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Research Article

Morphological and Chemical Characters of *Petroselinum crispum* (Mill) Subjected to Some Biostimulants

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Abstract

Background and Objectives: Parsley herb has various biological properties and used in food and pharmaceutical industries. Biostimulants represent one area of research that has the potential to increase medicinal and aromatic plants (MAP) productivity. The aim of this study was to evaluate the growth and chemical composition of parsley plant under some biostimulants treatments. **Materials and Methods:** Parsley plants were subjected to different concentrations of L-tryptophan (LTRYP, 100 and 200 mg L⁻¹), trans-cinnamic acid (TCA, 10 and 20 mg L⁻¹) and distilled water as control. Plant growth characters (PGC), plant height (cm), herb fresh weight (FW) and herb dry weight (DW), essential oil (EO), photosynthetic pigments (PSP), total carbohydrates (TCAR), total soluble sugars (TSS) and antioxidant enzymes [superoxide dismutase (SOD), catalase activity (CAT), peroxidase (POX)] were evaluated through various growth stages. The averages of data were statistically analyzed using two-way analysis of variance (ANOVA-2). **Results:** Obtained results reported that the treatment 200 mg L⁻¹ of LTRYP produced the highest values of PGC, EO yield and main components of EO (apiol, myristicin, α -pinene, β -pinene), PSP, TCAR, TSS, SOD, CAT and POX. **Conclusion:** Generally, the highest values of growth, yield and chemical composition of parsley plants were obtained with 200 mg L⁻¹ (LTRYP) treatment.

Key words: Parsley, L-tryptophan, trans-cinnamic acid, essential oil, antioxidant enzymes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Parsley (*Petroselinum crispum* (Mill)) belongs to family Apiaceae. It is an aromatic herb used in food and drug industries. The essential oil (EO) is present in various organs of the plant such as leaves, roots and mature seeds (fruits). Parsley EO is used as a natural additive (flavouring agent) in food products and as fragrance in cosmetics or perfumes. Different biological activities such as anti-microbial, diuretic and weak antioxidants were found in parsley EO¹⁻⁴. The major component (Myristicin) of parsley EO is a potential cancer chemo protective agent⁵.

Research into different methods to improve medicinal and aromatic plants (MAP) productivity must increase as demand for food and natural pharmaceutical raw material production increases. Biostimulants represent one area of research that has the potential to increase MAP productivity⁶.

Amino acids (AMAs) are the main source of organic nitrogen that use for building proteins, amines, purines, pyrimidines, alkaloids, vitamins, enzymes, terpenoids and others⁷. The AMAs are necessary for cell growth, maintain pH value in plant cell and protect the plants from the toxicity of ammonia and pathogens. They are a very important source of carbon and energy⁸. L-tryptophan (LTRYP) is known as an essential AMA which acts as a precursor of auxins in plants⁹. Plant growth characters (PGC), EO composition, total carbohydrates (TCAR) and total soluble sugars (TSS) of *Pelargonium graveolens* were gradually increased as LTRYP dose increase¹⁰. The influences of LTRYP on *Catharanthus roseus* plants were investigated by Talaat *et al.*¹¹ and they revealed that LTRYP rates caused significant increments in PGC, photosynthetic pigments (PSP), TSS and cytokinins contents. Exogenous application of LTRYP improved PGC and pod weight of Chickpea¹². Foliar application of LTRYP significantly increased PGC and bulb weight of onion¹³. Orabi *et al.*¹⁴ indicated that LTRYP promoted the values of fresh weight (FW) and dry weights (DW), EO (yield and major constituents) and enzyme activities of thyme (*Thymus vulgaris*). The effects of LTRYP on PGC and chemical composition of green onion were investigated by Abd El-wahed *et al.*¹⁵, they indicated that LTRYP resulted in significant increases of PGC, PSP and EO composition. The contents of PSP of *Philodendron erubescens* plants were significantly promoted with LTRYP doses¹⁶. Khattab *et al.*¹⁷ reported that LTRYP (300 mg L⁻¹) caused a significant increment in PGC of gladiolus plant (*Gladiolus grandiflorus*).

Phenolics are low molecular secondary metabolites, they have highly significant effects on PGC, soil and water

conservation, weed control, mineral nutrition, act as defense molecules against soil pests and pathogens and protect agents against biotic and abiotic stress¹⁸⁻²⁰. The roles of trans-cinnamic acid (TCA) as phenolic compound (PHC) in plants were reported by some investigators. The TCA plays important role in plants which grow with a biotic stress factors because it has antioxidant and antibacterial properties²¹. The TCA is a fundamental phenylpropanoid involved in the restoration of damage caused by various abiotic stresses^{22,23}. The TCA promoted PGC and PSP of quinoa²⁴. Responses of basil to TCA were studied by Talaat and Balaa²⁵ and they reported that PGC, EO, major constituents of EO and TCAR were increased with TCA levels. The TCA produced highly significant increases in PGC, EO composition and TSS of sweet marjoram²⁶. Various increments were found in *Lupinus termis* yield, PSP, oil content and TCAR under TCA treatments²⁷.

In this investigation, the possible effects of some biostimulants such as LTRYP (as AMA) and TCA (as PHC) on the PGC, EO, PSP, TCAR, TSS and antioxidant enzymes of parsley plants were studied.

MATERIALS AND METHODS

Experiments: Two pot experiments were conducted in the greenhouse of National Research Centre (NRC), Dokki, Cairo, Egypt, during two successive seasons of 2015/2016 and 2016/2017. The conditions of the greenhouse were adjusted to 31/15°C, 80/50% RH day/night and light intensity of approximately 3700 Lux. Parsley seeds were obtained from the Department of MAP, Ministry of Agriculture, Giza, Egypt. Ten seeds were sown in each pot (30 cm diameter) in the 3rd week of October during both seasons. Each pot was filled with 10 kg of air-dried soil. Physical and chemical properties of the soil used in this study were determined according to Jackson²⁸ and Cottenie *et al.*²⁹ and are presented in Table 1. Eight weeks after sowing, the seedlings were thinned and three plants/pot were left. Pots were divided into three main groups. The first group was exposed to different levels of AMA (LTRYP, 98% feed grade) at concentrations 100 and 200 mg L⁻¹. The second group was subjected to different levels of PHC (TCA, 99% HPLC grade) at concentrations 10 and 20 mg L⁻¹. The third group was subjected to distilled water (as control). The LTRYP and TCA were applied after 10 weeks from the sowing date as foliar spray. All agricultural practices were conducted according to the recommendations by the Egyptian Ministry of Agriculture. The experiments were carried out twice during two seasons because repeated the experiments lead to reduce the experimental errors and produce comprehensive results.

Table 1: Analysis of soil used

| Items | Values |
|--|--------|
| Mechanical properties (%) | |
| Sand | 30.2 |
| Silt | 19.5 |
| Clay | 50.3 |
| Chemical properties | |
| pH (1:2.5) | 7.9 |
| EC (dS m ⁻¹) | 1.5 |
| Organic matter (%) | 1.3 |
| CaCO ₃ (%) | 1.9 |
| Total N (%) | 67.8 |
| Soluble cations (mg/100 g soil) | |
| P | 14.9 |
| K | 18.6 |
| Fe | 24.6 |
| Mn | 12.1 |
| Zn | 6.9 |
| Cu | 17.8 |
| Ca | 45.8 |
| Mg | 11.2 |
| Na | 40.6 |
| Soluble anions (mg/100 g soil) | |
| HCO ₃ | 12.9 |
| Cl | 8.5 |
| CO ₃ | 11.4 |
| SO ₄ | 22.7 |
| NO ₃ | 6.8 |

PGC: Plant heights (cm), herb FW and herb DW (g plant⁻¹) were recorded during the vegetative stage, 120 days after sowing (120 DAS), flowering stage, 180 days after sowing (180 DAS) and fruiting stage, 225 days after sowing (225 DAS).

EO isolation: Fresh herbs (aerial part) were collected from each treatment during vegetative, flowering and fruiting stages; air dried and weighed to extract the EO, then 100 g from each replicate of all treatments was subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus³⁰. The EO content was calculated as a relative percentage (v/w). In addition, total EO as mL/1000 plants was calculated by using the herb DW. The EOs extracted from parsley herbs were collected from each treatment and dried over anhydrous sodium sulfate to identify the chemical constituents.

Gas chromatography-mass spectrometry (GC-MS): The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. DB-5 column (60 m×0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL min⁻¹). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min⁻¹ that was kept constant at 220°C for 10 min and followed by elevating the temperature to 240°C at a rate of 1°C min⁻¹. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra was recorded at 70 eV. Mass range was m/z 35-450.

GC analysis: The GC analysis was carried out using an Agilent 6890N GC system using flame ionization detector (FID) detect temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto injection was done on a duplicate of the same column at the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components: Identification of the EO components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their retention index (RI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Mass Finder 3 Library)^{31,32} and in-house "Başer Library of EO Constituents" built up by genuine compounds and components of known oils. Additionally, MS literature data^{33,34} were also used for the identification.

Determination of PSP: Chlorophyll A (Chl A), chlorophyll B (Chl B) and total carotenoids (TC) in fresh leaves collected at the vegetative and flowering stages of each treatment were determined as mg g⁻¹ using the methods described by the AOAC³⁵.

Determination of TCAR and TSS: The TCAR and TSS contents were determined from plant material (young leaves) collected at the vegetative and flowering stages of each treatment. The method of Dubois *et al.*³⁶ was used.

Extraction and assaying antioxidant enzymes activities: The method adopted in enzyme extraction was that described by Mukherjee and Choudhuri³⁷. Assay of catalase activity (CAT) EC 1.11.1.6 was assayed according to the method of Kar and Mishra³⁸. Assay of superoxide dismutase activity (SOD) EC 1.15.1.1 was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by Marklund and Marklund³⁹. Assay of peroxidase activity (POX) EC 1.11.1.7 was assayed following the method of Kar and Mishra³⁸ with slight modifications.

Statistical analysis: In this experiment, 2 factors were considered; Biostimulants {LTRYF (2 levels), TCA (2 levels) and control} and 3 growth stages. For each treatment there were four replicates. The experimental design followed a randomized complete block design (RCBD). According to De Smith⁴⁰ the averages of data of both seasons were statistically analyzed using two-way analysis of variance (ANOVA-2). Significant values were determined according to p-values (p<0.05 = significant, p<0.01 = moderate significant and p<0.001 = highly significant). The applications of that technique were according to the STAT-ITCF program⁴¹.

RESULTS

Effect of LTRYP and TCA on PGC: Biostimulants (LTRYP and TCA) and/or various growth stages (vegetative, flowering and fruiting) affected the all PGC {plant height (cm) herb FW and DWs (g/plant)} (Table 2). Thus, various PGC increased under the various biostimulants levels compared with control treatment. Greatest PGC were recorded at the treatment of 200 mg L⁻¹ (LTRYP) during the fruiting stage (225 DAS) with values of 106.7, 42.9 and 17.7. On the other hand all various PGC were increased towards fruiting stage. The increases various PGC were highly significant ($p < 0.001$) for biostimulants levels, growth stages and biostimulants \times growth stages (Table 2).

Effect of LTRYP and TCA on EO contents: Different variations were found in EO contents under biostimulants rates through all growth stages (Table 2). The highest percentage (0.4) of

parsley EO was obtained from aerial part at the flowering stage i.e. 180 DAS with the treatment of 20 mg L⁻¹ (TCA). On the other hand, greatest EO yield (mL/1000 plants) was recorded under 200 mg L⁻¹ (LTRYP) during the flowering stage (180 DAS) with the value of 120.3. The variations in EO percentage were highly significant ($p < 0.001$) for different growth stages while it was insignificant for biostimulants levels or biostimulants \times growth stages. Highly significant changes ($p < 0.001$) were obtained with biostimulants, growth stages and their interactions for EO yield.

Effect of LTRYP and TCA on EO constituents: Sixteen components were identified by GC-MS analysis under LTRYP and TCA treatments during vegetative, flowering and fruiting stages (Table 3, 4). Apiol, myristicin, α -pinene and β -pinene were identified as the major compounds which reflected the highest amount of parsley EO (more than 70%). The major constituents were increased towards the flowering stage and

Table 2: Effect of L-tryptophan and trans-cinnamic acid on plant growth characters and essential oil contents during various growth stages

| Growth stages | Treatments (mg L ⁻¹) | PGC | | | EO | |
|-----------------------------------|-------------------------------------|----------------------|-----------------------------|-------------|---------|---------------------------|
| | | Plant height (cm) | FW ----- (g/plant) ----- | DW ----- | % | Yield (mL/1000 plants) |
| 120 DAS | | | | | | |
| Control | | 26.0±2.0 | 3.6±0.3 | 2.4±0.4 | 0.2±0.1 | 7.2±0.2 |
| TCA | 10 | 31.0±1.0 | 7.3±0.3 | 3.0±0.5 | 0.3±0.1 | 21.9±0.1 |
| | 20 | 32.6±0.6 | 9.9±0.1 | 3.5±0.5 | 0.3±0.1 | 29.7±0.3 |
| TRYP | 100 | 29.8±0.4 | 6.7±0.2 | 2.4±0.1 | 0.3±0.1 | 20.1±0.1 |
| | 200 | 34.3±0.3 | 10.8±0.2 | 4.5±0.5 | 0.3±0.1 | 32.4±0.4 |
| Overall 120 DAS | | 30.7±1.1 | 7.7±1.2 | 3.2±0.6 | 0.3±0.1 | 22.3±2.1 |
| 180 DAS | | | | | | |
| Control | | 65.2±0.2 | 17.9±0.1 | 4.3±0.3 | 0.3±0.1 | 53.7±0.3 |
| TCA | 10 | 73.3±0.3 | 24.2±0.2 | 6.1±0.1 | 0.3±0.1 | 72.6±0.2 |
| | 20 | 87.7±0.2 | 30.6±0.6 | 7.6±0.4 | 0.4±0.1 | 96.8±0.1 |
| TRYP | 100 | 73.3±0.3 | 27.2±0.1 | 7.5±0.5 | 0.3±0.1 | 81.6±0.1 |
| | 200 | 88.7±0.7 | 40.1±0.1 | 9.1±0.1 | 0.3±0.1 | 120.3±0.3 |
| Overall 180 DAS | | 77.6±1.4 | 28.0±1.6 | 6.9±0.9 | 0.3±0.1 | 85.0±2.3 |
| 225 DAS | | | | | | |
| Control | | 79.7±0.7 | 19.7±0.3 | 8.8±0.2 | 0.1±0.0 | 17.9±0.1 |
| TCA | 10 | 88.3±0.3 | 30.8±0.1 | 9.1±0.1 | 0.1±0.0 | 30.8±2.0 |
| | 20 | 103.3±3.0 | 32.7±2.0 | 11.9±0.1 | 0.2±0.1 | 65.4±0.4 |
| TRYP | 100 | 98.7±0.3 | 33.8±0.2 | 10.6±0.4 | 0.2±0.1 | 67.6±0.2 |
| | 200 | 106.7±2.0 | 42.9±0.4 | 17.7±0.3 | 0.2±0.1 | 85.8±1.0 |
| Overall 225 DAS | | 95.3±1.4 | 32.0±1.7 | 11.6±1.3 | 0.2±0.1 | 53.5±2.3 |
| Overall treatments | | | | | | |
| Control | | 57.0±2.1 | 13.7±1.6 | 5.2±1.8 | 0.2±0.1 | 26.3±2.1 |
| TCA | 10 | 64.2±2.1 | 20.8±1.5 | 6.1±1.1 | 0.2±0.1 | 41.8±2.5 |
| | 20 | 74.5±2.2 | 24.4±1.9 | 7.7±1.6 | 0.3±0.1 | 64.0±2.1 |
| TRYP | 100 | 67.3±1.3 | 22.6±1.1 | 6.8±1.2 | 0.3±0.1 | 56.4±2.9 |
| | 200 | 95.3±1.6 | 31.3±1.4 | 10.4±1.8 | 0.3±0.1 | 79.5±2.3 |
| F-ratio | | | | | | |
| Treatments | | 355.6*** | 775.2*** | 199.4*** | 1.5 | 3606.6*** |
| Growth stages | | 10468.7*** | 5466.2*** | 1468.7*** | 12.0*** | 14139.2*** |
| Treatments \times growth stages | | 45.8*** | 77.9*** | 40.7*** | 0.5 | 383.0*** |

*Significant, **Moderate significant, ***Highly significant, LTRYP: L-tryptophan, TCA: Trans-cinnamic acid, DAS: Days after sowing, PGC: Plant growth characters, FW: Fresh weight, DW: Dry weight, EO: Essential oil, values are given as Mean \pm SD

Table 3: Effect of L-tryptophan and trans-cinnamic acid on essential oil constituents during various growth stages

| Compounds | RI | 120 DAS | | | | | | 180 DAS | | | | | | 225 DAS | | | | | | F-ratio |
|------------------------------|------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|--------|--|--|---------|
| | | TCA | | TRYP | | TCA | | TRYP | | TCA | | TRYP | | TCA | | TRYP | | | | |
| | | 0.0 | 10 | 20 | 100 | 200 | 0.0 | 10 | 20 | 100 | 200 | 0.0 | 10 | 20 | 100 | 200 | | | | |
| α -Thujene | 931 | 0.4±0.1 | 0.3±0.1 | 0.4±0.1 | 0.1±0.0 | 0.3±0.1 | 0.2±0.1 | 0.2±0.1 | 0.1±0.0 | 0.2±0.1 | 0.5±0.2 | 1.3±0.3 | 2.1±0.1 | 0.8±0.2 | 0.3±0.1 | 28.2*** | | | | |
| α -Pinene | 939 | 13.0±1.0 | 13.4±0.4 | 13.5±0.5 | 13.8±0.2 | 13.9±0.1 | 13.2±0.2 | 13.6±0.6 | 13.9±0.1 | 14.1±0.1 | 14.4±0.4 | 14.1±0.1 | 14.4±0.4 | 13.3±0.3 | 13.7±0.3 | 0.1 | | | | |
| Camphene | 953 | 2.3±0.3 | 2.1±0.1 | 1.6±0.2 | 1.7±0.2 | 1.1±0.1 | 2.4±0.4 | 2.1±0.1 | 1.3±0.3 | 1.3±0.3 | 1.1±0.1 | 2.4±0.4 | 3.2±0.2 | 1.9±0.1 | 0.5±0.1 | 9.2*** | | | | |
| Sabinene | 976 | 1.4±0.4 | 1.1±0.1 | 1.3±0.3 | 1.1±0.1 | 1.1±0.1 | 1.3±0.3 | 1.7±0.2 | 1.2±0.2 | 1.2±0.2 | 1.0±0.1 | 1.5±0.5 | 2.4±0.4 | 1.4±0.4 | 1.4±0.2 | 4.1*** | | | | |
| β -Pinene | 980 | 9.0±1.0 | 9.4±0.4 | 9.7±0.1 | 9.3±0.3 | 9.7±0.1 | 9.3±0.3 | 9.7±0.1 | 9.8±0.1 | 9.7±0.1 | 9.9±0.1 | 8.8±0.2 | 9.1±0.1 | 9.5±0.5 | 9.2±0.2 | 1.0 | | | | |
| Myrcene | 991 | 1.5±0.5 | 1.4±0.4 | 1.1±0.1 | 1.8±0.2 | 1.1±0.1 | 1.6±0.1 | 1.1±0.1 | 1.1±0.1 | 1.1±0.1 | 1.1±0.2 | 1.6±0.6 | 1.4±0.4 | 1.3±0.3 | 0.2±0.1 | 2.6* | | | | |
| β -Phellandrene | 1031 | 2.6±0.6 | 1.1±0.1 | 1.6±0.2 | 1.3±0.3 | 1.1±0.1 | 2.4±0.2 | 1.0±0.0 | 1.7±0.2 | 1.1±0.1 | 1.2±0.2 | 2.8±0.2 | 1.2±0.2 | 1.5±0.5 | 1.6±0.1 | 1.3±0.3 | 0.8 | | | |
| γ -Terpinene | 1062 | 2.1±0.1 | 2.6±0.3 | 2.7±0.3 | 2.3±0.3 | 1.4±0.2 | 2.1±0.5 | 2.2±0.2 | 2.5±0.5 | 2.1±0.1 | 1.1±0.1 | 2.3±0.3 | 2.4±0.4 | 2.5±0.5 | 2.6±0.4 | 2.7±0.3 | 4.0*** | | | |
| Myrtanal | 1193 | 3.2±0.2 | 2.4±0.4 | 2.3±0.3 | 1.9±0.1 | 1.7±0.3 | 3.0±0.5 | 2.1±0.1 | 2.1±0.1 | 1.7±0.2 | 1.1±0.1 | 3.1±0.1 | 2.2±0.2 | 2.4±0.4 | 1.8±0.2 | 1.8±0.2 | 0.9 | | | |
| β -Caryophyllene | 1418 | 1.9±0.1 | 1.5±0.5 | 1.2±0.2 | 1.1±0.1 | 1.6±0.1 | 1.7±0.2 | 1.4±0.1 | 1.1±0.1 | 1.2±0.2 | 1.5±0.5 | 2.2±0.2 | 1.6±0.1 | 1.5±0.5 | 1.4±0.4 | 1.7±0.2 | 0.3 | | | |
| Trans- α -Bergamotene | 1434 | 1.6±0.1 | 1.5±0.5 | 1.6±0.1 | 1.4±0.4 | 1.5±0.5 | 1.3±0.2 | 1.5±0.5 | 1.5±0.5 | 1.5±0.5 | 1.3±0.3 | 1.5±0.5 | 1.4±0.2 | 1.4±0.1 | 1.1±0.2 | 1.4±0.2 | 0.5 | | | |
| (Z)- β -Farnesene | 1443 | 2.3±0.3 | 2.8±0.2 | 2.5±0.5 | 2.5±0.5 | 2.2±0.2 | 2.6±0.2 | 2.7±0.3 | 2.3±0.3 | 2.4±0.4 | 2.1±0.1 | 2.1±0.1 | 2.9±0.1 | 2.6±0.1 | 2.3±0.3 | 2.3±0.3 | 1.0 | | | |
| Myristicin | 1520 | 25.0±2.0 | 25.8±0.8 | 26.4±0.4 | 26.7±0.2 | 27.1±0.4 | 25.2±0.2 | 25.9±0.5 | 26.8±0.8 | 26.9±0.3 | 27.2±0.2 | 24.7±0.7 | 25.1±0.1 | 25.9±0.1 | 26.2±0.2 | 27.0±0.1 | 0.2 | | | |
| Elemicin | 1554 | 1.7±0.2 | 2.1±0.1 | 1.3±0.3 | 1.5±0.5 | 1.8±0.2 | 1.5±0.5 | 2.2±0.2 | 1.2±0.2 | 1.3±0.1 | 1.7±0.3 | 1.8±0.2 | 2.0±0.1 | 1.3±0.3 | 1.4±0.4 | 2.1±0.1 | 0.3 | | | |
| Carotol | 1594 | 2.9±0.1 | 2.5±1.5 | 2.1±0.1 | 1.8±0.2 | 2.1±0.1 | 2.8±0.2 | 2.6±0.1 | 2.2±0.2 | 1.5±0.5 | 2.2±0.2 | 2.8±0.2 | 2.8±0.2 | 2.1±0.1 | 1.9±0.1 | 2.2±0.2 | 0.8 | | | |
| Apinol | 1680 | 29.0±1.0 | 29.5±0.5 | 30.6±0.6 | 31.4±0.4 | 31.8±0.2 | 29.4±0.4 | 29.8±0.2 | 30.8±0.2 | 31.8±0.2 | 32.4±0.4 | 28.5±0.5 | 28.7±0.3 | 29.1±0.1 | 30.7±0.2 | 31.5±0.5 | 0.9 | | | |
| MCH | | 32.3±0.3 | 31.4±0.4 | 31.9±0.1 | 31.4±0.4 | 29.7±0.3 | 32.4±0.4 | 31.7±0.3 | 31.8±0.2 | 31.5±0.5 | 30.0±1.0 | 32.6±0.4 | 32.7±0.3 | 33.6±0.4 | 32.5±0.5 | 29.6±0.4 | 1.0 | | | |
| MCHO | | 3.2±0.2 | 2.4±0.4 | 2.3±0.3 | 1.9±0.1 | 1.7±0.9 | 3.0±0.1 | 2.1±0.1 | 2.1±0.1 | 1.7±0.2 | 1.1±0.1 | 3.1±0.1 | 2.2±0.2 | 2.4±0.4 | 1.8±0.2 | 1.8±0.2 | 0.6 | | | |
| SCH | | 5.8±0.2 | 5.8±0.2 | 5.3±0.3 | 5.0±0.1 | 5.3±0.3 | 5.6±0.1 | 5.6±0.1 | 4.9±0.1 | 5.1±0.1 | 4.9±0.1 | 5.8±0.2 | 5.9±0.1 | 5.5±0.5 | 4.8±0.2 | 5.4±0.4 | 0.8 | | | |
| SCHO | | 58.6±0.4 | 59.9±0.1 | 60.4±0.4 | 61.4±0.4 | 63.1±0.1 | 58.9±0.2 | 60.5±0.5 | 61.0±0.1 | 61.5±0.5 | 63.5±0.5 | 57.8±0.2 | 58.6±0.4 | 58.4±0.4 | 60.2±0.2 | 62.9±0.1 | 3.0* | | | |
| Total identified | | 99.9 | 99.5 | 99.9 | 99.7 | 99.5 | 99.9 | 99.9 | 99.7 | 99.8 | 99.5 | 99.3 | 99.4 | 99.9 | 99.3 | 99.6 | | | | |

*Significant, **Moderate significant, ***Highly significant, RI: Retention index, LTRYP: L-tryptophan, TCA: Trans-cinnamic acid, DAS: Days after sowing, MCH: Monoterpenes hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes, values are given as Mean±SD

Table 4: Effect of L-tryptophan, trans-cinnamic acid and growth stages on essential oil constituents

| Compounds | RI | BREG (mg L ⁻¹) | | | | | GS | | | F-ratio | Growth stages |
|---------------------|------|----------------------------|----------|----------|----------|----------|----------|----------|----------|------------|---------------|
| | | TCA | | TRYP | | | DAS | | | | |
| | | 0.0 | 10 | 20 | 100 | 200 | 120 | 180 | 225 | Treatments | |
| α-Thujene | 931 | 0.3±0.2 | 0.6±0.5 | 0.8±0.9 | 0.3±0.4 | 0.3±0.1 | 0.3±0.1 | 0.2±0.1 | 1.0±0.7 | 37.3*** | 169.7*** |
| α-Pinene | 939 | 13.0±0.6 | 13.3±0.5 | 13.5±0.4 | 13.7±0.4 | 14.0±0.4 | 13.5±0.6 | 13.8±0.5 | 13.1±0.4 | 8.5*** | 11.0*** |
| Camphene | 953 | 2.4±0.3 | 2.5±0.5 | 1.3±0.3 | 1.6±0.3 | 0.9±0.3 | 1.8±0.5 | 1.6±0.6 | 1.8±0.1 | 76.4*** | 0.1 |
| Sabinene | 976 | 1.4±0.4 | 1.3±0.3 | 1.6±0.6 | 1.2±0.3 | 1.2±0.2 | 1.2±0.2 | 1.3±0.3 | 1.6±0.5 | 3.9* | 7.9*** |
| β-Pinene | 980 | 9.0±0.6 | 9.4±0.3 | 9.5±0.3 | 9.4±0.3 | 9.7±0.3 | 9.4±0.5 | 9.7±0.3 | 9.2±0.4 | 5.1*** | 6.6*** |
| Myrcene | 991 | 1.6±0.4 | 1.3±0.3 | 1.2±0.2 | 1.8±0.2 | 0.8±0.5 | 1.4±0.4 | 1.4±0.4 | 1.2±0.6 | 15.3*** | 1.0 |
| β-Phellandrene | 1031 | 2.6±0.4 | 1.1±0.1 | 1.6±0.3 | 1.3±0.3 | 1.2±0.2 | 1.5±0.6 | 1.5±0.5 | 1.7±0.7 | 46.5*** | 2.2 |
| γ-Terpinene | 1062 | 2.2±0.3 | 2.4±0.3 | 2.6±0.3 | 2.3±0.3 | 1.7±0.8 | 2.2±0.5 | 2.0±0.5 | 2.5±0.4 | 8.1*** | 7.2*** |
| Myrtenal | 1193 | 3.1±0.3 | 2.2±0.3 | 2.3±0.3 | 1.8±0.2 | 1.5±0.4 | 2.3±0.5 | 2.0±0.7 | 2.3±0.5 | 47.9*** | 6.0** |
| β-Caryophyllene | 1418 | 1.9±0.3 | 1.5±0.3 | 1.3±0.3 | 1.2±0.3 | 1.6±0.3 | 1.5±0.4 | 1.4±0.3 | 1.7±0.4 | 9.3*** | 4.6 |
| trans-α-Bergamotene | 1434 | 1.5±0.3 | 1.5±0.3 | 1.5±0.4 | 1.3±0.3 | 1.4±0.3 | 1.5±0.3 | 1.4±0.4 | 1.4±0.3 | 0.1 | 0.8 |
| (Z)-β-Farnesene | 1443 | 2.3±0.3 | 2.8±0.2 | 2.5±0.3 | 2.4±0.4 | 2.2±0.2 | 2.5±0.4 | 2.4±0.3 | 2.4±0.3 | 5.3*** | 0.1 |
| Myristicin | 1520 | 25.0±1.0 | 25.6±0.6 | 26.4±0.6 | 26.6±0.4 | 27.2±0.3 | 26.3±1.2 | 26.4±0.9 | 25.8±0.9 | 16.1*** | 3.4* |
| Elemicin | 1554 | 1.7±0.3 | 2.1±0.5 | 1.3±0.2 | 1.4±0.4 | 1.9±0.3 | 1.7±0.4 | 1.6±0.5 | 1.7±0.5 | 6.9*** | 0.5 |
| Carotol | 1594 | 2.8±0.2 | 2.6±0.3 | 2.1±0.1 | 1.7±0.3 | 2.2±0.2 | 2.3±0.5 | 2.3±0.5 | 2.4±0.4 | 30.6*** | 0.8 |
| Apiol | 1680 | 29.0±0.7 | 29.3±0.6 | 30.2±0.9 | 31.3±0.5 | 31.9±0.5 | 30.5±1.2 | 30.8±1.1 | 29.7±1.0 | 73.4*** | 26.2*** |
| MCH | | 32.5±0.3 | 31.9±0.4 | 32.3±0.9 | 31.6±0.7 | 29.8±0.6 | 31.3±1.0 | 31.5±1.2 | 32.1±1.1 | 1.9 | 1.7 |
| MCHO | | 3.1±0.5 | 2.2±0.3 | 2.3±0.3 | 1.8±0.2 | 1.5±0.6 | 2.3±0.6 | 2.0±0.7 | 2.3±0.2 | 18.2*** | 3.4* |
| SCH | | 5.7±0.2 | 5.8±0.2 | 5.3±0.3 | 4.9±0.5 | 5.2±0.3 | 5.5±0.5 | 5.2±0.3 | 5.5±0.5 | 9.3*** | 2.4 |
| SCHO | | 58.5±0.5 | 59.6±0.9 | 60.0±1.1 | 61.0±0.7 | 63.2±0.4 | 60.8±1.5 | 61.1±1.1 | 59.6±1.3 | 159.9*** | 50.2*** |
| Total identified | | 99.8 | 99.5 | 99.7 | 99.3 | 99.7 | 99.9 | 99.8 | 99.5 | | |

*Significant, **Moderate significant, ***Highly significant, RI: Retention index, LTRYP: L-tryptophan, TCA: Trans-cinnamic acid, DAS: Days after sowing, MCH: Monoterpenes hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes, values are given as Mean ± SD

then reduced at fruiting stage. Various constituents of parsley EO were classified to four chemical fractions. Oxygenated sesquiterpenes (SCHO) and monoterpene hydrocarbons (MCH) were the major fractions (more than 80%). Plants treated with 200 mg L⁻¹ of LTRYP (during the flowering stage) produced the highest amounts of major constituents that recorded the values of 32.4, 27.2, 14.4 and 9.9%. The greatest amount of MCH (33.6%) was recorded with the 20 mg L⁻¹ of TCA (during the fruiting stage) while the treatments of 10 mg L⁻¹ of LTRYP and 20 mg L⁻¹ of TCA produced the highest amount of MCHO (2.4%) during the vegetative and fruiting stages (Table 3). On the other hand 10 mg L⁻¹ of TCA and 200 mg L⁻¹ of LTRYP levels resulted in the highest amounts (5.9 and 63.5%) of SCH and SCHO at the fruiting and flowering stages (Table 3). Higher values were found in apiol, myristicin, α-pinene, β-pinene and SCHO (30.8, 26.4, 13.8, 9.7 and 61.1%) at the flowering stage than vegetative or fruiting stages (Table 4). Vegetative and flowering stages resulted in higher values of MCHO and SCH (2.3 and 5.5%) than fruiting stage. Fruiting stage obtained higher value in MCH (32.1) than both Vegetative and flowering stages. The variation in all constituents and chemical classes were insignificant for biostimulants × growth stages except the constituents of α-thujene, camphene, sabinene and γ-terpinene were highly significant (p<0.001) and myrcene and SCHO were significant

(p<0.05) (Table 3). Highly significant changes (p<0.001) were found in all components and chemical groups for biostimulants levels except trans-α-bergamotene and MCH were insignificant and sabinene were significant (p<0.05) (Table 4). Through various stages, the changes in α-thujene, α-pinene, sabinene, β-pinene, γ-terpinene apiol and SCHO were highly significant (p<0.001) while the variations in myrtenal were moderate significant (p<0.01) but it was significant of myristicin and MCHO (Table 4).

Effect of LTRYP and TCA on PSP: Applying both biostimulants (LTRYP or TCA) resulted in an increase in the accumulation of PSP i.e. Chl A, Chl B and TC during vegetative and flowering stages (Table 5). The greatest amounts of all PSP were obtained during the flowering stage (180 DAS) from the treatments of 200 and 20 mg L⁻¹ of LTRYP and TCA with values of 7.5, 4.6 and 1.2 mg g⁻¹. The highest of accumulation of PSP were higher during the flowering stage (6.8, 4.2 and 1.1 mg g⁻¹) than vegetative stage (4.8, 3.0 and 0.8 mg g⁻¹). The increments in various PSP were highly significant (p<0.001) for growth stages, biostimulants levels and their interactions.

Effect of LTRYP and TCA on TCAR and TSS: The accumulation of TCAR and TSS in parsley leaves during the vegetative and flowering stages was promoted by applying various levels of

Table 5: Effect of L-tryptophan and trans-cinnamic acid on photosynthetic pigments, total carbohydrates and total soluble sugars during various growth stages

| Growth stages | Treatments (mg L ⁻¹) | PSP (mg g ⁻¹) | | | TCAR ----- Mg g ⁻¹ ----- | TSS |
|----------------------------|-------------------------------------|---------------------------|-----------|-----------|--|-----------|
| | | Chl A | Chl B | TC | | |
| 120 DAS | | | | | | |
| Control | | 3.5±0.02 | 2.2±0.03 | 0.8±0.01 | 70.1±0.5 | 55.8±0.2 |
| TCA | 10 | 3.9±0.01 | 2.3±0.03 | 0.8±0.01 | 73.3±0.2 | 65.1±0.4 |
| | 20 | 5.7±0.02 | 3.5±0.03 | 0.9±0.01 | 84.4±0.4 | 67.8±0.3 |
| TRYP | 100 | 5.4±0.02 | 3.3±0.03 | 0.9±0.02 | 83.4±0.4 | 67.2±0.3 |
| | 200 | 5.7±0.02 | 3.5±0.04 | 0.9±0.02 | 99.7±0.6 | 78.5±0.2 |
| Overall 120 DAS | | 4.8±0.90 | 3.0±0.60 | 0.8±0.10 | 82.2±0.5 | 66.9±0.5 |
| 180 DAS | | | | | | |
| Control | | 5.7±0.10 | 3.5±0.10 | 1.0±0.02 | 108.1±0.3 | 64.1±0.3 |
| TCA | 10 | 6.5±0.01 | 4.1±0.02 | 1.1±0.01 | 130.0±0.2 | 69.2±0.3 |
| | 20 | 7.5±0.02 | 4.6±0.04 | 1.2±0.02 | 134.3±0.3 | 71.5±0.2 |
| TRYP | 100 | 6.9±0.02 | 4.2±0.02 | 1.1±0.01 | 139.3±0.3 | 79.3±0.3 |
| | 200 | 7.5±0.02 | 4.6±0.03 | 1.2±0.01 | 154.0±0.6 | 95.4±0.4 |
| Overall 180 DAS | | 6.8±0.7 | 4.2±0.40 | 1.1±0.10 | 132.8±0.3 | 75.9±0.3 |
| Overall treatments | | | | | | |
| Control | | 4.6±0.20 | 2.9±0.30 | 0.9±0.10 | 89.1±0.2 | 59.9±0.4 |
| TCA | 10 | 5.2±0.30 | 3.2±0.30 | 0.9±0.20 | 101.6±0.3 | 67.2±0.2 |
| | 20 | 6.6±0.10 | 4.1±0.40 | 1.0±0.20 | 109.3±0.4 | 69.6±0.2 |
| TRYP | 100 | 6.1±0.40 | 3.8±0.40 | 0.9±0.10 | 111.4±0.6 | 73.2±0.6 |
| | 200 | 6.6±0.40 | 4.1±0.20 | 1.0±0.10 | 126.9±0.4 | 86.9±0.3 |
| F-ratio | | | | | | |
| Treatments | | 4357.9*** | 1321.9*** | 84.1*** | 472.3*** | 6727.4*** |
| Growth stages | | 26352.9*** | 9033.4*** | 1572.6*** | 8239.5*** | 6860.7*** |
| treatments × growth stages | | 234.2*** | 140.3*** | 73.9*** | 36.8*** | 524.0*** |

*Significant, **Moderate significant, ***Highly significant, LTRYP: L-tryptophan, TCA: Trans-cinnamic acid, DAS: Days after sowing, PSP: Photosynthetic pigments, TCAR: Total carbohydrates, TSS: Total soluble sugars, Chl: Chlorophyll, TC: Total carotenoides, values are given as Mean ± SD

LTRYP and TCA (Table 5). The highest contents of TCAR and TSS were recorded during the treated with 200 mg L⁻¹ of LTRYP at flowering stage with the values of 154.0 and 95.4 mg g⁻¹. Lower values (82.2 and 66.9) were found in TCAR and TSS during the vegetative stage than those recorded at flowering stage (132.8 and 75.9 mg g⁻¹). The increases in TCAR and TSS were highly significant (p<0.001) for growth stages, biostimulants levels and their interactions.

Effect of LTRYP and TCA on antioxidant enzymes activities:

The effect of LTRYP and TCA on antioxidant enzymes such as SOD, CAT and POX were reported during vegetative and flowering stages (Table 6). Both of LTRYP and TCA levels caused an increment in all antioxidant enzymes activities at two stages. The highest accumulations of SOD, CAT and POX were obtained under the treatment of 200 mg L⁻¹ of LTRYP with the values of 3.3, 33.0 and 2.4 unit g FW min⁻¹. Flowering stage recorded higher values in SOD, CAT and POX (2.6, 28.4 and 2.3 unit g FW min⁻¹) than vegetative stage (1.7, 27.8 and 1.6 unit g FW min⁻¹) the increases in various antioxidant enzymes were highly significant (p<0.001) for different stages, biostimulants levels and biostimulants × stages.

DISCUSSION

In this investigation, the obtained results indicated that LTRYP and TCA caused significant changes in PGC, PSP, EO composition, TCAR, TSS and antioxidant enzymes activities during various growth stages.

The AMAs such as LTRYP can promote the PGC and development through their influence on gibberellin biosynthesis⁴², increase endogenous cytokinins rate which play an important role in enhancement of cell division and thereby increase branching, buds and antagonize auxin in apical dominance^{43,44}. The LTRYP which is the precursor of IAA improves different vegetative growth characters and plant production. The exogenous applications of LTRYP can activate the endogenous rate of IAA, thus PGC were improved under LTRYP levels^{10,11,45}. On the other hand, the increment in PGC under PHC was concomitant with high rates of endogenous growth promoting substances which increased cell division, cell enlargement, cell elongation, cell differentiation, enzymatic activities, protein synthesis and photosynthetic activity as well as increase the antioxidant capacity of plants⁴⁶⁻⁴⁸. It was reported that the increases in IAA and GA₃ in

Table 6: Effect of L-tryptophan and trans-cinnamic acid on antioxidant enzymes activities during various growth stages

| Growth stages | Treatments (mg L ⁻¹) | Antioxidant enzymes (unit FW g min ⁻¹) | | |
|---------------------------|-------------------------------------|--|-----------|-------------|
| | | SOD | CAT | POX |
| 120 DAS | | | | |
| Control | | 1.4±0.0 | 24.6±0.2 | 1.0±0.0 |
| TCA | 10 | 1.7±0.0 | 26.8±0.1 | 1.2±0.0 |
| | 20 | 1.9±0.0 | 28.6±0.2 | 2.1±0.0 |
| TRYP | 100 | 1.7±0.0 | 28.0±0.1 | 1.5±0.0 |
| | 200 | 2.0±0.0 | 31.1±0.2 | 2.2±0.0 |
| Overall 120 DAS | | 1.7±0.2 | 27.8±1.1 | 1.6±0.5 |
| 180 DAS | | | | |
| Control | | 2.2±0.0 | 25.7±0.2 | 2.0±0.0 |
| TCA | 10 | 2.3±0.0 | 27.0±0.1 | 2.3±0.0 |
| | 20 | 2.8±0.0 | 27.9±0.1 | 2.4±0.0 |
| TRYP | 100 | 2.4±0.0 | 28.4±0.2 | 2.3±0.0 |
| | 200 | 3.3±0.0 | 33.0±0.2 | 2.4±0.0 |
| Overall 180 DAS | | 2.6±0.4 | 28.4±1.6 | 2.3±0.2 |
| Overall treatments | | | | |
| Control | | 1.8±0.4 | 25.1±0.6 | 1.5±0.5 |
| TCA | 10 | 2.0±0.3 | 26.9±0.1 | 1.7±0.6 |
| | 20 | 2.4±0.4 | 28.2±0.4 | 2.2±0.1 |
| TRYP | 100 | 2.1±0.4 | 28.2±0.3 | 1.9±0.5 |
| | 200 | 2.6±0.7 | 32.1±1.1 | 2.3±0.1 |
| F ratio | | | | |
| Treatments | | 7242.1*** | 1431.1*** | 27079.3*** |
| Growth stages | | 58389.4*** | 96.4*** | 123395.5*** |
| Treatments×growth stages | | 981.5*** | 53.9*** | 8751.9*** |

*Significant, **Moderate significant, ***Highly significant, LTRYP: L-tryptophan, TCA: Trans-cinnamic acid, DAS: Days after sowing, SOD: Superoxide dismutase, CAT: Catalase, POX: Peroxidase, values are given as Mean±SD

sunflower herb was concomitant with an increase in growth rate due to the role of these endogenous hormones in stimulating cell division and/or the cell enlargement and subsequently growth measurements⁴⁹. The increase in PGC under TCA rates may be due to an important role of TCA in some physiological activities such as cellular expansion, membrane permeability, nutrient uptake and Chl synthesis²⁵.

Different variations were obtained in EO content or composition under various levels of LTRYP and TCA through different growth stages may be due to its effects on the enzymes activity and metabolism improvements of parsley EO⁵⁰. Similar component of parsley EO were found by Kurowska and Galazka⁵ in Poland.

The increase in PSP under LTRYP treatments may be due to LTRYP (AMA) is the source of nitrogen which has a main role in the biosynthesis of PSP molecules and transpiration rates^{51,52}. The increments in PSP with TCA (PHC) levels may be due its enhancing effects on Rubisco activities, content of pigments⁵³, CO₂ assimilation, photosynthetic rate and increased mineral uptake by the plant⁵⁴.

The high accumulation in TCAR and TSS by the application of LTRYP was concomitant with the chlorophylls rates that in turn affected TCAR content. As well as AMA are the building blocks for proteins that make as enzymes can

build carbohydrates⁷. As well as PHC increase the metabolism of carbohydrates accurate the incorporation of TSS into polysaccharides and inhibit polysaccharides-hydrolyzing enzyme⁵⁵.

Plants affected by many factors in their habitats, the antioxidants are main defense. The antioxidant enzymes include CAT, SOD and POX. Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), superoxide (O₂), hydroxyl radical (HO) and singlet oxygen, can damage the cells of the plant, loss the cell membrane integrity and that may led to senescence or aging of various plant organs^{56,57}. The increase in antioxidant enzymes with AMA may be due to reduce of harmful effects of ROS during growth stages⁵⁸. The increments in antioxidant enzymes under PHC due to its effect on capacity of the tissue to scavenging excess ROS and influence plant induction of antioxidant synthesis^{59,60}.

Present results in this study are confirmed with those obtained by some previous investigation. Significant increases in PGC, PSP and TSS were reported with treatments of *Catharanthus roseus*¹. The LTRYP doses caused significant increments in PGC, EO, TC and TSS of *Pelargonium graveolens*¹⁰. Orabi *et al.*¹⁴ reported that LTRYP promoted the PGC, major constituents of EO and antioxidant enzymes rates of thyme. The LTRYP levels caused higher values in PGC, PSP

and EO (contents and major constituents) on green onion than control treatments¹⁵. The TCA improved growth, yield, EO, major constituents of EO, PSP, TCAR and TSS of basil, sweet marjoram and *Lupinus termis*²⁵⁻²⁷. It may be concluded that the biostimulants such as LTRYP and TCA in the production system should be considered in the growth and chemical contents obtained from MAP.

CONCLUSION

It is concluded that LTRYP and TCA caused a positive effects on PGC and chemical composition of parsley plants. The treatment of 200 mg L⁻¹ (LTRYP) resulted in higher values in PGC, EO yield and major components of EO, PSP, TCAR, TSS and antioxidant enzymes activities (SOD, CAT and POX) than control and other treatments.

SIGNIFICANT STATEMENT

This investigation discovered that production of parsley plants under biostimulants conditions is required. LTRYP or TCA treatments resulted in various changes in the active principals (EO) extracted from parsley; so this trials help the producers, ministry of agriculture and drug companies to improve yield and active principal of parsley as a natural source of pharmaceutical and drug industries. It can be recommended that application of LTRYP or TCA is required to improve growth, yield and active principal of parsley plant.

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