Melatonin as Stress Marker in Fennel Plant

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Abstract. Medicinal plants are extremely important for their use in the treatment of diseases and chronic conditions. The fennel plant is one of the most important that humans have used for hundreds of years. It is an important annual herbal plant that contains many effective compounds, vitamins and minerals. Biotic and abiotic stresses affect the fennel plant's growth, yield, and the active compounds. Furthermore, the plants density is one of these stresses that affect the reduction of the crop quality. In order to solve this problem and increase production at the lowest economic cost, plant hormones were used to improve plant resistance to these stresses, including melatonin, which plays an important role in improving the defense systems of plants under any environmental or non-environmental stresses. Melatonin also works to regulate plant physiological processes such as photosynthesis and respiration with resistance to stress, thus improving the quality of secondary metabolism, which works to increase the active compounds and volatile oils in the fruits of the fennel.

1. Introduction
Recently, about 90% of the developing countries rely on medical plants for curing since they have the advantage of not showing side effects. Many studies revealed the medical importance of these plants, which brought them to investigation by studies in terms of producing medicinal drugs, cosmetics and perfumes [1]. Among these plants is the fennel plant Foeniculum fennel vulgar mill which belongs to Umbeliferae family. It is one of the most important aromatic annual plants in the world and especially in Iraq, as it combines medicinal and nutritional use. It also contains volatile oil reaching 6% composed of Anethole Limonene and α-Pinen. The main source responsible for the distinctive smell of the plant is the Fenchone in addition to many vitamins such as A, B, C and minerals [2]. It is also important in the treatment of many diseases such as respiratory and urinary diseases. It also facilitates digestion, expels gases, fights colds, infections and viruses [3]. In addition, studies have also revealed the compounds in this plant that treat hormone deficiency such as estrogen associated with the phenomenon of anxiety and stress removal through the GABA-ergic receptor system [4]. Globally, agricultural production is affected by various means of stress, whether vital or abiotic. Medicinal plants are among the plants that are directly affected by stresses. Among these stresses is the plant density, which is one of the most important methods in managing field crops because of its direct connection with plant growth and the vital processes that have significant effect on plant vegetative characteristics, yield composition and quality improvement [5]. Plant density is a stress factor with multiple dimensions, as plant density is an abiotic stress such as light stress or its shade, water deficit and mineral nutrients that affect plant growth and production. Plant density may be a major biological stress through increasing the activity of pathogens and insects. This requires studying and fixing this problem by finding modern means that help plants to resist high densities and give an economic yield of good quality. One of these recently used methods is the application of modern plant hormones [6]. Among these hormone is
melatonin or the so-called (N-acetyl-5 methoxytryptamin), a multifunctional molecule called the sleep hormone, which is secreted in the human body. It is found in all living organisms, also found in higher plants and is also referred to by another name, pheto-elatonin. The chemical structure consists of a cyclic compound (Indole), which leads to the formation of the amino acid tryptophan [7]. Tryptophan is converted to 5-hydrotryptophan in animals, while it is converted to tryptamine in plants, and the latter two are converted to serotonin and finally to melatonin, which works to resist stress within plants [8]. Therefore, recently it was demonstrated that the concentrations of the active compounds increase along with the concentrations of melatonin. For instance, [9] demonstrated the importance of melatonin in the treatment of biotic and abiotic stresses to maintain the composition of the active substance in the fruits of the Fennel.

2. Melatonin Biomolecule

2.1. Chemical Structure and Molecular Formula of Melatonin.
Melatonin (N-acetyl-5-methoxytryptamine) is a compound which consists of a cyclic compound (Indole) linked to an amino group (amine) to form an Indolemine compound. The molecular formula for melatonin is (C13H16N2O2) with a molecular weight of 232.3 gm. Mol-1 [10]. The following figure shows its molecular structure:

![Molecular Structure of Melatonin](image1)

Figure (1): The molecular structure of melatonin [10].

![Function of 5-Methoxytryptamine](image2)

Figure (2): The function of 5-methoxytryptamine in melatonin metabolism [11].
2.2. Plants density stress.
Although the plant density has been studied in detail, it has not gained the required scientific attention. For instance, we refer to the impact on gene expression and genetic engineering and the accompanying changes in the biological processes within the plant which directly affect the yield and its quality. It is clear that the use of administrative methods and practices in serving the crop is the optimal provision of plant growth requirements in terms of the necessary amount of water and nutrients, the availability of which is determined by the nature of the average number of plants per unit area (vegetation density) [12]. Agricultural distances are the main cause of two types of competition that affect the value of the crop. The first is a competition that occurs between plants over the amount of water, food and light, and the second is associated with the blooming process, and the competition peaks between plant organs to obtain metabolic materials. Therefore, it is preferable to reduce plant densities in arid and semi-arid areas and vice versa for temperate areas [13]. A previous study confirmed that growing plants in high plant densities increases the amount of plant consumption of most of the biological energy, which increases the height of the plant through the division and elongation of the internodes, which negatively affects most of the physiological and morphological characteristics [14]. Therefore, determining the optimal plant density according to the type of crop is an important factor, which most of the biotic and abiotic stresses are linked with. The optimum density gives high productivity by reducing the intensity of competition and plant shading, which allows the plant to obtain sufficient growth resources such as light, nutrients and water [15].

2.3. Relationship between melatonin and plant hormones.
Since the discovery of melatonin (N-acetyl-5-methoxytryptamine) in dinoflagellate single-celled flagella [16], and then in plants [17], as well as in algae [18], melatonin concentrations are in a strong contrast with the nanoscale ranging to 30 mg gm⁻¹ [19]; [20]. Recently, research has confirmed the presence of high concentrations of melatonin in medicinal plants [9]; [21]. It is worth to mention that melatonin is produced by the plant, and it interferes with the rest of the plant hormones that are naturally produced in the plant which has a significant effect, such as growth stimulation, decrease the salinity stress, drought, cold, toxicity of chemical pesticides, and resistance to fungi and pathogens. It is known that when seeds or plants are exposed to dormancy, plant hormones either stimulate germination, inhibit or kill the plant under their concentration increment. In other words, hormones activate physiological processes despite the suitability of dormancy to these seeds. However, melatonin has a direct or indirect effect by receiving some signals from the seeds when they are ready to grow; it stimulates some dormant enzymes and genes or the desire to remain in dormancy. Hence, melatonin maintains its dormancy [22]. In addition, melatonin has more effective functions than the rest of the plant hormones. The prerequisite for the classification of melatonin as a plant hormone is to determine its soluble fraction and recently special criteria have been discussed before it can be classified as a plant hormone [23]. In order to demonstrate the interaction of melatonin and hormones, a recent study demonstrated the interaction of melatonin with ethylene in regulating the secondary metabolism mechanism and converting them from fuel and food to energy and proteins. Although the mechanism of melatonin is not certainly clear, this interaction has proven its activity through metabolic pathways and genetic metabolites that form differentially accumulated metabolites (DAMS) that are secondary metabolites such as phenolic acids and flavonoids, whereby between the analysis pathway for RNA-seq, melatonin regulates the pathway of ethylene-assisted transport of hormones and the regulation of Vvdfr and Vvdfr2 gene expression (special genes that play a major role in regulating secondary metabolism). Melatonin also increases the ethylene through the accumulation of secondary metabolites [24].
2.4. **Common effects of melatonin on biotic and abiotic stresses.**

Studies revealed that the application of melatonin gave the plant the ability to resist infection with pathogens and fungi. The results also gave a lower number of leaf infestation and damage with a high content of chlorophyll and a more efficient photosynthesis process compared to the control plants that gave more infection and damage to leaves [25]. Melatonin and plant density depend on the nature of the activity of any effort to which the plant is exposed through increasing the plant’s tolerance to extreme environmental conditions as well as its role in physiological processes in stimulating cells located in the thin layer of the plant tissue between the dermis and phloem. It also increases the activity and growth of root hairs, as well as its effective role in the process of regulating and early flowering process and reducing its fall, which accelerates the harvest process and reduces economic losses [26].

2.5. **Melatonin mechanism on antioxidants balance.**

Melatonin is greatly important in the regulation the anti-stress responses in plants, as well as its ability to interact directly or indirectly with free radicals which are formed by two main types: oxygenic reactions (ROS) and nitrogenous reactions (RNS). These have a dual effect in causing oxidative damage and affect the stability in plant tissues. Also, they serve as an indicator by which the plant is exposed to any stress that affects physiological processes, thus a decrease in plant growth and economic yield [27]. In addition, there is a group of nitric oxide (NO) triggers that stimulate a range of responses to reduce stress and cellular damage by reprogramming the primary
and secondary metabolic pathways induced by melatonin [28]. Biological and abiotic stresses increase the concentration of melatonin through the synthesis of new vital genes. It is necessary to have a balance between the amount of free radicals compared with the antioxidants that have the ability to treat the risk of free radicals, which leads to infection with many diseases and fungi. Here comes the role of melatonin in maintaining that balance and preventing infection with any effort that the plant is exposed to, whether internal or external, vital or non-vital [29]. Melatonin and the study of stresses such as plant density can create a new approach in the development and increase of agricultural production for their role in genetic modification in vital gene expression provided that the restrictions imposed on the use of genetically modified plants are not exceeded to prove the effectiveness of these two factors to increase the plant's ability to scavenge free radicals by increasing the antioxidants actually present in plants. However, the composition and effectiveness of these antioxidants are affected when exposed to environmental stresses, but melatonin gives instructions to resist the stresses the plant was exposed to. The following figure reveals a diagram showing the effectiveness of melatonin in stress resistance [30].

Figure (4): The effect of biotic and abiotic stresses on the antioxidants in melatonin [27].

3. The effect of melatonin on physiological processes.

There is no doubt that biotic and abiotic stresses affect agricultural production for their impact on the physiological processes, thus they lead to infirmity in the structure and composition of biomass and may lead to plant death [31]. Therefore, it was revealed that the development of the plant physiology system is one of the most important economic means used to increase the yield using compounds that are effective in increasing the plant’s resistance to inappropriate conditions. Recent studies revealed the promising value of melatonin in plant’s resistance as it regulates the plant physiology, anti-oxidation, free radicals and the synthesis of new pathways involved in gene expression [32].

The main function of melatonin is growth stimulation and an integrated defense system within the plant [26]. Additionally, it protects plant's tissues, reduces aging, activates enzymes, which in turn preserves proteins and fats from damage and maintains their role in the metabolic processes within the plant and the production of secondary metabolic compounds, and increases their quantity [33].
4. Conclusion

In general, the main function of melatonin is to scavenge free roots and resist stress, but recent studies have shown that melatonin has the ability to interact with the rest of the plant’s growth regulators to give a chemical composition inside the plant through which genetic receptors can be organized. This maintains the amount of effective compounds, especially in medicinal plants that have become the future source of medications since they are safe from any toxic symptoms that affect human health. In this sense, melatonin can create a qualitative event in the progress of science and a new approach in the development of the study of medicinal plants in the world. We also conclude that the optimal plant density and its interaction with melatonin have the ability to preserve the plant from environmental stresses, and this leads to an increase in the proportion of active compounds found in the medicinal plant.

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