1	N-acetyl-5-methoxytryptamine as an enhancer of salinity tolerance via modulating physiological
2	and anatomical attributes in faba bean
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### Abstract

To meet the increasingly food demands, all environmental resources should be exploited in 43 sustainable manner. Soil, a major component in agricultural for production, is subjected to 44 salinization owing to climate changes. Growing crop plants in saline soil dramatically results in 45 significant yield declines. Hence, crops require external aids to obtain rational quantity and quality 46 of the economic product while achieving the land sustainability. Accordingly, a two-year field 47 experiment was implemented for outstanding the probable changes in physiology and anatomy 48 49 occurring in melatonin-treated faba bean plants under saline conditions. In randomized complete block design with three replicates, three levels of melatonin (0, 50 and 100 µM) were tested under 50 salty stress conditions. The result data illustrated that application of melatonin (50 or 100 µM) 51 maintained cell membrane stability index and leaf water content of salt-suffered faba bean plants. 52 Chlorophyll content, chlorophyll a fluorescence, total soluble sugars, proline content, total 53 antioxidant activity, ascorbate, glutathione and catalase were increased by 43.8, 5.0, 59.9, 15.6, 54 55.1, 19.7, 19.6, and 9.0%, respectively, owing to melatonin (100 µM) supply. Remarkable 55 increases in stomatal aperture area and stomatal length were obtained with spraying of melatonin. 56 Also, melatonin-treated faba bean plants at a rate of 100 µM showed increases of 1.75 and 1.33 57 folds in green pod yield and dry seed yield greater than the salt-suffered plants. In conclusion, 58 regulation of faba bean plant growth can be adjusted in favour of a healthy physiological status and 59 60 sustainable productivity under saline soils using melatonin at a rate of  $100 \,\mu$ M.

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Keywords: Lipid peroxidation, osmo-protectants, osmotic pressure, photosynthetic efficiency,
physiological stress, stomatal conductivity, Vicia faba.

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### 1. Introduction

Soil salinization is expected to increase over time owing to the possible climatic changes. It is 69 well known that stresses of different types represent a drastic impediment in agricultural production 70 71 (Saudy et al., 2021; Ansabayeva et al. 2025; Emam et al., 2025). Growth and yield of most crops 72 are dramatically depressed by biotic and abiotic stresses such as pests, extreme temperatures, drought, salinity, and nutrient deficiency (Saudy et al., 2022; Abou El-Enin et al., 2023; Ramadan 73 et al., 2023a; Abdo et al., 2024). Such various stresses stimulate the excess formation of free 74 radicals within plant cells, mainly expressed in reactive oxygen species (ROS), particularly, 75 superoxide radical (O2<sup>-</sup>), singlet oxygen (1O2), hydroxyl radical ('OH) and hydrogen peroxide 76 (H<sub>2</sub>O<sub>2</sub>), which are highly vulnerable and dramatically disturb plant metabolism while causing 77 serious negative impacts on crop productivity (El-Beltagi et al., 2022 a; Abd El-Mageed et al., 78 2022; Hadid et al., 2023). Additionally, osmotic stress and toxicity of ions are the two major 79 hazardous impacts of salinity (Van Zelm and Testerink, 2020; Selvanarayanan et al, 2024). 80 Accordingly, salt stress is associated with alteration in the expression of genes, stability of mRNA, 81 82 and translational regulation, disturbing proteins synthesis (Sharma and Dietz, 2009; Yashodha et al., 2025). It has been documented that these stress agents influence normal plant growth and 83 development, resulting in lower yield outcomes with poor quality (Shabbir et al., 2022). In faba 84 85 bean (Vicia faba, L.), such stresses have significantly negative impacts on growth, yield and quality, threatening food security (Saudy et al., 2020; El-Bially et al., 2023). In order to adapt to stresses, 86 plants have evolved intricate mechanisms involving enzymatic and non-enzymatic antioxidant 87 defines to maintain ROS within plant cells at a harmless level (Sharma et al., 2019; Ramadan et al., 88 89 2023). Furthermore, a variety of hormonal activities had a substantial action in regulation the 90 adaptive response of plants to the stresses (El-Beltagi et al., 2022b; Rizk et al., 2023).

Recently, melatonin has exhibited as a powerful and dynamic regulator of abiotic stress tolerance in plant biology (Pan *et al.*, 2023; El-Beltagi *et al.*, 2023). Melatonin is regarded as a significant molecule in a diverse plant physiological process, such as germination of seeds, root

development, movement of stomata and different responses to stresses (Pan et al., 2023; Jensen et 94 al., 2023). Melatonin acts as a signalling molecule, controlling the growth and development of 95 plants as well as the responses to ecological pressures (Altaf et al., 2021). Melatonin acts in 96 harmony with other phytohormones. Substantial relations between melatonin and various related-97 98 stresses plant hormones such as salicylic acid, indole acetic acid, ethylene, cytokines and abscisic acids have been documented (Wang et al., 2022; Yang et al., 2021; Yang et al., 2022). Based on the 99 physiological aspects, melatonin has the potential to affect salinity tolerance; however, the changes 100 101 in plant anatomy due to melatonin supply under salt stress did not sufficiently investigate. The current work hypothesized that melatonin could enhance the physiological status of faba bean plant 102 while modifying anatomical appearance of leaf. Therefore, the current work aimed to assess the 103 potential action of three levels of melatonin the physiological and anatomical traits of faba bean 104 grown in saline soil. 105

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## 2. Materials and Methods

109 2.1. Experimental site description

Two field trials were conducted over two succeeding seasons (2022/23 and 2023/24) at a private
field (latitudes 29°06' and 29°35' N, longitudes 30°26' and 31°05' E.), in Fayoum province of Egypt.
Primary physio-chemical properties of the investigated soil were measured in accordance with
(Klute and Dirksen, 1986) and (Page *et al.*, 1982) and data were presented in Table 1.

- 114
- **Table 1. Some initial physico- chemical characteristics of the studied soils**

Particle size (%)			Textur	Bd	ECe		ОМ	CaCO <sub>3</sub>	CEC
Sand	Sil	Clay	e class	(g cm <sup>-3)</sup>	(dS m <sup>-1</sup> )	рН	(%)	(%)	(cmol kg <sup>-1</sup> )
75.2	11.7	13.1	LS	1.56	7.83	7.61	1.02	4.47	10.25

LS: loamy sand Bd: soil bulk density, ECe: electrical conductivity, pH: soil acidity,
OM: organic matter content, CaCO3: calcium carbonate, CEC: cation exchange
capacity

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### 120 2.2. Agronomic management and treatments

Healthy seeds of faba bean, (*Vicia faba* L.) cv. Sakha 1, were sown on November 1 and 5 and harvested on March 26 and 31 for two winter consecutive seasons 2022/23 and 2023/24, respectively. Melatonin was used to spray plant foliage at three concentrations (0; tap water as a control, 50 and 100  $\mu$ M) three times 15 days intervals beginning from 20 days after sowing (DAS) to run-off. Tween-20 (0.1%, v/v), as a surfactant agent, was compiled with the sprays to ensure optimal penetration into leaf tissues (Figure 1).



145 The three tested melatonin concentrations were replicated three times in a random complete 146 block design, totalling 9 experimental plots. Each experimental plot had a 36.0 m2 net area with

three planting ridges and 0.8 m width and 15 m length. Faba bean seeds were sown in hills spaced 147 20 cm apart on two sides of the planting ridge, and seedlings thinned to two healthy and uniform 148 plants per hill four weeks after planting. Phosphorus (P) fertilizer with a rate of 75 kg P ha<sup>-1</sup> in the 149 form of calcium superphosphate (15.5%  $P_2O_5$ ) was basically added at planting and potassium (K) 150 fertilizer with a rate of 120 kg K ha<sup>-1</sup> in the form of potassium sulfate (48% K<sub>2</sub>O) was topdressed at 151 four weeks after planting. Nitrogen (N) fertilizer was added once as a starter dose 10 DAS with a 152 rate of 48 kg N ha<sup>-1</sup> in the form of ammonium nitrate (33.5% N). A drip irrigation system was sited, 153 154 and two drip lines were placed 25 cm apart in every elementary test plot.

155 2.3. Cell membrane stability and leaf water

At 75 DAS, relative water content (RWC %) and membrane stability index (MSI %) were assessed
according to (Hayat *et al.*, 2007) and (Premachandra *et al.*, 1990), respectively.

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## 159 2.4. Leaf photosynthetic traits

Leaf chlorophyll content was extracted and determined (in mg g<sup>-1</sup> fresh weight; FW) according to
the procedure of (Arnon, 1949). Chlorophyll a fluorescence (Fv/Fm) and performance index were
determined according to (Maxwell and Johnson, 2000) and (Clark *et al.*, 2000) by Handy PEA,
Hansatech Instruments (Ltd, Kings Lynn, UK).

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# 165 2.5. Osmolytes and antioxidants

The free proline content and total soluble sugars (TSS) (mg g<sup>-1</sup> FW) of fresh faba bean leaves were extracted and quantified using the procedures described by (Irigoyen et al., 1992). The 2, 2diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (DPPH RSA %) of the extract was calculated by DPPH free radical according to (Brand-Williams et al., 1995). The ascorbic acid (AsA;  $\mu$ mol g<sup>-1</sup> FW) and reduced glutathione (GSH;  $\mu$ mol g<sup>-1</sup> FW) contents in fresh leaf tissues of faba bean were determined using the techniques outlined by Mukherjee and Choudhuri (Mukherjee and Choudhuri, 1983). Plant cells were extracted following the technique of (Bradford, 1976) for use as a crude enzyme extract to measure CAT content (U  $mg^{-1}$  protein). CAT activity was established using the approach published by (Aebi, 1984).

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### 176 2.6. Stomatal performance

To estimate the guard cell dimensions, stomatal apertures area, and stomatal density measurements, 177 for this purpose, three plants per replicate (3 replicates for each treatment; total 9 plants) were 178 selected. The measurements were performed for one well expanded leaf (on 6th node from the shoot 179 apex) of each plant. From lower surface, the sample of epidermal cells was obtained (abaxial side) 180 by nail varnish technique (Agami et al., 2016). A small area of abaxial then was observed through a 181 light microscope (BX60, Olympus, Hamburg, Germany), equipped with a digital camera (Camedia 182 C4040, Olympus, Hamburg, Germany). Stomata dimensions, stomatal aperture area, and stomata 183 density were assessment with the software of AnalySIS®3.2 program for image analysis (Olympus, 184 Hamburg, Germany) and their frequency  $(n/mm^2)$ . 185

## 186 2.7. Agronomic attributes

At 90 DAS, ten plants were randomly obtained from every experimental plot and assessed for their 187 growth characteristics. First, plant height was recorded and then branches plant<sup>-1</sup> were counted. 188 Next, the total leaf area plant<sup>-1</sup> was measured using a digital plan meter Planix 7 (Sokkia Co., Ltd. 189 Kanagawa, Japan). The plant leaves and branches were dried in oven at 70 °C until constant weight 190 then the plant dry weight was recorded. Once faba bean pods attained commercial green maturation 191 for fresh pod human consumption, all green pods from all plants in one planting ridge from each 192 experimental plot were hand-harvested, and the obtained value was then converted to a hectare 193 basis (t ha<sup>-1</sup>). At the harvesting stage (full maturation) of dry pods, 10 plants randomly were 194 195 selected from each experimental plot and used to determine yield components, i.e., number of pods per plant and seed index. The faba bean dry seed yield on a 13% seed moisture basis was 196 appreciated after the threshing of the dried plants harvested from the remaining two planting ridges 197 in each plot. 198

#### 2.8. Statistical analysis 199

Data were statistically examined following (Gomez and Gomez, 1984) with a two-way analysis of 200 variance using the general linear model (GLM) procedure of GenStat statistical package (version 201 11) (VSN International Ltd, Oxford, UK) to test significant differences among treatments. While 202 replications were regarded as a random factor, the three tested Me-applied concentrations were 203 considered a fixed factor. When the F-value was significant (p < 0.05), significant differences among 204 treatments for each attribute were investigated using Fisher's protected LSD test. 205

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### 3. Results

#### 3.1. Cell membrane stability and leaf water 208

Different melatonin levels had substantial effects (p<0.05) on cell membrane stability index and leaf 209 water content of faba bean plants grown in saline soil (Fig 2). Application of melatonin at a rate of 50 210 211 or 100 µM surpassed the control treatment (without melatonin supply) for maintaining cell membrane stability index and leaf water content. The improvements were 9.5 and 12.4% in cell membrane 212 stability and 9.8 and 9.6 in leaf water content with supplying 50 and 100 µM, respectively. The 213 214 differences between 50 and 100  $\mu$ M treatments were not significant (p>0.05) in this respect.







in each bar followed by the same letter are not significantly different according to the LSD test  $(p \le 0.05)$ .

### 221 3.2. Photosynthetic traits

As shown in Fig 3, chlorophyll content, Fv/Fm and performance index of faba bean cultivated in saline soil affected significantly (p<0.05) by melatonin levels. In this context, melatonin at a rate of  $50 \mu$ M recorded the maximum values of all studied photosynthetic traits achieving increases of 40.6, 6.2 and 74.3% in chlorophyll content, Fv/Fm and performance index, respectively, compared to the control. Also, melatonin at a rate of  $100 \mu$ M increased chlorophyll content by 43.8% and Fv/Fm by 5.0%, higher than the control. There were no noticeable variations between 50 and 100  $\mu$ M in



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Figure 3. Chlorophyll content (mg g<sup>-1</sup> fresh weight), chlorophyll a fluorescence and performance index of salt-suffered faba bean as influenced by melatonin concentrations. FW: fresh weight. Values are means  $\pm$  standard error; Values in each bar followed by the same letter are not significantly different according to the LSD test (p $\leq 0.05$ ).

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## 268 *3.3. Osmolytes and antioxidants*

Application of melatonin caused significant (p<0.05) changes in osmolytes (Fig 4) and antioxidants of faba bean plants under salt-affected soil (Fig 5). Herein, as melatonin rate increases osmolytes and antioxidants increased. Melatonin at a rate of 100  $\mu$ M was the effective treatment for increasing total soluble sugars by 59.9%, proline content by 15.6%, total antioxidant activity (DPPH) by 55.1%, ascorbate (AsA) by 19.7%, glutathione (GSH) by 19.6% and catalase (CAT) by 9.0%, relative to the control treatment. The rate of 50  $\mu$ M was as similar as the rate 100  $\mu$ M in AsA and GSH.



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**Figure 4**. Proline and total soluble sugars (TSS) of salt-suffered faba bean as influenced by melatonin concentrations. FW: fresh weight. Values are means  $\pm$  standard error; Values in each bar followed by the same letter are not significantly different according to the LSD test (p $\leq$ 0.05).



**Figure 5.** The activity of total antioxidants (DPPH), ascorbate AsA), catalase (CAT) and glutathione (GSH) of salt-suffered faba bean as influenced by melatonin concentrations. FW: fresh weight. Values are means  $\pm$  standard error; Values in each bar followed by the same letter are not significantly different according to the LSD test (p≤0.05).

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## 314 *3.4. Stomatal performance*

To have a deep knowledge about the role of foliar application of melatonin in improving faba bean 315 growth, physio-biochemical, and yield-related traits, the stomatal traits in leaf of melatonin and 316 non-melatonin treated plants, were further analyzed. The anatomical measurements were performed 317 in the leaf of control and melatonin-treated plants. Data in (Table 2) showed that stomatal aperture 318 319 area and stomatal length were increased significantly (p<0.05). The highest increase was observed with melatonin at a rate of 100 µM treatment (31.1 and 10.7%) followed by the treatment of 320 melatonin at a rate of 50 µM (14.2 and 6.1%). However, stomatal density and stomatal width were 321 322 not significantly (p>0.05) changed by application of melatonin. Furthermore, it has been found that 323 melatonin application has a high impact on the stomatal aperture as shown in Fig. 6.

324 Table 2. Stomatal performance traits of salt-suffered faba bean as influenced by melatonin

Melatonin leve	Stomata aperture are	Stomata densit	Stomata lengtl	Stomata wid	
$(\mu M) \qquad \qquad (\mu^2)$		(no. mm <sup>-1</sup> )	(μ)	(μ)	
0	162.30±10.79b	8.36±0.35a	40.72±1.02c	26.72±0.60a	
50	185.40±7.10ab	7.67±0.36a	43.22±0.58b	27.96±0.72;	
100	212.70±13.6a	7.34±0.26a	45.07±0.63a	28.80±0.58;	

concentrations

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326 Values are means  $\pm$  standard error; Values in each column followed by the same letter are not

significantly different according to the LSD test ( $p \le 0.05$ ).



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**Figure 6.** Photographs of leaf abaxial surface of faba bean plant foliar sprayed with melatonin (A) control; (B) melatonin 50  $\mu$ M; (C) melatonin 100  $\mu$ M. gc, guard cells; ec; Epidermal cells. bars = 20  $\mu$ m.

Generally, all faba bean agronomic traits significantly (p<0.05) improved with melatonin treatment 362 363 compared to non-treatment (Table 3). The progressive increase in melatonin rate occurred increases in all agronomic parameters. Accordingly, melatonin at a rate of 100 µM was the remarkable 364 treatment significantly equalling (p>0.05) melatonin at a rate of 50  $\mu$ M in plant height, branches 365 number plant-1, pods number plant-1 and seed index. Melatonin at a rate of 100 µM achieved 366 increases of 1.24, 1.38, 1.71, 1.70, 1.63, 1.04, 1.75 and 1.33 folds in plant height, branches number 367 plant-1, leaf area plant-1, plant dry matter, pods number plant-1, seed index green pod yield and dry 368 seed yield, respectively, greater than the control treatment. As well, melatonin at a rate of 50 µM 369 increased plant height by 22.0%, branches number plant-1 by 47.7%, pods number plant-1 by 370 57.5%, seed index by 2.7%, green pod yield 30.5% and dry seed yield by 20.3%, compared to no 371 faba bean as influmelatonin supply. 372

373	Table 3. A	Agronomic	traits of	salt-su	ffered t	faba	bean as	s influe	nced by	melatonin	concentrations

Melatonin lev	Plant height	Branches number	Leaf area plant <sup>-1</sup>	Plant dry matter
(µM)	(cm)	plant <sup>-1</sup>	(dm <sup>2</sup> )	(g)
0	81.00±4.88b	3.67±0.17b	92.80±1.37b	140.40±2.07b
50	98.83±5.77a	5.42±0.33a	118.90±3.16b	179.80±4.78b
100	100.50±2.65a	5.08±0.47a	158.20±11.94a	239.30±18.05a
	Pod number plan	Seed index (g)	Green pod yield	Dry seed yield
	1		$(t ha^{-1})$	(t ha <sup>-1</sup> )
0	13.33±0.63b	94.80±0.22b	12.61±0.31c	2.61±0.01c
50	21.00±0.81a	97.36±1.08a	16.46±0.10b	3.14±0.00b
100	21.83±0.81a	98.63±0.71a	22.09±0.30a	3.49±0.02a

Values are means ± standard error; Values in each column followed by the same letter are not 374 significantly different according to the LSD test ( $p \le 0.05$ ). 375

### 4. Discussion

Salinization is a severe global issue, shrinking the crop productivity and causing substantial economic losses in agricultural production (Su *et al.*, 2021; Shaaban *et al.*, 2023; Hadid *et al.*, 2024). Salt stress has adverse impacts on plant growth since physio-biochemical processes are disturbed (Lasheen *et al.*, 2024; Ramadan *et al.*, 2024). However, melatonin as promising molecule could, at least partially, regulate the metabolism and mitigate the salt-associated injuries on faba bean crop cultivated in saline soils. It is possible to highlight this topic via the following explanation and exegeses.

The current study was performed in saline soil with electric conductivity of 7.83 dS  $m^{-1}$  (Table 1) 384 and showed negative effects on cell membrane stability and leaf water content of faba bean. Various 385 stresses mainly target the corruption of cell membrane (Saha et al., 2015). Because of salt stress, 386 cellular cytoplasmic constituents and viscidity are altered while lipid peroxidation enhanced (Ali et 387 al., 2020). Measuring cell membrane stability expresses substantially in the behaviour of diverse 388 plant cultivars toward the salt stress (Mahlooji et al., 2017). It has been reported that cell membrane 389 stability significantly associated with relative water content, potassium (K<sup>+</sup>) ions and osmotic 390 adjustment (Shekari et al., 2017). Further, the level of membrane fatty acids saturation and 391 membrane fluidity were changed under salt stress (Farooq and Azam, 2006). On the other hand, 392 water is the main component related to physio-biochemical reactions for growing plants. Reduction 393 394 in water absorption is realized under salts stress while hindering relative water content of plant cells (Saha et al., 2015; Meng et al., 2018). 395

There is a strong association between photosynthesis rate and plant pigments content. High salt content can adversely influence photosynthetic apparatus via chlorophyll degradation (Wu *et al.*, 2014). Plant leaves showed remarkable decline in different pigments (chlorophyll a, chlorophyll b and carotenoids) under saline conditions (Wu *et al.*, 2014). Chlorophyll disintegration under salinity stress could be ascribed to the activity of chlorophyllase enzyme (Neelesh and Veena, 2015), in addition to the abundant concentration of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-1</sup>) in plant leaves

(Gul et al., 2013). Also, salinity of soil conversely influences photosynthetic activity and induces 402 ROS production, thus declining plant growth and yield. Increased accumulation of ROS is an 403 indication of plant stress for several biochemical molecules involving pigments, lipids and proteins 404 405 (Mehmood et al., 2019). Furthermore, plants subjected to salinity showed low quantum efficiency in photosystem II (Acosta-Motos et al., 2017). A remarkable correlation has been reported between 406 the amount of Na<sup>+</sup> ion and chlorophyll fluorescence. Since the ions of Cl<sup>-1</sup> and Na<sup>+</sup> increased in soil 407 solution, their absorption by plants increased while the ions of  $K^+$ , calcium (Ca<sup>+2</sup>) and magnesium 408 (Mg<sup>+2</sup>) decreased (Hu et al., 2016; Mahmud et al., 2017). Based on the ion balance, Cl<sup>-1</sup> and Na<sup>+</sup> 409 toxicity prompt nutritional tumult while rise physiological drought stress by reducing the osmotic 410 potential of the soil solution. Owing to the fluctuation in water potential under salinity, plant 411 suffered osmotic stress (Khan et al., 2019). It has been documented that the assimilation of carbon 412 dioxide via photosynthesis declined with depressing in water potential (Aldesuguy et al., 2014). 413

Carbohydrates content was depleted in different plant parts owing to salinity stress. 414 Carbohydrates have to adjust cell osmosis since the accumulation of sugars increases via the 415 stimulation of enzymatic sugar hydrolysis under stress conditions (Wang et al., 2013). There was a 416 significant association between stress tolerance and sugar accumulation in several plants (Ali et al., 417 2018). On the other hand, proline acts a substantial effect for stimulating plant tolerance against 418 environmental stresses. Salinity stress can elevate proline levels and proline increases the enzymatic 419 antioxidant activities via enhancing superoxide dismutase and peroxidase (El-Beltagi and Mohamed 420 et al., 2010; Hu et al., 2016; Devnarain et al., 2016). In this respect, ROS enzymatically scavenged 421 by peroxidase, ascorbate peroxidase, superoxide dismutase, glutathione-synthesizing, catalase, and 422 glutathione reductase. Also, ascorbic acid restored by ascorbate peroxidase and reduced the 423 424 oxidation (El-Beltagi et al., 2022b; Khan et al., 2018).

As obtained in the current research findings, melatonin improved the cell membrane, leaf water, and photosynthetic traits and regulated the osmolytes, antioxidants activity and stomatal performance in faba bean plants grown in saline soil. In this context, as an adaptive performance

plant accumulate noticeable amounts of various osmo-protectants such as proline, soluble sugars 428 and free amino acids under salty stress (Farag et al., 2022). These molecules harmonically work to 429 readjust the osmotic potential of plant cells under stressed conditions, suppressing tissues 430 dehydration and stimulating water uptake (Ramadan *et al.*, 2022). The osmolytes biosynthesis and 431 metabolism under salinity were substantially stimulated with affording melatonin exogenously. 432 Furthermore, application of melatonin induced the activities of antioxidant enzymes, attenuating the 433 ROS accumulation, as well as increased K<sup>+</sup> content and K<sup>+</sup>/Na<sup>+</sup> ratio and Ca<sup>+</sup> content, hence 434 435 maintaining membrane stability of the stressed cells (El-Bially et al., 2022; El-Metwally et al., 2022). Melatonin regulated the transcription of genes responsible for leaf pigments, keeping 436 photosynthetic proteins and activating the xanthophyll cycle (Yang et al., 2022). Therefore, 437 application of melatonin under saline conditions achieved remarkable increase in leaf pigments 438 while raising the photosynthetic efficiency (Altaf et al., 2020). Since melatonin had a powerful 439 action to adjust of physiological and biochemical constituents of faba bean plants, growth and yield 440 were boosted under salty soil by melatonin supply (Figure 7). 441

Stomatal traits are important inductors that associated with stress tolerance ability in plants 442 and have critical role in nutrients and water movement in plants (Aebi, 1984; Mohammed et al., 443 2009; Agami et al., 2016). Melatonin foliar application improved performance of stomatal traits of 444 faba bean, since the leaf anatomical traits particularly stomatal aperture and area stomatal length 445 446 were improved. Melatonin foliar spray has important role in strengthening the cell wall and maintained cell expansion for improving the stomata anatomical traits and protecting the chloroplast 447 structure (Mohamed et al., 2020). This improvement in stomatal traits may be due to the role of 448 melatonin for activation of phytohormone that control maintenance of cell turgor pressure and 449 activity of antioxidant defiance system as well as control pro-cambial of vascular tissues (Mohamed 450 et al., 2020). The current results highlighted the great importance of melatonin in improving 451 nutrients and water translocation into active parts of faba bean through enhancing the stomatal 452 performance. Briefly, findings of the current research present obvious insights into the role of 453

melatonin as an enhancer in supporting yield traits through improving photosynthetic apparatus, osmos-regulators defensive antioxidants and stomatal system for sustaining the productivity of faba bean under salty soil situation. Practically, faba bean growers can safely exploit melatonin (100  $\mu$ M) to raise the resilience of salt-suffered plants, thus obtaining better productivity and quality.

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### 459 **5. Conclusions**

Briefly, findings of the current research present obvious insights into the role of melatonin as an 460 enhancer in supporting yield traits through improving photosynthetic apparatus, osmos-regulators 461 defensive antioxidants and stomatal system for sustaining the productivity of faba bean under salty 462 soil situation. Practically, faba bean growers can safely exploit melatonin (100 µM) to raise the 463 resilience of salt-suffered plants, thus obtaining better productivity and quality. Herein, application 464 of melatonin at 100 µM improved the economic outcomes of faba bean, i.e. green pod yield and dry 465 seed yield by 75.2 and 33.7%, respectively. Since the 100 µM, as the highest rate tested, revealed 466 the maximum improvement in faba bean performance, further investigations are neded to explore 467 the efficacy of melatonin at other application rates above the 100 µM, especially its effect at the 468 molecular level. 469

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475 Data availability

476 Data presented in current research work are available on request.

# 477 **Conflicts of interest**

478 The authors declare no conflict of interest.

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