Effect of Benzyl Adenine, Indole Acetic Acid and Gibberellic Acid on Vegetative Growth, Chemical Constituents and Volatile Oil Attributes of Sweet Basil Plants

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BENZYL ADENINE, indole acetic acid and gibberellic acid at different concentrations were sprayed to study their effects on growth, chemical components and volatile oils of sweet basil in 2018 and 2019 seasons.

Plant height, number of leaves/plant, average leaf area, and herb fresh and dry weights/plant greatly improved with the applied treatments especially with IAA treatments followed by GA, treatments. However, the number of lateral branches/plant and stem diameter were increased with BA treatments especially with 10 ppm than 5 ppm. Generally, the second cut was superior in the studied vegetative growth parameters than the first cut.

The highest values of N, P, K, Fe, and Mn were recorded with BA at 10 ppm, whereas higher Zn values were recorded with BA at 5 ppm. Active ingredients including total phenols, total flavones, total chlorophyll, and L-ascorbic acid greatly increased with IAA at 50 or 100 than the other treatments or the control. However, volatile oil percentage and volatile oil content/plant were high in IAA at 50 or 100 ppm followed by GA3 at 200 ppm. The second cut was superior to the first cut in promoting volatile oil percentage and volatile oil content/plant in both studied seasons. The main components in volatile oil were 1,8-cineole, linalool, α-trans-bergamotene, germacrene D and α-muurolol. The highest values of linalool, the main component in the volatile oil of sweet basil (50.90%) was recorded by IAA at 100 ppm.

Keywords: Plant growth regulators, BA, IAA, GA3, Sweet basil, Vegetative growth, Total phenols, Volatile oil.

Introduction

There are more than a hundred and fifty basil species belonging to the Ocimum genus (Javanmardi et al., 2002). The two most widely grown species for producing volatile oil are holy basil (Ocimum sanctum L.) and sweet basil (Ocimum basilicum L.). Basil oil is utilized for flavor and aroma in the foods, pharmaceutical, beauty products, and aromatherapy fields. The oil is considered antimicrobial (Elgayyar et al., 2001, Suppakul et al., 2003, Kristinsson et al. 2005 and Bozin et al., 2006) and insecticidal (Bowers & Nishida, 1980 and Aslan et al., 2004) agents. The action of sweet basil basic oil has been historically taken out from herb (stems, leaves, and flowers) by steam percolation (Trevisan et al., 2006). The ideal phase for volatile oil extraction is at blossom emergence, while the oil content material and fractions components are the highest (Zheljazkov, 2008).

Sweet basil (Ocimum basilicum L.) which investigated in this research is identified by high yields of raw fabric and active ingredients involved total phenols, total flavones, total chlorophyll, and L-ascorbic acid. As mentioned by Berbeć et al. (2003) who reported that the utilization of bio-stimulators can do the main function in production increasing and enhancing plant validity effect. Bio-stimulators transport essential nutrients to the plant, thereby growing its resistance to pests. In Western Europe, herb growers were use plant growth regulators on a
large scale for over a dozen years. They promote metabolic processes within the plant, improve growth seed and fruit production as well as raise resistance to fungal and bacterial disorders (Carvajal and Alcaraz, 1998).

The quantitative and qualitative synthesis of the phytochemicals in sweet basil leaves can differ according to many factors. Chemical plant nutrition is one of the essential factors influencing plant metabolism and the level of secondary metabolites. Flavonoids are considered vital secondary metabolites regulating the growth of plants (Winkel-Shirley, 2002), and are found in leaves, flowers, fruits, and roots. They are used natural medicines and healthful food. The pathway of flavonoids in flowers starts with the shikimate pathway, and intermediate marketplace, together with chalcone and isoflavonoids, are formed through the phenylpropanoid pathway (Dixon et al., 2002 and Koes et al., 2005). These flavonoids are then conjugated to other flavonoids and glycosyl to form final products inclusive of anthocyanin and flavonol glycoside.

Plant growth regulators (PGRs) are natural produced with the aid of plants or synthetically through chemists and affect in plant growth cycle (Davies, 2013). PGRs include cytokinins and auxins, that at low concentrations modify plant developmental strategies (Fu et al., 2011). However, benzyl adenine (BA) and naphthalene acetic acid (NAA) have both been carried out pre- and post-cut of crops to a lot of decorative plant species. Cytokinins including BA which encourages cellular elongation and division (Krug et al., 2006). BA has additionally been used to enhance the growth and development of aromatic flowers. Treating Lantana camara L. flora with BA increased unstable natural compound production as volatile oil contents (Affonso et al., 2007). Similarly, Zielińska et al. (2011) indicated that the production of volatile oil contents elevated with the utility of various PGRs, such as indole-3-acetic acid (IAA), thiadiazuron, kinetin, and BA, when applied to Agastache rugosa flowers grown in vitro. Auxins which include NAA coordinate many increase and behavioral procedures in vegetation cycles. Furthermore, NAA promotes cellular division and elongation, performs a key function in shoot improvement, and increases flower manufacturing (Fu et al., 2011). However, Saffari et al. (2004) in their working on Rosa damascena (Damask rose) indicated that the utilization of NAA at 25 mg/l to improve vase life, expanded flower longevity, plant height, flower yield, and flower oil composition. Another study by Bota and Deliu (2015) confirmed that the addition of 0.1 mg/l NAA and 1 mg/l BA to a cellular suspension culture of Digitalis lanata (Grecian foxglove) elevated the production of flavonoids, that are related to secondary metabolite production.

The volatile oil manufacturing does not relate only to plant genetics or developmental stage. The climate and its variations can have an impact on in a significant manner biochemical pathways and physiological approaches that alter plant metabolism and, therefore, the volatile oil biosynthesis (Sangwan et al., 2001).

Gibberellins as diterpenoids components make up a group of plant hormones which can ameliorate the plant development and improvement. GA3 has many physiological outcomes on plant life and it has confirmed that GA3 has an inductive effect on plant vigor and secondary metabolite biosynthesis (Taiz and Zeiger, 2010). However, Bais et al. (2001) reported that application of GA3 leads to hastening the growth of intact common chicory plant (Cichorium intybus L.) and the buildup of coumarin (phenolic compound). Gibberellic acid will increase the biosynthesis of several secondary metabolites like steroids, terpenoids, flavonoids, and anthocyanins (Srivastava and Srivastava, 2007). In this respect, Davies (2013) reported that GAs growth regulators are effective in developmental strategies as seed germination, stem elongation, leaf growth, trichome improvement and flower and fruit development.

It has been known that an increase in growth regulators in various agriculture practices is maximum favorable for promoting and enhancing plant-growth of different plant life (Eid and Abou-Leila, 2006.). The useful effect of gibberellic acid on different vegetative phases were recorded with the aid of Shedeed et al. (1991) on croton plant, Brooking and Cohen (2002) on Zantedeschia, Al-khassawneh et al. (2006) on black iris. They all concluded that gibberellic acid is utilized to regulating plant increase through increasing cellular department and cell elongation. GA3 sprays superior plant dry mass, leaf location, plant increase price and increased yield in Cymbogon martini (plamarosa) (Khan et al., 2015).

Auxins have numerous physiological results on the medicinal and aromatic plants included cellular elongation and differentiation, apical dominance phenomena, root initiation and fruit
development (Taiz and Zeiger, 2010, Khan et al., 2015). Indole-3-acetic acid (IAA) is the main detectable auxin in plants and plays many roles in plant growth (Georg et al., 2008). Moreover, the presence of indole-3-butyric acid (IBA) in plant tissues has demonstrated (Santner et al., 2009). Also, IAA inspired leaf increase in Cymbopogon Jwarancusa (Ansari et al., 1988).

Cytokinins are plant promoters which are utilized in agricultural practices for stimulation and synchronization of flowering and fruit set, promotion of rooting, reduction of vegetative growth, reduction of accommodations of agronomic crops, or defoliation (Briant, 1974). Essential plant hormones that regulate numerous tactics of plant growth and development including cellular division and differentiation, enhancement of leaf enlargement and nutrient mobilization (Shudok, 1994). The reaction of plants to cytokinins have been also mentioned by Eraki (1994) on Hibiscus sabdariffa L, plant referred to that utilization of BA appreciably improve plant height, number of branches as properly as fresh and dry weights of leaves than the control. However, Hassanine (1985) on Pelargonium graveolens, El-Sayed et al. (1989) on Polianthus tuberosa, Menesi et al. (1991) on Calendula officinalis and Mazrou et al. (1994) on candy basil, found that foliar utility of BA multiplied increase of various organs, active constituents production of these plant life and accelerated total carbohydrates content material on contrast to the untreated flowers. The pharmaceutical and beauty industries depend on the purity and heterogeneity of essential oils to meet the needs and necessities of the marketplace, within the aim to have a powerful organic activity (antifungal, antibacterial) or to enhance the cosmetics.

The main target of this study was to evaluate the effect of BA, IAA and GA3 on vegetative growth, macro- and micro-elements levels, chemical constituents, volatile oil production and volatile oil fractions of sweet basil plants.

**Materials and Methods**

The present work was carried out during 2018 and 2019 seasons in the Ornamental Nursery, Faculty of Agriculture, Ain shams Univ., Cairo, Egypt on sweet basil (Ocimum basilicum L.).

Seeds were sown on the mid of March 2018 and 2019 in seed bins filled with peat moss and sand (1:1). After 45 days from sowing (first of May) where the seeding reached 10-12 cm height with 8-10 leaves and 5 branches, they were transplanted to 30 cm diameter plastic pot filled with peat moss and sand (1:1). The seedlings of sweet basil were selected to be uniform in shape and exposed to all horticulture practices including irrigation, fertilization and pest managements as recommended in this respect.

Plant growth regulators were sprayed twice, on firs of June (after one month of transplanting) and again on the mid of July (the first cut) of both seasons. Seven treatments were applied as follows:

- Control as sprayed with tap water.
- Spraying benzyl adenine (BA) at 5 ppm.
- Spraying benzyl adenine (BA) at 10 ppm.
- Spraying indole acetic acid (IAA) at 50 ppm.
- Spraying indole acetic acid (IAA) at 100 ppm.
- Spraying gibberellic acid (GA3) at 100 ppm.
- Spraying gibberellic acid (GA3) at 200 ppm.

The experiment was designed in a randomized complete block design (RCBD) with 4 replicates for each treatment and each replicate contained 5 sweet basil plants (7 treatments x 4 replicates x 5 plants = 140 sweet basil plants) in each season. Herb of sweet basil plants was harvest twice, in the first cut when inflorescence shoots occurred (50% flowering) in mid-July in both seasons and after 45 days the second cut was done (first of September) (Tae et al., 2010). In each cut, the plants were cut at a height of 6-7 cm above the ground (Biesada and Kus, 2010).

The following data were recorded:

**Vegetative growth parameters**

At each cut, plant height (cm), number of lateral branches/plant, number of leaves/plant, stem diameter (cm) measured five cm above the ground, average leaf area (cm²), herb fresh weight/plant (g) and herb dry weight/plant (g) were estimated in both seasons.

**Mineral contents**

At the second cut of each season, the leaves were collected, washed and dried at 70°C until constant weight and then ground for determination the nutrient elements. Nitrogen was determined according to Guebel et al. (1991). Phosphorus was determined according to Bringham (1982) and potassium was measured according to Westerman (1990). Fe, Zn, and Mn were determined according to Chapman and Pratt (1982).
Chemical constituents
At the second cut of the two studied seasons, the herb was collected and total phenols (mg/g dry weight) was determined according to Singleton and Rossi (1965), total flavones (mg/g dry weight) was determined according to Zhishen et al. (1999), total chlorophyll (mg/100g fresh weight) was determined according to Moran and Porath (1980) and L-ascorbic acid (mg/100g fresh weight) was determined according to A.O.A.C (2005).

Volatile oil percentage and volatile oil content/plant
In the two cuts in both studied seasons, the essential oil was extracted by water distillation method according to Novak et al. (2002). The amount of obtained volatile oil from three plants for each replicate was measured and both oil percentage and volatile oil content/plant were measured according to Charles and Simon (1990).

Chemical constituents of the essential oil
It was determined in the second cut of the second season only, chemical constituents of the essential oil of the herbs were analyzed using the Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) (GC/MS) apparatus to determine their main constituents (Charles and Simon, 1990).

Statistical analysis
Results were statistically analyzed using the analysis of variance (ANOVA) as described by Snedecor and Cochran (1990). The method of Duncan’s multiple range tests was applied for the comparison between means according to Waller and Duncan (1969).

Results and Discussion
Vegetative growth parameters
Data in Tables 1, 2, 3 and 4 show the effect of BA, IAA and GA3 spraying on some vegetative growth parameters of sweet basil plant during 2018 and 2019 seasons.

As shown in Table 1, the highest values of plant height (73.1 and 71.4 cm) were obtained by IAA spraying at 100 ppm in the two studied seasons, respectively. However, the treatments of IAA at 50 ppm and GA3 at 200 ppm were similar in their effect on plant height of sweet basil without significant differences between them. The least values of plant height due to the used treatments were recorded by BA at 5 or 10 ppm which were similar to the control. The second cut of sweet basil recorded the highest significant values of plant height than the first cut in both seasons. The great effect to the interaction between the applied treatments and the two cuts on plant height was recorded with 100 ppm IAA in the second cut. However, the least interaction values were recorded in the first season with unsprayed plants in the first cut and in the second season with BA 10 ppm in the first cut.

The number of lateral branches/plant illustrated in Table 1 was greatly improved with all applied treatments than the control in both seasons of the study. The superior effect was more pronounced (34.3 and 32.0 branches/plant) with 10 ppm BA in the first and second seasons, respectively, however, 5 ppm of BA came next in this respect. The treatments of GA3 at 100 or 200 ppm recorded medium values of lateral branches/plant without significant differences between them. However, IAA at 50 or 100 ppm recorded the least effect on the lateral branches of sweet basil. Moreover, the second cut recorded the highest significant values of lateral branches than the first cut in both seasons. Interaction values showed that the highest values of lateral branches (36.5 and 33.4 branches/plant) were recorded in the second cut due to the applied of 10 ppm BA.

Data in Table 2 show that all applied treatments, GA3 at 200 ppm in the first season and IAA at 100 ppm and GA3 at 200 ppm in the second season, increased the number of leaves/plant than the control. However, BA at 5 ppm, IAA at 50 ppm and GA3 at 100 ppm exhibited similar non-significant values of the number of leaves/plant in both studied seasons. Also, the second cut produced the great number of leaves/plant than the first cut in the second season only. Interaction between the two studied factors did not follow a clear trend where the highest interaction value (260.5 leaves/plant) in the first season was obtained in the first cut with 10 ppm BA, whereas in the second season the highest value (254.9 leaves/plant) was recorded in the second cut with the same treatment.

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TABLE 1. Effect of BA, IAA and GA3 spraying on plant height and the number of lateral branches/plant of sweet basil in 2018 and 2019 seasons.

| Treatments       | Plant height (cm) | No. lateral branches/plant |
|------------------|-------------------|----------------------------|
|                  | 1st cut | 2nd cut | Mean    | 1st cut | 2nd cut | Mean    |
| Control          | 38.92 h | 46.6 fg | 42.7 E  | 12.6 h  | 15.3 gh | 14.0 F  |
| BA 5 ppm         | 44.7 g  | 51.4 efg| 48.1 D  | 25.7 cd | 30.4 bc | 28.1 B  |
| BA 10 ppm        | 45.6 g  | 49.7 fg | 46.2 DE | 32.1 ab | 36.5 a  | 34.3 A  |
| IAA 50 ppm       | 64.3 bc | 68.2 ab | 66.2 B  | 19.3 fg | 23.1 def| 21.2 DE |
| IAA 100 ppm      | 70.8 ab | 75.3 a  | 73.1 A  | 16.5 gh | 20.4 efg| 18.5 E  |
| GA3 100 ppm      | 53.2 ef | 57.1 de | 55.2 C  | 21.5 ef | 24.6 de | 23.1 CD |
| GA3 200 ppm      | 61.3 cd | 65.8 bc | 63.6 B  | 23.7 def| 27.3 bcd| 25.5 BC |
| Mean             | 54.1 B  | 59.1 A  | 54.2 D  | 14.7 f  | 17.9 ef | 16.2 E  |

2019 Season

| Treatments       | Plant height (cm) | No. lateral branches/plant |
|------------------|-------------------|----------------------------|
|                  | 1st cut | 2nd cut | Mean    | 1st cut | 2nd cut | Mean    |
| Control          | 41.9 ef | 48.4 e  | 45.2 D  | 14.7 f  | 17.9 ef | 16.2 E  |
| BA 5 ppm         | 44.7 ef | 46.2 ef | 45.5 D  | 24.8 ed | 27.1 bc | 26.0 B  |
| BA 10 ppm        | 40.2 f  | 44.7 ef | 42.5 D  | 30.5 ab | 33.4 a  | 32.0 A  |
| IAA 50 ppm       | 61.5 cd | 66.5 bc | 64.0 B  | 21.7 de | 24.8 ed | 23.3 BC |
| IAA 100 ppm      | 68.9 ab | 73.8 a  | 71.4 A  | 18.2 ef | 21.5 de | 19.9 D  |
| GA3 100 ppm      | 56.9 d  | 60.2 cd | 58.3 C  | 20.8 de | 23.3 ed | 22.1 CD |
| GA3 200 ppm      | 63.1 bc | 62.6 bcd| 62.9 BC | 24.2 cd | 25.4 bcd| 24.8 BC |
| Mean             | 53.8 B  | 57.5 A  | 53.8 B  | 22.1 B  | 24.77 A | 24.8 BC |

Values followed by the same letter (s) are not significantly different at 5% level.

It is clear from data in Table 2 that stem diameter was increased with all treatments than the control except IAA at both concentrations in the first season and IAA at 100 ppm only in the second season. BA treatments were effective than the other two growth regulators in increasing the stem diameter of sweet basil plant. However, 10 ppm of BA was more effective than 5 ppm in this respect. The second cut produced thicker plants than the first cut in both seasons of study with significant differences between them. Interaction between the studied factors was significant in most cases where the highest interaction values of stem diameter (2.83 and 2.65 cm) were obtained in the second cut with the plants sprayed with 10 ppm of BA in both seasons of study.

Regarding the average leaf area, Data in Table 3 clearly show that all applied treatments with plant growth regulators increased the leaf area except the treatments of 5 ppm BA in both studied seasons. However, the highest values of leaf area (11.15 and 10.53 cm²) were recorded by IAA at 100 ppm in both seasons. Generally, it could be noticed that IAA as promoting growth regulator was more effective than GA3 or BA in improving the leaf area of sweet basil plant. The spraying of BA was negligible and reduced leaf area than the control and this could be attributed to that BA as a cytokinin led to branching the treated plants and produced more leaves with small areas. No significant differences were detected between the two cuts in effecting the leaf area in both studied seasons. Interaction values showed that the highest values of average leaf area (11.34 and 10.53 cm²) were recorded with 100 ppm IAA in the second cut of both studied season.

Herb fresh weight/plant values (Table 4) were significantly increased with all spraying treatments than the control. The highest values (214.1 and 223.7 g/plant) were recorded with BA at 10 ppm in the first and second seasons, respectively. However, GA3 at 200 ppm came next in producing high values of herb fresh weight, whereas IAA at 50 ppm recorded the least values of herb fresh weight/plant in both studied seasons. Herb fresh weight/plant was significantly affected in the second cut than the first cut in both seasons. Interaction values showed that the highest values of herb fresh weight/plant (221.8 and 230.0 g) were recorded with BA in the second cut at 10 ppm in the first and second season, respectively.

Herb dry weight/plant values (Table 4) were followed a similar trend to those found in herb fresh weight values, where the highest herb dry weight (59.0 and 62.7 g/plant) were recorded with BA at 10 ppm in the first and second season, respectively. Moreover, the least herb dry weight was gained with IAA at 50 or 100 ppm in both studied seasons. Generally, it could be noticed...
that the herb dry weight of sweet basil greatly increased with BA at 10 ppm and GA, 200 ppm and this result is important to sweet basil producers where the herb dry weight is the main product of this medicinal plant. In addition, the second cut produced the highest values of herb dry weight of sweet basil than the first cut in both studied seasons. The highest interaction values (61.3 and 63.9 g/plant) were recorded by BA at 10 ppm in the second cut in both seasons of study.

In this respect, Eraki, (1994) mentioned that benzyl adenine (BA) and naphthalene acetic acid (NAA) have both been applied pre- and post-harvest to a lot of ornamental plant species. Cytokinins such as BA are used to enhance the quality and vase life of cut flowers. Moreover, BA increases cell elongation and division, decreases flower fall and increases flower yield. Moreover, NAA stimulates cell division and elongation, affects shoot development, and improves flower yield. A study on *Rosa damascena* (Damask rose) pointed that the utilization of NAA at 25 mg/l to rose plants, improved flower longevity, plant height, flower production and flower oil yield (Hassan and El-Queni, 1989). Also, Briant (1974) extracted that cytokinins are used in the agricultural industry for stimulation and synchronization of flowering and fruit setting, promotion of rooting, reduction of vegetative growth, reduction of lodging of agronomic crops, or defoliation.

Great effect of GAs was also mentioned by Khan and Chaudhry (2006) who reported that GA, utilization improved petiole length, leaf area and retarded petal abscission and color fading (senescence) by the hydrolysis of starch and sucrose into fructose and glucose. It has been known that growth regulators among the agriculture practices which is most favorable for enhancing and improving plant growth of different plants. The useful reaction of gibberellic acid on many plants were reported by Shedeed et al. (1991) on croton plant, Brooking and Cohen (2002) on *Zantedeschia*, Al-khassawneh et al. (2006) on black iris. They deduced that gibberellic acid is used to regulate plant growth through raising cell division and cell elongation. GA utilization improved plant dry mass, leaf area, plant growth rate and crop growth rate in mustard.

| TABLE 2. Effect of BA, IAA and GA3 spraying on number of leaves/plant and stem diameter of sweet basil plant in 2018 and 2019 seasons. |
| Treatments | Number of leaves/plant | Stem diameter (cm) |
|            | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control    | 178.4 e | 196.0 de | 187.2 CD | 1.46 f | 1.47 f | 1.47 E |
| BA 5 ppm   | 231.7 bc | 225.1 bc | 228.4 B | 2.19 b | 2.34 b | 2.27 B |
| BA 10 ppm  | 260.5 a  | 248.5 ab | 254.5 A  | 2.31 b | 2.83 a | 2.57 A |
| IAA 50 ppm | 218.1 cd | 211.7 cd | 214.9 B  | 1.58 def | 1.65 def | 1.62 DE |
| IAA 100 ppm| 178.5 e  | 183.5 e  | 215.1 D  | 1.51 e | 1.59 e | 1.53 E |
| GA3 100 ppm| 213.2 cd | 217.1 cd | 212.7 BC | 1.79 de | 1.74 def | 1.77 CD |
| GA3 200 ppm| 194.6 de | 203.8 de | 199.2 C  | 1.85 cd | 2.11 bc | 1.98 C |
| Mean       | 210.7 A  | 212.2 A  | 1.81 B   | 1.95 A |

| Treatments | Number of leaves/plant | Stem diameter (cm) |
| Control    | 185.6 fg | 190.4 fg | 188.0 D  | 1.42 g | 1.40 g | 1.41 E |
| BA 5 ppm   | 215.9 cde | 230.5 abc | 223.2 B  | 2.10 cd | 2.41 ab | 2.25 A |
| BA 10 ppm  | 250.2 ab | 254.9 a  | 252.6 A  | 2.23 bc | 2.65 a | 2.44 A |
| IAA 50 ppm | 225.4 bcd | 205.7 def | 215.6 BC | 1.65 cef | 1.77 ef | 1.70 CD |
| IAA 100 ppm| 170.4 g  | 195.7 ef  | 183.1 D  | 1.50 fg | 1.56 fg | 1.53 DE |
| GA3 100 ppm| 220.5 cd | 204.8 def | 212.7 BC | 1.84 de | 1.93 cde | 1.89 BC |
| GA3 200 ppm| 190.4 fg | 213.8 cde | 202.0 CD | 1.93 cde | 2.15 bc | 2.04 B |
| Mean       | 208.4 B  | 213.7 A  | 1.81 B   | 1.99 A |

Values followed by the same letter (s) are not significantly different at 5% level.
TABLE 3. Effect of BA, IAA and GA3 spraying on average leaf area of sweet basil plant in 2018 and 2019 seasons.

| Treatments     | 2018 Season |                    | 2019 Season |                    |
|----------------|-------------|--------------------|-------------|--------------------|
|                | 1st cut     | 2nd cut            | Mean        | 1st cut            | 2nd cut            | Mean        |
| Control        | 7.11 de     | 6.75 ef            | 6.93 D      | 6.54 de            | 7.25 cd            | 6.90 C      |
| BA 5 ppm       | 6.58 ef     | 6.26 ef            | 6.42 DF     | 6.11 de            | 6.55 de            | 6.33 C      |
| BA 10 ppm      | 6.03 ef     | 5.49 f             | 5.76 F      | 5.74 e             | 6.17 de            | 5.96 C      |
| IAA 50 ppm     | 9.72 abc    | 10.15 ab           | 9.94 B      | 9.54 ab            | 9.84 ab            | 9.69 AB     |
| IAA 100 ppm    | 10.96 a     | 11.34 a            | 11.15 A     | 10.14 ab           | 10.53 a            | 10.34 A     |
| GA3 100 ppm    | 9.18 bc     | 9.72 abc           | 9.45 BC     | 9.25 ab            | 9.54 ab            | 9.40 B      |
| GA3 200 ppm    | 8.51 cd     | 8.93 bc            | 8.72 C      | 8.75 bc            | 9.07 ab            | 8.91 B      |
| Mean           | 8.30 A      | 8.38 A             | 8.02 A      | 8.43 A             |                    |             |

Values followed by the same letter (s) are not significantly different at 5% level.

TABLE 4. Effect of BA, IAA and GA3 spraying on herb fresh and dry weights/plant of sweet basil in 2018 and 2019 seasons.

| Treatments     | Herb fresh weight/plant (g) |                    | Herb dry weight/plant (g) |                    |
|----------------|----------------------------|--------------------|---------------------------|--------------------|
|                | 1st cut                    | 2nd cut            | Mean                      | 1st cut            | 2nd cut            | Mean        |
| Control        | 129.1 g                    | 154.6 f            | 141.9 E                   | 35.7 g             | 42.8 f             | 39.3 D      |
| BA 5 ppm       | 183.5 de                   | 192.7 bcd          | 188.1 C                   | 51.6 cde           | 53.4 cd            | 52.5 B      |
| BA 10 ppm      | 206.3 abc                  | 221.8 a            | 214.1 A                   | 56.7 abc           | 61.3 a             | 59.0 A      |
| IAA 50 ppm     | 155.9 f                    | 174.6 ef           | 165.3 D                   | 43.5 f             | 48.4 e             | 46.0 C      |
| IAA 100 ppm    | 177.5 de                   | 185.2 de           | 181.4 C                   | 50.7 de            | 51.3 de            | 51.0 B      |
| GA3 100 ppm    | 187.3 cde                  | 194.4 bcd          | 190.9 C                   | 52.1 cde           | 54.0 bcd           | 53.1 B      |
| GA3 200 ppm    | 195.3 bcd                  | 211.7 ab           | 203.5 B                   | 54.6 bcd           | 58.8 ab            | 56.7 A      |
| Mean           | 176.5 B                    | 190.8 A            | 190.3 B                   | 49.3 B             | 52.9 A             |             |

Values followed by the same letter (s) are not significantly different at 5% level.
Macro- and micro-elements

Data in Table 5 show the effect of spraying of BA, IAA, and GA₃ on the levels of N, P, K and Fe, Zn, Mn of sweet basil leaves in 2018 and 2019 seasons.

Nitrogen recorded 1.91 and 1.84% with unsprayed sweet basil plants in the first and second seasons, respectively whereas N reached 2.57% due to the application of plant growth regulators. However, the highest values of N (2.57 and 2.38%) were recorded with BA at 10 ppm in the two studied seasons. However, 5 ppm of BA and GA₃ at 200 ppm greatly increased N and came next after 10 ppm BA treatments. P and K percentages were increased with applied treatments except IAA at 100 ppm and GA₃ at 100 ppm with P in both studied seasons. However, all applied treatments increased K in sweet basil leaves than control except IAA at 50 or 100 ppm and GA₃ at 100 ppm which did not differ significantly than control in both studied seasons. Generally, it could be concluded that BA at 10 ppm treatment was effective in increasing N, P and K in sweet basil leaves and consequently improved the nutritive value of sweet basil herbs.

The effect of growth regulators spraying on Fe, Zn and Mn content of sweet basil leaves was clear during the two studied seasons. All applied treatments increased Fe content than the control, where the highest Fe content values were obtained by BA at 10 ppm and GA₃ at 100 ppm in both studied seasons. On the contrary, the treatment of IAA 100 ppm exhibited similar non-significant values to the control in both seasons. Data of Zn content were fluctuated in both seasons, where in the first season the higher values were recorded by BA at 5 ppm whereas, in the second season the highest values were recorded by BA at 10 ppm and GA₃ at 200 ppm. However, the treatment of IAA at 100 ppm recorded similar values of Zn content to the control in both seasons. Mn content data show that all treatments were negatively affected Mn content except IAA at 100 ppm or GA₃ at 100 ppm to content in both seasons. The highest values of Mn contents were recorded with BA at 10 ppm in both seasons, whereas the least values were recorded by IAA at 100 ppm in both seasons. Generally, it could be concluded that spraying of BA at 10 ppm increased Fe and Mn in sweet basil leaves, whereas BA at 5 ppm was effective in increasing Zn content.

TABLE 5. Effect of BA, IAA and GA₃ spraying on some macro- and micro-elements of sweet basil leaves of the second cut in 2018 and 2019 seasons.

| Treatments | Macro-elements (%) | Micro-elements (ppm) |
|------------|--------------------|----------------------|
|            | N                  | P                    | K        | Fe       | Zn       | Mn       |
| 2018 Season |                    |                      |          |          |          |
| Control    | 1.91 d             | 1.23 d               | 0.56 d   | 246.3 d  | 52.44 d  | 74.11 d  |
| BA 5 ppm   | 2.34 ab            | 1.54 b               | 0.67 bc  | 312.4 c  | 78.07 a  | 97.24 ab |
| BA 10 ppm  | 2.57 a             | 1.73 a               | 0.78 a   | 381.7 a  | 74.21 ab | 103.16 a |
| IAA 50 ppm | 2.06 cd            | 1.46 bc              | 0.60 cd  | 305.8 c  | 58.93 c  | 87.15 bc |
| IAA 100 ppm| 1.97 cd            | 1.31 cd              | 0.54 d   | 274.2 d  | 57.61 cd | 78.39 cd |
| GA₃ 100 ppm| 2.13 bc            | 1.37 cd              | 0.61 cd  | 325.7 bc | 63.45 c  | 83.12 cd |
| GA₃ 200 ppm| 2.28 b             | 1.60 ab              | 0.73 ab  | 354.8 ab | 70.38 b  | 94.75 ab |
| 2019 Season |                    |                      |          |          |          |
| Control    | 1.84 d             | 1.17 c               | 0.50 c   | 227.4 c  | 54.71 d  | 79.31 d  |
| BA 5 ppm   | 2.29 ab            | 1.61 a               | 0.63 ab  | 307.5 b  | 66.12 b  | 95.46 b  |
| BA 10 ppm  | 2.38 a             | 1.68 a               | 0.69 a   | 365.9 a  | 75.41 a  | 108.32 a |
| IAA 50 ppm | 2.05 c             | 1.39 b               | 0.56 bc  | 288.3 b  | 62.51 bc | 90.87 bc |
| IAA 100 ppm| 1.93 cd            | 1.24 bc              | 0.52 c   | 253.4 c  | 58.94 cd | 83.88 cd |
| GA₃ 100 ppm| 2.11 bc            | 1.25 bc              | 0.52 c   | 310.6 b  | 60.63 bc | 85.62 bcd|
| GA₃ 200 ppm| 2.26 ab            | 1.56 ab              | 0.64 ab  | 342.7 a  | 72.19 a  | 91.80 bc |

Values followed by the same letter(s) are not significantly different at 5% level.

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Chemical constituents

It is clear from data in Table 6 that chemical constituents of sweet basil herb which expressed as active ingredients were increased with the used growth regulators but in varied trend. However, total phenols were increased with all used treatments than the control, and the highest values were recorded by IAA at 100 ppm, IAA at 50 ppm and BA at 10 ppm in both studied seasons without significant differences between them. Total flavones were increased with all treatments than the control except the treatment of GA3 at 100 ppm in the first season and BA at 5 ppm and GA3 at 100 ppm in the second season. The values of total flavones increased from 10.75 mg/g d.wt in the control to 15.98 mg/g d.wt in IAA at 100 ppm (data of the first season). Total chlorophyll values were superior with both treatments of IAA in both seasons, whereas the treatments of BA at 5 ppm, GA3 at 50 at 100 exhibited similar non-significant values to the control in both seasons.

However, L-ascorbic acid values greatly improved due to the growth regulator application on sweet basil plant in both seasons. However, L-ascorbic acid increased from 18.54 mg/100 g fresh weight in the control to 27.15 mg/100 g fresh weight in IAA 100 ppm treatments (data of the first season). However, BA at 5 ppm exhibited similar non-significant values to the control in both seasons, whereas the highest values of L-ascorbic acid were recorded with both treatments of IAA and GA3 at 200 ppm without significant differences between them in both studied seasons.

Traditionally, sweet basil has been used as a medicinal plant in the therapy of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction (Kaurinovic et al., 2011). However, it has been found that sweet basil leaves are rich in phenolic compounds (rosmarinic, chloric, caeic, and caftaric), flavonol (quercetin, kaempferol) glycosides and anthocyanins (Ghasemzadeh et al., 2014).

Winkel-Shirley (2002) reported that the quantitative and qualitative components of the phytochemicals in basil leaves can vary within wide ranges and depend on both cultivation conditions and the variety of basil. Mineral plant nutrition is one of the main factors influencing plant metabolism and the level of secondary metabolites. Flavonoids, which are important secondary metabolites in plants, are involved in resist in environmental stress and regulating the growth of plants. Also, Koes et al. (2005) explained that abundant values in leaves, flowers, fruits, and roots, play important roles in herbal medicines and healthy food products. The biosynthesis of flavonoids in plants begins with the shikimate pathway, and intermediate products, such as chalcone and isoflavonoids, are formed via the phenylpropanoid pathway.

In another study by Bota and Deliu (2015) showed that the addition of 0.1 mg/l NAA and 1 mg/l BA to a cell suspension culture of *Digitalis lanata* (Grecian foxglove) increased the production of flavonoids, which are related to secondary metabolite production. Furthermore, Sharma and Kumar (2011) claimed that gibberellic acid increased the biosynthesis of several secondary metabolites like steroids, terpenoids, and anthocyanins.

**Volatile oil percentage and volatile oil content**

Data in Table 7 show that volatile oil percentage in sweet basil herb greatly affected with the applied treatments than the control where it increased from 0.12% in the control to 0.38% in the used treatments. The highest values of volatile oil (0.38 and 0.44%) were recorded with IAA at 100 ppm in the first and second seasons, respectively. Generally, it could be noticed that the effect of applied plant growth regulators on volatile oil percentage of sweet basil herb was varied, where IAA was superior followed by GA3, where BA came later in this respect. In addition, the second cut of sweet basil herbs produced high volatile oil percentage than the first cut with significant differences between them in both seasons.

Interaction values showed that the highest values of volatile oil percentage (0.42 and 0.46%) were recorded with IAA at 100 ppm in the second cut of both seasons.

The productivity of volatile oil content per plant exhibited a similar trend to those found in volatile oil percentage. The highest values of volatile oil content/plant (0.62 and 0.59 ml/plant) were recorded with IAA at 100 ppm treatments in both first and second seasons, respectively.
Generally, it could be concluded that the application of plant growth regulators on sweet basil plants was effective in increasing volatile oil content per plant but IAA at both used concentrations was more effective than GA₃ or BA in this respect. The second cut was superior to the first one in increasing volatile oil content/plant with significant differences between them in both studied seasons. Interaction values showed that IAA at 100 produced the highest values of volatile oil content (0.66 and 0.63 ml/plant) in the second cut of both studied seasons.

Volatile oil components

Data presented in Table 8 show the effect of plant growth promoters applied to sweet basil plant on volatile oil fractions in the second cut of the second season only.

All applied treatments exhibited high values of total components than the control. The main components, in general, were 1,8-cineole, linalool, bergamotene, germacrene, and muurolol. The highest values of 1,8 cineole (5.90%) was recorded by GA₃ at 100 ppm, whereas Linalool, the main active substance in sweet basil plant (50.90%) was superior in IAA at 100 ppm. The highest value of bergamotene (8.20%) was recorded by GA₃ at 200 ppm. Germacrène (3.50%) was recorded by IAA at 50 ppm and the highest value of muurolol (4.60%) was recorded by both treatments of IAA. Generally, it could be concluded that linalool as main active ingredient of sweet basil was increased by all applied treatments than control.

The essential oils distilled from various basil cultivars can involve linalool, methyl chavicol, 1,8-cineole, eugenol, methyl eugenol, methyl isoeugenol, thymol, methyl cinnamate, citral, and camphor (Zabka et al., 2014). On another study by Marotti et al. (1996) who found that the high economic value of basil oil is attributable to the existence of phenyl propanoids, like eugenol, chavicol and their derivatives or terpenoids like monoterpen alchole linalool, methyl cinnamate, and limonene.

Affonso et al. (2007) mentioned that BA has also been used to enhance the growth and expansion of aromatic plants. Utilization Lantana camara L. plants with BA at 0.44 and 4.4 μmol/L raised volatile organic ingredient (VOC) production, which was detected by solid phase micro-extraction. Similarly, Zielińska et al. (2011) pointed the volatile oil yield increased with the utilization of many PGRs, such as indole-3-acetic acid (IAA), thidiazuron, kinetin, and BA when applied to Agastache rugosa. It is regular that essential oils are the most important raw substances of the fragrance and aroma industry. They are also used in the food and pharmaceutical industries due to their therapeutic, antimicrobial and antioxidant activities. Nevertheless, they have biological activities that make them able to be used as herbicides, pesticides and anticancer compounds (Burfield and Reekie, 2005). However, Taiz and Zeiger (2010) reported that the essential oils are related to plant defense and pollinator attraction among other ecological functions. As other secondary metabolites groups, these compounds play an important role in the plant’s fitness under environmental variation. For this reason, a common problem that occurs in aromatic plants cultivation is the quantitative and qualitative variation in response to the environment.

The content and composition of basic oils are highly impacted by outer factors and cultural practices. In Morocco, the Ocimum genus and specifically O. gratissimum L species are widely used in traditional medicine. Basic oils of this species play a very important role in international oil marketing. The antibacterial action of basil essential oil raises by the increase in levels of methyl chavicol (Pessoa et al., 2002). The heterogeneity of oils may also be obtained by the synergistic effect of the different compounds (Ngassoum et al., 2003).

Salah El-Deen (1996) studied the effect of GA, IAA, and kinetin on yield and composition of basic oils of O. basilicum. They showed that the GA leads to the decrease in essential oil yield while kinetin and IAA increased the yield. This change is accompanied by a decrease in levels of the main compound (methyl chavicol) for all treatments (from 75.16% in the control to 74.1%, 73.2% and 70.7% in kinetin, IAA and GA respectively). The same observation was made by Fraternale et al. (2003) who found that spraying plants Thymus mastichina by cytokinin caused an increase in the concentration of essential oils.
### TABLE 6. Effect of BA, IAA and GA3 spraying on total phenols, total flavones, total chlorophyll and L- ascorbic acid of sweet basil plants of the second cut in 2018 and 2019 seasons.

| Treatments  | Total phenols (mg/g d.w.) | Total flavones (mg/g. d.w.) | Total chlorophyll (mg/100g f.w.) | L- ascorbic acid (mg/100g f.w.) |
|-------------|---------------------------|----------------------------|----------------------------------|---------------------------------|
| **2018 Season**                       |                           |                             |                                  |                                 |
| Control     | 16.82 e                   | 10.75 d                     | 7.09 cd                          | 18.54 d                         |
| BA 5 ppm    | 21.48 cd                  | 12.36 c                     | 7.88 bc                          | 20.17 cd                        |
| BA 10 ppm   | 25.11 ab                  | 13.86 b                     | 8.25 b                           | 23.08 bc                        |
| IAA 50 ppm  | 25.94 ab                  | 14.71 b                     | 9.21 a                           | 25.20 ab                        |
| IAA 100 ppm | 27.62 a                   | 15.98 a                     | 9.77 a                           | 27.15 a                         |
| GA3 100 ppm | 19.57 d                   | 11.51 cd                    | 7.13 cd                          | 21.94 c                         |
| GA3 200 ppm | 23.75 bc                  | 14.93 ab                    | 6.25 d                           | 24.84 ab                        |
| **2019 Season**                       |                           |                             |                                  |                                 |
| Control     | 18.12 e                   | 11.23 c                     | 6.87 d                           | 16.29 d                         |
| BA 5 ppm    | 20.06 de                  | 12.21 c                     | 7.69 cd                          | 17.84 cd                        |
| BA 10 ppm   | 24.77 ab                  | 14.42 ab                    | 8.45 bc                          | 21.15 b                         |
| IAA 50 ppm  | 24.51 ab                  | 14.06 ab                    | 9.58 a                           | 24.58 a                         |
| IAA 100 ppm | 26.75 ab                  | 15.02 a                     | 10.13 a                          | 25.27 a                         |
| GA3 100 ppm | 21.95 cd                  | 12.15 c                     | 7.54 cd                          | 20.36 bc                        |
| GA3 200 ppm | 23.35 bc                  | 13.54 b                     | 7.11 d                           | 22.47 ab                        |

Values followed by the same letter (s) are not significantly different at 5% level.

### TABLE 7. Effect of BA, IAA and GA3 spraying on volatile oil percentage and volatile oil content of sweet basil plant in 2018 and 2019 seasons.

| Treatments  | Volatile oil (%) | Mean | Volatile oil content (ml/plant) | Mean |
|-------------|------------------|------|-------------------------------|------|
|              | 1st cut          | 2nd cut |                                |      |
| **2018 Season** |                   |       |                                |      |
| Control     | 0.11 g           | 0.13 g | **0.12 E**                     | 0.21 g | 0.22 g | **0.22 E** |
| BA 5 ppm    | 0.19 f           | 0.22 f | **0.21 D**                     | 0.30 fg | 0.35 ef | **0.33 D** |
| BA 10 ppm   | 0.24 ef          | 0.27 de | **0.23 CD**                    | 0.38 def | 0.43 de | **0.41 C** |
| IAA 50 ppm  | 0.34 bc          | 0.38 ab | **0.36 A**                     | 0.54 bc | 0.62 ab | **0.58 AB** |
| IAA 100 ppm | 0.37 ab          | 0.42 a | **0.38 A**                     | 0.58 ab | 0.66 a  | **0.62 A** |
| GA3 100 ppm | 0.24 ef          | 0.28 de | **0.26 BC**                    | 0.37 ef | 0.44 de | **0.41 C** |
| GA3 200 ppm | 0.30 cd          | 0.35 bc | **0.33 AB**                    | 0.48 cd | 0.55 bc | **0.52 B** |
| Mean        | **0.25 B**       | **0.29 A** | **0.26 E**                     | 0.41 B   | **0.47 A** |

**Mean** values followed by the same letter (s) are not significantly different at 5% level.

**Values followed by the same letter (s) are not significantly different at 5% level.**
### TABLE 8. Effect of BA, IAA and GA3 spraying on volatile oil fractions of sweet basil plant in the second cut in 2019 season.

| No. | Component name          | Area % | RI  | Control | BA 5 ppm | BA 10 ppm | IAA 50 ppm | IAA 100 ppm | GA3 100 ppm | GA3 200 ppm |
|-----|-------------------------|--------|-----|---------|----------|-----------|-------------|-------------|-------------|-------------|
| 1.  | á-pinene                | 936    | 1.04| 1.02    | 0.90     | 1.13      | 1.21        | 1.08        | 1.03        |
| 2.  | Sabinene                | 975    | 0.43| 0.50    | 0.60     | 0.68      | 0.70        | 0.62        | 0.66        |
| 3.  | β-pinene                | 977    | 1.33| 1.42    | 1.50     | 1.28      | 1.43        | 1.30        | 1.28        |
| 4.  | Myrcene                 | 995    | 0.90| 0.88    | 1.00     | 1.07      | 1.13        | 1.03        | 1.12        |
| 5.  | 1,8-cineole             | 1037   | 5.88| 5.86    | 5.83     | 5.00      | 5.33        | 5.90        | 4.93        |
| 6.  | Linalool                | 1110   | 48.80| 48.20  | 49.20    | 49.20     | 50.90       | 49.60       | 50.70       |
| 7.  | Camphor                 | 1145   | 0.88| 0.91    | 0.88     | 1.12      | 1.20        | 1.14        | 1.28        |
| 8.  | á-terpineol             | 1170   | 0.42| 0.43    | 0.52     | 0.46      | 0.48        | 0.47        | 0.42        |
| 9.  | á-terpineol             | 1192   | 1.80| 1.82    | 1.80     | 1.68      | 1.73        | 1.73        | 1.80        |
| 10. | Bornyl acetate          | 1288   | 0.74| 0.73    | 0.72     | 0.82      | 0.88        | 0.84        | 0.83        |
| 11. | Eugenol                 | 1348   | 1.12| 1.10    | 1.73     | 1.86      | 1.88        | 1.63        | 0.60        |
| 12. | á-clemene               | 1389   | 1.92| 1.90    | 1.83     | 1.88      | 1.98        | 1.68        | 1.73        |
| 13. | á-trans-bergamotene     | 1434   | 7.40| 7.36    | 7.20     | 6.60      | 6.80        | 7.80        | 8.20        |
| 14. | á-guaiene               | 1439   | 0.72| 0.70    | 0.63     | 0.81      | 0.88        | 0.72        | 0.76        |
| 15. | á-humulene              | 1455   | 0.72| 0.63    | 0.72     | 0.71      | 0.80        | 0.62        | 0.63        |
| 16. | á-farnesene             | 1462   | 1.30| 1.40    | 1.30     | 1.21      | 1.33        | 1.42        | 1.43        |
| 17. | Muurola-4(14),5-diene   | 1465   | 0.83| 0.82    | 0.80     | 0.92      | 0.93        | 0.88        | 0.89        |
| 18. | Germacrone D            | 1488   | 3.32| 3.43    | 3.20     | 3.50      | 3.30        | 3.33        | 3.40        |
| 19. | Bicyclogermacrene       | 1502   | 0.77| 0.70    | 0.82     | 0.90      | 0.93        | 0.82        | 0.81        |
| 20. | á-bulnesene             | 1510   | 1.30| 1.40    | 1.20     | 1.32      | 1.23        | 1.41        | 1.42        |
| 21. | Y-cadinene              | 1512   | 1.62| 2.41    | 2.10     | 2.33      | 2.50        | 2.20        | 1.80        |
| 22. | á-sesquiphellandrene    | 1520   | 0.43| 0.40    | 0.42     | 0.49      | 0.48        | 0.38        | 0.36        |
| 23. | Spathulenol             | 1579   | 0.76| 0.70    | 0.80     | 0.83      | 0.83        | 0.81        | 0.84        |
| 24. | 1,10-di-epi-cubenol     | 1618   | 1.12| 1.10    | 1.00     | 1.12      | 1.10        | 1.00        | 1.12        |
| 25. | á-muurolol              | 1644   | 3.40| 3.80    | 4.30     | 4.60      | 4.60        | 4.30        | 4.20        |
| 26. | á-cudesmol              | 1650   | 0.68| 0.63    | 0.80     | 0.88      | 0.73        | 0.82        | 0.84        |
|     | Total                   | 89.63  | 90.25| 91.80  | 92.40    | 91.80     | 95.29       | 93.53       | 93.00       |
|     | Unknown                 | 10.37  | 9.75 | 8.20   | 7.60     | 8.20      | 4.71        | 6.47        | 7.00        |

**Conclusion**

It could be concluded that spraying of growth regulators like BA, IAA, and GA, were effective in improving growth, active substances and volatile oil productivity of sweet basil plants. However, BA at 5 or 10 ppm were effective in improving vegetative growth parameters and leaf mineral contents, whereas IAA at 50 or 100 ppm were superior in increasing active substances, volatile oil percentage and volatile oil content/plant. Additionally, IAA at 100 ppm exhibited higher values of volatile oil fractions especially linalool.
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Tam رش بنيزل أدينين وأندول حمض الخليك وحمض الجبريلك بتركيزات مختلفة لدراسة تأثير على النمو وخصائص الزيت الطيار لنباتات الريحان

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