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Improvement of germination, sprout weight and nutraceutical potential of mung bean (*Vigna radiata* (L.) R. Wilczek), sprouts by melatonin, as an elicitor

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Improvement of germination, sprout weight and nutraceutical potential of mung bean (*Vigna radiata* (L.) R. Wilczek), sprouts by melatonin, as an elicitor

Abstract: Using elicitors is one of the innovative techniques being currently applied for improving phenolic content, antioxidant capacity, and bioactive compounds in ready-to-eat sprouts. Therefore, this experiment was designed to evaluate the effect of melatonin (MT), as an elicitor, on mung bean germination and its sprout's yield and nutraceutical quality for a 5-day period during seed germination. Results showed that the processes involved on the 5 th day of germination led to an increase in total soluble protein (TSP), free amino acids (FAA), vitamin C, and antioxidant activity in the treated mung bean sprouts. In contrast, as time goes by, the rate of titratable acidity (TA), total phenolic content (TPC), flavonoid compounds, and reducing power decreased. Moreover, MT increased the percentage of seed germination (18.84 %) and sprout mass (25 %), as compared to the controls. Eliciting the seed of mung bean with MT resulted in enhancing total soluble solids (17.47 %) (TSS), TSP (23-37 %), FAA (20-38%), and reducing power (27-78%). In the sprouts of mung beans, similarly, it increased the TPC (13-28 %), flavonoid compounds (24-46 %), vitamin C (18-41 %), and antioxidant potential (13-34 %). In conclusion, using MT could change the phytochemical profiles of ready-to-eat sprouts and improve the health-promoting potential of produced sprouts during germination.

Key words: antioxidant activity, ascorbic acid, elicitation, flavonoids, legume

Izboljšanje kalitve, mase poganjkov in hranilne vrednosti kalčkov zlatega fižola (*Vigna radiata* (L.) R. Wilczek) z melatoninom kot elicitorjem

Izvleček: Uporaba elicitorjev je novejša tehnika, ki se v zadnjem času uporablja za izboljšanje vsebnosti fenolov, antioksidacijske sposobnosti in vsebnosti bioaktivnih snovi v kalčkih pripravljenih za prehrano. V ta namen je bil opravljen poskus za ovrednotenje učinka melatonina (MT) kot elicitorja na kalitev zlatega (mungo) fižola, pridelek kalčkov in njihove hranilne vrednosti v petdnevnem obdobju kalitve semen. Rezultati so pokazali, da je obravnavanje zlatega fižola peti dan kalitve vodilo k povečanju vsebnosti celokupnih topnih beljakovin (TSP), prostih amino kislin (FAA), vitamina C in antioksidacijske aktivnosti kolčkov. V nasprotju so se s časom parametri kot so titrabilna kislost (TA), vsebnost celokupnih fenolov (TPC) in flavonoidov ter redukcijska moč zmanjševali. Dodatek melatonina je povečal odstotek kalitve (18,84 %) in maso kalčkov (25 %), v primerjavi s kontrolo. Elicitiranje semen zlatega fižola z melatoninom je povečalo vsebnost topnih snovi (17,47 %), topnih beljakovin (23-37 %) in prostih amino kislin (20-38 %), a zmanjšalo redukcijsko moč (27-78 %). Podobno je obravnavanje z melatoninom v kalčkih zlatega fižola povečalo vsebnost celokupnih fenolov (13-28 %), flavonoidov (24-46 %), vitamina C (18-41 %), in antioksidacijski potencial (13-34 %). Zaključimo lahko, da bi uporaba melatonina med kalitvijo lahko spremenila fitokemični profil kalčkov pripravljenih za prehrano in s tem povečala njihov potencial za izboljšanje zdravja.

Ključne besede: antioksidacijska aktivnost, askorbinska kislina, elicitacija, flavonoidi, stročnice

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1 INTRODUCTION

Legumes are a valuable group of nutritious foods rich in carbohydrates, dietary fibers, proteins, lipids, minerals and antioxidant compounds (Lin and Lai, 2006). In legumes, mung bean (*Vigna radiata* (L.) R. Wilczek) because of its edible bean has remarkably been receiving the attention of consumers for a long time. The seed of mung beans is rich in nutrients and phytochemicals (Syed et al., 2011; Tang et al., 2014). In recent years, a great deal of research has shown that seed germination can enhance biological activities in the seed and increase its nutritional value (Shohang e al., 2012; Fouad and Rehab, 2015; Nkhata et al., 2018 Araújo et al., 2023). During the germination process, starch and complex proteins are converted into simple carbohydrates and free amino acids, and this facilitates digestive mechanisms in the human body (Nkhata et al., 2018). Also, seed germination improves the amount of vitamins, minerals, secondary metabolites and antioxidant compounds in the sprout (Xu et al., 2005; Khalil et al., 2007; Świeca et al., 2012), while reducing specific anti-nutrient compounds such as phytic acid (Khalil et al., 2007). Currently, there is increasing interest in consuming seed sprouts, either on their own or as ingredients in other products, due to their superior health benefits and nutritional composition (Ebert, 2020).

Elicitors are biotic and abiotic compounds which induced physiological changes in plants. By activating different defense mechanisms and responses, they affect the metabolism of plants and change the synthesis of phytochemicals (Baenas et al., 2014b). Accordingly, using various elicitors has reportedly improved plant growth and bioactive compounds in edible sprouts (Kim e al., 2006; Pérez-Balibrea et al., 2011; Limón et al., 2014; Świeca, 2015; Peñas et al., 2015). Treating edible chia sprouts with salicylic acid and hydrogen peroxide remarkably augments antioxidant compounds and conversely reduced obesity-related oxidative stress in laboratory mice (Gómez-Velázquez et al., 2021). Furthermore, phytohormones such as methyl jasmonate and jasmonic acid have reportedly acted as elicitors and assisted in increasing glucosinolate contents in edible *Brassica* sprouts (Baenas et al., 2014a). Melatonin (MT) is a bioactive molecule, with several biological roles in plants (Arnao and Hernández-Ruiz, 2018). In higher plants, MT is available in almost all tissues and organs. It is involved in various physiological processes such as seed germination, rooting, flower development, photosynthesis, maturity, senescence, osmotic regulation and resistance to abiotic stress (Arnao and Hernández-Ruiz, 2018). In plants, MT is served as an activator of antioxidant systems, and this mitigates the destructive effects of oxidative stress (Zhang et al., 2014; Lei et al., 2021). So far, exogenous MT has reportedly reduced chilling-induced damage, delayed aging, maintained sensory qualities and benefited nutritional values in fruits and vegetables in the postharvest stage (Jannatizadeh, 2019; Tan et al., 2020; Ma et al., 2021) and improved seed germination of *different* plant species under abiotic stressors (Zhang et al., 2014; Cao et al., 2019; Li et al., 2019; Lei et al., 2021; Yin et al., 2022). The current study aimed to determine the effects of exogenous MT, as an elicitor, on germination, sprout mass, qualitative features and nutritional characteristics of edible mung bean sprouts.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS AND TREATMENTS

One-year old seeds of mang bean (Parto cultivar) were obtained from Pakan Bazr Co. (Isfahan, Iran). Before germination, mung bean seeds were soaked in distilled water for 8 hours. The seeds were disinfected with 2 % sodium hypochlorite solution for two minutes and then washed with distilled water until a neutral pH was reached. Then, the seeds were divided into two groups: one group was placed in distilled water (as control) and another group was placed in 150 µM MT solution (elicitor solution) for 20 minutes. The concentration of 150 µM MT was selected according to preliminary experiments. The seeds were maintained at room temperature for 2 hours so that extra water would evaporate from their surface. The seeds (80 g) were placed in plastic containers and were allowed to germinate for 5 days. The conditions were made suitable for seed germination, with darkness, an appropriate temperature (25 °C) and a high level of relative humidity (80 %). In total, 30 plastic containers were used in the experiment, which included the control and MT treatments, each with 3 replications. Sampling was performed over a period of 5 days. The containers were irrigated daily with distilled water (25 ml). On each of the 5 days during the germination process, the samples were weighed (sprout mass), frozen in liquid nitrogen and stored at -80 °C for further measurements.

2.2 GERMINATION PERCENTAGE

After applying the MT treatment, which served as an elicitor, and having a control treatment, the germination rate was recorded separately. For this purpose, 5 batches of 100 seeds were considered for each treatment. The seeds were placed in Petri dishes (9 x 9 cm) containing two layers of Whatman filter paper, which were moistened with distilled water (10 ml). The Petri dishes were incubated for 5 days in the dark at 25 °C in a growth chamber (Memmert, Germany). The seeds were evaluated daily for germination, and germinated seeds were recorded. Each seed was considered as germinated when its seminal root emergence reached 2 mm. Germination rate was evaluated based on the number of germinated seeds from the total number of seeds (Peñas et al., 2015).

2.3 CHEMICAL ANALYSIS OF MUNG BEAN SPROUTS

2.3.1 Soluble solids and titratable acidity

Soluble solids (TSS) and titratable acidity (TA) were measured according to a method used by Chen et al. (2018) with slight modifications. For each treatment, a number of germinated mung beans (2 g) were randomly selected and their extract was prepared. The filtered extract was used for measuring TSS by a hand-held refractometer (N-50E, Atago, Japan), and the results were expressed as % Brix. Measuring the TA involved extracting one gram of sample using deionized water (3 ml). Then, the diluted extract (10 ml) was titrated using sodium hypochlorite (2 mM) and with phenolphthalein (1 %) as an indicator. Based on the consumed amount of solution during the titration process, the TA of the sprouts was reported as the percentage of malic acid.

2.3.2 Protein and free amino acids

The total soluble protein (TSP) was measured according to the Bradford method using bovine serum albumin as a standard (Bradford, 1976). Finally, the TSP was expressed as mg/g fresh mass. Free amino acids (FAA) were measured by the colorimetric method, using a ninhydrin reagent (Rosen, 1957). The amounts of FAA were calculated based on the glycine standard curve, and were ultimately reported as mg/g fresh mass.

2.3.3 Phenols and flavonoids

Total phenolic content (TPC) of sprouts was measured using Folin–Ciocalteu's reagent, as described by Singleton and Rossi (1965). Briefly, 2 grams of frozen sprouts were crushed using HCl-methanol-water solution (10 ml) at a ratio of (1:80:19) and placed on a shaker for 16 hours at room temperature. Then, the samples were centrifuged at 12,000 rpm for 15 minutes. The resultant extract was used for measuring TPC, flavonoids and antioxidant capacity.

Flavonoids content was determined according to a method used by Hasperué et al. (2016) with slight modifications. Accordingly, 100 µl NaNO2 (5 %) was added to 1 ml extract, and the solution was stored at room temperature for 5 minutes. Then, 50 µl $AlCl_3$ was added to the solution and, after 5 minutes, 250 µl NaOH (1M) was added. The absorbance of the solution was read at 510 nm using a spectrophotometer (Analytik Jena, Germany), and the flavonoid content was calculated based on a quercetin standard curve. The results were reported as mg g FM^{-1}.

2.3.4 Antioxidant activity

Determining the reducing power involved measuring the efficiency of extracts in reducing Fe^{+3} by relevant evaluations according to a method used by S'wieca et al. (2012). Briefly, 1 ml extract was mixed with 2.5 ml phosphate buffer (0.2 M) and 2.5 ml potassium ferricyanide (1 %). This solution was incubated for 30 minutes in a hot water bath (50 °C). Then, 2.5 ml trichloroacetic acid (10 %) (w/v) was added to the solution, and the samples were centrifuged at 3000 rpm for 10 minutes. The supernatant (2.5 ml) was mixed by 2.5 ml distilled water and 0.5 ml iron chloride (0.1 %), the absorption of samples was read at 700 nm. Higher absorption values per sample indicate greater reducing potential, which was expressed as quercetin equivalent (Q) in micrograms per gram of fresh mass.

In determining antiradical activity, each sample was evaluated in terms of free radical scavenging capacity, using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. 100 µl of extract was mixed with 2900 µl of DPPH solution. Immediately, the absorbance of each sample was read at 517 nm using a spectrophotometer. Then, the samples were placed in the dark for 30 minutes, and their absorbance values were reread. Using the following equation, the antiradical activity (%) was calculated (Khang et al., 2016).

$$\text{Antiradical activity} = [(Abs_{conrol} - Abs_{sample})/Abs_{conrol})] \times 100$$

$Abs_{extract}$ is absorbance of extracts, and Abs_{blank} is absorbance of water.

2.3.5 Ascorbic acid

Vitamin C content in mung bean sprouts was measured using 2,6-dichloroindophenol (DIP) reagent, according to a method used by Nielsen (2017) with slight modifications. Briefly, 1 gram of mung bean sprout was pulverized using 10 ml of cold metaphosphoric acid (2 %). The samples were centrifuged at 3000 rpm for 5 minutes, and the extract solution was filtered. The filtered extract was titrated with 2,6-dichloroindophenol solution until the solution began to turn pink. The ascorbic acid content was reported as mg/g of fresh samples.

2.3.6 Statistical analysis

This experiment was conducted in a completely randomized design (CRD) with factorial arrangement of treatments and 3 replications. All data were statistically analyzed by analysis of variance (ANOVA) using SAS software (version 9.1). Means comparisons were assessed by Duncan Multiple Range test at the significance level of $p < 0.05$. All experiments were performed in triplicate.

3 RESULTS AND DISCUSSION

All measured traits of the mango bean sprouts were affected following 5-day period during seed germination. The analysis of variance (ANOVA) for all traits indicates significant difference among sprouting time, MT elicitation and their interaction, except for TSS (data not shown).

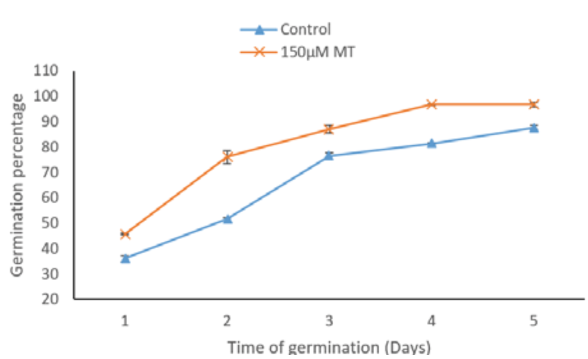

Figure 1: Impact of germination time and melatonin (MT) elicitation on germination percentage of mung bean sprouts. Error bars in the figure were standard deviations of triplicate experiments.

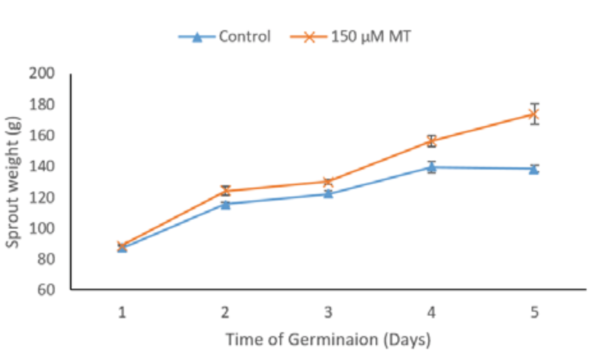

Figure 2: Impact of germination time and melatonin (MT) elicitation on weight of mung bean sprouts. Error bars in the figure were standard deviations of triplicate experiments.

3.1 EFFECTS OF MELATONIN AS AN ELICITOR ON SEED GERMINATION AND SPROUT MASS

Changes in seed germination and mass of mung bean sprouts are shown on different days of the experiment (Fig. 1 and 2). Through time, the percentage of seed germination increased and, thus, the mass of mung bean sprouts increased. Meanwhile, elicitation by MT increased the germination percentage and mass of mung bean sprouts. On the 5th day of elicitation, MT caused the sprout mass to increase by 25.57 %, compared to the control treatment (Fig. 1 and 2). Results from previous research showed that MT treatment not only increased the germination percentage, but also promoted subsequent growth when plants were exposed to environmental stresses (Li et al., 2019; Lei et al., 2021; Yin et al., 2022). The lipophilic and hydrophilic nature of MT, associated with its fluidity in crossing morpho-physiological barriers, usually result in rapid transfers and effectiveness of MT in all biological tissues (Garcia et al., 2014). Meanwhile, seed priming with MT can lead to optimal starch metabolism in seeds, thereby improving food supply in young seedlings while maintaining turgor pressure for tissue expansion during the germination process (Cao et al., 2019). MT is also known for its auxin-like activity, similar to IAA, which stimulates vegetative growth in plants (Arnao and Hernández-Ruiz, 2018). According to the results above presented, parallel to the increase in germination and initial seedling growth, the mass of sprouts also increases, and therefore, sprout production would stage higher levels of economic output.

3.2 EFFECTS OF MELATONIN AS AN ELICITOR ON TSS, TA AND VITAMIN C

Contents of TSS and TA were affected by MT and germination times treatments (Table 1). The results showed that the amount of TSS increased until the second day of germination, but then decreased significantly. On the other hand, as germination progressed, there was an increase in TA (Table 1). During the germination process, carbohydrates in the seed are metabolized into simpler sugars by digestive enzymes and are made available for the energy consumption of growing seedlings (Fouad and Rehab, 2015). There was a decrease in the TSS content of mung bean seeds after germination (Table 1), which can be explained by the consumption of sugars as an energy source for seedling growth. Results showed that on the 2th day of the experiment, elicitation by MT increased the TSS content and TA of mung bean sprouts, compared to the control (Table 1). Similarly, Bai et al. (2020) showed that cotton seeds pre-soaked with 100µM MT caused an increase in the amounts of soluble sugars in both normal and drought-stressed conditions. Another report indicated that MT regulated reserve mobilization (Lei et al., 2021) and that MT treatment increased the activity of starch hydrolyzing enzymes (α-amylase and β-galactosidase), thereby providing nutrients for seed germination (Chen et al., 2021). These results showed that MT elicitation provided energy for more efficient seed germination, which involved changing the metabolic patterns of sugars and organic acids in seeds and seedlings.

According to the results, as the germination time progressed, the vitamin C content increased significantly and reached its highest level (1.93 mg g FM^{-1}) up to the fourth day, although it decreased from the 4h to 5th day of germination. Compared to the control treatment, MT elicitation caused a significant increase in vitamin C content in mung bean sprouts on all days of germination, except the first day (Fig. 3). It has been reported that the application of exogenous MT in various species improved vitamin C as well as plant growth and development (Sardar et al., 2023; Iqbal et al., 2023). Using specific elicitors such as salicylic acid and chitosan (Pérez-Balibrea et al., 2011) and light (Xu et al., 2005) increased the vitamin C content in edible sprouts. Seemingly, the activity of key enzymes in ascorbic acid biosynthesis can be regulated by elicitors and, thus, could lead to changes in ascorbic acid content in edible sprouts (Xu et al., 2005).

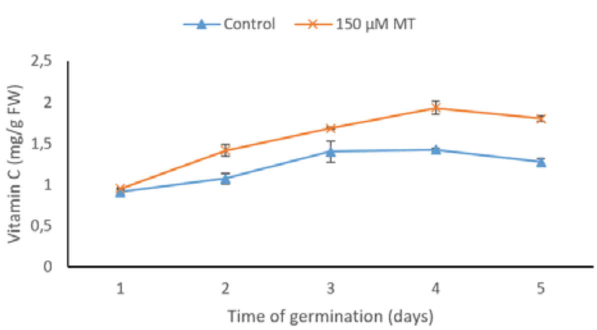

Figure 3: Impact of germination time and melatonin (MT) elicitation on vitamin C content of mung bean sprouts. Error bars in the figure were standard deviations of triplicate experiments.

3.3 EFFECTS OF MELATONIN AS AN ELICITOR ON TOTAL PROTEIN AND FREE AMINO ACIDS

Through the germination process, metabolic enzymes such as proteases are usually activated and, with the release of specific amino acids, new proteins begin to be synthesized (Diaz-Mendoza et al., 2019). As a result, sprouting may increase the nutritional quality of legumes, since the mechanism of sprouting is comprised of pathways that increase protein digestibility and increase biological value of proteins (Khalil et al., 2007; Nkhata et al., 2018). In the current research, as germination gradually progressed, especially on the 4th and 5th day, the TSP

Table 1: Influence of germination time and melatonin (MT) elicitation on total soluble solid (TSS), titratable acidity (TA), total soluble protein (TSP) and free amino acids (FAA) of mung bean sprouts.

Time of germination (Days)	Elicitor	TSS (% Brix)	TA (%)	TSP (mg g^{-1} FM)	FAA (mg g^{-1} FM)
1-day old	Control	11.33de	0.238e	8.74de	2.92e
	150 µM MT	13.00bcd	0.280e	7.78e	3.24de
2-day old	Control	13.33bc	0.293e	10.32cd	3.62cd
	150 µM MT	15.66a	0.393d	9.37cde	3.56d
3-day old	Control	13.33bc	0.413d	8.17de	3.47d
	150 µM MT	14.33ab	0.486ab	9.79cde	4.17b
4-day old	Control	10.33ef	0.433bcd	9.51cde	4.05bc
	150 µM MT	11.66cde	0.533a	13.04ab	5.59a
5-day old	Control	8.66fg	0.480abc	11.67bc	3.56d
	150µM MT	8.33g	0.426cd	14.43a	4.49b

Values are the means of three replicates, and different letters differ significantly by Duncan test. Mean significant at the $p < 0.05$ probability level.

and FAA increased significantly in mung bean sprouts (Table 1). Similarly, previous research indicated an increase in the amounts of protein and amino acids during the germination process, which may be caused by the synthesis of some enzymes (such as proteases), changes in the seeds composition or hormonal levels during seed swelling (Syed et al., 2011; Limón, et al., 2014; Fouad and Rehab, 2015).

The results showed that the MT elicitor had a significant effect on TSP and FAA of mung bean sprouts. In the early days of germination (until the 3rd day), the MT treatment had no significant effect on TSP, compared to the control. However, elicitation with MT caused an increase in TSP (37.11 and 23.65 %) on the 4th and 5th day, respectively, compared to the control. On all days of the experiment, except for the first and second days, MT-treated mung bean sprouts had higher FAA than non-elicited sprouts of the control (Table 1). This finding is in agreement with previous results by Li et al. (2019) where 200 µM of MT increased the efficiency of nutrient utilization and synthesis of new proteins to increase the germination of *Limonium bicolor* (Bag.) Kuntze seeds under salt stress conditions. Also, it has been reported that MT pretreatment improved α-amylase activity, soluble sugars and released amino acids, as well as, enhanced reserve mobilization, increased the germination rate and initial growth of wheat seeds under chromium stress (Lei et al., 2021). Therefore, it can be concluded that the MT treatment can provide energy for seed germination by changing amino acid contents and by regulating the degradation and biosynthesis of proteins.

3.4 EFFECTS OF MELATONIN AS AN ELICITOR ON PHENOLIC COMPOUNDS AND FLAVONOIDS

Phenolic compounds in plants have received much research focus because of their antioxidant properties and potential for human health (Świeca and Gawlik-Dziki, 2015). During the seed germination process, various changes occur in phenolic compounds, which depend not only on the genotype of the seed but also on the environmental conditions and germination time (Baenas et al., 2014b). The results showed that through germination time, TPC decreased (Fig. 4). The flavonoid content increased until the third day after germination and then suddenly decreased (Fig 5). Likewise, a significant decrease in phenolic compounds (e.g. tannin, phenolic acids, flavonoids and total phenolics contents) occurred in lentils seed during germination (Świeca et al., 2012). Similar conditions led to a gradual decrease in phenolic content in lentil sprouts after germination (Świeca, 2015), which are consistent with the results of the present research. In contrast, some cases of research indicated an increase in phenolic compounds in legumes during germination (Świeca and Gawlik-Dziki, 2015, Fouad and Rehab, 2015). In another relevant study, Shohag et al. (2012) reported that when the results were expressed based on fresh mass, germination caused a decrease in phenolic compounds because of the dilution effect, and on the contrary, the results were expressed based on dry mass, the phenolic content increased with the germination process.

Chemical elicitors provide a practical approach for increasing the nutritional quality and phytochemical compounds of edible sprouts (Baenas et al., 2014b). In the present research, using MT for the seed elicitation increased phenolic and flavonoid compounds in mung bean sprouts. Through the process of germination, the efficiency of elicitation increased. Accordingly, the highest MT effect on TPC and flavonoids content were observed on the fifth day of the experiment (Fig. 4 and 5). In pre-

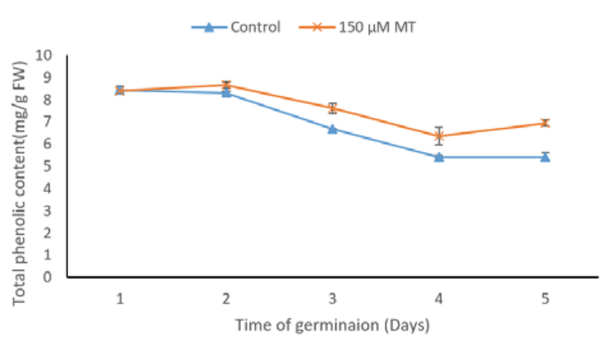

Figure 4: Impact of germination time and melatonin (MT) elicitation on total phenolic compounds of mung bean sprouts. Error bars in the figure were standard deviations of triplicate experiments.

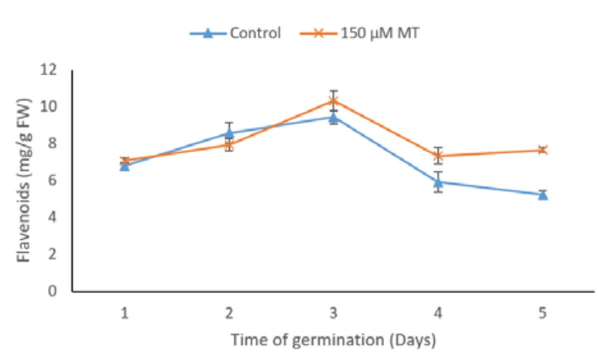

Figure 5: Impact of germination time and melatonin (MT) elicitation on flavonoids content of mung bean sprouts. Error bars in the figure were standard deviations of triplicate experiments.

vious research, several chemicals were used as elicitors, e.g. ascorbic acid, folic acid and chitosan/glutamic acid (Peñas et al., 2015), salicylic acid and hydrogen peroxide (Gómez-Velázquez et al., 2021) and methyl jasmonate (Kim et al., 2006), which enhanced the biosynthesis of phenolic and flavonoid compounds in the edible sprouts. While the current research indicated similar outcomes, Yin et al. (2022) reported that MT, can protect sprouts from NaCl stress by increasing antioxidant enzyme activities and phenolic acids accumulation (Yin et al., 2022). The application of MT in plants usually results in an accumulation of phenols by activating the phenylpropanoid pathway, which is actively associated with phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) activities (Jannatizadeh, 2019). Also, the application of exogenous MT on barley reportedly increased gene expression and activity of phenylalanine ammonia-lyase and cinnamate-4-hydroxylase. Since these enzymes are involved in the biosynthesis of phenols, the exogenous MT triggered an increase in the amounts of phenolic compounds and phenolic acids in barley sprouts (Yin et al., 2022). It is commonly apparent that a higher level of phenolic compounds can contribute to the antioxidant capacity and, thus, would improve the nutritional value of edible sprouts (Świeca et al., 2012).

3.5 EFFECTS OF MELATONIN AS AN ELICITOR ON ANTIOXIDANT CAPACITIES

With the aim of evaluating the roles of time and elicitors on biological changes in mung bean sprouts, the antioxidant properties of the sprouts were measured by two methods, DPPH and reducing power. In the control treatment, as the germination time progressed, the reducing potential showed a trend of decrease, but the antioxidant activity (DPPH) increased until the 4th day, but thereafter decreased until the 5th day of germination (Table 2). Changes in the antioxidant content of edible sprouts during germination have reportedly occurred in previous experiments. In lentil sprouts, for example, free radical scavenging, metal chelating and reducing properties gradually decreased through time in the germination process (S´wieca et al., 2012). In several cultivars of soybean and mung bean, the FRAP method was used for measuring the antioxidant capacity which decreased through germination time (Shohag et al., 2012). In contrast, other leguminous species showed an increase in antioxidant capacity during germination (Aguilera et al., 2015). In contrast, other species showed an increase in antioxidant compound during seed germination (Aguilera et al., 2015; Araújo et al., 2023). Such results may depend on the genotypes of each species and on the conditions of cultivation in each experiment.

The results showed that MT caused a significant increase in the antioxidant capacity and reducing power in edible mung bean sprouts, compared to the control treatment (Table 2). The results of similar research showed that using chemical agents such as salicylic acid, methyl jasmonate and hydrogen peroxide, as elicitors, increased the antioxidant potential of edible legume sprouts (Kim et al., 2006; Świeca, 2015; Gómez-Velázquez et al., 2021). In other words, MT is regarded as a powerful antioxidant that scavenge free radicals (Arnao and Hernández-Ruiz, 2018). In cucumber plants, exogenous MT reduced oxidative damage by increasing antioxidant gene expression

Table 2: Influence of germination time and melatonin (MT) elicitation on reducing power and antiradical activity of mung bean sprouts.

Time of germination (Days)	Elicitor	Reducing power (µg g FM^{-1})	Antiradical activity (%)
1-day old	Control	4.61ab	51.97de
	150 µM MT	4.49b	50.20e
2-day old	Control	4.70ab	59.80d
	150 µM MT	5.43a	80.58ab
3-day old	Control	3.10cd	77.45bc
	150 µM MT	3.96b	87.95a
4-day old	Control	2.13e	84.64ab
	150 µM MT	3.81bc	85.19ab
5-day old	Control	2.52de	70.83c
	150 µM MT	4.18b	83.76ab

Values are the means of three replicates, and different letters differ significantly by Duncan test. Mean significant at the $p < 0.05$ probability level.

and improved seed germination under salt stress conditions (Zhang et al., 2014). In another study, Aguilera et al. (2015) reported that enriching sprouts with MT caused antioxidant functions and free radical scavenging. Enhancements in antioxidant activity in plants, via elicitors, can be related to improvements in bioactive compounds that neutralize free radicals and protect plants against oxidative damage. Enhancements in antioxidant activity in plants, via elicitors, can be related to improvements in bioactive compounds that neutralize free radicals and protect plants against oxidative damage (Guru et al., 2022). In previous researches, it was observed that phenolic compounds, vitamin C and flavonoids had significant, positive relationships with antioxidant capacity in plants (Shohag et al., 2012, Peñas et al., 2015). Thus, the function of MT in increasing the antioxidant activity of mung bean sprouts can be attributed to the increase in flavonoids and ascorbic acid.

4 CONCLUSIONS

In conclusion, the results of this research showed that several biological features such as antioxidants, amino acids, proteins, soluble solids and vitamin C increased significantly through mung bean sprouting. As an elicitor, melatonin affected amino acid content and total protein, increased the germination percentage of seeds and, thus, improved the fresh mass of mung bean sprouts, compared to the control treatment. Also, using melatonin on mung bean seeds increased the measurable values of phenolic compounds, total flavonoids, antioxidant capacity, reducing power and vitamin C content, thereby improving the nutritional value of edible mung bean sprouts.

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