

# Responses of different quality parameters of Chia to arbuscular mycorrhiza and plant growth regulator

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All relevant data are within the paper and its  
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**Abstract:** Field experiment was conducted to evaluate the influence of arbuscular mycorrhiza fungi (AMF) and foliar spray of plant growth regulators (PGRs) and their interaction on vegetative growth, seed yield and yield attributes and some biochemical criteria of chia (*Salvia hispanica* L.), in a split plot design with three replications. Plants grown in absence or presence of AMF were sprayed every 2 weeks with benzyl adenine (BA), CPPU [N-(2-chloro-4-pyridinyl)-N'-phenylurea], common name forchlorfenuron, and Naphthalene acetic acid (NAA) at 50, 20 and 50 ppm respectively, while control plants were sprayed with tap water. The results revealed that, inoculation with AMF generally caused significant augmentation in all studied growth, yield and yield attributes, total chlorophylls and carbohydrates content in leaves, augmentation in nutritional values of seeds like carbohydrates %, macronutrient, micronutrients, proteins %, total flavonoids, oil % compared to non-inoculated plants. In absence or presence of AMF, application of PGRs generally caused significant increases in the studied parameters compared to control. The interaction between NAA and AMF was more effective since gave higher increases in the studied parameters. It can be concluded that, cultivation of chia plant in presence of mycorrhiza with foliar application of NAA at 50 ppm is recommended for enhancing growth, and nutritional values of seed yield.

## 1. Introduction

*Salvia hispanica*, ordinarily known as chia, is an annual herbaceous plant which belongs to Lamiaceae family. It is native to southern Mexico and Northern Guatemala; the word of Chia comes from the Nahuatl word "chian" with means oily. The name *Salvia hispanica* was specified by the Swedish botanist Carl Linnaeus, who discomfited the wild-growing plant coming from the new world with a regional plant from Spain. It grows up to 1-m tall with leaves of about 4-8 cm long and 3-6 cm wide. Chia flowers are white or purple containing oval seed mottle-colored with brown, gray, black, and white with size ranging from 1 to 2 mm. it grows naturally in tropical and subtropical environments; it is optimally established from 400 to 2500 m and considered to be a short-day plant with a threshold of

12-14 h. Chia acquired acceptance owing to the high nutritional value of its seeds. The main components of seeds are polyunsaturated fatty acids Omega-3 (PUFA  $\omega$ 3) (58-64% of total lipids) and Omega-6 ( $\omega$ 6), protein with a ratio (16-24%), carbohydrates (26-41%) lipids (31-35 %), and fiber (34-56 %). in addition to some minerals, vitamins and high level of antioxidants (Baginsky *et al.*, 2016; Sosa *et al.*, 2016; Marcinek and Krejpcio, 2017).

Arbuscular mycorrhiza fungi (AMF) are a category of soil microorganisms which form a symbiotic association with plants. Not only it could enhance uptake of mineral elements and water by plants that promote plant growth, but also increase crop yield, quality properties and active ingredients (Dupre *et al.*, 2008; Singh *et al.*, 2010). The positive effect of inoculation with AMF on physiological and biochemical changes in different medicinal and aromatic plants has been reported in a number of species such as enhancing growth performance and seed yield (Gashgaril *et al.*, 2020; Bilalis *et al.*, 2020), increase photosynthetic pigments and carbohydrates content (Amiri *et al.*, 2017), increased nutrient status in plant oranges (Chaudhary *et al.*, 2008), protein content (Ouzounidou *et al.*, 2015), antioxidants activity (Golubkina *et al.*, 2020), fixed oil (Moghith, 2019), promoted concentration of essential oil (Chaudhary *et al.*, 2008; Al-Amri *et al.*, 2016), enhanced primary and secondary metabolism and bioactive compounds (Gashgaril *et al.*, 2020).

Plant growth regulators (PGRs) have been defined as one of the major factors affecting plants growth and their primary and secondary metabolites, NAA is an organic compound that is synthetic plant hormone in the auxin family. it is known to enhance cell elongation, cell division, elongation of shoot, vascular tissue, photosynthesis, RNA synthesis, membrane permeability and water uptake is also involved in many physiological processes such as fruit set, delayed senescence, leaf chlorophyll content, stimulates flowering and increases yield (Davies, 1987). Foliar application of NAA on different medicinal and aromatic plants have been reported to improve growth and yield attributes, photosynthetic pigments, total carbohydrate and oil yield (Rohamare *et al.*, 2013), enhance nutrient status in plant oranges, essential oil %, polyphenols and flavonoids content and antioxidant activity (Atteya and El Gendy, 2018). Moreover, it has been reported for ameliorating the harmful effects of salinity (Abou El-ghit, 2015). Cytokinins include benzyl adenine (BA) that promotes

cellular elongation and division (Krug *et al.*, 2006). Foliar spray of BA has been reported to increase the growth and development of medicinal and aromatic plants (Matter, 2016; Moussa, 2019). It was reported to improve photosynthetic pigments content, total carbohydrate oil percentage and oil yield (Abdel-Rahman and Abdel-Kader, 2020), contents of macronutrient and micronutrient, total phenols and total flavones (Abdel-Hamid, 2020), enhance protein concentration (Prins *et al.*, 2013) and antioxidant activity (Sidkey, 2020).

Likewise, CPPU is one of PGRs. It is a cytokinin like substance that has strong cytokinin activity by stimulating fruit set and fruit quality, it plays a role in cell division and cell wall elongation (Nickell, 1985; Zhang and Whiting, 2011). It has been reported to improve growth and yield attributes or protein percentage of medicinal plants (Abbas and Zahwan, 2016).

Although the valuable roles of AMF and PGRs on medicinal and aromatic plants and their useful influence on enhancing growth and production has been formerly evaluated. However, there are not enough available data about their activity on the growth and production of Chia plants. Therefore, the objective of this study was to evaluate the impact of AMF and foliar spray of PGRs (BA, CPPU and NAA) and their interactions on vegetative growth, seed yield and yield attributes, and some chemical compositions of *Salvia hispanica* plants.

## 2. Materials and Methods

Open field experiment was carried out during the two successive seasons of 2018 and 2019 at Experimental area of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza Governorate (latitude 30°01'13.44"N, longitude 31°12'30.24"E, altitude 22 m a.s.l.). The aim of this work was to evaluate the effects of foliar application of different plant growth regulators (PGRs) such as 6-BA (6-Benzylaminopurine) benzyl adenine, CPPU [N-(2-chloro-4-pyridyl)] and NAA (Naphthaleneacetic Acid) in absence or presence of arbuscular mycorrhizal fungi (AMF) on vegetative growth, seed yield and its attributes and some biochemical parameters of Chia (*Salvia hispanica* L.) plant.

### Experimental procedure

Seeds of *Salvia hispanica* plants were obtained from experimental farm of Faculty of Pharmacy,

Cairo University. On 1<sup>st</sup> October and 15<sup>th</sup> October (in the two seasons, respectively), seeds were sown in the nursery at the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza. After 45 days old from seeds sowing (on the 15<sup>th</sup> of November, and 1<sup>st</sup> December, in both seasons, respectively), uniform seedlings, with an average plant height of 15-18 cm, were transplanted in the experimental open field with a distance of 70 cm among rows, 50 cm spacing between plants in plots (3.5×3.5 m).

The physical and chemical properties of the experimental soil were determined according to Jackson (1973), and the data are recorded in Table 1.

AMF inoculum contained roots, hyphae, spores and growth media from a pot culture of onion plants colonization with *Glomus mosseae* NRC31 and *Glomus fasciculatum* NRC15, and were obtained from Agricultural Microbiology Department, National Research Center. Inoculum material consisted of 275 spores g<sup>-1</sup> oven dry bases in addition to the colonization roots pieces (the infectivity 10<sup>4</sup> propagola). AMF inoculation treatments was achieved by mixing 5 g of it with 10 g of chia seed before sowing and repeated monthly after transplanting by injection inoculum material into the soil in roots area from five sides at 5 g/seedling.

Table 1 - Some physical and chemical characteristics of experimental soil during the two seasons

Parameter	2018	2019
Soil texture	Clay	Clay
Clay[%]	40.50	41.60
Silt [%]	35.10	34.00
Fine sand[%]	21.00	20.00
Coarse sand [%]	3.40	4.40
Field capacity [% V]	67.30	69.37
pH	7.12	7.19
EC [dS/m]	1.67	1.55
CEC [meq/100g]	39.40	35.72
Organic matter [%]	1.60	1.75
CaCO <sub>3</sub> [%]	1.65	1.75
K <sup>+</sup> [ppm]	65.85	67.95
Mg <sup>++</sup> [ppm]	39.83	40.94
Available N [ppm]	93.35	98.75
Available p [ppm]	20.25	22.35
Available Fe [ppm]	2.11	2.19
Available Mn [ppm]	3.12	3.99
Available Zn [ppm]	1.59	1.53

pH= soil acidity, EC= Electrical conductivity, CEC= cation exchange capacity, CaCO<sub>3</sub>= calcium carbonate.

PGRs treatments were initiated after 15 days from the transplanting, by foliar spraying the plants every 2 weeks with Sytonine 4%® [commercial name, consists of 6- BA (6-Benzylaminopurine) benzyl adenine 4 %], Cytovac® (CPPU) and Fast tonic® [commercial name, consists of Naphthaleneacetic Acid 25% + Sodium nitrophenolate 0.6%]. The three commercial products were obtained from Bio Green Company, the regional representative of the Jordanian company, Elite Company for the manufacture of agricultural fertilizers development, Egypt. The concentrations of BA and NAA were 50 ppm for each one, while CPPU was applied at 20 ppm and the untreated control plants were sprayed with tap water. Freshly prepared solutions of PGRs (50 mL containing Tween 20 at 1 mL/L (0.1%) as surfactant agent) were sprayed using plastic automizer until run off point. The common horticulture practices (such as irrigation, manual weeds control, fertilization) were carried out when needed.

The experimental design was split plot design with eight treatments [2 AMF (absence or presence) x 4 PGR<sub>s</sub> concentrations (including the control)] with 3 replicates, each consisting of 16 plants (2 plants from each treatment). AMF assigned to the main plots in a randomized complete blocks design and PGRs concentrations were allocated at random in sub-plots.

#### Measurement of vegetative growth and yield parameters

Vegetative growth parameters were recorded after 90 days from transplanting (On 15<sup>th</sup> of February to 1<sup>st</sup> March). In both seasons plant samples were taken for measurements growth characters in terms of plant height (cm), number of leaves/plant, stem diameter (cm, at 5 cm above the soil surface), number of branches/plant, fresh and dry weights of the herb (g/plant), root length (cm), number of root/plant and fresh and dry weights of the roots (g/plant). Whereas, at harvesting stage yield and yield attributes including number of inflorescence/plant, number of seeds/plant, weight of seeds (g /plant), weight of 1000 seeds (g), and seeds yield (Kg/Fed.) were recorded.

Seeds yield per feddan was obtained according to the equation:

$$\text{Seeds yield per feddan} = \frac{(\text{Weight of seeds (g/plant)} \times \text{number of plants/fed}) / 1000}{\text{Number of plants/fed} = (100 \times 100 \times 4200) / (50 \times 70) = 12000 \text{ plant/fed.}}$$

Some chemical parameters were determined in the seeds as carbohydrates %, some macro & micro elements, proteins %, phenols, flavonoids content

and antioxidant activity.

#### Chemical analysis

Total chlorophylls in fresh leaf samples were determined by using chlorophyll meter (Model SPAD 502 Minolta Co. Japan) as described by Netto *et al.* (2005). The total carbohydrates content in leaves and seeds (% of dry matter) was determined in dried samples according to Dubois *et al.*, (1956). A known weight (0.1 g) of the dried samples was completely hydrolyzed with 10 ml sulphuric acid (67%) in a test tube on a boiling water bath for one hour. The solution was decolorized and the filtrate was completed to 100 ml with distilled water. A known volume (1 ml) of the extract was taken in a test tube, to which 1 ml phenol solution (5%) was added, followed by 5 ml of concentrated sulphuric acid. The optical density of the resulting color was measured at 490  $\mu\text{m}$ , using a spectrophotometer, against a blank reagent. The standard curve of glucose was used to calculate the total carbohydrates concentration in the extract. Dried seeds samples were digested to extract nutrients and the extract was analyzed to determine concentrations of N, P, K, Ca, Mg (% of dry seeds), Fe and Zn (ppm) which were determined according to Estefan *et al.* (2013). Nitrogen concentration was determined by using the micro-Kjeldahl method. Phosphorus was determined calorimetrically by using the chlorostannous molybdophosphoric blue colour method in sulphuric acid. Potassium was determined by using the flame photometer apparatus (CORNING M 410, Germany). The concentrations of Ca, Mg, Fe and Zn were determined using atomic absorption spectrophotometer with air-acetylene and fuel (Pye Unicam, model SP-1900, US). Protein content in seeds was estimated by multiplying N values by 5.71 (conversion factors). Total phenolics content was determined by using the Folin Ciocalteu's reagent colorimetric method while total flavonoids content was estimated by the aluminum chloride colorimetric method and results are expressed as milligram Catechin equivalent per gram of seeds dry weight extract (mg CE/g DW) (John *et al.*, 2014). The antioxidant activity of seeds extract and standard antioxidant was assessed on the basis of the radical scavenging effect on DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals (Brand-Williams *et al.*, 1995).

Fixed oil %: the clean air dried seeds of chia were separately crushed in a Willey mill, then extracted in Soxhlet apparatus, samples of 10 g of seeds were moved into Soxhlet apparatus in 100 ml of N-hexane and the extraction period extended to 6 hours (30-36

syphon cycle approx.). The N-hexane extract was dried over anhydrous sodium sulfate, then filtered and the oil was obtained by distillation under vacuum. Fixed oil % was calculated according to the equation:

$$\text{Fixed oil \%} = \frac{\text{Extracted fixed oil weight (g)}}{\text{seeds sample weight (g)}} \times 100.$$

#### Statistical analysis

The means of all obtained data were subjected to statistical analysis of variance (ANOVA) in split plot design. Combined analysis of the two growing seasons was carried out. Means of data were compared by using Duncan's multiple range tests at 5% level Snedecor and Cochran (1989).

### 3. Results and Discussion

#### Vegetative growth parameters

It is evident from data in Table 2 and 3 that under the same treatments of PGRs, chia plants grown in presence of AMF had significant increase in studied vegetative growth parameters (*viz.*, plant height, number of leaves, stem diameter, number of branches/plant, fresh and dry weights of herb, root length, number of roots, root fresh and dry weights) compared to those grown in absence of AMF. The obtained increases in vegetative growth attributes due to AMF inoculation are in agreement with reports of several researches on medicinal and aromatic plants including *Artemisia annua* (Chaudhary *et al.*, 2008), *Salvia officinalis* (Geneva *et al.*, 2010; AbdelKader *et al.*, 2014), *Corianderum sativum* (Al-Amri *et al.*, 2016; Oliveira *et al.*, 2016), *Origanum majorana* (Engel *et al.*, 2016), *Pelargonium graveolens* (Amiri *et al.*, 2017), *Salvia miltiorrhiza* (Yang *et al.*, 2017), *Salvia hispanica* (Moghith, 2019), *Artemisia dracuncululus* and *Hyssopus officinalis* (Golubkina *et al.*, 2020) and *Foeniculum vulgare* (Mohamed, 2020).

The stimulatory influence of AMF on vegetative growth traits could be explained by AMF form symbiotic relationship with the host increase of root surface, which led to promote root uptake of nutrients. Thus, it could significantly augment growth parameters of tested plant (Jian-heng *et al.*, 2016). Furthermore it was indicated that the ability of AMF to enhance the availability of essential elements macro- and micronutrients in the rhizospheric soil that induce its uptake and the accumulation in plant (Gashgaril *et al.*, 2020).



Results in Table 2 and 3 also indicate that, in absence or presence of AMF, treating plants with different concentrations of PGRs resulted in significant increase in tested vegetative growth parameters compared to control. Among PGRs, application of NAA (at 50 ppm) appeared to be the most effective one particularly in presence of AMF since recorded the highest values in most cases. The results of the pronounced increase in growth parameters due to application of NAA are in accordance with findings of

previous studies on medicinal plants (Alam et al., 2012; Rohamare et al., 2013; Danesh-Talab et al., 2014; Abou El-ghit, 2015; Rostami and Movahedi, 2016; Dheeraj and Saravanan, 2018). Moreover, numerous studies reported increase in growth attributes of medicinal plants owing to either BA (Matter, 2016; Moussa, 2019; Abdel-Rahman and Abdel-Kader, 2020 and Abdel-Hamid, 2020) or CPPU treatments (Abbas and Zahwan, 2016).

The stimulation effect of NAA on morphological

Table 2 - Mean square for the effect of Arbuscular mycorrhiza fungi (AMF), plant growth regulators (PGRs) and their interaction on vegetative growth, yield and yield parameters of *Salvia hispanica*

Traits	Source of variation						
	Treatment			Error		Cv	
	AMF (A)	PGRs (B)	A × B	A	B	A	B
Plant height (cm)	233.750 **	514.534 ***	24.278 ***	1.182	1.037	1.322	1.239
No. of leaves	213.010 **	194.372 ***	5.844 *	2.042	2.222	4.527	4.723
Stem diameter (cm)	0.375 **	0.124 ***	0.011 *	0.004	0.005	7.036	8.386
No. of branches/plant	37.500 ***	36.301 ***	1.701 **	0.035	0.189	1.685	3.921
Herb fresh weight (g)	27.307 ***	18.106 ***	2.681 ***	0.003	0.090	0.433	2.412
Herb dry weight (g)	25.627 ***	21.694 ***	1.288 ***	0.002	0.072	0.609	3.991
Root length (cm)	145.042 ***	96.486 ***	12.042 ***	0.087	0.746	1.979	5.808
No. of roots/plant	32.667 ***	34.375 ***	1.167 *	0.135	0.448	4.576	8.322
Root fresh weight (g)	38.760 ***	20.386 ***	1.412 ***	0.020	0.030	2.531	3.068
Root dry weight (g)	4.770 **	5.794 ***	0.228 **	0.050	0.035	7.233	6.039
No. of inflorescence /plant	145.042 *	70.972 ***	7.903 *	0.510	1.313	4.844	7.767
No. of seeds / plant	18897.29 ***	57551.63 ***	283.104 ***	1.439	5.467	0.219	0.426
Weight of 1000 seeds (g)	1.321 ***	0.371 **	0.001 *	0.145	0.061	20.73	13.45
Weight of seeds (g/plant)	13.024 **	40.227 ***	0.556 *	0.290	0.367	7.02	7.89
Seeds yield (Kg/Fed.)	1875.494 **	5792.741 ***	80.027 *	5.041	21.110	2.44	4.99

\*, \*\*, \*\*\* significant at P≤0.05, P≤0.01, P≤0.001, respectively.

Table 3 - Vegetative growth parameters of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two seasons)

Treatment		Plant height (cm)	No. of leaves	Stem diameter (cm)	No. of branches/plant	Herb fresh weight (g)	Herb dry weight (g)	Root length (cm)	No. of roots/plant	Root fresh weight (g)	Root dry weight (g)
AMF	PGRs										
-	0	67.53±0.44 h	23.67±0.60 d	0.57±0.07 d	7.00±0.29 f	9.77±0.03 f	3.53±0.15 g	8.50±0.060 f	4.00±0.58 e	2.67±0.07 g	1.57±0.09 f
	BA at 50 ppm	78.50±0.50 f	27.17±0.33 c	0.77±0.03 c	9.93±0.35 d	11.83±0.07 cd	5.87±0.12 e	12.33±0.67 d	7.50±0.29 c	4.07±0.03 f	2.30±0.06 e
	CPPU at 20 ppm	81.83±0.67 e	28.00±0.58 c	0.87±0.03 c	11.10±0.06 c	11.67±0.23 d	6.63±0.28 d	14.00±0.50 c	7.50±0.29 c	4.83±0.15 e	3.27±0.09 d
	NAA at 50 ppm	88.50±1.15 c	35.50±0.29 b	0.83±0.03 c	11.37±0.105 c	12.33±0.19 c	6.67±0.13 d	14.83±0.73 c	8.50±0.50 c	5.93±0.03 d	3.50±0.06 cd
+	0	70.83±0.68 g	26.67±0.60 c	0.80±0.02 c	8.00±0.29 e	10.43±0.09 e	4.37±0.07 f	10.67±0.44 e	5.17±0.17 d	3.87±0.15 f	2.03±0.2 e
	BA at 50 ppm	90.67±0.60 b	34.00±0.58 b	1.00±0.06 b	13.30±0.15 b	13.67±0.12 b	8.17±0.15 c	17.17±0.17 b	9.67±0.44 b	6.53±0.13 c	3.60±0.1 c
	CPPU at 20 ppm	86.17±0.33 d	35.00±1.00 b	1.03±0.03 b	13.60±0.20 b	13.80±0.10 b	8.70±0.10 b	17.83±0.17 b	10.33±0.73 b	8.00±0.06 b	3.93±0.12 b
	NAA at 50 ppm	93.67±0.73 a	42.50±0.29 a	1.20±0.06 a	14.50±0.15 a	16.23±0.32 a	9.73±0.09 a	23.67±0.33 a	11.67±0.44 a	9.27±0.12 a	4.63±0.03 a

Each value represents the mean ± standard error of three replicates.

Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

attributes may be due its ability to increase membrane permeability and water uptake which accompanied by elements absorption, synthesis of chlorophyll and carbohydrates contents that achieving the highest dry weight of plants (Atteya and El Gendy, 2018).

*Yield and yield attributes*

Data in Table 4 revealed that under the same treatments of PGRs, the values of yield and yield attributes (*viz.*, number of inflorescence /plant, number of seeds/plant, weight of seeds, weight of 1000 seeds, and seeds yield) were significantly higher in plants grown in the presence of AMF than corresponding values in the absence of AMF. The present increase in number of inflorescence/plant due to AMF inoculation is supported by the results of previous report (Engel *et al.*, 2016). Moreover, recent study (Mohamed, 2020) on *Foeniculum vulgare* revealed that AMF inoculation significantly increased seeds yield parameters.

Exogenous application of PGRs in absence or presence of AMF resulted in significant increase in yield and yield parameters compared to control. Among PGRs, NAA was the most effective one particularly when interacted with presence of AMF. In this concern, application of NAA at 50 ppm has been reported to increase yield attributes (Rohamare *et al.*, 2013; Danesh-Talab *et al.*, 2014; Khodus *et al.*, 2017; Venkatesan and Shakila, 2017). Other study (Kassem *et al.*, 2011) found that, foliar spray of NAA at 75 mg/l or CPPU at 10 mg/l caused significant increase in yield and yield attributes of *Ziziphus jujuba* compared with the control. Moreover, increases in num-

ber of inflorescence /plant owing to application of NAA are in accordance with findings of earlier studies (Atteya and El Gendy, 2018; Dheeraj and Saravanan, 2018). While increasing yield and its attributes due to either BA application are in harmony with the findings of various studies (Mousa *et al.*, 2001; Matter, 2016; Abdel-Rahman and Abdel-Kader, 2020) or CPPU (Abbas and Zahwan, 2016).

The augmentation in yield and its attributes due to PGRs treatments may be due to its role in enhanced absorption of nutrients which promoted photosynthesis rate and translocation of photosynthates and other metabolites to the sinks that leading to increase yield and its attributes in present study (Alam *et al.*, 2012).

*Total chlorophylls and total carbohydrates*

Data in figure 1 indicate that under the same treatments of PGRs, plants grown in the presence of AMF had significantly higher values of total chlorophylls in leaves and total carbohydrates in leaves and seeds compared to corresponding values in the absence of AMF in most cases. These results run parallel with those obtained by earlier reports on medicinal plants that reported increase in chlorophyll and carbohydrates content in plants inoculated with AMF compared to controls (Amiri *et al.*, 2017; Gashgaril *et al.*, 2020; Moghith, 2019; Mohamed, 2020).

Results also show that, the recorded values were significantly increased in plants grown in absence or presence of AMF as a result of spraying tested PGRs compared to control. In most cases, plants sprayed with NAA in the presence of AMF had the highest values of the tested components. The results of increase

Table 4 - Yield and yield attributes of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two seasons)

Treatments		No. of inflorescence/ plant	No. of seeds/ plant	Weight of seeds (g/plant)	Weight of 1000 seeds (g)	Seeds yield (Kg/Fed.)
AMF	PGRs					
-	0	9.17±0.44 f	409.17±0.58 h	3.43±0.20 f	1.23±0.03 e	41.20±2.45 f
	BA at 50 ppm	12.67±0.88 e	477.17±1.11 f	7.47±0.01 d	1.69±0.09 d	89.64±0.14 d
	CPPU at 20 ppm	12.33±0.33 e	573.67±1.48 d	7.32±0.38 d	1.73±0.15 cd	87.88±4.52 d
	NAA at 50 ppm	15.00±0.58 d	622.17±1.21 c	9.53±0.15 bc	1.76±0.18 bcd	114.40±1.79 b
+	0	11.00±0.58 e	448.50±0.76 g	4.64±0.03 e	1.70±0.21 d	55.68±0.36 e
	BA at 50 ppm	17.17±0.6 c	546.83±1.72 e	8.57±0.13 c	2.16±0.17 abc	102.84±1.54 c
	CPPU at 20 ppm	19.00±0.58 b	638.33±1.15 b	9.71±0.26 b	2.19±0.11 ab	116.48±3.17 b
	NAA at 50 ppm	21.67±0.67 a	673.00±1.15 a	10.74±0.18 a	2.23±0.192 a	128.84±2.22 a

Each value represents the mean ± standard error of three replicates.

Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

of total chlorophylls or total carbohydrates due to application of NAA are similar to those obtained by previous studies (Alam et al., 2012; Rohamare et al., 2013; Rostami and Movahedi, 2016; Venkatesan and Shakila, 2017; Atteya and El Gendy, 2018), whereas the obtained increase due to either BA treatments are in close conformity with the findings of previous reports (Matter, 2016; Moussa, 2019; Abdel-Hamid, 2020; Abdel-Rahman and Abdel-Kader, 2020) or CPPU treatments (Kassem et al., 2011; Abbas and Zahwan, 2016).

### Content of macronutrients in seeds

Results of chemical analysis of dried seeds of

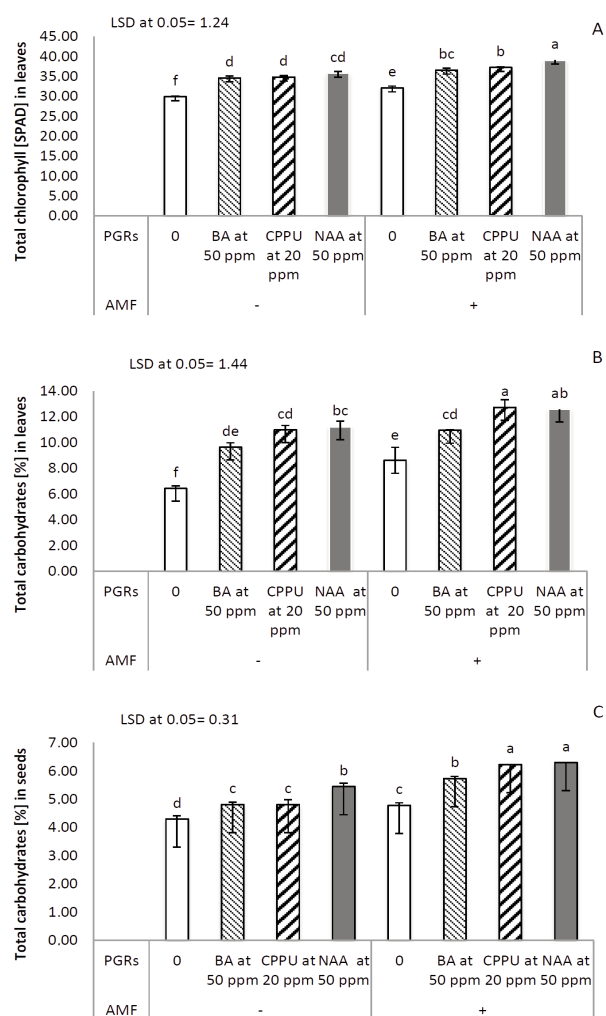


Fig. 1 - Total chlorophyll in leaves (A), total carbohydrates in leaves (B), and total carbohydrates in seeds (C) of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two season). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

*Salvia hispanica* plants (Table 5 and 6) disclosed that, under the same treatments of PGRs the uptake and accumulation of macronutrients in seeds of plants inoculated with of AMF were significantly higher in most cases compared to non-inoculated plants. The only exception to this general trend was observed in the case of P and K% as in the presence of AMF with control treatments they recorded insignificantly higher values compared to corresponding values in the absence of AFM. The obtained results are in agreement with those obtained by various researchers that reported the potential effects of AMF on the accumulation of macronutrients in medicinal plant oranges (Chen and Zhao, 2009; Karagiannidis et al., 2011; AbdelKader et al., 2014; Vafadar et al., 2014; Oliveira et al., 2016; Yang et al., 2017; Moghith, 2019; Mohamed, 2020).

The enhanced mineral absorption by AMF inoculated plants could be elucidated by the efficiency of AMF to boost mineral affinities, reduce the critical concentration of elements absorption, augment the area of uptake and decrease the area of diffusion (Gashgaril et al., 2020).

Data in Table 6 also indicate that, in absence or presence of AMF foliar spraying with any concentrations of PGRs resulted in significant increase in macronutrient in seeds in most cases, compared to control. With some exceptions recorded in absence of AMF as foliar application of CPPU resulted in insignificantly higher values of P% than the control, also in absence of AMF, foliar application of three tested PGRs resulted in insignificantly higher values of Ca % compared to control. Among PGRs, application of NAA was the most effective treatment especially in presence of AMF. The results of increasing macronutrients accumulation due to NAA treatments are accordance with findings of previous studies (Alam et al., 2012; Atteya and El Gendy, 2018), while the obtained increase in macronutrients due to BA is similar to those described in numerous reports (Matter, 2016; Moussa, 2019; Abdel-Hamid, 2020; Abdel-Rahman and Abdel-Kader, 2020).

### Content of micronutrients in seeds

As shown in figure 2 that under the same treatments of PGRs plants inoculated with AMF had significantly higher values of Fe and Zn in their seeds than those grown in the absence of AMF. These results are in the same line of the findings of earlier authors (Chaudhary et al., 2008; Golubkina et al, 2020).

Data in figure 2 also showed that, in the absence or presence of AMF foliar application of any concen-

trations of PGRs resulted in significant increase in tested micronutrients (Fe and Zn) in seeds compared to control. The increases in the recorded values were more evident in the presence of AMF mostly with application of NAA. Such increase in Fe and Zn content due to BA treatments is in good agreement with those elicited by prior works (Baydar and Erdal, 2004; Abdel-Hamid, 2020).

**Total protein**

Data in Table 7 displayed that under the same PGRs treatments total protein percentage was significantly higher in plants grown in the presence of AMF than those grown in the absence of AMF. These results are in conformity with that recorded by previous studies (Ouzounidou *et al.*, 2015; Al-Amri *et al.*, 2016). Increasing protein due to AMF may be due to

Table 5 - Mean square for the effect of Arbuscular mycorrhiza fungi (AMF), plant growth regulators (PGRs) and their interaction on some chemical constituents of *Salvia hispanica*

Traits	Source of variation						
	Treatment			Error		Cv	
	AMF (A)	PGRs (B)	A × B	A	B	A	B
Total chlorophylls in leaves (SPAD)	36.630 **	46.448 ***	0.517 *	0.782	0.428	2.53	1.87
Total carbohydrates in leaves (%)	16.368 **	25.279 ***	0.242 *	0.167	0.878	3.94	9.02
Total carbohydrates in seeds (%)	5.042 ***	1.914 **	0.221 *	0.007	0.037	1.58	3.61
N (%)	0.855 ***	0.824 ***	0.003 *	0.001	0.004	1.21	2.39
P (%)	0.014 **	0.008 ***	0.001 *	0.00	0.00	3.14	4.01
K (%)	0.005 *	0.006 **	0.001 *	0.000	0.001	8.20	11.28
Mg (%)	0.001 NS	0.002 *	0.000 *	0.001	0.001	13.82	9.71
Ca (%)	0.013 *	0.001 *	0.001 *	0.001	0.000	6.49	3.20
Fe (ppm)	62.210 *	32.204 ***	2.316 *	1.148	1.473	1.66	1.88
Zn (ppm)	29.704 *	67.935 ***	0.778 *	2.251	0.445	3.34	1.48
Total protein (%)	27.907 ***	26.881 ***	0.083 *	0.038	0.145	1.22	2.39
Total phenol (µg CE/g)	0.917 *	1.774 **	0.119 *	0.158	0.204	9.36	10.65
Total flavonoid (µg CE/g)	60.770 *	63.393 ***	2.161 *	0.621	0.473	1.83	1.60
Antioxidant (DPPH IC50 (µg/ml))	8.143 **	23.270 ***	1.989 *	0.619	0.843	1.19	1.38
Fixed oil %	93.102 *	139.584 ***	0.509 *	2.833	2.978	6.39	6.55

\*, \*\*, \*\*\* significant at P≤0.05, P≤0.01, P≤0.001, respectively; NS= Not significant at p=0.05.

Table 6 - Macronutrients in seeds of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two seasons)

AMF	Treatment PGRs	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
-	0	2.21±0.03 f	0.33±0.01 d	0.19±0.02 f	0.21±0.01 d	0.40±0.01 c
	BA at 50 ppm	2.40±0.02 e	0.37±0.01 c	0.23±0.02 d	0.25±0.02 b	0.43±0.02 bc
	CPPU at 20 ppm	2.73±0.08 c	0.35±0.00 cd	0.21±0.01 e	0.24±0.00 c	0.43±0.01 bc
	NAA at 50 ppm	3.080±0.01 b	0.41±0.01 b	0.25±0.02 c	0.26±0.03 b	0.42±0.00 bc
+	0	2.60±0.02 d	0.36±0.00 cd	0.20±0.01 f	0.23±0.01 c	0.45±0.01 ab
	BA at 50 ppm	2.77±0.03 c	0.41±0.01 b	0.27±0.02 ab	0.27±0.01 a	0.47±0.01 a
	CPPU at 20 ppm	3.15±0.02 b	0.43±0.00 b	0.25±0.01 c	0.25±0.01 b	0.47±0.01 a
	NAA at 50 ppm	3.40±0.01 a	0.46±0.01 a	0.28±0.02 a	0.27±0.01 a	0.48±0.01 a

Each value represents the mean ± standard error of three replicates.

Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.



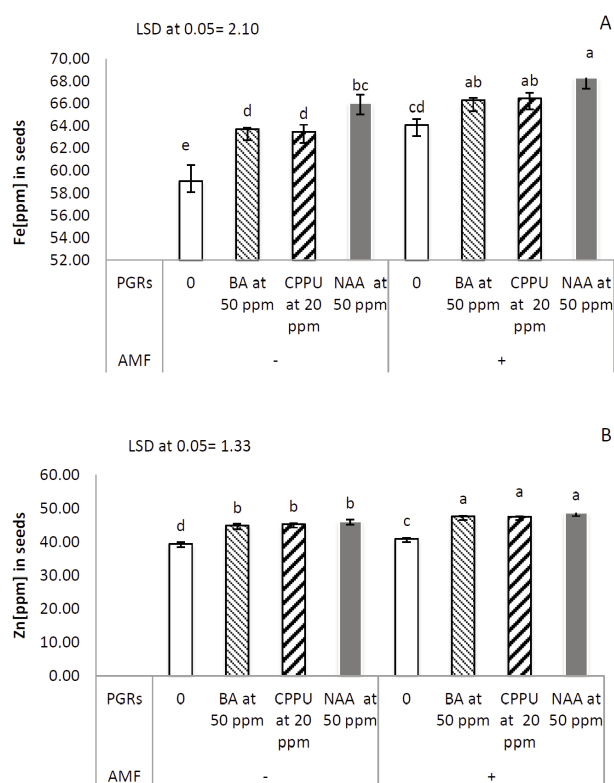


Fig. 2 - Fe (A) and Zn (B) in seeds of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two season), column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

its role on inducing  $\text{NH}_4^+$  and  $\text{NO}_3^-$  absorption, and assimilation of these molecules into free amino acids that are involved in protein synthesis (Gashgaril et al., 2020).

The data in Table 7 also exhibited that in the absence or presence of AMF, spraying of plants with PGRs resulted in significant increase in total protein content compared to control. Among the tested PGRs NAA was superior in its effect predominately in the presence of AMF. The results of increasing protein content due to application of BA are in good agreement with those elicited by Prins et al., 2013, while such increase owing to CPPU treatments is coincided with those obtained by Abbas and Zahwan, 2016.

*Total phenol content (TPC), Total flavonoid content (TFC) in in seeds*

The data presented in Tables 7 showed that under the same treatments of PGRs TPC and TFC in seeds were higher in plants inoculated with AMF compared to non-inoculated plants; however such increase was statically insignificant in the case of TPC compared to corresponding values in absence of AMF. The results of increasing TPC or TFC due to inoculation with AMF are analogy with that recorded by earlier workers (Amiri et al., 2017; Gashgaril et al., 2020).

Data outlined in Table 7 also indicate that in the absence or presence of AMF TPC and TFC in seeds were significantly higher in seeds of plants foliar sprayed with any concentrations of PGRs compared to control, with superiority of BA for increasing TPC and NAA for TFC especially in the presence of AMF. The present augmentations in TPC or TFC owing to NAA are in harmony with those obtained by previous author (Atteya and El Gendy, 2018), whereas increasing in tested components due to BA are in the same line with the findings of earlier study (Abdel-Hamid,

Table 7 - Total protein, total phenol, total flavonoid and antioxidants in seeds as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF), (mean of two seasons)

Treatments		Total protein (%)	Total phenol (µg CE/g)	Total flavonoid (µg CE/g)	Antioxidant (DPPH IC50 (µg/ml))
AMF	PGRs				
-	0	12.62±0.18 f	3.18±0.12 c	37.56±0.06 f	63.14±0.60 d
-	BA at 50 ppm	13.68±0.13 e	4.15±0.49 ab	40.74±0.13 d	66.18±0.58 c
-	CPPU at 20 ppm	15.57±0.45 c	4.55±0.03 a	43.18±0.05 c	67.54±0.02 bc
-	NAA at 50 ppm	17.57±0.06 b	4.31±0.04 ab	44.22±0.07 bc	66.34±0.6 c
+	0	14.87±0.10 d	3.68±0.34 bc	39.3±1.15 e	64.18±0.58 d
+	BA at 50 ppm	15.82±0.17 c	4.83±0.09 a	45.33±0.13 b	66.25±0.43 c
+	CPPU at 20 ppm	17.97±0.12 b	4.56±0.19 a	46.74±0.08 a	68.31±0.17 ab
+	NAA at 50 ppm	19.41±0.07 a	4.70±0.32 a	47.06±0.01 a	69.12±0.57 a

Each value represents the mean ± standard error of three replicates. Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

2020; Sidkey, 2020).

#### Antioxidant activity in seeds

As shown from data listed in Table 7 that under the same treatments of PGRs antioxidant activity in seeds were higher in plants inoculated with AMF compared to corresponding values of non-inoculated plants, however such increase was insignificant in most cases. The results of increasing antioxidant activity due to mycorrhizal treatments is supported by the results of other authors (Geneva *et al.*, 2010; Amiri *et al.*, 2017; Golubkina *et al.*, 2020; Gashgaril *et al.*, 2020).

Data recorded in Table 7 also indicate that in the absence or presence of AMF antioxidant activity was significantly higher in seeds of plants sprayed with PGRs concentrations compared to control, with superiority of NAA especially in the presence of AMF, since recorded the highest values compared to control. The obtained increase in antioxidant activity due to NAA are in harmony with those obtained by previous author (Atteya and El Gendy, 2018), while the augmentation due to BA are in harmony with the finding of recent study (Sidkey, 2020).

#### Fixed oil percentage

It is obvious from data listed in figure 3 that under the same treatments of PGRs, the values of fixed oil % for plants grown in presence of AMF was significantly higher than corresponding values in the absence of AMF. The results of increasing fixed oil % owing to inoculation with AMF are in sequence with the findings of earlier author (Moghith, 2019).

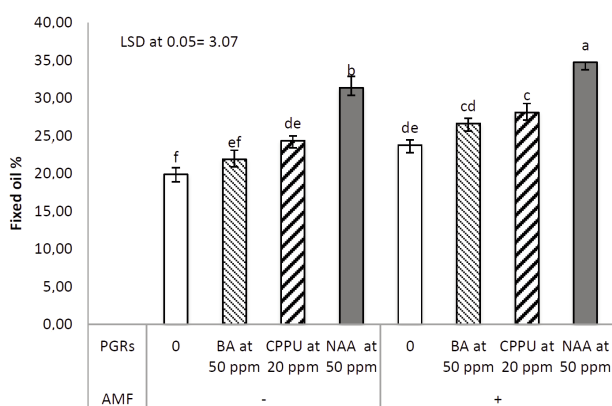


Fig. 3 - Fixed oil % of *Salvia hispanica* as affected by the interaction between plant growth regulators (PGRs) in absence (-) or presence (+) of Arbuscular mycorrhiza fungi (AMF) (mean of two season), column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates.

Data in figure 3 also showed that, in the absence or presence of AMF in general application of PGRs resulted in significant increase in fixed oil % compared to control. These increases in the recorded values were more evident in the presence of AMF particularly with application of NAA. Such augmentation in fixed oil due to application of BA is in agreement with those reported by previous study (Mousa *et al.*, 2001).

#### 4. Conclusions

Based on the outcome of the present research it could be concluded that, cultivation of chia plants in presence of mycorrhiza with foliar application of NAA at 50 ppm is recommended for enhancing growth, and nutritional values of seed yield.

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