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Melatonin: Possible mechanism of commercial production by cyanobacteria for human welfare and sustainable agriculture

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ABSTRACT

Melatonin (MT), a multifunctional signaling molecule is endogenously produced in different microorganisms such as bacteria, cyanobacteria, algae, fungi, and plants, animals including human beings. In addition to controlling the sleep cycle, MT has gained popularity in treating various human diseases, including cancer, COVID-19, and neurological and psychiatric disorders. It plays an important role in abiotic stress tolerance in all living beings including plants and cyanobacteria. In comparison to plants and other eukaryotes, MT is less studied in cyanobacteria. Cyanobacteria are the first photosynthetic oxygen-evolving microorganisms. They play an important role as natural biofertilizers in the agriculture fields, hence widely used for human welfare and environmental sustainability. The current review emphasizes the biosynthetic mechanisms, the function of MT in cyanobacteria under abiotic stress conditions, and the application of MT in human welfare and sustainable agriculture. A possible method for commercial production of MT with the support of a biotechnology approach where cyanobacteria can be used as a natural source has been discussed in brief.

1. INTRODUCTION

Melatonin (MT) (N-acetyl-5-methoxytryptamine) is a signaling molecule present in almost all living organisms in nature. As an indoleamine, it has a diffusive amphiphilic character [1] and plays an essential role in several biological processes of animals, plants, and cyanobacteria [2,3]. In recent years, MT has been used in different industries such as dairy [4], biofuel [5,6], pharmaceuticals [7,8], and so on. According to the World Health Organisation, as the population continues to grow, food production will need to expand by 50% by 2029 to feed the population [9]. To achieve this goal the application of conventional chemical fertilizers will be necessary, which negatively affects soil nutrients and makes agricultural land infertile. Because chemical fertilizers have detrimental effects on the environment and human health, it is advised to use bio-fertilizers to prevent any health and environmental risks.

Cyanobacteria are emerging microorganisms used as bio-fertilizers that improve the quality and production of crops [10]. Currently, cyanobacteria have offered hope for more economic and environmentally friendly MT production. The detrimental effects of industrial pollution on agriculture have drawn more interest in MT. Cyanobacteria are prokaryotic photosynthetic microorganisms present in various habitats such as soil, freshwater, bare rock, and the ocean [11]. They are the first oxygenic photosynthetic organisms on the Earth that originated approximately ~3,500 million years ago during the Precambrian period and play a significant role in CO_2 and N_2 fixation. The most effective cyanobacteria for nitrogen fixation include *Anabaena variabilis, Tolypothrix* sp., *Aulosira fertilisima, Nostoc linkia*, *Scytonema* sp., *Calothrix* sp., and so on. About 20–30 kg of N ha^{-1} and organic matter are added to soil by cyanobacteria and they are typically found in the areas where rice is cultivated [12].

Numerous other researches have demonstrated how cyanobacteria can be used in agriculture to boost yield, dry weight, and root and stem growth in rice and wheat [13]. The second most crucial nutrient for plants and soil microbes is phosphorus, after nitrogen. Cyanobacteria can increase the bioavailability of phosphorus to plants by solubilizing and mobilizing the insoluble organic phosphates present in the soil with the help of phosphatase enzymes [14]. Cyanobacteria such as *Anabaena* sp*.*, *Anabaenopsis* sp*.*, *Calothrix* sp., and so on, produce plant hormones such as auxins, cytokinin, and gibberellin. Hence, cyanobacteria increase the amount of nutrients that plants require and promote germination, growth, and development [13,14]. Exopolysaccharides are released by cyanobacteria in the upper crust of the soil, keeping soil particles together and hence enhancing the water-holding capacity and organic matter of the soil. This improved soil moisture and organic matter favours the growth and development of cyanobacteria that support plant growth. Consequently, cyanobacterial growth improves the chemical and physical properties of soils [15].

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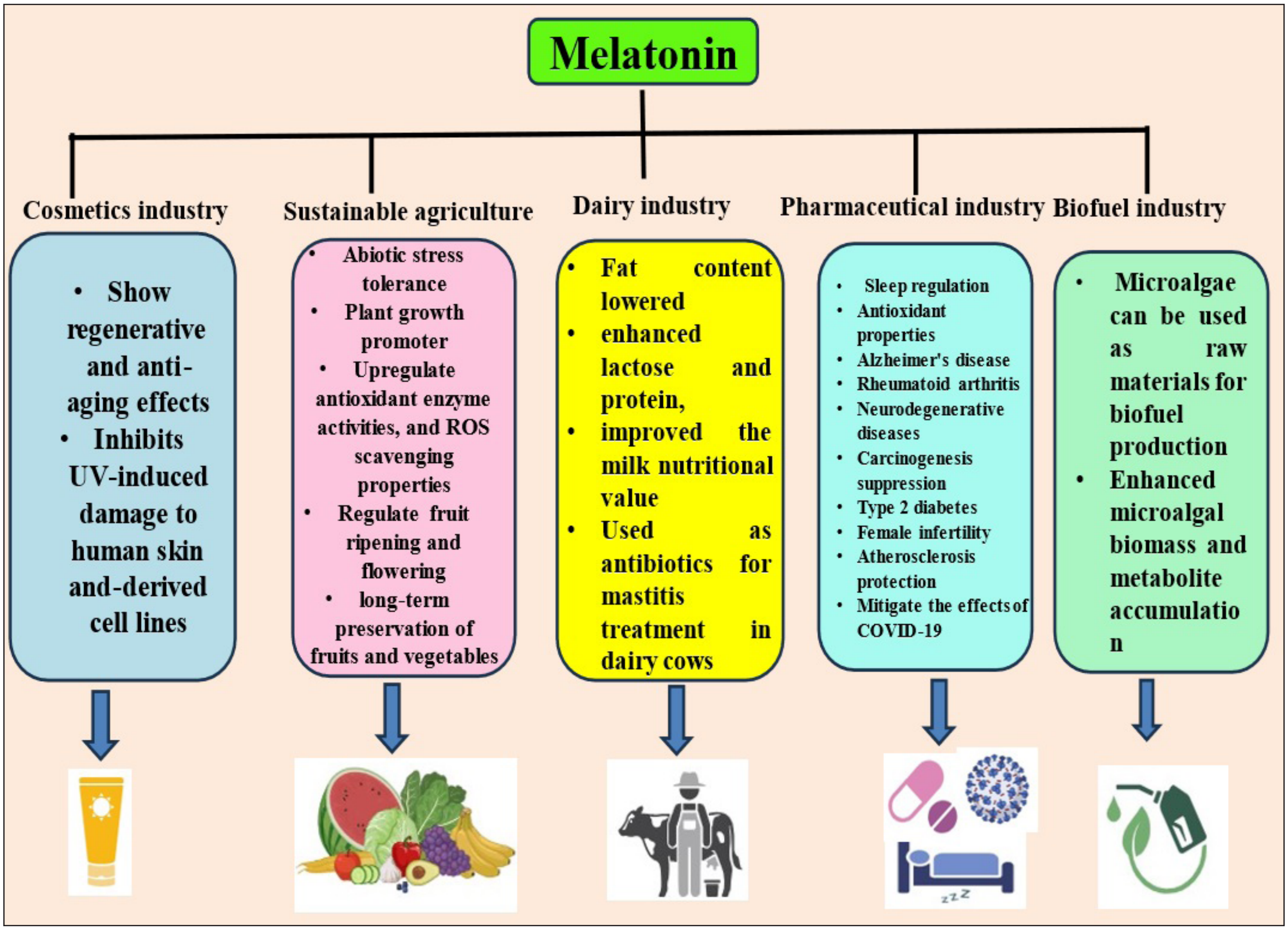

Figure 1. Melatonin application in human welfare.

Cyanobacteria have a higher growth rate than plants and eukaryotic algae. They produce several essential bioactive compounds widely used for industrial and therapeutic applications [13,16]. One such bioactive compound produced by cyanobacteria is MT [17]. It is an emerging signaling molecule that regulates stress tolerance and several metabolic processes in cyanobacteria and plants [3]. By scavenging reactive oxygen species (ROS), neutralizing free radical species, and activating the antioxidant defense system, it plays a very important role in abiotic stress alleviation [18]. Although in previous studies, MT application in abiotic stress alleviation in plants and algae has been significantly discussed; however, it is not adequate in the case of cyanobacteria. Hence, a review that highlights current research on MT-mediated abiotic stress tolerance in cyanobacteria is of great importance. To the best of our knowledge, this review is the first to discuss the alleviatory role of MT in cyanobacteria exposed to abiotic stress, the biosynthetic pathway of MT in cyanobacteria, and the commercial production of MT from cyanobacteria (Figs. 1 and 2). In addition to this, the protective functions of MT against a range of abiotic stressors, and its applications in human welfare have been outlined in this comprehensive review to improve knowledge of sustainable agriculture practices and the application of MT in human welfare.

2. MT AN ANCIENT SIGNALLING ANTIOXIDANT MOLECULE

MT is a pleiotropic molecule that has a wide range of physiological and cellular effects in all living creatures. It functions as a neurohormone in animals and humans and was first discovered in the bovine pineal gland in 1958 [19]. It has a molecular mass of 232.28 and a density of 1.269 g cm^{-3}, and the chemical formula is $C_{13}H_{16}N_2O_2$. The majority of notifications received by the European Chemicals Agency about the classification, labeling, and packaging of chemicals and mixtures led it to conclude that there were no classified hazards associated with MT. In animals, this indoleamine level regulates a wide range of physiological events that impact various biological processes, such as circadian rhythms such as sleep-wake cycles, mood, motor activity, body temperature fluctuations, retinal physiology, reproductive physiology, and so on, discussed in Figure 1 [20,21]. Like in mammals, MT controls various functions in plants, and cyanobacteria [3,18,22] (Fig. 3). It is a versatile signaling biomolecule widely present in animals, plants, humans, bacteria (aerobic photosynthetic bacteria), cyanobacteria, macroalgae, and fungi [17,22–26].

With the significant capacity to enhance stress tolerance by scavenging ROS, MT acts as an antioxidant molecule, hence its exogenous application recovers several physiological and biochemical functions of cyanobacteria under different abiotic stress situations [2,3] (Fig. 4). Certain studies have demonstrated that exogenous MT can mitigate abiotic stresses such as high light [27] and salt [28] in microalgae and *Nostoc flagelliforme* under salt stress [3]. It also enhances protein accumulations, chlorophyll contents, photosynthetic efficiency, and up-regulates the antioxidant defense system hence promoting stress tolerance and activating many signaling pathways in microalgae [28] (Fig. 4). As a result, there is growing research interest in the exogenous

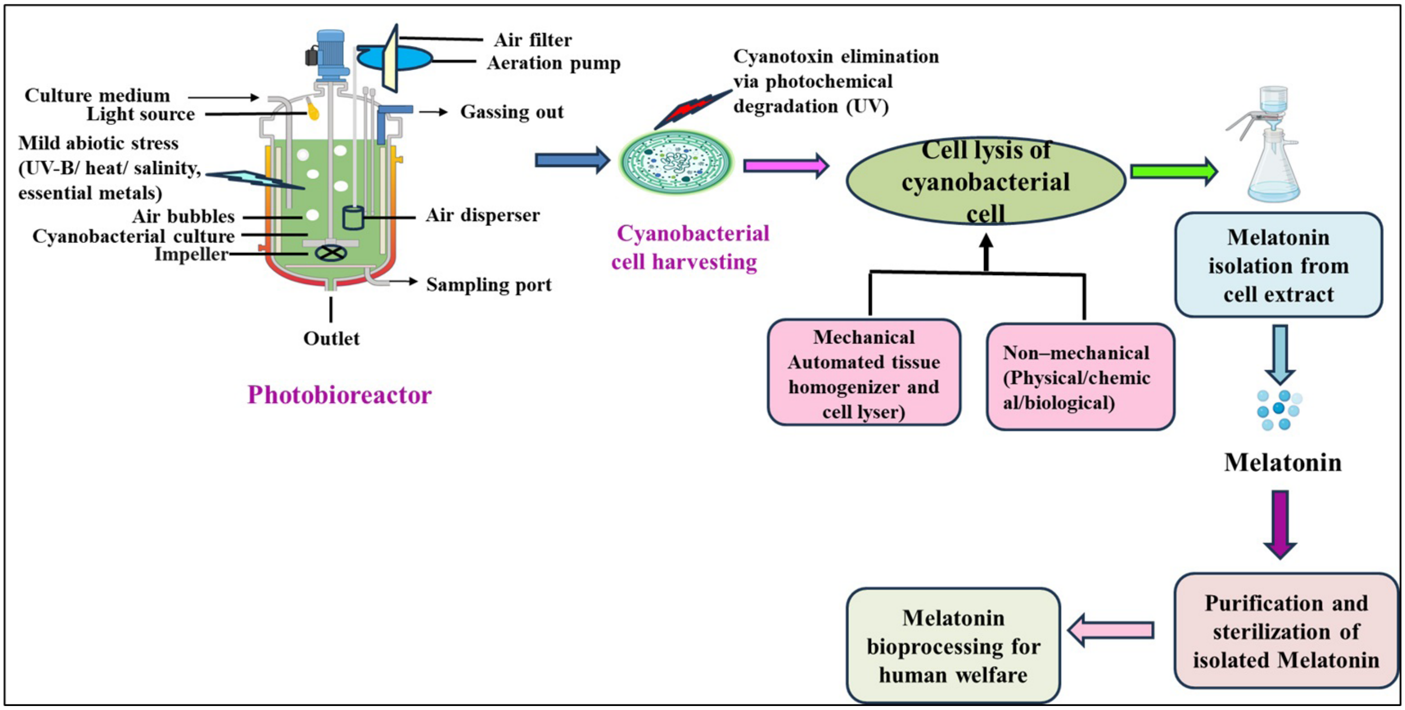

Figure 2. Proposed model for melatonin production by cyanobacteria.

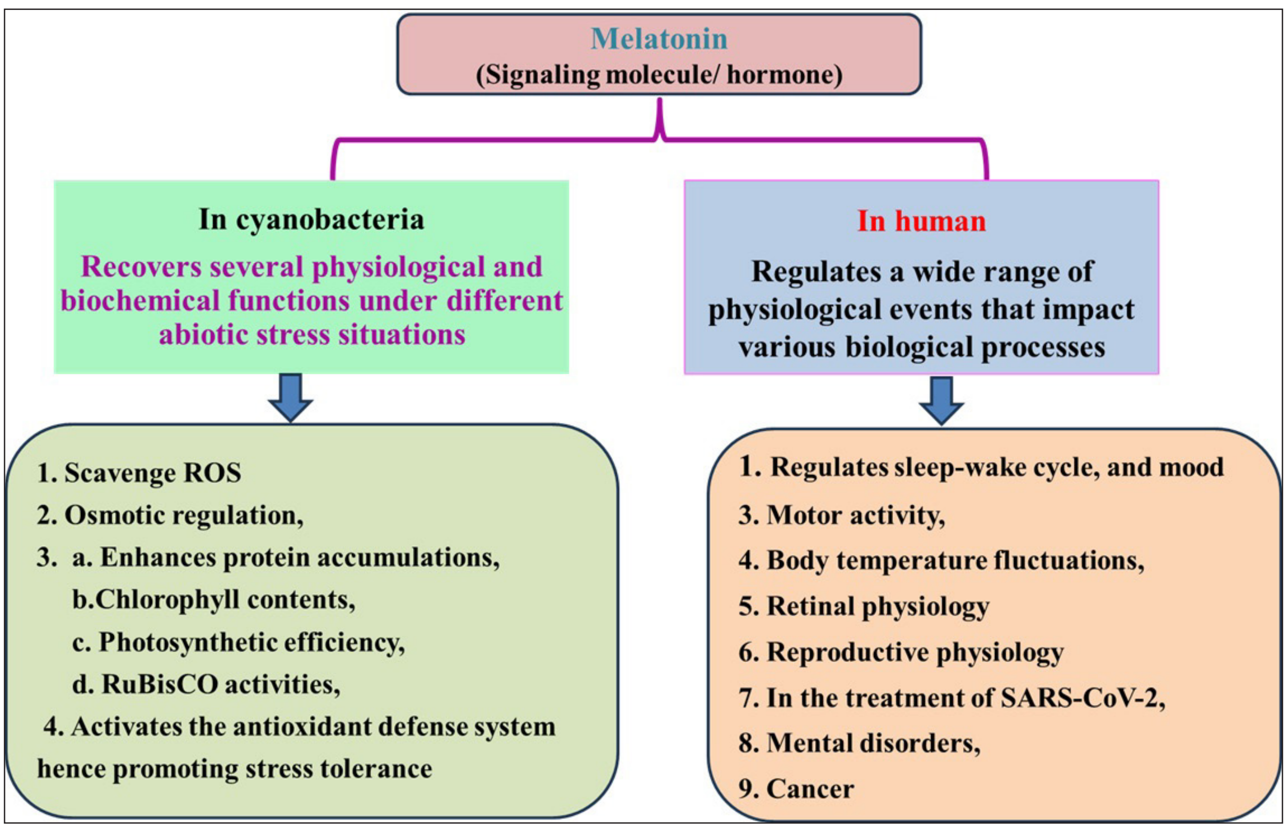

Figure 3. Impact of melatonin on cyanobacteria and human.

application of MT as a growth-promoting and stress-protecting molecule [22,29]. Therefore, compared to other synthetic compounds MT application to plants, algae, cyanobacteria, and humans could have various benefits [9,25–27].

3. BIOSYNTHESIS OF MT IN CYANOBACTERIA

It is considered that chloroplasts originated from photosynthetic cyanobacteria, whereas mitochondria evolved from ingested α-proteobacteria. Both organelles have probably continued

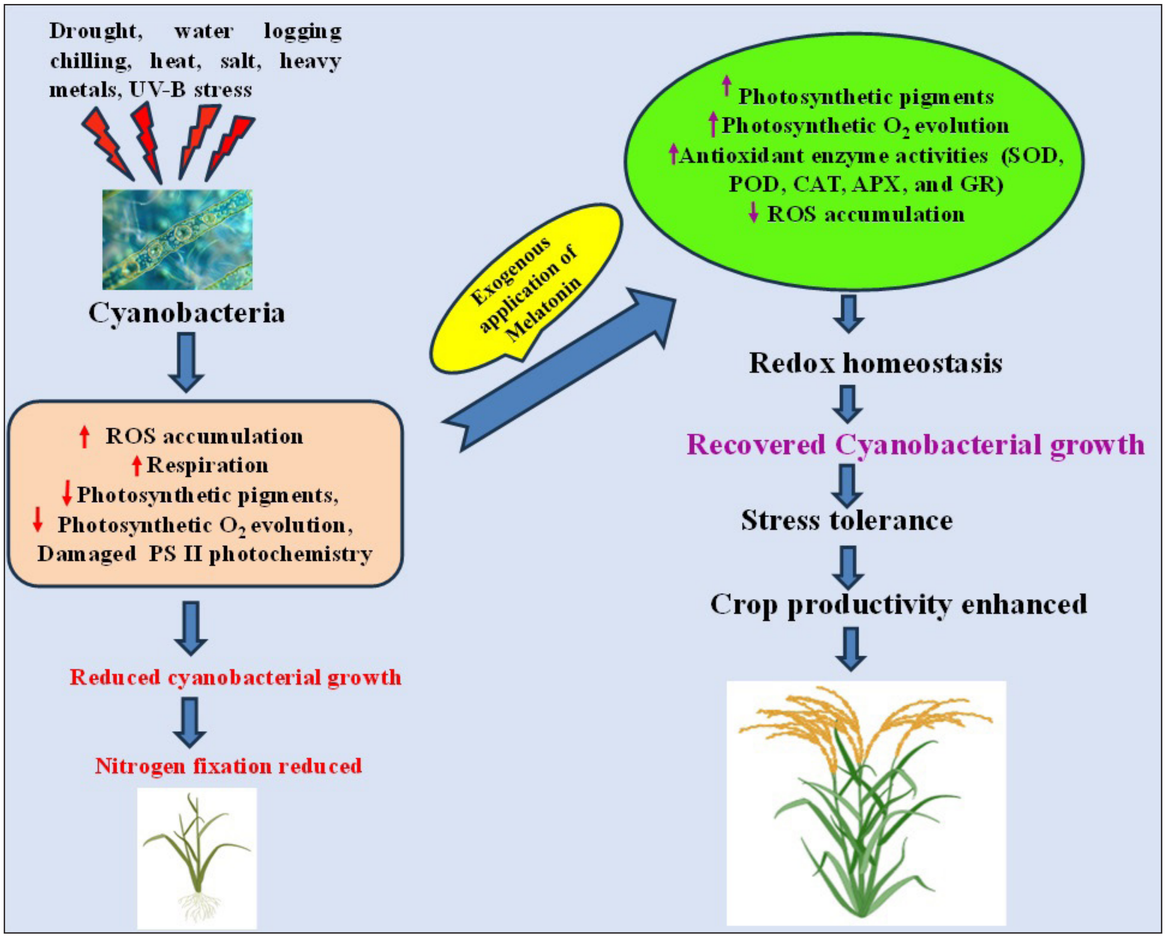

Figure 4. Melatonin mediated abiotic stress alleviation.

synthesizing MT throughout their evolutionary history [17]. MT is synthesized by enzymatic processes that are facilitated by enzymes in several cellular compartments, such as the cytoplasm, endoplasmic reticulum, chloroplasts, and occasionally in the mitochondria [22]. According to Gao *et al.* [30] MT biosynthesis is catalyzed by a minimum of six enzymes, which are tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), *N*-acetyl serotonin methyltransferase (ASMT), and caffeic acid *O*-methyltransferase. These various synthetic pathways and enzymatic activities are essential for maintaining MT levels, which regulate plant development and environmental adaption [31]. Zhao *et al*. [29] has suggested that the MT biosynthesis mechanism in microalgae is similar to that of higher plants. Previous research shows a considerable amino acid sequence homology between the main enzyme, SNAT, and its analogous enzyme in cyanobacteria. SNAT catalyzes N-hydroxytryptamine to N-acetyl-5-hydroxytryptamine in higher plants [32].

However, cyanobacteria and bacteria may use a similar pathway to synthesize MT. In the cyanobacteria, the TPH enzyme converts tryptophan into 5-hydroxytryptophan. Then TDC converts 5-hydroxytryptophan into 5-hydroxytryptamine (serotonin). Furthermore, SNAT changes into N-acetylates serotonin. ASMT, a hydroxy indole-O-methyltransferase, subsequently methylates N-acetyl serotonin to produce MT [26] (Fig. 5). Acetylated serotonin is the source of MT. Tryptophan amino acid synthesizes both Indolic amines, this biosynthetic pathway has been investigated considerably in plants and animals [33,34] but it is less explored in cyanobacteria. Previous research has indicated that MT regulates the production and accumulation of secondary metabolites in cyanobacteria similar to plants and microalgae and improves their resistance to stress [29].

4. MT-MEDIATED ENVIRONMENTAL STRESS TOLERANCE IN CYANOBACTERIA

Environmental stresses are currently one of the biggest issues imposing challenges for crop plants [29]. According to dos Reis *et al*. [35], one or more abiotic stressors can affect 90% of arable land and result in yield losses of up to 70% for major food crops. The productivity of important crops, such as rice, wheat, and maize, is expected to continue declining, according to estimates based on the integration of crop yield models and climate change [36,37,38]. The various abiotic stresses primarily affect cyanobacteria's physiology and metabolic behaviors [3,10,39]. Heat [40], chilling [41], salt [42], and heavy metal stress [43], can be better tolerated by plants after exogenous application of MT.

Though the possible impact of MT on plants and microalgae is significantly reported but less explored in cyanobacteria. For instance, it reduced oxidative damage caused due to salt stress by increasing the activity of antioxidant enzymes with enhanced expression of antioxidant genes (*NfCAT, NfSOD,* and *NfGR*) and scavenging hydrogen peroxide (H_2O_2) in the *Nostoc flagelliforme* [3] (Table 1). Exogenous MT can stimulate the endogenous biosynthesis of MT in response to various abiotic stressors, thereby scavenging H_2O_2 and

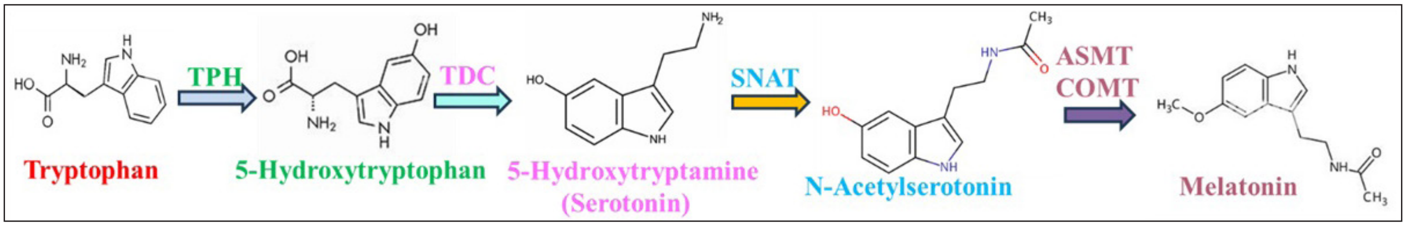

Figure 5. Biosynthetic pathway of melatonin in cyanobacteria.

$O_2^{\bullet-}$. In this way, it efficiently controls damage to cell membranes. MT application enhances photosynthesis, the main mechanism by which MT improves tolerance to various abiotic stresses [3,29,55] (Fig. 4).

The initial step in detoxification of oxidative stress is to scavenge free radicals using the antioxidant enzyme superoxide dismutase (SOD), which converts $O_2^{\bullet-}$to O_2 and H_2O_2. Furthermore, catalase (CAT) removes H_2O_2 by breaking down H_2O_2 to H_2O. The enzymatic action of ascorbate peroxidase can decrease the build-up of H_2O_2 in the ascorbate-glutathione cycle. According to Wang *et al.* [56], glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), thus maintaining the GSH/GSSG ratio in the organism. As a result, algal cells are protected from free radical damage by the actions of antioxidant enzymes and the redox state of primary antioxidants [27]. MT acts as the first line of defense against abiotic stress because it is a strong antioxidant [57]. The findings demonstrated a strong correlation between enhanced ROS detoxification and decreased cellular damage caused by exogenous MT. This was directly associated with the CAT, SOD, and GR pathway, which are important for the up-regulation of antioxidant enzymes [58].

Interaction between MT and other plant hormones focused on auxin because of their similarity in molecular structure [59,60]. Nevertheless, several studies have demonstrated intriguing connections between MT and almost every known plant hormone, including jasmonic acid, salicylic acid (SA), polyamines, strigolactones, brassinosteroids, auxin, abscisic acid (ABA), cytokinins, and gibberellin [59] and also with various signaling molecules such as gamma-aminobutyric acid (GABA), Ca^{2+} and so on (Table 1). Additionally, through the Ca^{2+}regulatory signal genes such as *CIPK3* and *CIPK9*, the Ca^{2+} signal pathway contributes to MT-mediated abiotic stress tolerance [61]. When MT and Ca^{2+} were applied together ROS-generating enzymes (NADPH oxidase and glucose oxidase) were decreased [62].

5. CYANOBACTERIA IN MT PRODUCTION

MT is a hot commodity in today's anxious world. However, as yearly demand rises, a sustainable and effective synthesis technique is required to meet market demand. Currently, it is predicted that 50–70 million US citizens are thought to suffer from a sleep or circadian disorder. The MT market was valued at USD 1 billion in 2020 and is estimated to grow to 3.4 billion USD by 2026. It was recently reported that over 300 million Chinese citizens experience sleeping disorders [45]. The previous study by Arnao *et al*. [26] discussed synthetic MT production from different microorganisms. Bacteria and fungi are heterotrophic and hence need an external supply of energy sources, i.e., carbohydrates, and other supplements. Further to prevent contamination aseptic conditions are required which increases the production costs. Considering this, MT production from cyanobacteria appears to be the most promising sustainable approach (Fig. 2) due to the photoautotrophic nature of cyanobacteria.

In addition to this, it might be a safe and cost-effective method compared to bacteria and fungi and a similar approach has been suggested in the earlier study of Mahawar *et al*. [63] for commercial production of GABA by cyanobacteria. Cyanobacteria are sustainable systems and MT accumulation can be enhanced under mild abiotic stress conditions such as UV-B, temperature, salinity, heavy metals, and so on. The proposed schematic model for commercial production of natural MT includes the mass scale production of cyanobacteria in a self-sustaining bioreactor under mild stress conditions, thereafter harvesting of cyanobacteria cells which are followed by cell lysis, separation, and purification of MT (Fig. 2).

5. MT IN HUMAN WELFARE AND SUSTAINABLE AGRICULTURE

5.1. MT as medicine

MT has multiple healthcare benefits. It is commonly used to treat immunity, circadian rhythms, and sleep-related disorders [64–66]. Its beneficial effects have also been demonstrated recently in the treatment of many cancers, especially when combined with chemical and radiation therapy [17], and also used for carcinogenesis suppression [67]. MT consumption reduces the toxicity of chemotherapy drugs in cancer patients [68]. According to Daryani *et al.* [69], it has a therapeutic effect in treating parasitic infections, primarily caused by parasitic protozoa. MT is thought to improve the host's immune response to infections including giardiasis, amoebiasis, malaria, Chagas disease, and so on. Due to the multifunctional properties of MT as an anti-inflammatory, antioxidant, and antiviral against certain viruses, it may be prescribed to patients with severe acute respiratory syndrome coronavirus-2 either alone or as the complement of other medications [70–72]. MT is also used in the treatment of asphyxia [72], Alzheimer's disease [73], rheumatoid arthritis [74], neurodegenerative diseases [75], temporal organization improvement during aging [76], type-2 diabetes [77], female infertility [78] protection against vascular disease and atherosclerosis development [79].

5.2. MT Up Regulates Lipid Production

The limited supply of non-renewable fossil fuels will run out in a few decades. Numerous researchers are inspired to investigate novel renewable energy sources. One of the greatest options for this is thought to be biodiesel [80]. Microalgae can synthesize significant amounts of lipids under unfavorable conditions, such as nutritional stress, high light intensity, salinity, and heavy metal stress. Hence, they are potential raw materials for biofuel generation [6]. The use of microalgae as a sustainable feedstock for biodiesel production has expanded due to the enhanced lipid content of microalgae. MT can be applied to increase microalgal biomass and metabolite accumulation [5]. In *Monoraphidium* sp. QLY-1, the lipid content has been increased 1.22 times when exogenous MT is added under stress conditions [81]. Cyanobacteria also serve as model organisms for biofuel production by genetic manipulation [81,82].

Table 1. Regulatory role of melatonin in abiotic stress mitigation in microalgae and cyanobacteria.

S.No.	Organism	Abiotic stress	Melatonin role in stress alleviation	MT interaction with signalling molecules/ phytohormones	References
1.	*Monoraphidium* sp. QLY-1	Salinity	Lipid accumulation is regulated by upregulating the transcription levels of lipogenesis-related genes hence alleviating salt-induced oxidative stress	-	Zhao *et al*. [28]
2	*Haematococcus pluvialis*	High light	Upregulates carotenogenesis, lipogenesis and antioxidant enzymes, and modulates autophagy and ROS signaling	-	Zhao *et al*. [44]
3.	*Haematococcus pluvialis* LUGU	Nitrogen limitation and high light condition	Stimulation of NO and SA production hence astaxanthin accumulation enhanced	Nitric oxide , SA	Ding *et al.* [27]
4.	*Monoraphidium* sp. QLY-1	Nitrogen deficiency	Alleviated oxidative damage and enhanced lipid accumulation by positively upregulating GA biosynthesis and ABA catabolism	GA, ABA	Zhao *et al.* [45]
5.	*Nostoc flagelliforme*	Salinity	Salt tolerance was enhanced by activating the antioxidant system	-	Yuan *et al* [3]
6.	*Haematococcus pluvialis*	Nitrogen deficiency and high light conditions	MT and Ca^{2+} synergistically enhanced the coproduction of astaxanthin and lipids by regulating carotenogenic gene levels and GABA and ROS signaling.	Ca^{2+} GABA	Cui *et al.* [46]
7.	*Haematococus pluvialis*	Nitrogen starvation and highlight	Prevented the ROS burst and limited cell damage induced by abiotic stress through activation of antioxidant enzymes	-	Ding *et al.* [47]
8.	*Chlamydomonas reinhardtii*	Nitrogen deficiency	Reduced chlorophyll loss, enhanced antioxidants, reduced lipid peroxidation	-	Meng *et al.* [48]
9.	*Monoraphidium* sp. QLY-1	High light	ROS reduced, lipid biosynthetic enzyme regulation	-	Li *et al*. [5]
10.	*Acutodesmus* sp.	Hight light	Antioxidant enzyme-related genes enhanced, reduced oxidative stress, Ethylene and GABA synthesis regulation	Ethylene and GABA	Zhu *et al.* [49]
11	*Dunaliella bardawil*	High light	Pigment content regulation	-	Xie *et al.* [50]
12.	*Haematococcus pluvialis*	High light and salinity, nitrogen deficiency	Carotenogenesis and lipogenesis promoted, GABA synthesis regulation	GABA	Xing *et al.* [51]
13	*Haematococcus pluvialis*	High light	Regulated carotenogenic, lipogenic, antioxidant systems, glycolysis, TCA cycle, and GABA shunt pathways	GABA	Zhao *et al.* [52]
14	*Microcystis aeruginosa*	H_2O_2	Photosynthetic and antioxidant enzymes activities enhanced; oxidative stress reduced	-	Anam *et al.* [53]
15	*Chlorella vulgaris*	Dairy wastewater	Photosynthetic pigments, MUFAs and PUFAs synthesis promoted	-	Samani and Mansouri [54]

5.3. MT Downregulates Oxidative Stress

MT has a beneficial effect on plants' fresh and dry biomass and helps them to cope up with abiotic stress conditions [40] such as salinity, cold, heat, drought, heavy metals, and UV stress. MT-mediated stress tolerance mechanism controls antioxidant enzyme activities and exhibits ROS-scavenging properties [40,83]. MT acts as a fruit ripening and flowering regulator, and plant growth promoter by regulating the redox network or by interacting with other phytohormones [22]. In abiotic stress conditions, the up-regulation of multiple anti-stress genes against abiotic stress in MT-treated plants thereby downregulates oxidative stress. Under stress, the stimulation of endogenous MT has established its crucial role as a signaling molecule [22].

Hence, MT application efficiently regulates plant development and improves plant tolerance against abiotic stress [81] by promoting anti-oxidant activities, photosynthetic performance, and the accumulation of different osmolytes. It is important in crop improvement as a potential bio-stimulator for enhancing agricultural yields [84]. According to Wang *et al*. [85], it is biodegradable and safer for use in organic farming. It also makes certain fruits and vegetables easier to store after harvest. The quantity of H_2O_2 in roots can be considerably reduced by exogenous MT, delaying the development of symptoms associated with post-harvest physiological deterioration, which is caused by damage incurred during harvest and treatment. It allows for the long-term preservation of fruits and vegetables such as peppers, onions, cabbage, cucumbers, beans, and carrots [86,87]. Therefore, MT can be utilized in agricultural crop plants for sustainable agriculture [88].

5.4 MT in Dairy Industry

Dairy cows' milk productivity is measured using the dairy herd improvement (DHI) standard [89]. The characteristics of DHI measures, which indicate the quality of milk [90], include the milk somatic cell number (SCC), milk yield, protein, fat, lactose, and dry matter [83]. The SCC is one of the most significant indicators of the DHI's measured parameters, reflecting the cows' breast health and the quality of their milk [91]. According to Rohda *et al*.[92], milk quality decreases with increasing SCC. The cow is diagnosed with mastitis when the milk SCC is greater than 200,000/ml. A significant breast infection in cows is always indicated by an abnormally high SCC [93]. MT can drastically lower the SCC and improve the DHI in the milk of Holstein cows that have high SCC [4]. By lowering fat content with enhanced lactose and protein levels, the MT treatment increased the nutritious value of milk [4,93] (Fig. 1). It may be a good alternative to antibiotics for mastitis treatment in dairy cows. More research is necessary for this purpose. Since MT is safe and has no negative impact on the health of humans or animals the development of MT products and their application in dairy farming can enhance the industrial benefits [4].

5.5. MT as Anti-Aging Compound

Endogenous MT may have regenerative and anti-aging effects [20]. MT is a powerful signaling molecule that protects mitochondria [95,96]. Age-related degradation in mitochondrial functional activity is mostly caused by chronic and cumulative oxidative damage[97]. Consequently, MT's protective effect on mitochondria may be able to delay the aging process [98]. MT inhibits UV-induced damage to human skin and-derived cell lines (fibroblasts and keratinocytes) [99,100]. Recently, an MT-based emulsion with effective benefits for skin photoprotection has been developed [37].

6. FUTURE PERSPECTIVES

In 2019, approximately 4,000 tonnes of synthetic MT were produced worldwide worth about 1.3 billion USD. It was estimated in the next five years, the MT market was anticipated to expand at a compound annual growth rate of more than 10%. The insomnia issues brought on by the COVID-19 epidemic have become extremely relevant in light of this significant increase in demand. North America consumes the most of MT produced, followed by Europe. A few large corporations, including Baden Aniline and Soda Factory, Aspen Pharmacare, Nature's Bounty, Pfizer, Natrol LLC, Aurobindo Pharma, and Biotics Research, dominate the global MT market. About half of the synthetic MT produced is used for medical purposes; the remaining portion is used in chemical and industrial processes. This enormous business is entirely supported by the chemical MT, whose manufacturing is extremely profitable, efficient, and inexpensive. However, many toxic byproducts are produced during the chemical synthesis of MT, which has occasionally resulted in serious diseases in humans. Furthermore, prior research suggests that in the future genetically modified organism (GMO) *E. coli* strains could serve as the foundation for the use of microbial cell factories in the manufacturing of biological MT. However, when the objective is to introduce a natural product to consumer who is sensitive or anti-GMO, the utilization of transgenic organisms to manufacture compounds for human consumption can provide challenges. MT produced by cyanobacteria will be more widely accepted than MT produced by chemical synthesis techniques, and GMOs. Thus, cyanobacteria offer possible a bio-agent for sustainable MT commercial production.

7. CONCLUSIONS

Currently, MT is a very important and interesting biological tool in every field especially in sustainable agriculture and human welfare. It enhances abiotic stress tolerance in plants and microbes, thus this characteristic makes it an important molecule. This review discusses the biosynthetic mechanisms of MT in cyanobacteria. Furthermore, cyanobacteria are ubiquitous in nature and have the ability to promote commercial production of MT with biotechnological advances to meet future demand in a sustainable manner.

8. ABBREVIATIONS

ASMT	*N*-acetylserotonin methyltransferase
CAT	catalase
GABA	gamma-aminobutyric acid
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
H_2O_2	hydrogen peroxide
MT	melatonin
ROS	reactive oxygen species
SA	salicylic acid
SNAT	serotonin N-acetyltransferase
SOD	superoxide dismutase
T5H	tryptamine 5-hydroxylase
TDC	tryptophan decarboxylase
TPH	tryptophan hydroxylase

9. ACKNOWLEDGMENTS

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10. AUTHORS CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

11. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

12. ETHICAL APPROVALS

As this is a review article based on previously published literature, no ethical approval, or informed consent was required.

13. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

14. USE OF ARTIFICIAL INTELLIGENCE-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

15. PUBLISHER'S NOTE

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