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Effect of Exogenous Application of Tryptophan and Methyl Jasmonate on Some Metabolites and Antioxidant Activities of Feverfew

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HIGHLIGHTS

- Active metabolites of *T. parthenium* were affected by TRP and MeJA treatments
- MeJA application enhanced the SER level in the flowering period
- TRP application enhanced SER and MEL levels in the flowering period
- TRP and MeJA treatments did not positively affect PRT amount.

Abstract: *Tanacetum parthenium* (L.) Schultz-Bip (feverfew) is among the important medicinal and aromatic plants due to its tryptophan (TRP), serotonin (SER), melatonin (MEL), and parthenolide (PRT) content. In recent studies, have reported TRP, MEL, and (PRT) are effective in the treatment of COVID-19, thus increasing the popularity of feverfew, which is rich in these valuable molecules. This study investigated the possible effects of exogenous foliar applications of methyl jasmonate (MeJA 0.5 mM) and TRP (20 mM) on plant TRP, SER, MEL, and PRT levels. During the pre-flowering period, endogenous TRP was measured as 128.9 µg/mL and endogenous PRT as 1.53% mg/g in the leaves of the control group. During the flowering period, the MEL level was measured as 1.38 µg/mL in the leaves of the TRP application group. In addition, in the pre-flowering period, MeJA-induced increases of 94.51% were determined in DPPH antioxidant activity and the total flavonoid content was 38.76 mg QE/g, whereas the highest total phenolic content of 51.63 mg GAE/g was found in flower samples of the control group. However, neither the developmental periods nor the treatments significantly affected the total phenolic content in the leaves.

Keywords: *Tanacetum parthenium*; serotonin; melatonin; parthenolide; total phenol; flavonoid.

INTRODUCTION

Feverfew (*Tanacetum parthenium*), belonging to the Asteraceae family, has been widely used for treatment of migraine, rheumatism, arthritis, insect bites, spasms, and gynecological problems [1,2,3]. The plant is well known for its rich sesquiterpene lactone content. Of those metabolites, parthenolide (PRT) is among the most important [3,4]. It is found in higher amounts in the flowers and leaves, with trace amounts

contained in the stems and roots. The PRT originates from the germacranolide lactone skeleton and is derived from its precursor, costunolide, which is most likely synthesized through the mevalonic acid pathway [4]. Parthenolide has been well-documented as possessing antileishmanial, anti-inflammatory, and antitumor activity, e.g., against bone tumors, breast cancer, melanoma, mesenchymal tumors, and prostate cancer. In addition, its strong ability to inhibit nuclear factor kappa B (NF-κB) led to its patenting as a cancer inhibitor in the year 2005 [5,6]. Furthermore, PRT is the first small compound to selectively kill acute myelogenous leukemia stem cells (LSCs) without causing any damage to normal stem cells [6,7]. In a recent report, Bahrami and coauthors [8], claimed that PRT, may be one of the herbal candidates for clinical evaluation in supporting reduced mortality from COVID-19. The IL-6 cytokine storm plays a significant role in diabetes mellitus and cardiovascular diseases as principal comorbidities, whereas PRT reduces L-1, IL-2, IL-6, IL-8, and TNF-α production pathways.

Noteworthy among the other important molecules, melatonin, its precursor serotonin, and tryptophan are. Tryptophan (TRP) is one of the essential amino acids and is synthesized through the same pathway precursors of auxin, phytoalexins, glucosinolates, terpenoid indole alkaloids, and tryptamine derivatives. TRP plays significant roles in plant development, pathogen defense systems, plant-insect interactions, and pollination biology. The biosynthetic pathway of TRP in plants was exhaustively investigated in the late 1980s using molecular and physiological applications in the characterization of the pathway in wild-type and mutant plants [9,10]. Plant growth parameters have been positively induced with TRP treatments [11,12,13,14,15]. In addition to growth and development, the carotenoid content, total soluble sugars, and total free amino acids in *Philodendron erubescens* have been augmented with TRP application [16]. Serotonin (SER) is synthesized from TRP. The physiological roles of SER in the regulation of morphogenesis, flowering, ion permeability, delaying senescence, and seed germination have been reported, in addition to its protective role as an antioxidant against unfavorable conditions [17]. The presence of SER has been revealed in all plant tissues in varying amounts, and is especially more abundant in fruits, seeds, and the vascular parenchyma [18]. Many reports have been published in previous studies concerning the SER content in foods because of its association with feelings of happiness and well-being and its role in preventing both anxiety and depression. However, studies carried out with aromatic plants have been limited.

Melatonin (MEL) is one of the significant compounds in *T. parthenium*. It is produced from the TRP-SER pathway. As a potent antioxidant compound, MEL has a role in scavenging reactive oxygen species and regulating the circadian rhythm and photoperiodism. Moreover, the role of the compound in the regulation of plant growth and development in response to environmental stress factors has been well-documented [19]. In a recent study, the expression of genes implicated in TRP metabolism was elevated in COVID-19 patients, and thus, tryptophan-rich sources could be beneficial for treating COVID-19 [20]. Researchers have suggested that MEL, alone or in combination with other drugs, be given consideration for prophylactic use or treatment for COVID-19 [21]. In Spain, a clinical study was designed with MEL use in COVID-19 patients and results showed that it had a positive effect in the treatment of COVID-19 symptoms [22]. According to recent reports, on the global market, MEL was worth 700 million USD in 2018 and was expected to reach 2,790 million USD by 2025, growing at a compound annual growth rate (CAGR) of 18.9% between 2019 and 2025 [23]. Research focusing on the MEL, SER, and TRP content of foods, drinks, and medicinal plants is rapidly increasing because of the rising demand for synthetic MEL.

Although synthetic antioxidants have been widely used, health problems occurring over time attract attention. Herbal antioxidants can protect the human body from free radicals, foods against lipid oxidative rancidit and play role in prevention and treatment of diseases. There is an increasing demand to replace these synthetic antioxidants with natural ones. Natural antioxidants from plants include phenolic compounds, vitamins and carotenoids. Phenolic compounds are contain simple molecules (e.g., ferulic acid, vanillin, gallic acid, caffeic acid) and polyphenols like tannins and flavonoids [24, 25, 26]. Flavonoid compounds donate a hydrogen atom from their hydroxyl group and thereby scavenge the free radical groups. The radical-scavenging activity is noteworthy because of the harmful role of free radicals play in foods and in biological systems. Studies on the free radical scavenging and antioxidant activities of medicinal plants have revealed the importance of plant species efficiency [24, 27]. Biotic and abiotic stresses induce changes in both primary and secondary plant metabolism, leading to the generation of phenols and other valuable compounds. Elicitors may stimulate specific biosynthetic pathways and thus enhance levels of stress metabolites [28].

Recently, Gharaghanipor and coauthors [29] demonstrated that hormone signal transduction pathways regulate the expression of a network of transcription factors (TFs) mediating abiotic-stress responses in plants. They also highlighted the role of phytohormones such as cytokinins, auxins, ABA, jasmonic acid, salicylic acid, gibberellins, and ethylene in regulating plant defense under abiotic stress conditions. Therefore, the exogenous application of these phytohormones triggers various signaling pathways associated with particular stress and developmental responses [30]. Nonetheless, the exogenous applications of salicylic

acid and jasmonates are widely used to elicit the production of bioactive compounds in herbal plants. These hormones are known to elicit different species-specific specialized metabolic pathways. Methyl jasmonate (MeJA)-mediated changes in volatile compounds [31] and terpenoid pathways [32] have been reported. These include MeJA-induced increments in glucosinolates, a group of secondary metabolites [33,34,35,36,37], and increments in antioxidant activity and phenolic compounds in basil [38,39,40], phenolic acids in *Mentha spicata* [41], and artemisin in *Artemisia annua* L. [42].

This study examined the way in which metabolic profiles were affected by the exogenous foliar application of the two elicitors MeJA and TRP. We also investigated the relationship between these induced metabolic profiles and the production of PRT, TRP, SER, MEL, and total phenolic and flavonoid contents in different parts of *T. parthenium* L. In this context, the treatments were applied at two different developmental stages: the pre-flowering and flowering periods.

MATERIAL AND METHODS

Plant material

Tanacetum parthenium (L.) Schulz Bip. seeds were purchased from Swallowtail Garden Seeds. Seeds were sterilized with 1% sodium hypochlorite solution for 2-3 min and then washed with sterile water [40]. The seeds were then planted in plastic pots filled with a mixture of peat, perlite and soil (3:1:1 w/w, respectively). The plants were grown until four true leaves were produced. When the seedlings reached the stage of four fully expanded leaves and afterwards for every 15 days until harvest, foliar spraying was carried out with 100 mg/L liquid manure mixed with a commercial fertilizer (EC Fertilizer, Magnum) containing w/w N (20 %), Cu (0.05%), Fe (0.05%), Mn (0.05%), Mo (0.001%), and Zn (0.05%). Seedlings were then transplanted into 1-L pots and placed in a growth chamber with a fluorescent lamp generating about 100 µmol m^{-2} s^{-1} light at 23 ± 2 °C, and 40-60% RH, following a photoperiod regime of 16:8 h (L:D). Plants were regularly irrigated using tap water, with field capacity measured at 80%. Foliar spray application of the plant growth regulator solutions of 0.5 mM MeJA (dissolved in 1% ethanol solution) and 20 mM TRP (dissolved in warm distilled water) were carried out 15 days before harvesting. Both sides of the leaves were completely saturated with the fertilizer and elicitor spray. Control groups were sprayed with distilled water only. The MeJA groups were grown in a separate chamber to prevent the volatile MeJA from contaminating the other plants. Concentrations of the elicitors were determined as recommended in previous reports [40,43]. After harvest, the plant material was dried under room temperature conditions. A complete randomized block design was used for each treatment, corresponding to nine plants and replicated three times. Nearly six months was needed to reach the flowering period. Plants were harvested at pre-flowering and full flowering stages.

Chemicals

All of the analytical grade solvents were purchased from Merck. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was procured from Aldrich, the L-tryptophan, SER, MEL, and MeJA from Sigma-Aldrich and, the Folin-Ciocalteu reagent (2N), gallic acid (GA), and quercetin (Q) from Sigma. The butylated hydroxytoluene (BHT) and aluminum chloride were obtained from Fluka and the PRT from Cayman.

Sample preparation

For sample preparation, 2 g dried plant material was crushed in a porcelain mortar, put into a Duran bottle to which 60 mL of methanol-water (2:1) was added, and mixed with a magnetic stirrer (INTTLAB) for 20 min to achieve homogenization. The samples were then placed in an ultrasonic bath (United Jewelry Tool Supplies) for 1 h and centrifuged for 10 min at 4,025 x g (Centrifugal, Hettich Zentrifugen Micro 220 R, Germany) [44]. After centrifugation, the samples were preserved in a freezer (-20 C) until subsequent analyses. For TRP, SER, and MEL analyses, samples were incubated in the freezer for two weeks. Prior to the HPLC analysis, plant extracts were passed through an HPLC filter membrane (0.2-25 µm, Millipore). The same extractions were used for PRT and antioxidant activity analyses.

HPLC analysis

Plant extracts were identified using an HPLC system (Agilent Technologies, Inc., Santa Clara, CA, USA) with four ECOM pumps (Prague, Czech Republic) equipped with a UV detector (Hewlett-Packard 1100 model). Analysis was performed on an ACE 5-C18 column (4.6 × 250 mm). Standards were prepared in 1.95 to 250 µg/mL concentrations at maximum resolution: TRP (dissolved in warm double-distilled water- ddH_2O),

SER (dissolved in 0.1 M HCL- ddH_2O), and MEL (dissolved in 80% methanol). The injections were performed in triplicate. For TRP and derivates, the mobile phase used 0.1% formic acid and 0.1 M KH_2PO_4 buffer in ddH_2O / acetonitrile (ACN) (20:80), at a flow rate of 1 mL/min. The total analysis time was 35 min. Column heat was adjusted to 28 °C and the UV detector to 280 nm [44]. The SER, TRP, and MEL peaks were seen at 3.9, 4.5, and 28.3 min, respectively. For the PRT analyses, the PRT was dissolved with DMSO at 1.95 - 250 µg/mL concentrations. The mobile phase used isocratic elusion with 55% ACN and 45% water. The total analysis time was 22 min at a 1.5 mL/min flow rate [45]. The peaks were analyzed at 210 nm using the UV detector. The 20 µL samples were injected into the HPLC system and the PRT peak was seen at 4.7 min.

Total phenolic compounds

Total phenolic content was measured using the Folin-Ciocalteu procedure modified by Fonseca and coauthors [45]. Folin-Ciocalteu reagent (2 mL) was added to a 400 µL sample. After 3 min, 1.6 mL of sodium carbonate (7.5%) was added and the mixture was allowed to stand for 30 min. Absorption was measured at 765 nm using a spectrophotometer (Agilent Rochester, NY, USA). Total phenolics were expressed as gallic acid (GA) units. A multipoint linear curve was obtained with the GA standard (Sigma) ranging from 20 to 400 mg/L. The results were inserted into the equation (y = 0.0083x + 0.3223) and calculated as mg/g GA.

Total flavonoid compounds

Total flavonoid content was measured using the procedure modified by Subedi and coauthors [46], in which 100 µL (2%) $AlCl_3$ were added to 100 µL extract, two drops of CH_3COOH were added to the mixture, and the total volume adjusted with methanol to 5 mL. The mixture was allowed to stand for 40 min and was measured at 415 nm by the spectrophotometer. Quercetin (Q) was used as the standard (6.125-200 mg/L) and the absorbance was calculated using the equation (y = 0.0022x + 0.0054) as mg/g Q.

Antioxidant activity

The antioxidant activity of the extracts was determined by using the radical scavenging activity of DPPH. The positive control BHT (1 mM) and DPPH (1 mM) were dissolved in methanol. Extract samples (50 µL) were put into plastic spectrophotometer cuvettes and 3 mL DPPH were added to each sample, which was then kept in the dark for 30 min. After the 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. The percent of DPPH free radical inhibition was calculated as: radical scavenger effect (%): {(A0-A1/A0) × 100}, where A0 represents the absorbance of the control without the sample and A1 represents the absorbance of the test solution.

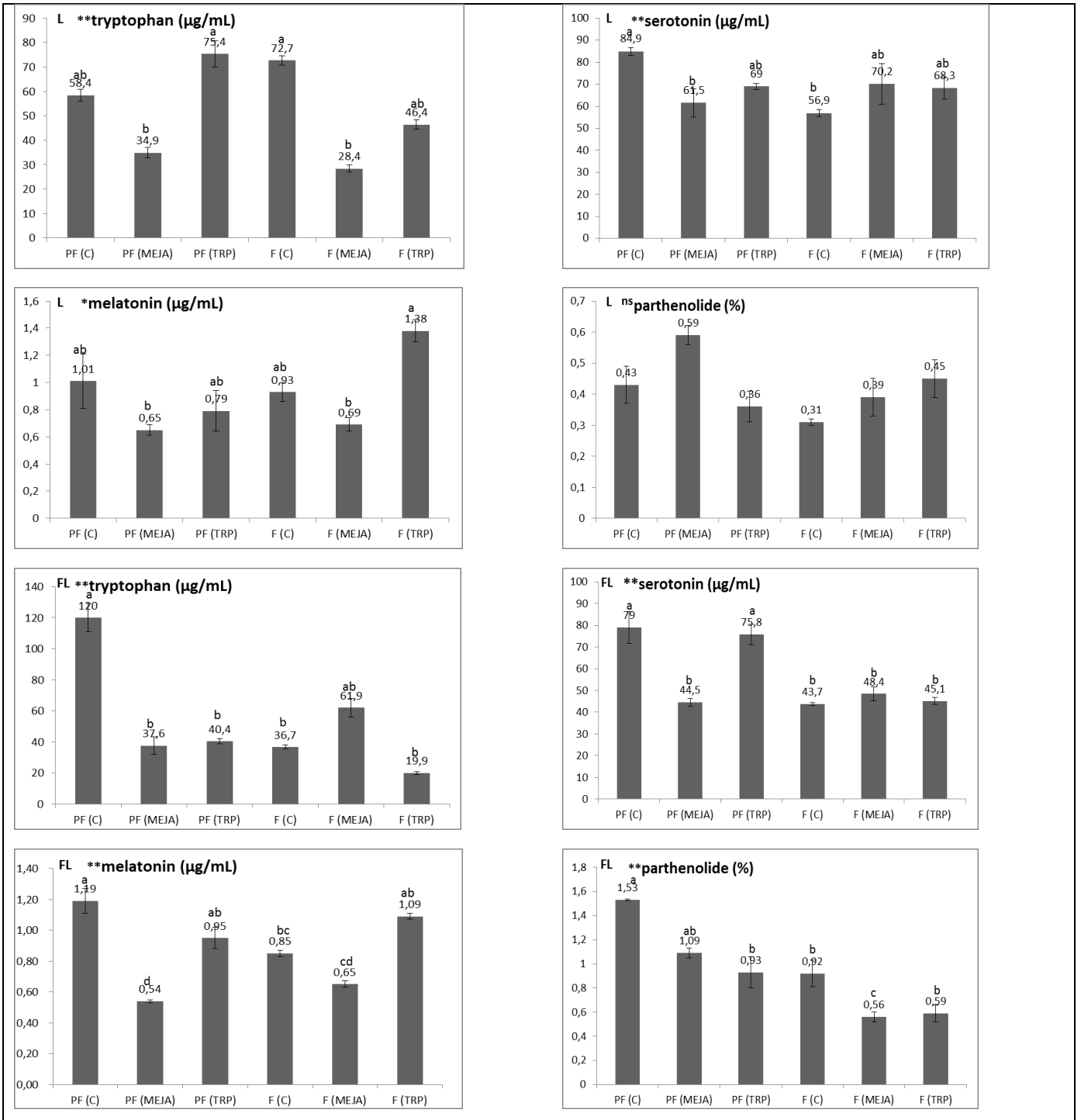
Statistical analysis

All measurements were performed in three replicates and the results were represented as means. SPSS 15 statistical program was used to determine statistical significance levels by employing the independent one-way analysis of variance (ANOVA) followed by the Tukey HSD multiple range test and the differences between individual averages were considered to be statistically significant at $p < 0.05$. The results were expressed as mean and standard deviation.

RESULTS AND DISCUSSION

Hplc Analysis of Tryptophan, Serotonin, Melatonin and Parthenolide Content

Changes in the endogenous content of TRP and its derivates SER and MEL were analyzed after exogenous MeJA and TRP applications. The results are represented in Figure 1. The chromatogram of feverfew extract is also given in Figure 2a. All treatments significantly influenced the content of the compounds in the flowers (p <0.01), but not all parameters affected the leaves at different developmental stages. With the TRP treatments, the TRP content of leaf samples at the pre-flowering stage was enhanced, but it decreased at the flowering stage in comparison with the control. Under all circumstances, TRP-mediated changes in the endogenous TRP content of the leaf tissue were higher than with the MeJA treatments, which decreased the endogenous TRP amount of the leaves. However, the TRP content peaked at the pre-flowering stages in the TRP application group. Interestingly, the flower TRP content decreased at the flowering stage by approximately 50% with TRP treatments, whereas MeJA treatment caused an increase in TRP content in the flower samples compared to the non-treated and TRP-treated groups.



Data represent means ± SD, n = 3. Different letters indicate a significant difference according to a Tukey's multiple range test (*p < 0.05, ** p < 0.01). L: Leaf, FL: Flower, PF: Pre-flowering period, F: Flowering period, C: Control, ns: not significant

Figure 1. Mean values and standard deviation of metabolites in leaves and flowers of *T. parthenium* at different developmental stages with MeJA and TRP treatments

The TRP treatments enhanced the SER and MEL content in leaves and flowers in the flowering period but reduced the endogenous TRP content. This might be explained by the conversion of TRP to its products, SER and MEL. This inference was supported by Zhao and coauthors [47], who reported that TRP decarboxylase gene (PaTDC) expression was positively related to the MEL production in cherry. The SER content of the flowers and leaves exhibited significant differences (p <0.01), ranging between 43.7 and 84.9 µg/mL in the plant tissues. Treatments triggered the biosynthesis of SER content in leaves at the flowering stage, whereas a decrease was observed at the pre-flowering stage in comparison with the control.

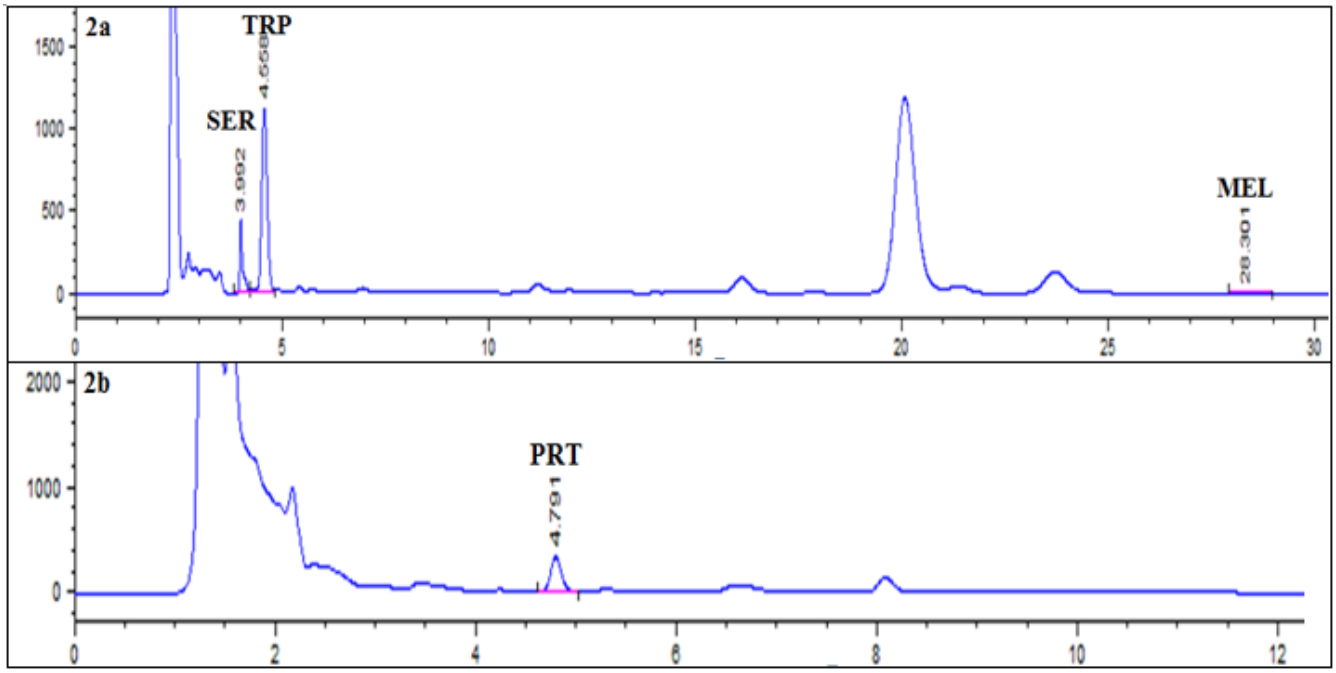


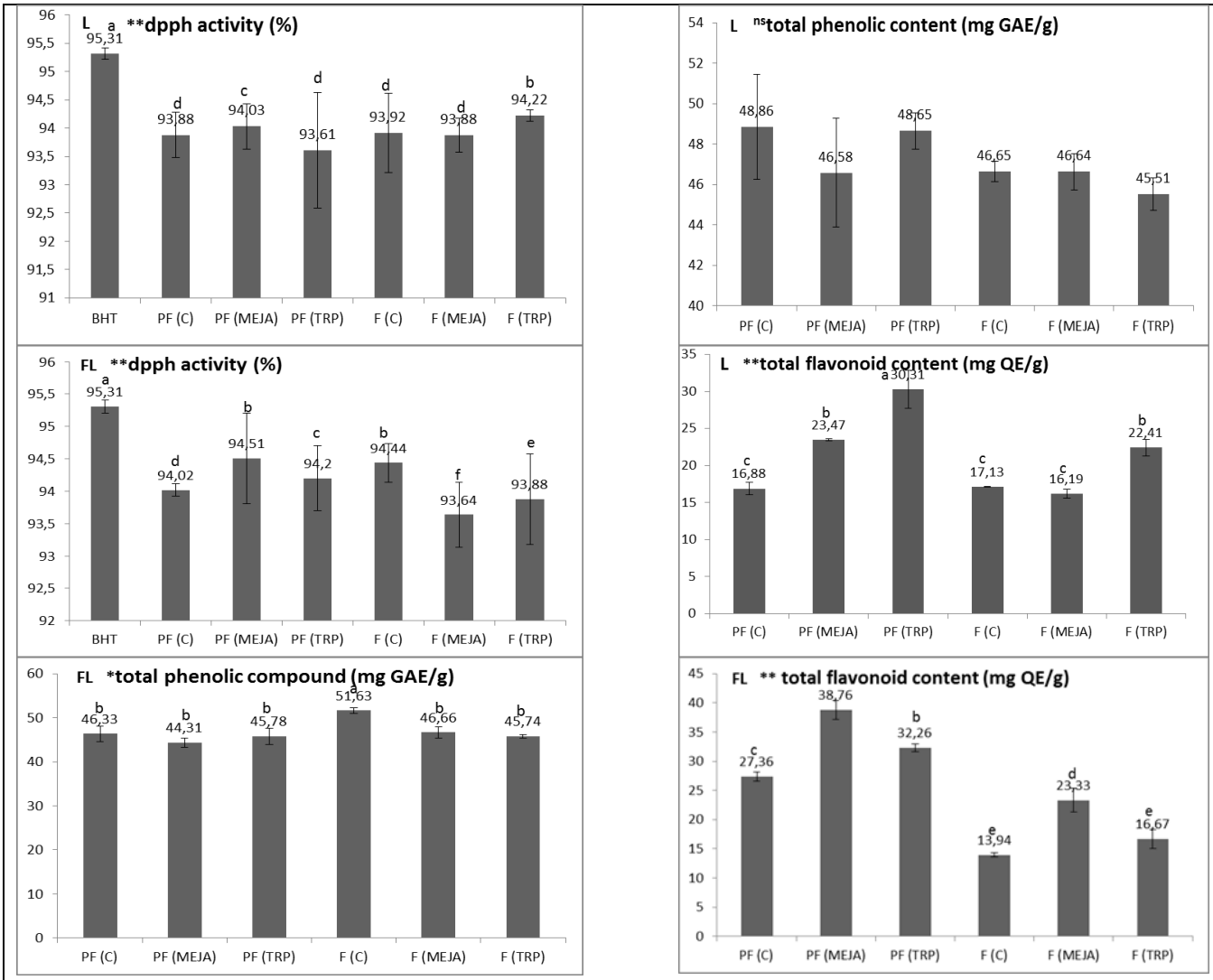
Figure 2a. HPLC chromatograms of TRP, SER and MEL in plant extracts
Figure 2b. HPLC chromatogram of PRT in plant extracts

The phytohormone MEL increased in the flowering period with TRP applications for both leaves (1.38 µg/mL) and flowers (1.09 µg/mL) compared to the control groups (0.93 µg/mL, 0.85 µg/mL). The highest MEL content was observed in leaf samples at the flowering stage with TRP treatment, whereas MeJA suppressed the biosynthesis of MEL in leaf samples at both the pre-flowering and flowering stages. However, the highest MEL content in the flowers was observed at the pre-flowering stage in the control group, followed by groups treated with TRP and MeJA, respectively. Similar to the MEL content, in the leaf samples, the highest content was observed at the flowering stage with TRP treatments, whereas MeJA again caused a decline in the content in the flowers. Likewise, in *Hypericum perforatum* germplasm, exogenous TRP application enhanced the endogenous MEL, SER, and TRP content in roots and shoots, but depended on the concentration of the application [48]. During the flowering period, the endogenous TRP, SER, and MEL content of the flowers decreased once compared with the pre-flowering control group. This decline might be attributed to the consumption of TRP for the protein biosynthesis required by the flower reproductive organs. Similar findings were also reported in *Datura metel*, with SER and MEL contents progressively decreasing as the flower buds and fruits matured [49]. During the flowering period, TRP content was increased, whereas MEL content exhibited a decline in the leaves of control groups compared to the pre-flowering period. Korkmaz and coauthors [50] reported similar results for eggplant, in which lower MEL contents in the leaves and roots were detected at the flowering stage, whereas TRP levels increased dramatically from the beginning of the flowering stage. As with all biological systems, plant systems are relatively complex and susceptible to any environmentally induced stimuli, exogenous treatments, or post-harvest practices, etc. In this study, we also examined the biochemical pathways including the primary metabolite TRP and its conversion to secondary metabolites such as the phytohormones SER and MEL. One of the well-known major metabolites of *T. parthenium* flowers is PRT, as was determined in the leaf and flowers of the plant (Figure 1; Figure 2b). The PRT content was more pronounced during the pre-flowering period than in the flowering period. The samples of control groups during the two vegetation periods had higher amounts of PRT content (1.53%-0.92%) in the flowers than did the treatment group samples (1.09%, 0.93%-0.56%, 0.59%). In the leaves, TRP applications enhanced the PRT content in the flowering period, whereas MeJA applications increased the PRT content in both the pre-flowering and flowering periods, although this effect was not statistically significant. Furthermore, exogenous application of 2,4-D enhanced the PRT level [51,52], and PRT biosynthesis was significantly increased with the addition of 2 mM Mg up to 50 µM Mn [53]. Applications with a combination of α-naphtalenacetic acid and kinetin induced higher PRT amounts in shoots than in roots in 30-day-old tissues, and also in younger tissues (9, 20, and 24 days) [54]. The MeJA applications were effective only in the first 24 h, after which increases were not seen in the *T. parthenium* leaves [55]. In the study of Malarz and coauthors [56], a sesquiterpene lactone group reached the maximum (over 200% compared to the control) in 72 h after jasmonate treatment of the hairy roots of *Cichorium intybus*. Rowe and coauthors [57] reported that MeJA-treated sunflower plants had a lower sesquiterpene lactone production,

although MeJA has been successfully used to induce secondary metabolite production in many plants, such as the artemisin level in *Artemisia annua* [58] and the xanthumin level in *Xanthium strumarium* [59], the rosmarinic and caffeic acid in *Ocimum basilicum* [39,40] and *Salvia miltiorrhiza* [60], the saponin content in *Calendula officinalis* [61], and the jacaranone amount in *Jacobaea vulgaris* and *Jacobae aquatic* [62].

Analysis of Antioxidant Activity, Total Phenolic and Total Flavonoid Contents

Phenolic compounds, including flavonoids, play multiple regulatory and protective roles in plant and human physiology including defense, antioxidant activity, free radical scavenging, signaling, and mediating auxin transport [63]. The average total phenolic contents, total flavonoid concentrations and antioxidant activity of feverfew grown with applications of different plant growth regulators are presented in Figure 3.



Data represent means ± SD, n = 3. Different letters indicate a significant difference according to a Tukey's multiple range test (*p < 0.05, ** p < 0.01). L: Leaf, FL: Flower, PF: Pre-flowering period, F: Flowering period, C: Control, ns: not significant

Figure 3. Mean values and standard deviation of total phenolic and flavonoid contents and DPPH antioxidant activity in leaves and flowers of *T. parthenium* at different developmental stages with MeJA and TRP treatments

Antioxidant capacity was determined according to the DPPH method. Extracts of leaves (93.61%-94.22%) and flowers (93.64%-94.51%) exhibited significant differences from the positive control BHT (p <0.01). Feverfew showed strong free radical-scavenging activity, especially in the flowering period in leaves and in the pre-flowering period in flowers. In previous studies, *T. parthenium* extracts showed high DPPH scavenging activity 84.4% [64] and 81.12% [65], and 83.66% and 88% in leaves and flowers, respectively [66]. The results obtained herein are consistent with the previous studies. Total phenolic contents in the pre-flowering period ranged from 44.31 to 45.78-46.33 mg GAE/g dry weight (DW) for feverfew flowers treated

with MeJA and TRP applications and for the control group and in the flowering period, from 46.66 to 45.74-51.63 mg GAE/g DW, respectively. Total flavonoid content ranged from 16.19 to 30.31 mg QE/g in the leaves, and from 13.94 to 38.76 QE/g in the flowers. In the current study, no statistically significant effects from the treatments were exhibited on the total phenolic contents in the leaves of the plant (p >0.05) or in the flowers during the pre-flowering period. However, in comparison with control groups, decreases were observed in the flowering period. The results of the current study could indicate that TRP and MeJA might have given rise to the increased activity of the enzymes that caused the degradation of the phenolic compounds [67].The TRP and MeJA applications enhanced the total flavonoid contents significantly in both leaves and flowers compared to the control groups (p <0.01). In the flowers, total flavonoid content was more pronounced in the pre-flowering period than in the flowering period. The MeJA treatment groups had more abundant total flavonoid content than the others. However, it was observed that the TRP applications were more effective in the leaves.

The TRP application significantly enhanced the total phenol and flavonoid contents of *Citrullus colocynthis* L. [68] and the total phenolic compounds of lupine seeds [69], although induced total phenolic content inhibited the total flavonoid content in basil leaves, depending on the application dose [11]. The MeJA applications stimulated a group of flavonoids known as anthocyanins in cell cultures of ohelo (*Vaccinium phalae*) [70], enhanced the total phenol and flavonoid contents of basil leaves [40] and the total phenolic content and antioxidant capacity of 7-day buckwheat cultures [71]. Danaee and coauthors [72] reported that MeJA treatments increased the flavonoid, phenolic yield, and antioxidant activity, depending on concentration, in *Phyllanthus pulcher* callus. According to Chung and coauthors [73], jasmonic acid-elicited hairy root cultures of *Momordica charantia* produced significantly higher amounts of phenolic compounds, total phenolic and flavonoid content, and DPPH activity. Açıkgöz and coauthors [74] stated that, MeJA applications neither enhanced nor affected total phenolic content and neither decreased nor affected total flavonoid content in *Achillea gypsicola* cell suspensions, depending on application dose. In *Achillea millefolium* aerial parts, the total flavonoid amount was shown to increase and the DPPH antioxidant activity to either increase or remain unchanged with different concentrations of MeJA application [75]. Duran and coauthors [76] reported that the flavonoid production of *Thevetia peruviana* cell suspension cultures was enhanced by MeJA application. Several reports have shown that the application of different concentrations of MeJA led to changes such as lower or higher antioxidant capacity in different tissues of the plants at harvest time.

Correlation and Multiple Regression Analysis of the Metabolites

According to the correlation analysis, moderate and strong negative correlations were observed between PRT-Leaf and TRP-Leaf; TPC-Flower and SER-Leaf; TPC-Flower and SER-Flower; TPC-Flower and PRT-Leaf; TPC-Leaf and DPPH-Leaf; DPPH-Leaf and PRT-Leaf; DPPH-Leaf and PRT-Flower; TFC-Leaf and MEL-Leaf; TFC-Leaf and MEL-Flower; TFC-Leaf and TPC-Flower; TFC-Flower and TPC-Flower.

The remaining correlation coefficients between other parameters were low or positive. The PRT content was found to be negatively correlated with the DPPH scavenging activities of the extracts. Moreover, the total phenolic content was clearly determined to be negatively correlated with the SER content in the plant tissues, as was the total flavonoid content with the MEL content in the plant tissues (Table 1).

Correlation coefficients of the metabolites estimated in flowers at pre-flowering and flowering stages with MEL and TRP treatment (r=0.52), SER and TRP (r=0.60), SER and PRT (r=0,56), as well as TRP and PRT (r=0.60). It is worthy to note that all correlation coefficients are positive directed (Table 2).

However, no statistically significant correlations were found between the metabolites examined in leaf samples. In contrast to flower samples, the correlation coefficients were negatively directed except for the correlations between MEL and SER (0.08) and SER and PRT (0.04) (Table 3).

Table 1. Correlation coefficients of the variables in leaves and flowers of *T. parthenium* at different developmental stages with MeJA and TRP treatment

	TRP-Leaf	TRP-Flower	SER-Leaf	SER-Flower	MEL-Leaf	MEL-Flower	PRT-Leaf	PRT-Flower	DPPH-Leaf	DPPH-Flower	TPC-Leaf	TPC-Flower	TFC-Leaf	TFC-Flower
TRP-Leaf		0.03	-0.05	0.49	0.12	0.54	-0.64	0.33	0.04	0.68	0.02	0.33	-0.40	0.21
TRP-Flower	0.03		0.83	0.67	-0.07	0.40	-0.04	0.74	0.11	-0.07	0.48	-0.13	-0.24	0.02
SER-Leaf	-0.05	0.83		0.76	0.20	0.62	0.04	0.49	0.22	-0.38	0.32	-0.49	-0.32	0.24
SER-Flower	0.49	0.67	0.76		-0.11	0.59	-0.21	0.63	0.18	0.27	0.32	-0.47	-0.14	0.59
MEL-Leaf	0.12	-0.07	0.20	-0.11		0.72	-0.09	-0.12	-0.11	-0.60	0.01	0.28	-0.78	-0.28
MEL-Flower	0.54	0.40	0.62	0.59	0.72		-0.35	0.33	0.09	-0.21	0.16	0.03	-0.80	0.10
PRT-Leaf	-0.64	-0.04	0.04	-0.21	-0.09	-0.35		0.20	-0.72	-0.33	0.62	-0.44	0.62	0.41
PRT-Flower	0.33	0.74	0.49	0.63	-0.12	0.33	0.20		-0.48	0.34	0.87	-0.01	0.02	0.38
DPPH-Leaf	0.04	0.11	0.22	0.18	-0.11	0.09	-0.72	-0.48		-0.09	-0.81	-0.10	-0.33	-0.35
DPPH-Flower	0.68	-0.07	-0.38	0.27	-0.60	-0.21	-0.33	0.34	-0.09		0.09	0.20	0.31	0.32
TPC-Leaf	0.02	0.48	0.32	0.32	0.01	0.16	0.62	0.87	-0.81	0.09		-0.09	0.21	0.44
TPC-Flower	0.33	-0.13	-0.49	-0.47	0.28	0.03	-0.44	-0.01	-0.10	0.20	-0.09		-0.48	-0.73
TFC-Leaf	-0.40	-0.24	-0.32	-0.14	-0.78	-0.80	0.62	0.02	-0.33	0.31	0.21	-0.48		0.51
TFC-Flower	0.21	0.02	0.24	0.59	-0.28	0.10	0.41	0.38	-0.35	0.32	0.44	-0.73	0.51	

Table 2. Correlation coefficients of the metabolites estimated in flowers at pre-flowering and flowering stages with MeJA and TRP treatment

	Mean	Std deviation	Melatonin	Serotonin	Tryptophan	Parthenolide
Melatonin	0.88	0.25	1.00	0.52	0.31	0.27
Serotonin	56.12	16.23	0.52	1.00	0.60	0.56
Tryptophan	54.10	42.24	0.31	0.60	1.00	0.60
Parthenolide	0.94	0.38	0.27	0.56	0.60	1.00

Table footnote: Marked correlations are significant at p < ,05000; N=18

Table 3. Correlation coefficients of the metabolites estimated in leaves at pre-flowering and flowering stages with MeJA and TRP treatment

	Mean	Std deviation	Melatonin	Serotonin	Tryptophan	Parthenolide
Melatonin	0.90	0.40	1.00	0.08	-0.06	-0.19
Serotonin	68.54	14.56	0.08	1.00	-0.17	0.04
Tryptophan	52.75	35.27	-0.05	-0.17	1.00	-0.01
Parthenolide	0.42	0.23	-0.19	0.04	-0.01	1.00

Table footnote: Marked correlations are significant at p < ,05000; N=18

CONCLUSION

Recently, many studies have been conducted on the exogenous application of phytohormones and their responses. This study investigated the role of the plant growth regulators TRP and MeJA on changes in the secondary metabolite content and antioxidant activity of *T. parthenium*. Active metabolites of *T. parthenium* were affected by TRP and MeJA applications, dependent on the vegetation period and plant tissue involved. The MeJA treatment was observed to enhance the SER level, whereas the TRP treatment enhanced the SER and MEL levels in the flowering period. Furthermore, the PRT content was not positively influenced by the treatments. None of the treatments enhanced the main metabolites. That could be attributed to the formation of different intermediate metabolites, indicating that different metabolic pathways might play a role in the conversion of the products to the target molecules. Different valuable plant native products have been collected from specific specialized structures. By creating an infrastructure, this study facilitates opportunities for future studies that can provide new prospects for enhancing and diversifying the manufacture of highly valuable bioactive metabolites.

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REFERENCES

1. Awang DV, Dawson BA, Kindack DG, Crompton CW, Heptinstall S. Parthenolide content of feverfew (*Tanacetum parthenium*) assessed by HPLC and 1H-NMR spectroscopy. J Nat Prod. 1991 Nov; 54(6):1516-21.
2. Liu Q, Manzano D, Tanić N, Pesic M, Bankovic J, Pateraki I, Bouwmeester H. Elucidation and in planta reconstitution of the parthenolide biosynthetic pathway. Metab. Eng. 2014 May; 23: 145-53.
3. Mannelli LDC, Tenci B, Zanardelli M, Maidecchi A, Lugli A, Mattoli L, Ghelardini C. Widespread pain reliever profile of a flower extract of *Tanacetum parthenium*. Phytomedicine. 2015 July; 22(7-8): 752-8.
4. Majdi M, Liu Q, Karimzadeh G, Malboobi MA, Beekwilder J, Cankar K, et al. Biosynthesis and localization of parthenolide in glandular trichomes of feverfew (*Tanacetum parthenium* L. Schulz Bip.). Phytochem. 2011 Oct; 72:1739-50.
5. Tiuman TS, Ueda-Nakamura T, Cortez DAG, Dias Filho BP, Morgado-Díaz JA, De Souza W, et al. Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium.* Antimicrob. Agents Chemother. 2005 Jan; 49(1):176-82.
6. Long J, Ding YH, Wang PP, Zhang Q, Chen Y. Protection-group-free semisyntheses of parthenolide and its cyclopropyl analogue. J. Org. Chem. 2013 Sep; 78(20):10512-8.
7. Guzman ML, Rossi RM, Karnischky L, Li X, Peterson DR, Howard DS, et al. The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood. 2005 Jun; 105(11):4163-9.
8. Bahrami M, Kamalinejad M, Latifi SA, Seif F, Dadmehr M. Cytokine storm in COVID-19 and parthenolide: preclinical evidence. Phytother Res. 2020 May; 34(10):2429–30.
9. Vered T, Galili G. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants, Mol plant. 2010 Nov; 3(6): 956-72.
10. Radwanski, ER, Last RL. Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. The Plant Cell. 1995 Jul; 7(7): 921-34.
11. Kahveci H, Bilginer N, Diraz-Yildirim E, Kulak M, Yazar E, Kocacinar F, et al. Priming with salicylic acid, β-carotene and tryptophan modulates growth, phenolics and essential oil components of *Ocimum basilicum* L. grown under salinity. Sci. Hortic. 2021 Apr; 281:109964.
12. Rahmatzadeh S, Khara J, Kazemitabar SK. The study of in vitro regeneration and growth parameters in *Catharanthus roseus* L. under application of tryptophan. Mater. Energy. 2014; 14(3): 249-60.
13. Abdel Aziz NG, Mazher AAM, Farahat MM. Response of vegetative growth and chemical constituents of *Thuja orientalis* L. plant to foliar application of different amino acids at Nubaria. J. Am. Sci. 2010; 6(3):295-301
14. Dawood MG, Sadak MS. Physiological response of canola plants (*Brassica napus* L.) to tryptophan or benzyladenine. Lucrari stiintifice. 2007; 50:198-207.
15. Talaat IM, Bekheta MA, Mahgoub MH. Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. Int J Agric. Biol. 2005 Feb; 7(2): 210-3.
16. Dahab TA, El-Aziz NGA. Physiological effect of Diphenylamin and Tryptophan on the growth and chemical constituents of *Philodendron erubescens* plants. World J. Agric.Sci. 2006 Jan-Mar; 2(1):75-81.
17. Ramakrishna A, Giridhar P, Ravishankar GA. Phytoserotonin a review. Plant Signal Behav. 2011 Jun 6(6): 800-9.
18. Erland LA, Murch SJ, Reiter RJ, Saxena PK. A new balancing act: the many roles of melatonin and serotonin in plant growth and development. Plant Signal. Behav. 2015 Dec; 10(11):1-14.

19. Arnao MB, Hernández-Ruiz J. The Potential of phytomelatonin as a nutraceutical. Molecules. 2018 Jan;23(1):238-57.
20. Essa MM, Hamdan H, Chidambaram SB, Al-Balushi B, Guillemin GJ, Ojcius DM, Qoronfleh MW. Possible role of tryptophan and melatonin in COVID-19. Int. J. Tryptophan Res. 2020 Jan-Dec; 13: 1-2.
21. Reiter RJ, Abreu-Gonzalez P, Marik PE, Dominguez-Rodriguez A. Therapeutic algorithm for use of melatonin in patients with COVID-19. Front. Med. 2020 May; 7: 226-43.
22. Acuña-Castroviejo D, Escames G, Figueira JC, De la Oliva P, Borobia AM, Acuña-Fernández C. Clinical trial to test the efficacy of melatonin in COVID-19. J. Pineal Res. 2020 Agust; 69(3): 1-4.
23. Juhnevica-Radenkova K, Moreno DA, Ikase L, Drudze I, Radenkovs V. Naturally occurring melatonin: Sources and possible ways of its biosynthesis.Compr. Rev. Food Sci. Food Saf. 2020 Sep;19(6):4008-30
24. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules. 2019 Nov; 24(22): 4132.
25. Elmastaş M, Ozturk L, Gokce I, Erenler R, Aboul-Enein HY. Determination of antioxidant activity of marshmallow flower (*Althaea officinalis* L.). Anal. Lett. 2004 Oct; 37(9): 1859-69.
26. Elmastaş M, Telci İ, Akşit H, Erenler R. Comparison of total phenolic contents and antioxidant capacities in mint genotypes used as spices. Turkish J. Biochem. 2015 Nov; 40(6), 456-62.
27. Demirtas I, Erenler R, Elmastas M, Goktasoglu A. Studies on the antioxidant potential of flavones of *Allium vineale* isolated from its water-soluble fraction. Food Chem. 2013 Jan; 136(1), 34-40.
28. Lucho SR, Do Amaral MN, López-Orenes A, Kleinowski AM, Do Amarante L, Ferrer MÁ, et al. Plant growth regulators as potential elicitors to increase the contents of phenolic compounds and antioxidant capacity in *Stevia* plants. Sugar Tech. 2019 Jul; 21: 696-702.
29. Gharaghanipor N, Arzani A, Rahimmalek M, Ravash R. Physiological and transcriptome indicators of salt tolerance in wild and cultivated barley. Front. Plant Sci. 2022 Apr; 13, 1-22
30. Haghighi R, Sayed-Tabatabaei BE, Maibody M, Arzani A, Omidi M, Talebi M. Expression of flowering repressor gene cssvp, carbohydrates, and antioxidants affected by plant growth regulators in saffron. J. Plant Growth Regul. 2022 Mar; 1-15.
31. Kondo S, Setha S, Rudell DR, Buchanan DA, Mattheis JP. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. Postharvest Biol. Technol. 2005 Apr; 36(1): 61-8.
32. Külheim C, Jones CG, Plummer JA, Ghisalberti EL, Barbour L, Bohlmann J. Foliar application of methyl jasmonate does not increase terpenoid accumulation, but weakly elicits terpenoid pathway genes in sandalwood (*Santalum album* L.) seedlings. Plant Biotechnol. 2014; 31(5): 585-91.
33. Bodnaryk RP. Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. Phytochemistry. 1994 Jan; 35(2): 301-5.
34. Kim HS, Juvik JA. Effect of selenium fertilization and methyl jasmonate treatment on glucosinolate accumulation in broccoli florets. J. Am. Soc. Hortic. Sci. 2011 Jul; 136(4): 239-46.
35. Ku KM, Choi JH, Kushad MM, Jeffery EH, Juvik JA. Pre-harvest methyl jasmonate treatment enhances cauliflower chemoprotective attributes without a loss in postharvest quality. Plant Foods Hum. Nutr. 2013 May; 68: 113-7.
36. Liu Z, Mohsin A, Wang Z, Zhu X, Zhuang Y, Cao L, Yin Z. Enhanced biosynthesis of chlorogenic acid and its derivatives in methyl-jasmonate-treated gardenia jasminoides cells: a study on metabolic and transcriptional responses of cells. Front. Bioeng. Biotechnol. 2020 Jan; 8: 1-19.
37. Wei X, Vrieling K, Kim HK, Mulder PP, Klinkhamer PG. Application of methyl jasmonate and salicylic acid lead to contrasting effects on the plant's metabolome and herbivory. Plant Sci. 2021 Feb; 303: 110784.
38. Złotek U, Szymanowska U, Karaś M, Świeca M. Antioxidative and anti-inflammatory potential of phenolics from purple basil (*Ocimum basilicum* L.) leaves induced by jasmonic, arachidonic and β-aminobutyric acid elicitation. Int. J. Food Sci. Tech. 2016 Oct; 51(1):163-70.
39. Kim HJ, Chen F, Wang X, Rajapakse NC. Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 2006 Feb; 54(6): 2327-32.
40. Koca N, Karaman Ş. The effects of plant growth regulators and l-phenylalanine on phenolic compounds of sweet basil. Food Chem. 2015 Jan; 166(1): 515-21.
41. Yousefian S, Lohrasebi T, Farhadpour M, Haghbeen K. Effect of methyl jasmonate on phenolic acids accumulation and the expression profile of their biosynthesis-related genes in *Mentha spicata* hairy root cultures. PCTOC. 2020 May; 142: 285-97.
42. Wang H, Ma C, Li Z, Ma L, Wang H, Ye H, Liu B. Effects of exogenous methyl jasmonate on artemisinin biosynthesis and secondary metabolites in *Artemisia annua* L. Ind. Crops. Prod. 2010 Mar; 31(2): 214-8.
43. Pérez-Balibrea S, Moreno DA, García-Viguera C. Improving the phytochemical composition of broccoli sprouts by elicitation. Food Chem. 2011 Nov; 129(1): 35-44.
44. Ansari M, Rafiee K, Yasa N, Vardasbi S, Naimi SM, Nowrouzi A. Measurement of melatonin in alcoholic and hot water extracts of *Tanacetum parthenium, Tripleurospermum disciforme* and *Viola odorata*. Daru. 2010 Apr; 18(3):173–78.
45. Fonseca JM, Rushing JW, Rajapakse NC, Riley M. Phenolics and parthenolide levels in feverfew (*Tanacetum parthenium*) are inversely affected by environmental factors. J. Appl. Hortic. Jun 2008; 10(1): 36-9.
46. Subedi L, Timalsena S, Duwadi P, Thapa R, Paudel A, Parajuli K. Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. J Tradit Chin Med. 2014 Oct; 34(5): 584-90.

47. Zhao Y, Tan DX, Lei Q, Chen H, Wang L, Li QT, et al. Melatonin and its potential biological functions in the fruits of sweet cherry. J. Pineal. Res. 2013 Jan; 55(1): 79–88.
48. Erland LA, Shukla MR, Singh AS, Murch SJ, Saxena PK. Melatonin and serotonin: mediators in the symphony of plant morphogenesis. J. Pineal Res. 2018 Mar; 64(2):1-24.
49. Murch SJ, Alan AR, Cao J, Saxena PK. Melatonin and serotonin in flowers and fruits of *Datura metel* L. J. Pineal Res. 2009 Sep; 47(3): 277-83.
50. Korkmaz A, Yakupoğlu G, Köklü Ş, Cuci Y, Kocacinar F. Determining diurnal and seasonal changes in melatonin and tryptophan contents of eggplant (*Solanum melongena* L.). Turk J Botany. 2017 Jan; 41(4): 356-66.
51. Fonseca JM, Rushing JW, Rajapakse NC, Thomas RL, Riley MB. Parthenolide and abscisic acid synthesis in feverfew are associated but environmental factors affect them dissimilarly. J. Plant Physiol. 2005 May; 162(5): 485-94.
52. Majdi M, Charnikhova T, Bouwmeester H. Genetical, developmental and spatial factors influencing parthenolide and its precursor costunolide in feverfew (*Tanacetum parthenium* L. Schulz Bip.). Ind. Crops. Prod. 2013 May; 47: 270-6.
53. Farzadfar S, Zarinkamar F, Behmanesh M, Hojati M. Magnesium and manganese interactively modulate parthenolide accumulation and the antioxidant defense system in the leaves of *Tanacetum parthenium*. J. Plant Physiol. 2016 Sep; 202: 10-20.
54. Trujillo AN, González LB, Morales CG, Guerrero AR, Sosa FC, Zuñiga ME. Phenolic compounds and parthenolide production from in vitro cultures of *Tanacetum parthenium*. Rev Mex Ing Quim. 2017 May-Aug; 16(2): 371-83.
55. Majdi M, Abdollahi MR, Maroufi A. Parthenolide accumulation and expression of genes related to parthenolide biosynthesis affected by exogenous application of methyl jasmonate and salicylic acid in *Tanacetum parthenium*. Plant Cell Rep. 2015; 34: 1909-18.
56. Malarz J, Stojakowska A, Kisiel W. Effect of methyl jasmonate and salicylic acid on sesquiterpene lactone accumulation in hairy roots of *Cichorium intybus*. Acta Physiol. Plant. 2007 Jan; 29:127-32.
57. Rowe HC, Dae-kyun R, Loren H, Rieseberg LH. Response of sunflower (*Helianthus annuus* L.) leaf surface defenses to exogenous methyl jasmonate. PLoS One. 2012 May; 7(5):1-11.
58. Wang H, Ma C, Li Z, Ma L, Wang H, Ye H, Liu B. Effects of exogenous methyl jasmonate on artemisinin biosynthesis and secondary metabolites in *Artemisia annua* L. Ind. Crops. Prod. 2010 Mar; 31(2): 214-8.
59. Li C, Chen F, Zhang Y. GA3 and other signal regulators (MeJA and IAA) improve *Xanthumin* biosynthesis in different manners in *Xanthium strumarium* L. Molecules. 2014 Aug; 19(9):12898-908.
60. Xiao Y, Gao S, Di P, Chen J, Chen W, Zhang L. Methyl jasmonate dramatically enhances the accumulation of phenolic acids in *Salvia miltiorrhiza* hairy root cultures. Physiol. Plant. 2009 Sep; 137(1): 1-9.
61. Ghanati F, Bakhtiarian S. Effect of methyl jasmonate and silver nanoparticles on production of secondary metabolites by *Calendula officinalis* L (Asteraceae). Trop. J. Pharm. Res. 2014 Sep; 13(11):1783-9.
62. Wei X, Vrieling K, Kim HK, Mulder PP, Klinkhamer PG. Application of methyl jasmonate and salicylic acid lead to contrasting effects on the plant's metabolome and herbivory. Plant Sci. 2021 Feb; 303: 110784.
63. Kiani R, Arzani A, Mirmohammady Maibody SAM. Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, *Aegilops cylindrica* and their amphidiploids. Front. Plant Sci. 2021 Mar; 12: 646221.
64. Wu C, Chen F, Wang X, Kim HJ, He GQ, Haley-Zitlin V, Huang G. Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. Food. Chem. 2006 May; 96(2): 220-7.
65. Fraisse D, Felgines C, Texier O, Lamaison JL. Caffeoyl derivatives major antioxidant compounds of some wild herbs of the Asteraceae family. Food Nutr Sci. 2011Apr; 2(3): 181-92.
66. Dıraz E. Determination of the morphological features, chemical composition and biological activity of *Tanacetum* L. taxa from Kahramanmaraş region. [PhD thesis]. Kahramanmaraş: University of Kahramanmaraş Sütçü İmam, Graduate School of Natural and Applied Sciences; 2015. 157 p.
67. Rizzi GP, Boekley LJ. Observation of ether-linked phenolic products during thermal degradation of ferulic acid in the presence of alcohols. J. Agric. Food Chem. 1992 Sep; 40(9): 1666-70.
68. Sanikhani M, Akbari A, Kheiry A. Effect of phenylalanine and tryptophan on morphological and physiological characteristics in colocynth (*Citrullus colocynthis* L.). J. Plant Proc. Func. 2020 May; 9:317-28.
69. Yasser AMK, Gamal F, El-Naem A, Mohamed AM. Effect of Tryptophan and Ascorbic Acid on Yield and Some Chemical Constituents of Lupine (*Lupines termis* L.) Plants. Egypt. J. Agron. 2020; 42: 47-61.
70. Fang Y, Smith MAL, Pépin MF. Effects of exogenous methyl jasmonate in elicited anthocyanin-producing cell cultures of ohelo (*Vaccinium phalae*). In Vitro Cell. Dev. Biol– Plant. 1999; 9(35): 106-13.
71. Kim HJ, Park KJ, Lim JH. Metabolomic analysis of phenolic compounds in buckwheat (*Fagopyrum esculentum* M.) sprouts treated with methyl jasmonate. J. Agric. Food Chem. 2011 Mar; 59(10): 5707–13.
72. Danaee M, Farzinebrahimi R, Kadir MA, Sinniah UR, Mohamad R, Taha RM. Effects of MeJA and SA elicitation on secondary metabolic activity, antioxidant content and callogenesis in *Phyllanthus pulcher.* Rev. Bras. Bot. 2015 Jul; 38: 265-72.
73. Chung Ill-Min, Thiruvengadam M, Rekha K, Rajakumar G. Elicitation enhanced the production of phenolic compounds and biological activities in hairy root cultures of bitter melon (*Momordica charantia* L.). Braz Arch Biol Technol. 2016 Jan-Dec; 59:e16160393.

74. Açikgöz MA, Kara ŞM, Aygün A, Özcan MM, Ay EB. Effects of methyl jasmonate and salicylic acid on the production of camphor and phenolic compounds in cell suspension culture of endemic Turkish yarrow (*Achillea gypsicola*) species. Turk J Agric For. 2019 Jun; 43(3):351-9.
75. Ghanati F, Bakhtiarian S, Parast BM, Behrooz MK. Production of new active phytocompounds by *Achillea millefolium* L. after elicitation with silver nanoparticles and methyl jasmonate. Biosci Biotechnol Res Asia. 2014 Sep;11(2): 391-9.
76. Durán MDL, Zabala MEA, Londoño GAC. Optimization of flavonoid production in plant cell culture of *Thevetia peruviana* elicited with methyl jasmonate and salicylic acid. Braz Arch Biol Technol. 2021 Jun; 64: e21210022