 cm. After seedling emergence, plants were thinned and three healthy seedlings were maintained per pot. After thinning, basal application of 1.5 g of urea, 0.5 g of diammonium phosphate, and 0.5 g of muriate of potash was applied per pot. Plants were irrigated once in every 5 days intervals until the pot attaints 100 % field capacity. Drought was imposed on the 56th day after sowing (ear head initiation) for 12 days by the withholding of water. During the drought stress period, the soil moisture content was measured by using an ML2 theta probe moisture meter (Delta-T Soil moisture kit - Model: SM150, Delta-T Devices, Cambridge). Two days after stress (on the 58th day) melatonin-loaded nanoformulation was given through foliar application to the plants. The physiological, biochemical, and yield traits were recorded on the 5th day of the stress period initiation for the following treatments: T_1: No spray, T_2: 60 µM conventional formulation (CF) of melatonin (MT), T_3: Surfactant alone, T_4: 20 µM nanoformulation (NF) of melatonin (MT), T_5: 40 µM NF of MT, T_6: 60 µM NF of MT were used. Each treatment was replicated four times, with three seedlings in each replication.

2.4 Traits Recorded

2.4.1 Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and chlorophyll fluorescence

On bright, sunny days between 9:00 and 11:00 a.m., the portable photosynthesis equipment (LI-6400 XT, LicorInc, Nebraska, USA) was employed, and four replications of each treatment were measured and expressed as µmol CO_2 m^{-2} s^{-1}. The fluorescence meter was used to measure chlorophyll fluorescence in fully expanded leaves that had been dark-adapted for 30 mins (Plant PAM-210, Heinz Walz, Germany). The key fluorescence parameters, Fo (initial fluorescence), Fv (variable fluorescence), Fm (maximal fluorescence), and the Fv/Fm ratio, were all measured. Fv/Fm is a ratio that depicts the proportion of quantum yield about a high degree of photosynthesis.

2.4.2 Catalase and superoxide dismutase enzyme activity

Catalase activity was determined as per the [16] method and expressed in enzyme unit µg H_2O_2 g^{-1} min^{-1}. According to [17], SOD activity was measured using the nitro blue tetrazolium (NBT) salt method and expressed in enzyme unit mg $protein^{-1}$ min^{-1}.

2.4.3 Yield and yield components

The ear heads were harvested when they reached their physiological maturity and kept for sun-drying for 2-3 days, then measured the weight of individual ear head (g) and grain yield per plant (g).

2.5 Statistical Analysis

All data were statistically analyzed in Microsoft Excel for statistical means, standard deviation, and standard error. The completely randomized block design (CRD) was used and significant differences between treatments were analyzed using Analysis of Variance (ANOVA) as described by [18].

3. RESULTS AND DISCUSSION

3.1 Photosynthetic rate (Pn) and Fv/Fm Ratio

Our result showed (Fig 1.) that drought stress caused a significant reduction in Pn and Fv/Fm ratio. However, melatonin nanoformulation significantly ($P<0.05$) increased the photosynthetic rate and Fv/Fm ratio in finger millet under drought stress conditions. Among different melatonin treatments, 60 µM NF of MT has recorded the highest photosynthetic rate (19.4 %) and Fv/Fm ratio (2.6 %) as compared to 60 µM conventional formulation of melatonin (MT). Drought stress causes a series of effects, such as disruption of the thylakoid membranes, inhibition of the light-harvesting system (PS I and PS II), reduction of the rubisco activity and disruption of the photosynthetic apparatus [19]. A previous study [20] demonstrated that foliar application of melatonin could reduce the drought effect and showed increased photosynthetic rate and photosystem II activity in rice seedlings. Our results revealed a positive relationship between Pn and Fv/Fm ratio, increasing of Fv/Fm ratio in melatonin-treated plants showed plant photosystem II (PS II) sensing low drought stress. Under drought circumstances, the flow of electrons via PS II is a good indicator of the total rate of photosynthesis, this might be a reason for increasing the net photosynthetic rate of the plant.

Melatonin treatment in apple seedlings under drought was reported to improve the photosynthetic rate and stomatal conductance, this could enable the supply of more photoassimilates to sink tissues [21]. Melatonin activates PS I and PS II reaction center and increases the photosynthetic efficiency of the photosystems. Exogenous melatonin application improved photosystem II efficiency (Fv/Fm ratio) in drought-stressed maize [22]. The above findings were incorporated into our current study.

3.2 Catalase and Superoxide Dismutase Enzyme Activity

Drought stress actuates the enzymatic antioxidant activities. However, the results (Fig 2.) revealed melatonin application on finger millet significantly ($P<0.05$) increased catalase and superoxide dismutase enzyme activity in leaves under drought. Foliar spray of 60 µM NF of MT enhanced the CAT enzyme activity up to 7.5 % and SOD enzyme activity about to 3.8% as compared to 60 µM CF of MT. However, drought stress increases the production and accumulation of O_2^- and H_2O_2 radicles and decreases the antioxidant enzyme activity. Enzymatic antioxidant defense system, SOD is a key enzyme that destroyed O_2^- to O_2 and H_2O_2, while CAT and POD can break down H_2O_2 to H_2O through a different pathway in plant cells [23]. Reactive oxygen species (ROS) caused negative effects on cellular membranes, oxidation of carbohydrates, breakdown of chlorophyll pigments, and impaired enzyme activity [24]. Melatonin scavenges the free radicals directly or indirectly and also improved the mechanisms of the cellular defense system against drought stress [25,26]. Melatonin treatments scavenge free radicals by an electron donation process that forms melatonin cation and a hydrogen donation reaction that forms nitrogen atoms [27]. Exogenous application of melatonin enhanced the antioxidant enzyme activity and alleviate the drought stress-induced ROS accumulation. Several studies have found that under stress conditions melatonin treatment increased the enzymatic antioxidants like CAT activity in maize [28], cucumber leaves [25], and buckwheat [29]. Our current study was consistent with previous findings regarding the increased activity of the antioxidant system (CAT and SOD) under drought conditions.

3.3 Yield Traits

Drought stress had a negative effect on ear head weight and grain yield plant^{-1}(Fig 3). Even though, the exogenous application of melatonin-loaded nanoformulation showed a pronounced effect on ear head weight and plant yield. A significant ($P<0.05$) result was observed in 60 µM NF of MT followed by 40 µM NF of MT. Melatonin-loaded nanoformulation was recorded highest plant yield (6.5 %) and ear head weight (9.2 %) when compared to the conventional formulation of melatonin. The occurrence of drought stress at the time of the heading stage reduced millet grain yield. It lowers the number

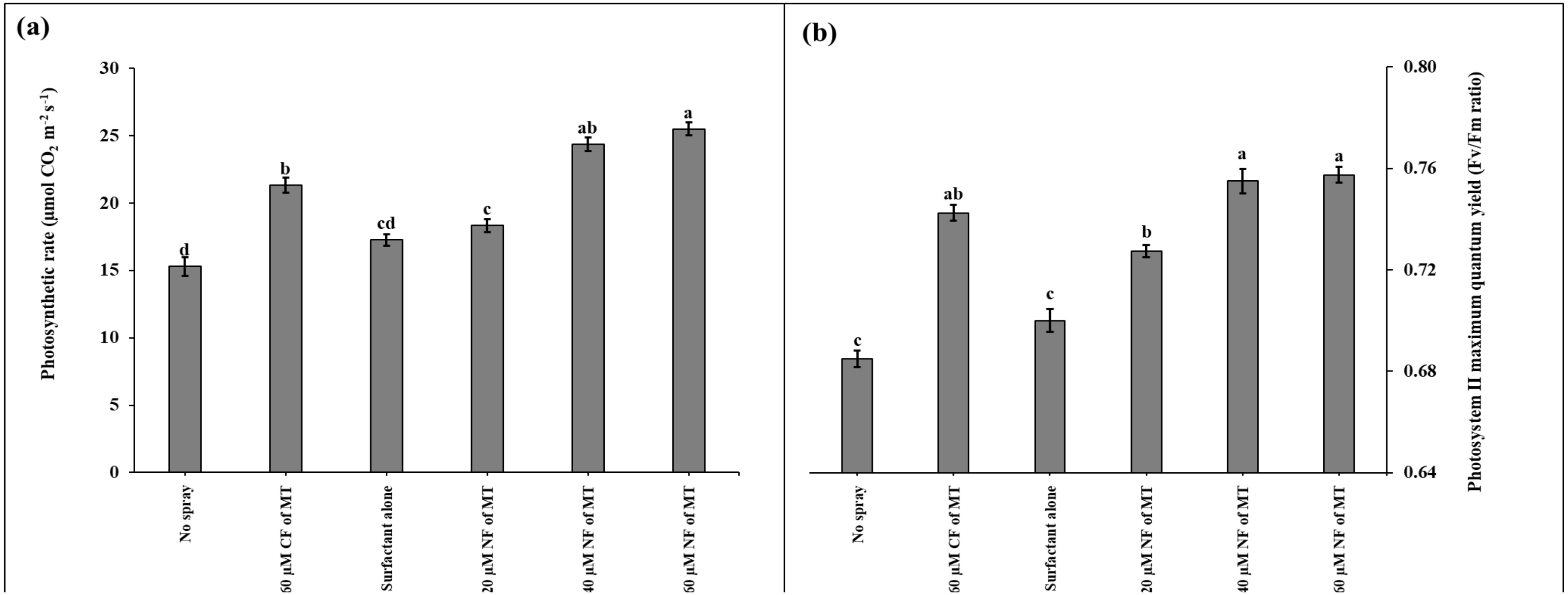

Fig. 1. Effects of melatonin nanoformulation on photosynthetic rate and Fv/Fm ratio on finger millet at 5th day of drought stress. Values are the mean of four replications ± S.E. Values with different letters are significantly different at *P*<0.05

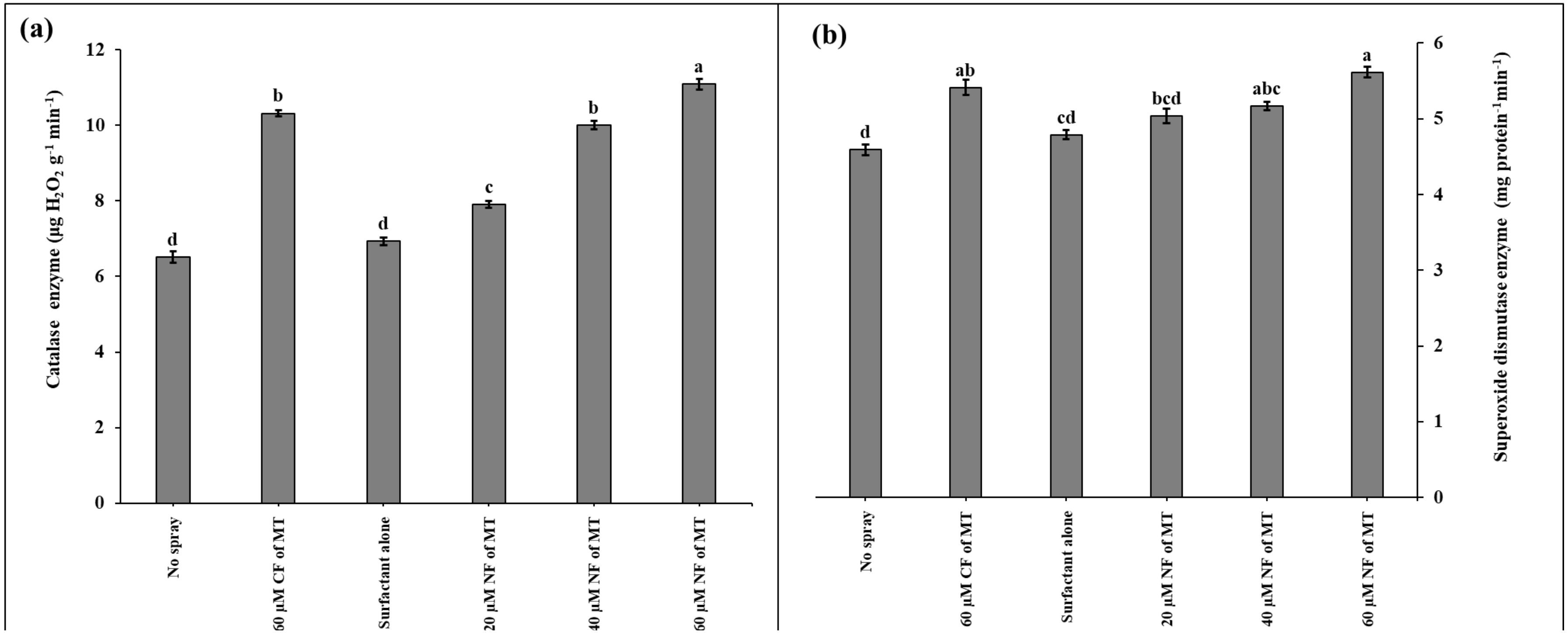

Fig. 2. Effects of melatonin nanoformulation on (a) catalase enzyme and (b) superoxide dismutase enzyme activity in finger millet on 5th day of drought stress. Values are the mean of four replications ± S.E. Values with different letters are significantly different at P<0.05

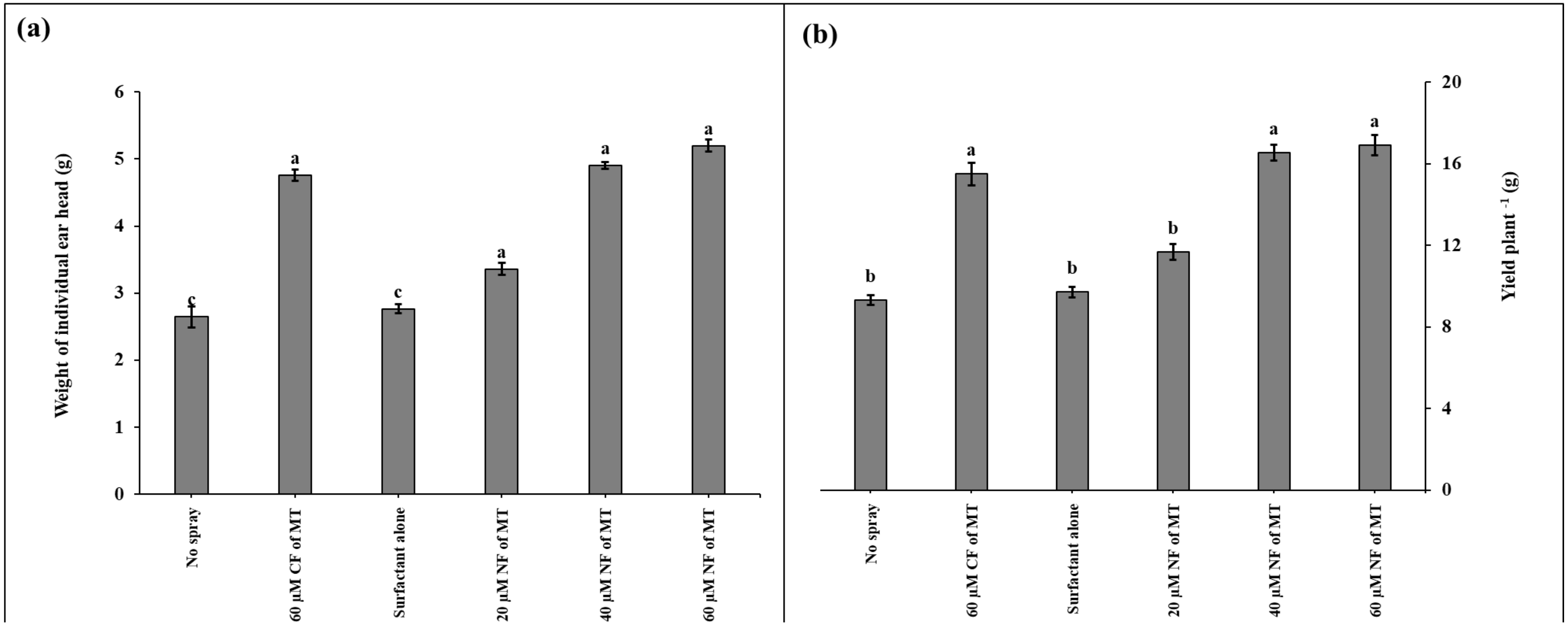

Fig. 3. Effects of melatonin nanoformulation on (a) weight of individual ear head (g) and (b) yield plant^{-1} (g) in finger millet on 5th day of drought stress. Values are the mean of four replications ± S.E. Values with different letters are significantly different at *P*<0.05

of panicles, grains per panicle, and grain yield [5]. Melatonin could improve the source activity, sink strength and translocation of assimilates. In previous findings, melatonin enhanced the performance of finger millet under drought stress conditions by maintaining high water status through the photosystem II activity and increased the antioxidant enzyme activity, which might have contributed to increasing the activity of source and the sink strength, the whole process could be responsible for higher ear head weight and plant yield in finger millet under drought condition. A similar result has been found in melatonin-treated corn seeds where melatonin-treated plants had larger cobs than untreated plants, resulting in a 20 % higher yield than control plants [30]. Application of melatonin increased maize grain yield [31] and soybean plant yield [32] under drought conditions.

4. CONCLUSION

The findings of the current study suggested that exposure of finger millet to drought stress inhibits the PS II system activity thereby it reduces the photosynthetic activity. It was clear that melatonin application through foliar spray improved the performance of finger millet under drought conditions by increasing photosynthetic activity, effective antioxidant system, and improved carbohydrate assimilation and translocation, which might be responsible for higher yield in finger millet under drought conditions. study overall, it could be concluded that photosynthetic efficiency, PS II maximum quantum yield, antioxidant enzyme activity, and grain yield are significantly increased by the application of melatonin-loaded nano-formulation under drought conditions. Further, synthesized melatonin nanoformulation showed positive results in alleviating the effects of drought stress in finger millet as compared to the application of conventional formulations of melatonin. Melatonin nanoformulation can be an effective solution for farmers to improve productivity and enhance drought stress tolerance in crop plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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