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Effect of Methyl Jasmonate, Salicylic Acid and Their Combination on Growth and Bioactive Constituents in *Cichoriun intybus* L.

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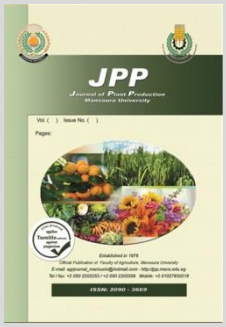

ABSTRACT

The current study was conducted to evaluate the efficiency of seedling's roots-feeding of the elicitor solution of methyl jasmonate (MeJ), salicylic acid (SA) and their combination as an elicitation approach for enhancing the biosynthesis of cichory's main bioactive secondary metabolites. The long-lasting elicitation effect of both MeJ and SA was also evaluated through comparing the level of bioactive compounds in treated plants of the first harvest with those of the second one. Results indicated that MeJ, SA, and their combination decreased growth of cichory plants whereas increased their potential to biosynthesize their characteristic bioactive secondary metabolites. Shoots and roots of MeJ-, SA- and MeJ+SA-treated plants contained significantly higher contents from ascorbic acid as well as total antioxidants, total phenols as well as flavonoids and inulin compared with untreated plants of either the first or the second harvest. The effect of MeJ surpassed that of SA in inducing contents of the secondary metabolites. Control plants of the second harvest had, generally, higher contents from ascorbic acid, total antioxidants, total phenols total flavonoids and inulin compared with plants of the first harvest. It could be concluded that seedlings' roots-feeding of the elicitor solution is feasible, easy and cost-effective approach for long-lasting elicitation effect, and that MeJ is superior to SA in eliciting cichory's bioactive secondary metabolites.

Keywords: *Cichoriun intybus*; elicitation; methyl jasmonates; salicylic acid; secondary metabolites

INTRODUCTION

Chicory (*Cichorium intybus* L.) is a plant species belonging to the Asteraceae family with many commercial uses. Chicory was grown by the ancient Egyptians as a medicinal plant, coffee substitute, vegetable crop, and occasionally for animal forage (Munoz, 2004). The root, leaf and seeds contain a number of medicinally important compounds such as inulin, sesquiterpene lactones, coumarins, flavonoids and vitamins thereby, these organs were traditionally used to cure various ailments. The phytochemical composition and several pharmaceutical properties of *C. intybus* have been extensively reviewed (Azay-Milhau *et al*., 2013; Morales *et al*., 2014). The plant has immunomodulator (Watzl *et al*., 2005) and anticancer properties (Hughes and Rowland, 2001) and preventive effect against colon cancer (Watzl *et al*., 2005). In addition, it has also antihepatotoxic, antiulcerogenic, anti-inflammatory, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, digestive stomachic, depurative and diuretic effects (Rastogi and Mehrotra, 1994). These biological properties could be attributed to inulin, vitamins, and specialized metabolites such as sesquiterpene lactones, flavonoids, coumarins, hydroxycinnamic acids as well as alkaloids that are present in the different parts of *C. intybus* (Das *et al*., 2016). Inulin is a reserve carbohydrate, present in higher amounts in the roots of chicory which is reported to be useful for fat and sugar replacement (Franck, 2002).

Secondary metabolites are produced by plant cells in response to environmental stimuli or as defensive mechanisms against invading pathogens. Different approaches have been utilized for the aim of enhancing the accumulation of PSM. These approaches include the use and optimization of the tissue/cell cultures conditions (Isah *et al*., 2018), Nutrient and precursor feeding, Immobilization of plant cells (Murthy *et al*., 2014), Metabolic engineering (Lu *et al*., 2017), and elicitation (Zhao *et al*., 2005). Elicitation aims to misguide the cells or tissues for a possible biotic/abiotic attack by using agents that trigger the defense responses at the biochemical and molecular level via upregulation of genes (Zhao *et al*., 2005). The elicitors can be biotic or abiotic and may comprise signaling molecules like methyl jasmonate, salicylic acid, microbial cell wall extracts (e.g., yeast extract, chitosan), inorganic salts, heavy metals, physical agents (e.g., UV radiation) among others (Ramirez-Estrada *et al*., 2016). Methyl jasmonates and its related signal molecules, and salicylic acid are probably the most extensively used elicitors (Giri and Zaheer, 2016). Enhanced PSM accumulation in response to MeJ (Ali *et al*., 2005; Ali *et al*., 2007; Yousefan *et al*., 2020) and SA (Ali *et al*., 2007; Norozi *et al*., 2019) was reported. For a comprehensive review about MeJ and SA as elicitors of PSM, the publications of Ho *et al*. (2020) and Ali (2021), respectively, may be consulted.

Elicitation is mainly adopted to enhance the biosynthesis of bioactive compounds in in vitro-grown plant cells and tissues and, afterwards, these bioactive compounds are separated and converted to pharmaceutical products. Cell suspension culture is the most used culture

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system for elicitation of secondary metabolites (Giri and Zaheer, 2016). However, the efficiency of in vivo elicitation through seedlings' roots-feeding with the elicitor solution, as a cost-effective approach was poorly-explored and less understood. If we managed, otherwise, to elicit bioactive compounds in vivo, we will provide the consumer, directly, with health-promoting plant products. This approach is more feasible in case of vegetable crops which could be transplanted by seedlings raised in the nursery, as the provision of the elicitor in this stage is effort-conserving and cost-effective. This could be done through treating the seedlings with the elicitors either as a foliar spray or as roots-feeding. As provision through spraying could be constrained by leaf surface's absorption and climatic conditions, the addition through roots-feeding would be more feasible approach, therefore, this route of elicitors' supplementation was adopted in the current study.

So, the current study was conducted for achieving the following objectives: 1) To evaluate the efficacy of seedlings' roots-feeding of the elicitor solution as an elicitation approach, 2) To compare the elicitation potential of the joined application of MeJ and SA with that of either one applied individually, and 3) to test the long-lasting elicitation effect of both MeJ and SA through pursuing the elicitation effect in the second harvest's plants.

MATERIALS AND METHODS

Experimental conditions and cultivate

Seeds of *Cichorium intybus* L. subsp. *divaricatum* cv Catalogna were obtained from the Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Seeds were sterilized for 30 min in 5% NaOCl containing 0.5 % Tween 20. After rinsing three times with sterile distilled water, seeds were cultivated in four germination plates containing peat moss as germination medium on Nov. 15th, 2018. The peat moss of one plate was moistened by 400 mL of MeJ (100 µM) solution whereas the other was moistened by the same volume of SA (1mM) solution. A solution of similar volume prepared from the mixture of 100 µM MeJ + 1mM SA (1: 1, V : V) was used to moisten the peat moss of a third plate. Peatmoss of the fourth tray, that is designated to contain the control plants, was moistened by 400 mL of distilled water. Seeds were mixed with pre-sterilized sand and broadcasted over the moistened peatmoss and covered by a 1.0 – 1.5 cm layer of peatmoss and sprinkled also with 100 mL of the respective solution. Germination trays were kept in the green house at 25 ± 2 ℃ and 80 ± 5 RH for four weeks and sprinkled twice a week by the 500 mL of the respective solution. Four-week-old plants were uprooted and transplanted in the field in plots, 2 X 3 m^2 containing four rows, with 50 cm distance between rows and 30 cm distance between plants. The physical and chemical characteristics of the experimental soil were determined according to Hoddinott and Lamb (1990) and presented in Table (1). The experiment was laid out in a randomized complete block design with four replicates. Nitrogen (ammonium nitrate, 33.5% N), phosphorus (calcium superphosphate, 15.5% P_2O_5) and potassium (potassium sulphate, 48% K_2O) were applied in the rates of 100, 75, 70 kg fed^{-1}, respectively. Calcium superphosphate was thoroughly mixed within the upper soil layer (25 cm) before planting. Nitrogen was added at two equal doses, the first 20 days after transplanting (DAT) whereas the second 30 DAT. Potassium was added as a single application, 30 DAT. Plants were hand weeded fortnightly and irrigated whenever required to avoid the plants being water-stressed.

Table 1. Mechanical and chemical analysis of the experimental soil.

CS %	FS %	S %	C %	$CaCO_3$ %	OM %	N%	P ppm	K ppm	TSS %	pH
11.0	28.6	27.8	32.6	2.6	2.4	0.17	16	251	0.16	7.6

***CS, Coarse sand; FS, Fine sand; S, Silt; C, Clay; OM, Organic matter; N, total N; P, available P; K, exchangeable K; TSS, total soluble salts; determinations were according to Hoddinott and Lamb (1990).**

Plants were harvested 60 DAT by cutting the foliage 5 cm above soil surface, and the plants were maintained for another 60 days to take a second harvest. From each harvest, three plants were selected randomly from each replicate to determine growth traits and bioactive constituents. Shoots and the main root length was measured and the fresh as well as dry weight of shoots and roots were determined with drying being naturally in a well-ventilated space out of direct sunlight.

Biochemical analyses of bioactive compounds

Ascorbic acid was extracted by homogenizing a sample of 0.3 g in 1 mL trichloroacetic acid (0.6 %, w/v) and determined spectrophotometrically at 525 nm using a T60 U UV-VIS Spectrophotometer (PG Instruments, Leicestershire, England) according to Kampfenkel *et al*. (1995). Total antioxidants were estimated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according to Brand-Williams *et al*. (1995). An aliquot of 0.2 mL of the methanolic extract was added to 2.8 mL 40 µM DPPH prepared in methanol and the reduction in the number of DPPH free radicals was measured through reading the absorbance at 517 nm after 30 min at room temperature. The percent inhibition was calculated as: % Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] × 100

Extraction of Phenols and flavonoids was carried out by soaking 1 g of plant material in 70% methanol for 16 h at 4 ℃, followed by centrifugation at 13000 xg for 20 min. Total phenols in the methanolic extract was estimated by the method described by Shad *et al.* (2012) whereas total flavonoids was determined by Al Cl_3 method according to Michalaska *et al*., (2007). Extraction and determination of inulin was determined according to Gibson *et al.* (1994) in which an amount of 200 mg dried plant material was extracted in 10 ml of warm water. To 1 ml of extract, equal volume of conc. HCl and 0.1 ml resorcinol were added and the mixture was made up to 10 ml with distilled water. The mixture was warmed on a water bath for 10 min and the absorbance was read at 490 nm. Inulin content was calculated as mg g^{-1} DW from the calibration curve established with fructose concentrations in the range of 0.5 – 20 µg mL^{-1}.

Statistical Analysis: The results were statistically analyzed as a factorial experiment comprising two independent variables, namely elicitors and harvests as well as their interaction with four replicates. The data were subjected to two way ANOVA and the means with significant difference at 95% confidence level ($p \leq 0.05$) were separated using Tukey's multiple range test. All the statistical tests were performed using the GraphPad Prism statistical software (version 9.0).

RESULTS AND DISCUSSIONS

Results

1-Growth parameters

All recorded growth traits were significantly affected by elicitors and harvests (Table 2). In addition, the traits of roots length, shoots fresh weight, roots fresh weight, roots dry weight, and leaf number were significantly affected by the interaction between elicitors and harvests. Growth traits of control plants of the SH were significantly higher than those in plants of the FH, except length of the root system (Fig. 1). Compared with the control, growth attributes of MeJ-treated plants were significantly lower during either the FH or SH. Similar effect to that of MeJ was obtained with SA, but with a lower magnitude. However, SA-induced decrease was insignificant regarding root system's DW during the FH as well as shoot system's DW during the SH. The combination treatment was also inhibitory to all growth parameters, except root system's DW during the FH as well as DW of both the shoot and root systems during the SH. Generally, there were no significant differences between growth parameters of plants treated with the combined treatment and those of plants treated with either MeJ or SA alone (Fig. 1).

Table 2. Results of the two way ANOVA on data of growth traits

	Shoots length	roots length	shoots FW	roots FW	shoots DW	roots DW	leaves number	leaf area
Elicitors (E)	****	****	****	****	***	***	****	****
Harvests (H)	****	****	****	****	****	****	****	****
E X H	NS	**	**	*	NS	*	**	NS

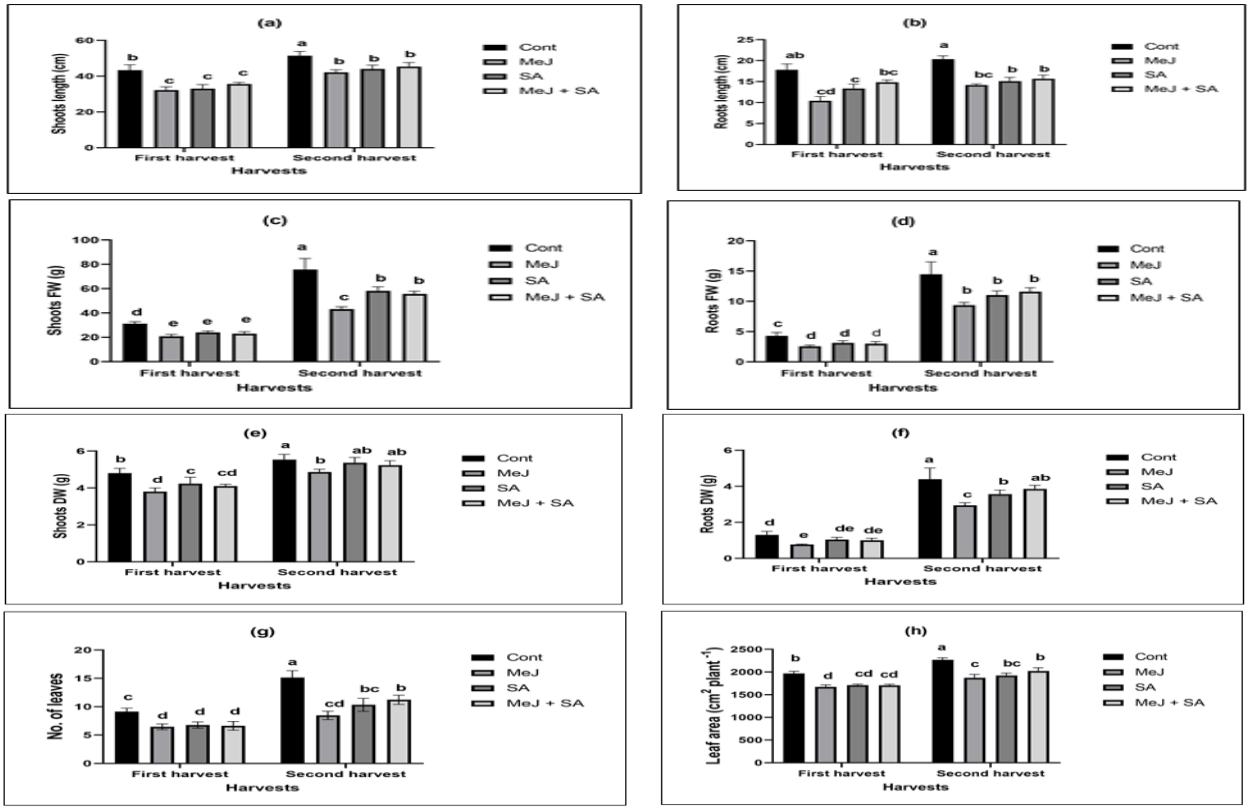

Fig .1. Shoots length (a), roots length (b), shoots FW (c), roots FW (d), shoots DW (e), roots DW (f), leaves number (g), and leaf area (h) of plants treated with methyl jasmonate (MeJ), salicylic acid (SA) and their combination (MeJ+SA) compared with untreated plants (cont); columns represent mean ± SE (n = 4). Different letters above columns indicate significant difference between means at $p \leq 0.05$ according to Tukey's multiple range test.

2- The content from AsA and DPPH scavenging activity

Cichory shoots contained higher levels from ascorbic acid and total antioxidants compared with roots (Fig. 2). The contents of AsA as well as total antioxidants estimated as DPPH scavenging activity in both shoots and roots were significantly affected by Elicitors (Table 3) . In addition, DPPH scavenging activity in shoots as well as in roots was significantly affected by harvests. The content from AsA was significantly affected by harvests in shoots, but not in roots. On the other hand, these traits in either shoots or roots did not significantly affected by the interaction between elicitors and harvests. Control plants of the two harvests contained insignificantly different DPPH scavenging activity in both shoots and roots as well as insignificantly different content from AsA in shoots (Fig. 2). On the other hand, roots of control plants of the SH contained significantly higher content from AsA compared

with that in the roots of the FH's plants. During both harvests, AsA content and DPPH scavenging activity in either shoots or roots of MeJ-, SA-, and MeJ+SA-treated plants were significantly higher than those in respective organs of control plants. The highest increase in both parameters was obtained in response to MeJ during both harvests. The content from AsA and the DPPH scavenging activity of the combined treatment was, generally, significantly lower than that in MeJ only-treated plants, but was insignificantly different from that in SA only-treated plants during the FH. However, in the SH, there were no significant differences between AsA content as well as DPPH scavenging activity, either in shoots or in roots, of plants treated with the combined treatment and those in either MeJ- or SA- treated plants (Fig. 2).

Table 3. Results of the two way ANOVA on AsA and DPPH scavenging activity

	AsA		DPPH scavenging activity	
	Shoots	**Roots**	**Shoots**	**Roots**
Elicitors (E)	****	****	****	****
Harvests (H)	***	NS	****	*
E X H	NS	NS	NS	NS

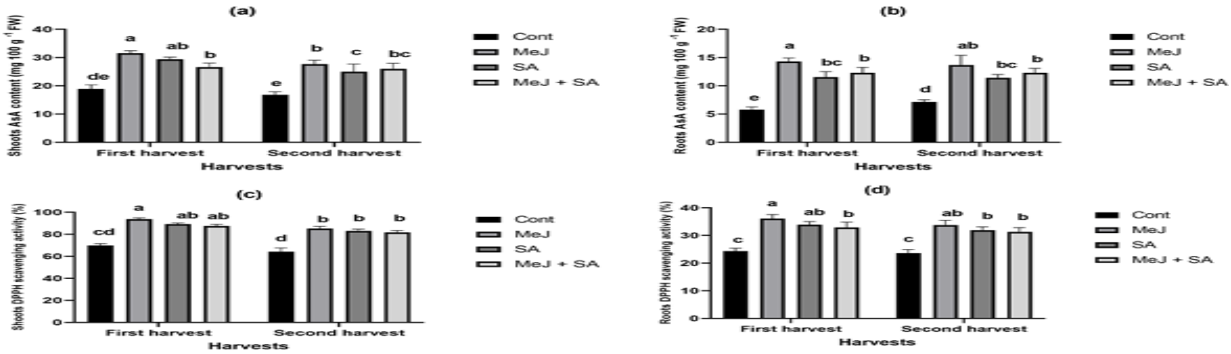

Fig .2. Content of AsA in shoots (a) and roots (b) as well as total antioxidants determined as DPPH scavenging activity in shoots (c) and roots (d) in plants treated with methyl jasmonate (MeJ), salicylic acid (SA) and their combination (MeJ+SA) compared with untreated plants (cont). Different letters above columns indicate significant difference between means at p ≤ 0.05 according to Tukey's multiple range test.

3- Total phenols and flavonoids

Cichory shoots contained higher levels from phenolic compounds compared with roots (Fig. 3). Elicitors significantly affected total phenols and flavonoids in shoots as well as in roots (Table 4). Harvests affected significantly total flavonoids content in both shoots and roots as well as total phenols content in the roots only. The interaction between elicitors and harvests had no significant effect on all these traits, except total flavonoids in shoots, which was significant at $P \leq 0.05$. Control plants of the SH contained significantly higher flavonoids content in both shoots and roots compared with control plants of the FH. On the other hand, total phenols in both shoots and roots of the control plants during the SH were not significantly different from those during the FH (Fig. 3). Treatment with MeJ and SA increased total phenols and total flavonoids in both shoots and roots during both harvests. Higher contents from both total phenols and total flavonoids were obtained in response to MeJ than those obtained in response to SA in both shoots and roots during both harvests. Likewise, the combined treatment (MeJ+SA) increased both parameters in shoots and roots during both harvests, however, the increase recorded regarding shoots total phenols was insignificant in the SH. Though the combined treatment increased total phenols and total flavonoids relative to control, these contents were, generally, significantly lower than those obtained in response to MeJ, but insignificantly different than those obtained in response to SA (Fig. 3).

Table 4. Results of The two way ANOVA on total phenols and flavonoids contents

	total phenols		total flavonoids	
	Shoots	**Roots**	**Shoots**	**Roots**
Elicitors (E)	****	****	****	****
Harvests (H)	NS	***	****	****
E X H	NS	NS	*	NS

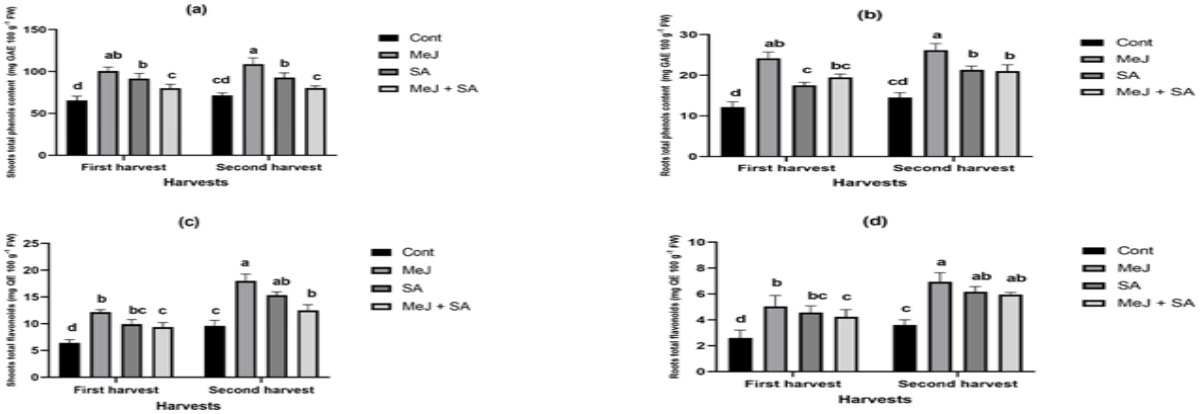

Fig .3. Content of total phenols in shoots (a) and roots (b) as well as total flavonoids in shoots (c) and roots (d) in plants treated with methyl jasmonate (MeJ), salicylic acid (SA) and their combination (MeJ+SA) compared with untreated plants (cont); columns represent mean ± SE (n = 4). Different letters above columns indicate significant difference between means at p ≤ 0.05 according to Tukey's multiple range test.

4- Inulin

Inulin content in the roots were considerably higher than those in shoots (Fig. 4). Both elicitors and harvests affected significantly inulin content in both shoots and roots (Table 5). However, inulin content was not affected by the interaction between elicitors and harvests. Inulin contents in shoots and roots of the SH's plants did not differ significantly from those in respective organs of the FH's plants (Fig. 4). Individual application of MeJ and SA significantly increased inulin contents in the shoots and roots of the plants of the two harvests. The enhancing effect of MeJ on inducing inulin content prominently surpassed that of SA. The increases recorded in inulin content were 38.3, 44.4; 25.5, 39.4 and 26.0, 30.0; 16.4, 28.5 % in shoots of the first, second harvest; roots of the first, second harvest, in response to MeJ and SA, respectively. In addition, the combined treatment increased inulin contents in shoots and roots of the two harvests' plants. However, the increase recorded in response to the combined treatment with respect to shoots inulin content was not significant during the FH. Similarly, the enhancing effect of the combined treatment on shoots' inulin content was lower than that of either MeJ or SA during both harvests. However, with respect to roots' inulin content, the increase recorded in response to the combined treatment was significantly higher than that obtained in response to SA alone, and also higher than the MeJ-induced increase, but insignificantly.

Table 5. Results of the two way ANOVA on Inulin content

	Inulin	
	Shoots	**Roots**
Elicitors (E)	****	****
Harvests (H)	**	*
E X H	NS	NS

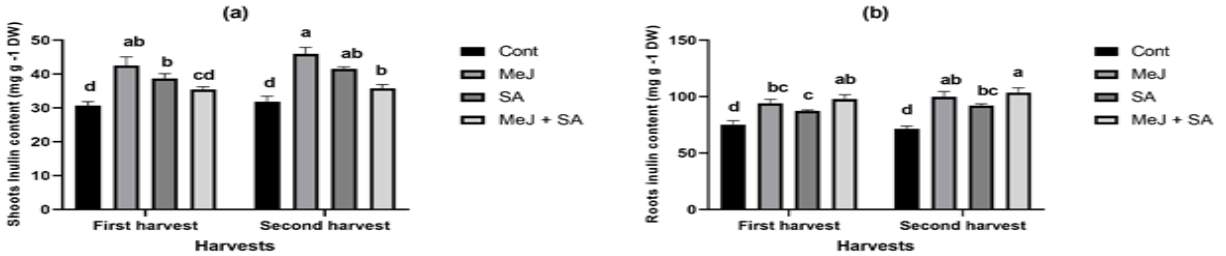

Fig .4. Content of inulin in shoots (a) and roots (b) in plants treated with methyl jasmonate (MeJ), salicylic acid (SA) and their combination (MeJ+SA) compared with untreated plants (cont).

Discussion

Growth and Secondary metabolites in the FH's versus those of the SH's plants

Results of the present study indicated that not only growth attributes but also bioactive constituents of the second harvest plants were higher than those of the first harvest plants. This may be due to the more favorable environmental conditions with respect to both temperature and light prevailing during the duration of the SH compared with the first one. The SH duration spans from Feb. 15 till April 15, which is characterized by warmer temperature and higher degree of sun-light exposure compared with the first one, which spans from Dec. 15 till Feb. 15, according to the characteristic Egyptian climatic conditions. Higher duration of exposure to photosynthetic radiation means higher photosynthetic capacity and higher contents from photosynthates available for growth and biosynthesis of primary as well as secondary metabolites. In addition, the higher temperature prevailing during the SH period will be manifested in higher rates of bioprocesses including cell division, cell expansion and enzymatic activities. According to Yang *et al.* (2018), PSM accumulation is strongly dependent on a variety of environmental factors including light and temperature. In *Perilla frutescens* (Lamiaceae), leaf net photosynthetic rates were increased as Photosynthetic photon flux density increased in both the green and red varieties, accompanied with higher leaves' dry weight as well as higher contents from perillaldehyde and rosmarinic acid (Lu *et al*., 2017). Likewise, the content of anthocyanins in *Pinus contorta* seedlings growing under long sunlight duration was notably higher than that in seedlings growing under the short duration of sunlight (Camm *et al*., 1993). Moreover, *Taraxacum officinale* plants produced more taraxinic acid β-D glucopyranosyl ester (a sesquiterpene lactone) under higher temperature (Huang *et al*., 2020).

Effect of MeJ and SA on growth

It is evident that treatment with MeJ, SA or their combination decreased growth attributes of cichory plants (Fig. 1). Jasmonates have functions in a remarkable number of plant developmental processes, including primary root growth, reproductive development, and leaf senescence (Huang *et al*., 2017). The inhibiting effect of jasmonates on vegetative growth of plants was thoroughly documented (Song *et al*., 2014; Huang *et al*., 2017). In addition. Indirect evidence for the negative effect of jasmonates on plant growth came from the finding that mutations in Jasmonic acid biosynthesis pathway promote vegetative growth and development (Riemann *et al*., 2003). The inhibitory effect of JA on growth promotes survival in natural environments by allowing plants to concentrate on defending themselves against various stresses (Huang *et al*., 2017). It seems that inhibition of cell mitosis is the main mechanism by which jasmonates suppresses plant growth of roots and shoots (Zhang and Turner, 2008).

Salicylic acid had been implicated in the growth and development of plants. It has controversial roles in plant growth and development depending on its concentration and plant growth conditions and developmental stages (Rivas-San Vicente and Plasencia, 2011). Generally, high levels of SA negatively regulate plant development and growth depending on plant species. Nevertheless, the application of optimal concentrations of

SA showed beneficial effects. Fariduddin *et al*. (2003) reported that the dry matter accumulation was significantly enhanced in *Brassica juncea* when lower concentrations of salicylic acid were sprayed, however, higher concentrations of SA had an inhibitory effect.

Effect of MeJ and SA on biosynthesis of bioactive secondary metabolites

The results of the present study revealed that either MeJ, SA or their combination evidently increased the contents from the antioxidants (Fig. 2) and phenolic compounds (Fig. 3) as well as inulin (Fig. 4) in both shoots and roots of plants during either the first or the second harvest, and that the elicitation effect of MeJ surpassed that of SA. Jasmonates-induced secondary metabolites benefit plants not only in fine-tunning their developmental processes, but also in activating their defense responses against both abiotic and biotic challenges (Yan *et al*., 2013). In accordance with the results of the present study, jasmonates were reported to increase total glucosinolates content in leaves of *Brassica napus* up to 20-fold (Doughty *et al*., 1995), anthocyanin accumulation in the *Arabidopsis* shoots (Lorenzo *et al*., 2004), Nicotine in *Nicotiana tabacum* (Shoji *et al*., 2008) and artemisinin in *Artemisia annua* as well as vinblastine in *Catharanthus roseus* (De Geyter *et al*., 2012). All pathways of jasmonates-elicited nicotine, glucosinolates, artemisinin, and vinblastine is regulated by JA-signaling components such as COI1, MYC2, ERF1 and JAZs (De Geyter *et al*., 2012). On the other hand, jasmonates-induced anthocyanin biosynthesis was mediated by up-regulation of the late anthocyanin biosynthetic genes DFR, LDOX, and UF3GT and, concomitantly, activation of anthocyanin biosynthetic regulators such as transcription factors PAP1, PAP2, and GL3 (Shan *et al*., 2009). Protein phosphorylation, lipid oxidation, enhanced antioxidant enzyme activity (SOD, G-POD, APX, CAT), and the activation as well as the de novo biosynthesis of transcription factors as a consequence of jasmonates-signal transduction pathways leading to up-regulation of secondary metabolites' biosynthetic genes have been documented (Santino *et al*., 2013).

As a small molecule with a vital role in plant defense regulatory systems, SA is known to induce systemic acquired resistance (SAR) to many pathogens, during which a rapid SA accumulation in the infection site triggers a hypersensitive response, spreading the signal to other parts of the plant to induce a wide range of defense responses including the production of PSM. Accordingly, SA was widely applied as a secondary metabolism elicitor (Duˇcaiová *et al*., 2013). Several studies documented the direct and indirect involvement of SA in inducing synthesis of secondary metabolites in plants (Ali *et al*., 2007; Idrees *et al*., 2013).

Similar SA-inducing effect on the biosynthesis of PSM that recorded in the present study was reported in previous studies. Concentration of AsA in tomatoes (Javaheri *et al*., 2012) and strawberry (Aghaeifard *et al*., 2015) was higher in SA-treated compared with untreated plants. The induction of AsA in response to SA treatment was ascribed to its enhancing effect on the activity of ascorbate peroxidase (Wiśniewska and Chełkowski, 1999). In addition, SA was reported to induce phenolic compounds in sweet cherry (Valero *et al*., 2011) and grapes (Ranjbaran *et al*., 2011; Khalil, 2014), paclitaxel (diterpene alkaloid) in cell suspension cultures of *Taxus chinensis* and *T. baccata* (Wang *et al*., 2007), taxol in *Corylus avellana* L. cell cultures (Rezaei *et al*., 2011), podophyllotoxin in *Linum album* cell cultures (Yousefzadi *et al*., 2010), Ginsenoside in *Panax ginseng* adventitious roots (Tewari and Paek, 2011), sesquiterpenes in cell suspension cultures of *Ginkgo biloba* (Kang *et al*., 2006), xanthonescadensin G and paxanthone in *Hypericum spp.* cell suspension and hairy root cultures (Zubrická *et al*., 2015), Stilbene in cell suspension cultures of *Vitis vinifera* (Xu *et al*., 2015), withanolides in adventitious roots of *Withania somnifera* (Sivanandhan *et al*., 2012), and dicentrine in cell cultures of *Stephania venosa* (Kitisripanya *et al*., 2013).

As opposed to the expected, the elicitation effect of MeJ alone was higher than that of its combination with SA. Similar results were reported in elicitation of *Rehmannia glutinosa* hairy root cultures where the combination of MeJ and SA though resulted in higher levels of secondary metabolites compared to the control, these levels were lower than those observed in response to MeJ alone (Piątczak *et al*., 2016). In addition, their study revealed that SA alone was less efficient in enhancing PSM production compared with MeJ alone, in accord with the results of the present study. The superiority of the eliciting effect of MeJ only over the combined treatment may be due to jasmonates' signalling-induced inhibition of SA biosynthesis and accumulation (Yang *et al*., 2019).

CONCLUSIONS

Cichory shoots contained higher levels from ascorbic acid and total antioxidants as well as phenolic compounds compared with roots whereas inulin content in roots was considerably higher than that in shoots. Cichory seedlings' roots-feeding was ascertained as a feasible, easy and cost-effective approach for long-lasting elicitation effect. Through this route of elicitor delivery, solutions of methyl jasmonates and salicylic acid enhanced biosynthesis of bioactive constituents in either the shoots or the roots of cichory. Methyl jasmonates was better than salicylic acid for eliciting cichory's secondary metabolites. Also, in the context of the present study, elicitation based on the action of MeJ alone was better than that of SA alone , and also better than that of its combination with SA.

ABBREVIATIONS

MeJ, methyl Jasmonate, SA, salicylic acid; PSM, plant secondary metabolites; AsA, ascorbic acid; TP, total phenols; TF, total flavonoids; ROS, reactive oxygen species; RNS, reactive nitrogen species; First harvest, FH; Second harvest, SH.

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تأثير ميثايل حامض الجاسمونيك و حامض السالسيلك و مخلوطهما معآ على النمو و المحتوى من مركبات التمثيل الثانوى ذات النشاط الحيوى فى نبات الشيكوريا *Cichoriun intybus* L.

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أجريت الدراسة لتقييم كفاءة امتصاص الجذور لمحاليل ميثايل حامض الجاسمونيك (MeJ) و حامض السالسيلك (SA) و مخلوطهما معآ كطريقة لتحفيز مركبات التمثيل الثانوى ذات النشاط الحيوى فى نبات الشيكوريا. و لقد تم أيضاً تقييم التأثير المحفز طويل المدى للمعاملات من خلال مقارنة مستوى هذه المركبات فى نباتات الحشة الثانية Second harvest بمستواها فى نباتات الحشة الأولى First harvest. و لقد أوضحت النتائج أن معاملات MeJ، SA، و مخلوطهما معآ قد أدت الى نقص فى قياسات النمو بينما أدت الى زيادة فى المحتوى من مركبات التمثيل الثانوى الحيوية. فلقد احتوى كل من المجموع الخضرى و الجذرى للنباتات المعاملة بأى من MeJ، SA، أو مخلوطهما معآ سواء فى الحشة الأولى أو الثانية على محتويات من حامض الأسكوربيك، المواد المضادة للأكسدة الكلية، الفينولات، الفلافونيدات و الأنثيولين تزيد معنوياً عن نظائرها فى النباتات غير المعاملة. و لقد أوضحت النتائج أيضاً أن التأثيرالمحفز الناتج عن المعاملة ب MeJ أعلى من نظيره فى حالة المعاملة ب SA و أن محتويات مركبات التمثيل الثانوى الحيوية المختبرة كانت، بصفة عامة، تزيد فى أعضاء نباتات الحشة الثانية بالمقارنة بنظائرها فى أعضاء نباتات الحشة الأولى. و بناء على نتائج الدراسة يمكن الاستنتاج بأن الطريقة المستخدمة فى الدراسة لإمداد النباتات بالمواد المحفزة من خلال الامتصاص بواسطة الجذور فى مرحلة البادرة هى طريقة تتميز بالكفاءة و البساطة و انخفاض التكلفة و أن استخدام MeJ يفضل على SA لتحفيز تخليق مركبات التمثيل الثانوى ذات النشاط الحيوى فى نبات الشيكوريا.