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# Effect of Planting Spacing and Some Growth Regulators on Growth, Yield and Antioxidant Activity of Flax

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## Abstract

An experimental study has been carried out in the 2024 growing season for the impact of planting distances and some growth regulators on the growth, productivity and antioxidant activity of the flax plant. The factors investigated included: Planting distance between roots (5 cm and 15 cm), spraying with growth regulators (distilled water, IAA with a concentration of 2 mg L<sup>-1</sup>, GA with a concentration of 200 mg L<sup>-1</sup>, and its combination). The results indicated the superiority of the 15 cm distance in terms of maximum plant height (87.25 cm), number of branches (13.70 branches per plant), stem diameter (1.9667 mm), number of leaves (124.85 leaves per plant), leaf area (0.9303 cm<sup>2</sup>), total chlorophyll content (5.261 mg 100 g<sup>-1</sup> fresh weight), number of capsules (36.72 plant capsules<sup>-1</sup>), 1000 seeds weight (8.556 g), total seed yield (2.669 tons ha<sup>-1</sup>), oil percent (34.944%), total oil yield (94.4 kg ha<sup>-1</sup>), total phenols (15.17 mg g<sup>-1</sup> dry weight), total flavonoids (9.55 mg g<sup>-1</sup> dry weight), total carotenoids (2.784 mg g<sup>-1</sup>), total antioxidant capacity (62.44 mg g<sup>-1</sup> dry weight), and free radical scavenging efficiency (66.07%). The combined treatment of IAA and GA<sub>3</sub> also proved to be superior by obtaining the high-

est number of branches (19.42 branches plant<sup>-1</sup>), number of leaves (154.92 leaves plant<sup>-1</sup>), total chlorophyll content (5.332 mg g<sup>-1</sup> dry weight), number of capsules (46.06 capsules plant<sup>-1</sup>), weight of 1000 seeds (9.790 g), total seed yield (3.548 tons ha<sup>-1</sup>), oil percent (35.283%), total oil yield (125.4 kg ha<sup>-1</sup>), total phenols (16.06 mg g<sup>-1</sup> dry weight), total flavonoids (10.25 mg g<sup>-1</sup> dry weight), total carotenoids (2.960 mg g<sup>-1</sup> dry weight). The total antioxidant capacity was 63.45 mg g<sup>-1</sup> dry weight and the free radical scavenging efficiency was 67.58%. The treatment P15×IAA2+GA200 had the highest number of branches (19.94 branches<sup>-1</sup>), number of leaves (159.80 leaves<sup>-1</sup>), number of capsules (49.05 capsules<sup>-1</sup>), weight of 1000 seeds (10.407 g), total seed yield (3.822 tons ha<sup>-1</sup>), percentage of oil (36.090%), total oil yield (137.9 kg ha<sup>-1</sup>), total phenolic content (17.24 mg g ha<sup>-1</sup>), total phenolics content (17.24 mg g<sup>-1</sup> dry weight), total flavonoids (11.33 mg g<sup>-1</sup> dry weight), total carotenoids (3.241 mg g<sup>-1</sup> dry weight), total antioxidant capacity (64.30 mg g<sup>-1</sup> dry weight) and free radical scavenging efficiency (68.80%).

**Keywords:** Flax, planting distances, auxin (IAA), gibberellin (GA<sub>3</sub>)

## 1. INTRODUCTION

Flax is an economically valuable winter crop from the Linaceae family grown in many areas around the world. Flax is grown for the production of oil and fibers since its seeds contain 35-47% oil and 20-25% proteins. Flax seeds are known for the high content of unsaturated fatty acids, among which the most important are linoleic acid of omega-3 group, and linolenic acid of omega-6 group which have a significant role in preventing and treating some diseases, such as thrombosis, heart and arterial diseases [1]. It also includes oleic acid of the omega-9 group that can be useful for improving insulin sensitivity and fighting inflammation, while flaxseed oil is fit for human use [2]. About 80% of flaxseed oil is used in industries to make paints, varnishes and printing ink due to its drying properties, whereas the solid residue after extraction of the seeds, i.e., cassava, is used in producing feeds for animals because it contains approximately 36% of protein, along with being used as organic fertilizer due to its high percentage of nitrogen (5%) and phosphorus ( $P_2O_5$ ) (1.4%), and potassium ( $K_2O$ ) (1.8%). Besides, some residues of cassava are used in manufacturing insulation materials [3]. Agricultural practices and especially the arrangement of plant spacing is an important criterion that affects the growth of plants and their productivity. The optimal agricultural practices of the distances between lines and roots are essential in determining the space available to the plant, and size of the soil where the root system can grow and develop successfully. Thus, this agricultural organization creates an appropriate space to trap the solar rays and convert them into chemical energy that is invested to synthesize the dry matter and accumulate it in reproductive parts or other developing organs of the plant, which has a positive effect on the physiological performance and productivity of the crop [4]. Growth regulators are defined as non-nutritive organic compounds having the same impacts on plant physiology as natural hormones, but these chemicals are synthesized outside of plant tissues in order to affect plant growth and physiological activity with the aim of maximizing crop yields and quality [5]. Foliar application of growth regulators at certain concentrations is the effective technique that regulates growth processes of plants, either by promoting or inhibiting, or maintaining the normal rate of metabolic activity of the plant [6]. Auxin, particularly IAA, is a growth regulator of plant cells. Usually, it is unevenly distributed in plant tissues responding to various environmental stimuli like light or gravity, thereby causing the bending of a plant towards them. On a cellular level, auxin stimulates cell elongation through inducing activity of cell wall degradation factors, for example, expansins [7]. In case of environmental stress, auxin participates in root system restructuring along with ABA and signal transduction factors like DRO1 and SAURs, providing dense and deep root growth ensuring higher plant resistance to adverse environmental conditions. Gibberlin, namely  $GA_3$ , promotes cell elongation in stem and leaves and increases inter-nodal length resulting in the increase of plant height and productivity [8]. Gibberellin plays a crucial role in the process of secondary cell wall synthesis (xylogenesis) as it enhances activity of cambium and gene expression responsible for cellulose synthesis pathways including CESA3, CESA4, and CESA7 [9]. Hence, the main goal of this study is to find out the optimal plant distance for flax cultivation depending on the experiment conditions as well as the optimal concentration of growth regulators auxin and gibberellin that increase vegetative growth indexes, seed yield, oil content, secondary metabolites, total antioxidant capacity and free radical scavenging efficiency.

## 2. MATERIALS AND METHODS

Field trial was carried out in the Horticulture and Nursery Development Station in Umm Gharagher, Karbala Agricultural Directorate, latitude 44.12E and longitude 32.71 N using RCBD, Factorial design with three replications. Ten samples were randomly taken from the soil of both experimental areas before the planting for determining physical and chemical characteristics of soil. Soil preparation was through cultivation, smoothing and leveling and then application of manure at 32 t ha<sup>-1</sup> and supercomplex fertilizer (15:15:15:15 NPK) at 200 kg ha<sup>-1</sup> [10]. Then, division of the field into three sectors with a distance between sectors 2 m apart and corridors to isolate the experimental units, and each sector is divided into 24 experimental units, each unit was 3 m<sup>2</sup> and the distances between each unit is 30 cm from all sides. The distance of planting in rows was 30 cm apart, and there is a 1 m distance between units for ensuring that no spray flies. Flax seeds were planted on 11/15/2024 [11], obtained from the Abu Ghraib Agricultural Research Department of the Ministry of Agriculture. Two factors were used for this experiment: the first factor: planting distance between roots [12] 5 cm (200 plants/trial unit) and 15 cm (65 plants/trial unit) and the second factor: spraying of growth regulators [13] consisting only of distilled water and spraying auxin IAA2 at a concentration of 2 mg L<sup>-1</sup>, gibberellin  $GA_3$  at a concentration of 200 mg L<sup>-1</sup> and IAA2 at a concentration of 2 mg L<sup>-1</sup> × gibberellin  $GA_3$  at a concentration of 200 mg L<sup>-1</sup>. Spray was done twice; first in 30 days after planting when (4-6) leaves appear and the second time in 60 days after appearance of (8-10) leaves [14]. The following traits were measured 70 days after planting when the first flowers appear: plant height, number of main branches, main stem diameter, total number of leaves, leaf area, total chlorophyll content, number of capsules, number of seeds per capsule, weight of 1000 seeds, total seed yield, total seed yield, oil percentage and total oil yield. Total phenolics were measured according to the method adopted by Folin-Ciocalteu reagent, 5 g of seeds were taken from each trial unit and ground well then add 50 ml of 80% methanol and put in a water bath of 45°C for three hours then put in a rotary evaporator to reduce the sample volume to 5 ml, take 100 µL of sample extract and add 500 µL of Folin-Ciocalteu

reagent and 1.5 ml of sodium carbonate solution and put it in a Vortex blender and then add distilled water until it reaches a total of 10 ml then leave for two hours. Then put it in a Vortex blender again then add distilled water until it reaches a total of 10 ml then leave for two hours, measure the optical absorbance reading at wavelength 765 nm and Gallic acid is used for titration the total phenols in mg. In addition to measuring total flavonoids, total flavonoids were determined by the Aluminum chloride method  $AlCl_3$  using the same sample extract used to determine total phenolic as follows: Take 250  $\mu$ L of sample extract then add 1.25 ml of distilled water and 75  $\mu$ L of 5%  $NaNO_2$  solution then incubate for 5 minutes then add 150  $\mu$ L of 10% Aluminum Chloride and filter the mixture using Whatman-3 filter paper and finally add 500  $\mu$ L of 1 ai NaOH solution and 750  $\mu$ L distilled water, and finally measure the optical absorbance at wavelength 510 nm. Estimating the content of carotenoids at wavelength 480 nm using the method described by Goodwin [15] and calculate according to the following formula:

$$\text{Total Carotenoid} = (O.D \times T.VOL \div E_{cm1\%} \times 100) \times 1000000 \text{mg},$$

$$\text{Total Carotenoid} = (Ex250ml \div 2300 \times 100) 100 \text{mg/lg}.$$

Where, O.D= optical absorbance value at wavelength of 480 nm. T.Vol = total volume of the extract (ml). E = Extinction coefficient of carotenoids = 2300. Values are converted into units of mg/g of fresh weight of plant tissues. The total antioxidant capacity was calculated according to the method of estimating the total antioxidant capacity described by Budrat and Shotipruk [16] with some modifications as follows:

1- Solutions used:

Solution (1) Potassium hydroxide (0.1):

This solution was prepared by dissolving 0.5611 g of potassium hydroxide in a certain amount of distilled water, then the volume was completed to 100 ml with distilled water.

Solution (2) Potassium acetate (0.1):

This solution was prepared by mixing 1.2 ml of ice acetic acid and 1 ml of deionized distilled water then adjusting its pH value to 4.7 using potassium hydroxide solution described above and then complete the volume to 100 ml with deionized distilled water.

Solution (3) ABTS(2,2) azionbis-3-ethylbenzothiazoline-6-sulfonicacid radical in a concentration of 1 Mm and potassium persulfate in concentration of 2.45 m by dissolving 0.132 g of potassium persulfate in 0.1 mL of potassium acetate solution then adding 0.11 g of ABTS radical to this solution, after dissolving add distilled water to reach a volume of 100 mL.

2- Estimation Method:

1- Preparation of dilution series for Mg/Al-OG-LDH and ZnO-OG hybrid nanocomposites in addition to Trolox standard antibody and free OG in the dimethyl sulfoxide solvent (compounds under study concentrations limited between 1 mg/mL to 5 micrograms/mL).

2- Diluting the ABTS solution for obtaining an absorbance value (0.70.02) at wavelength 734 nm after zeroing the instrument using potassium acetate structure and then add 3 ml of the previous solution to 0.3 ml of each concentration of the compounds studied above.

3- Shake the tubes using Vortex shaker then incubate the mixture at room temperature for 10 minutes.

4- Measure the absorbance at wavelength 734 nm.

Estimation of inhibition of ABTS radical according to the following equation:

$$PI(\%) = [1 - (At/Ar)] * 100,$$

where At and Ar are the absorbances of the sample and ABTS radical, respectively. Free radical scavenging efficiency was estimated using the method described by Koleva et al. [17]. The reagent 1,1-Diphenyl-2-picrylhydrazyl (DPPH)  $C_{18}H_{12}N_5O_6$  in a concentration of 0.1 mmol is prepared by taking 39.4 mg of the reagent and adding 100 mL of methanol  $CH_3OH$  gradually with continuous shaking until it is completely dissolved. 4 mg of the reagent and add 100 mL of  $CH_3OH$  methanol gradually with continuous shaking until it is completely dissolved while measuring the optical absorbance of the reagent at wavelength 517 nm until reaching a value of ( $\pm 0.1$ ), then take 1 ml of each sample plant extract and add 1 ml of the reagent then mix well and put in a dark place for four minutes at laboratory temperature then measure the optical absorbance of 517 nm wavelength using a spectrophotometer, measure also the optical absorbance of the blank sample tube then determine the percentage of inhibition of the free radicals in grain using the following equation: Free radical scavenging efficiency (%) = (light absorbance of blank sample - light absorbance of plant sample  $\times 100$  / Light absorbance of blank sample).

### 3. RESULTS AND DISCUSSION

### 3.1. VEGETATIVE GROWTH CHARACTERISTICS

From the results presented in Table 1, planting distances, treatment with IAA solution 2 mg L<sup>-1</sup> and GA3 solution 200 mg L<sup>-1</sup>, and their interaction led to an increased vegetative growth traits. The highest plant height (87.25 cm), number of main branches (13.70 branches -1), stem diameter (1.9667 mm), number of leaves (124.85 leaves -1), leaf area (0.9303 cm<sup>2</sup>), leaf content (0.9303 cm<sup>2</sup>), and total chlorophyll content (5.261 mg 100 g<sup>-1</sup> wt<sup>-1</sup>) were observed at 15 cm planting distance than 5 cm planting distances which recorded 85.37 cm, 11.26 branches, 1.9005 mm, 119.59 leaves, 0.8858 cm<sup>2</sup> and 5.163 mg 100 g<sup>-1</sup> wt<sup>-1</sup>, respectively. This might be attributed to the increased planting distance (15 cm), which led to increased absorption capacity of nutrients and water by roots leading to increased physiological efficiency by plants, and as such there is increased photosynthetic ability, followed by leaf area and finally increased stem and branch length.

**Table 1.** *Effect of Planting Distance and Some Growth Regulators on Growth Parameters of Flax*

| Treats            | PH    | MB    | MSD     | LN     | LA      | TCH    |
|-------------------|-------|-------|---------|--------|---------|--------|
| P5                | 85.37 | 11.26 | 1.9005  | 119.59 | 0.8858  | 5.163  |
| P15               | 87.25 | 13.70 | 1.9667  | 124.85 | 0.9303  | 5.261  |
| LSD (0.05)        | 0.970 | 0.883 | 0.02079 | 1.569  | 0.01422 | 0.1434 |
| GR0               | 74.60 | 6.70  | 1.3183  | 97.28  | 0.9412  | 4.998  |
| IAA2              | 97.94 | 9.91  | 1.5620  | 115.34 | 0.9453  | 5.250  |
| GA200             | 82.28 | 13.88 | 2.7800  | 121.33 | 0.9177  | 5.267  |
| IAA2 + GA200      | 90.42 | 19.42 | 2.0740  | 154.92 | 0.8278  | 5.332  |
| LSD (0.05)        | 1.372 | 1.249 | 0.02939 | 2.219  | 0.02011 | 0.2029 |
| P5 × GR0          | 72.11 | 6.16  | 1.3000  | 95.66  | 0.9207  | 4.967  |
| P5 × IAA2         | 97.87 | 9.90  | 1.5073  | 111.84 | 0.9453  | 5.107  |
| P5 × GA200        | 82.19 | 10.07 | 2.7600  | 120.81 | 0.9177  | 5.267  |
| P5 × IAA2 +GA200  | 89.30 | 18.89 | 2.0347  | 150.05 | 0.7593  | 5.310  |
| P15 × GR0         | 77.09 | 7.25  | 1.3367  | 98.91  | 0.9617  | 5.030  |
| P15 × IAA2        | 98.01 | 9.92  | 1.6167  | 118.84 | 0.9453  | 5.393  |
| P15 × GA200       | 82.37 | 17.70 | 2.8000  | 121.85 | 0.9177  | 5.267  |
| P15 × IAA2 +GA200 | 91.54 | 19.94 | 2.1133  | 159.80 | 0.8963  | 5.353  |
| LSD (0.05)        | 1.940 | 1.766 | 0.04157 | 3.138  | 0.02843 | 0.2869 |

PH = Plant Height (cm), MB = Main Branch No., MSD = Main Stem Diameter (mm), LN = Leaves No., LA = Leave Area (cm<sup>2</sup>), TCH = Total Chlorophyll (mg 100g FW)

Moreover, the higher air circulation between the plants in the low density helped improve the chlorophyll metabolism efficiency; hence, the chlorophyll content was highest compared to the narrow farming distance (5 cm). It limited the space available for growth and led to high competition between plants [18]. It is evident from the results that the combination of IAA and GA3 yielded the highest number of branches (19.42 branches-1), number of leaves (154.92 leaves-1), and total chlorophyll content (5.332 mg 100 g<sup>-1</sup> fresh weight). On the other hand, the spraying of indole acetic acid at 2 mg L<sup>-1</sup> gave the highest plant height (97.94 cm) and leaf area (0.9453 cm<sup>2</sup>) compared to distilled water where plant height was lowest (74.60 cm), number of main branches (6.70 branches-1), stem diameter (1.3183 mm), number of leaves (97.28 leaves-1), and total chlorophyll content (4.998 mg 100 g<sup>-1</sup> fresh weight). The efficacy of IAA+GA3 could be credited to the interaction of the two hormones and the increase in efficiency of cell division and elongation processes, photosynthesis and chloroplasts pathway. This leads to higher growth and chlorophyll index and increases the metabolic capacity (synthesis of carbohydrates) to support the leaf formation and increased branching through auxin and cytokinin interactions [19]. In case of IAA alone, it is known to regulate cell expansion and phalanx elongation, which helps in the increase in stem length and leaf tissue expansion [20]. P15 × IAA2 +GA200 resulted in the highest number of main branches (19.94 branches-1) and number of leaves (159.80 leaves-1). In contrast, P15 × IAA2 was the best in plant height (98.01 cm) and total chlorophyll content (5.393 mg 100 g<sup>-1</sup> fresh weight). The P5 × GR0 cross-interaction registered the lowest plant height (72.11 cm), number of main branches (6.16 branches-1), stem diameter (1.3000 mm), number of leaves (95.66 leaves-1), and total chlorophyll content (4.967 mg 100 g<sup>-1</sup> fresh weight).

### 3.2. CHARACTERISTICS OF YIELDING

Results in Table 2 indicated that planting distance of 15 cm achieved the maximum values in terms of number of capsules (36.72 plant capsules-1), weight of 1000 seeds (8.556 g), total seed yield (2.669 tons ha<sup>-1</sup>), percentage of oil (34.944%) and total oil yield (94.4 kg ha<sup>-1</sup>), whereas the minimum levels were reached when using 5 cm planting distance (31.20 plant capsules-1, 7.567 g, 2.105 t ha<sup>-1</sup>, 32.573%, and 69.5 kg ha<sup>-1</sup>, respectively). The explanation of such superiority in productive properties for 15 cm planting distance over 5 cm planting distance could be based on the reduced

competition for nutrients, water and light, thereby ensuring a full utilization of resources for the development of branches and leaves and physiological capability to form capsules. Additionally, greater spacing allows increasing exposure of the leaves to sunlight, improving the process of photosynthesis and accumulation of carbohydrates, which are the main sources of energy needed for the reproduction organ (capsule) formation, thus enabling plants to direct more nutrients for seed growth and their increase in weight and oil content, positively influencing the overall yield of seeds and oil. The results revealed that IAA2 + GA200 treatment combination resulted in maximum levels of all analyzed characteristics of plants: number of capsules (46.06 capsules plant<sup>-1</sup>), weight of 1000 seeds (9.790 g), total seed yield (3.548 tons ha<sup>-1</sup>), percentage of oil (35%) and total oil yield (125.4 kg ha<sup>-1</sup>), whereas the lowest figures were recorded for 0 mg L<sup>-1</sup> (GR0) treatment (25.95 plant capsules<sup>-1</sup>, 6.875 g, 1.275 tons ha<sup>-1</sup>, 31.930%, 40.7 kg ha<sup>-1</sup>, respectively). Such superiority in productivity properties of the IAA2 + GA200 treatment could be explained by the fact that IAA acts as an important hormone controlling cell elongation through gene regulation of polymers (e.g. cellulose and pectin) and activation of H<sup>+</sup> - ATPase enzyme, which leads to the extension of cell wall. In turn, GA<sub>3</sub> stimulates stem and reproductive organs growth through the activation of hyaluronidase and xylanase enzymes promoting cell elongation and tissue differentiation [21]. The interaction between IAA and GA<sub>3</sub> could enhance the capability of the plant to accumulate carbohydrates and ensure efficient transfer of sugars to the capsules, thus contributing to increased weight of seeds and oil content, which positively influenced total yield [22]. According to the results obtained from the interference test, there was a significant impact of the factors studied on the characteristics of yielding. The highest number of capsules (49.05 plant<sup>-1</sup> capsules), weight of 1000 seeds (10.407 g), total seed yield (3.822 tons ha<sup>-1</sup>), oil percentage (36.36%), and total oil yield (137.9 kg ha<sup>-1</sup>) were demonstrated by the P15 × IAA2 + GA200 interference treatment. Meanwhile, the P5 × GR0 intercross resulted in the minimum values of these characteristics (25.13 capsules, 6.827 g, 1.230 tons ha<sup>-1</sup>, 31.140%, 38.3 kg ha<sup>-1</sup>, respectively).

**Table 2.** Effect of Planting Distance and Some Growth Regulators on Seed, and Oil Yield Parameters of Flax

| Treats             | CN    | SN     | WS     | SY     | OP     | OY    |
|--------------------|-------|--------|--------|--------|--------|-------|
| P5                 | 31.20 | 8.713  | 7.567  | 2.105  | 32.573 | 69.5  |
| P15                | 36.72 | 8.273  | 8.556  | 2.669  | 34.944 | 94.4  |
| LSD (0.05)         | 1.853 | 0.1813 | 0.0983 | 0.1494 | 0.0444 | 5.23  |
| GR0                | 25.95 | 7.143  | 6.875  | 1.275  | 31.930 | 40.7  |
| IAA2               | 28.18 | 9.823  | 7.315  | 2.028  | 33.858 | 68.8  |
| GA200              | 35.64 | 9.118  | 8.265  | 2.697  | 33.963 | 92.7  |
| IAA2 + GA200       | 46.06 | 7.888  | 9.790  | 3.548  | 35.283 | 125.4 |
| LSD (0.05)         | 2.621 | 0.2564 | 0.1391 | 0.2114 | 0.0629 | 7.40  |
| P5 × GR0           | 25.13 | 7.177  | 6.827  | 1.230  | 31.140 | 38.3  |
| P5 × IAA2          | 28.09 | 9.647  | 7.103  | 1.927  | 32.317 | 62.3  |
| P5 × GA200         | 28.51 | 9.743  | 7.163  | 1.990  | 32.360 | 64.4  |
| P5 × IAA2 + GA200  | 43.07 | 8.287  | 9.173  | 3.274  | 34.477 | 112.9 |
| P15 × GR0          | 26.78 | 7.110  | 6.923  | 1.319  | 32.720 | 43.2  |
| P15 × IAA2         | 28.28 | 10.000 | 7.527  | 2.130  | 35.400 | 75.4  |
| P15 × GA200        | 42.78 | 8.493  | 9.367  | 3.404  | 35.567 | 121.1 |
| P15 × IAA2 + GA200 | 49.05 | 7.490  | 10.407 | 3.822  | 36.090 | 137.9 |
| LSD (0.05)         | 3.706 | 0.3626 | 0.1967 | 0.2989 | 0.0889 | 10.46 |

CN = Capsules No. (Capsules plant<sup>-1</sup>), SN = Seed No. (Seed Capsules<sup>-1</sup>), WS = Weight 1000 seed (g), SY = Seed Yield ( $\mu\text{g ha}^{-1}$ ), OP = Oil (%), OY = Oil Yield (Kg ha<sup>-1</sup>)

### Chemical properties

It was found from the results in Table 3 that the planting distance between roots 15 cm achieved the highest values of total phenol 15.17 mg g<sup>-1</sup> dry weight, total flavonoid 9.55 mg g<sup>-1</sup> dry weight, total carotenoid 2.784 mg g<sup>-1</sup> dry weight, total antioxidant activity 62.44 mg g<sup>-1</sup> dry weight, and free radical scavenging activity of 66.07% compared with the planting distance between roots 5 cm. Values obtained with agronomic distance between roots 15 cm were 15.17 mg g<sup>-1</sup>, 9.55 mg g<sup>-1</sup>, 2.784 mg g<sup>-1</sup>, 62.44 mg g<sup>-1</sup> dry weight, and 66.07% compared with agronomic distance between roots 5 cm recorded low values 12.19 mg g<sup>-1</sup>, 7.46 mg g<sup>-1</sup>, 2.400 mg g<sup>-1</sup>, 59.61 mg g<sup>-1</sup> dry weight, and 61.69%. This may be because the use of an agronomic distance between roots of 15 cm decreases plant competition for nutrients leading to a high rate of photosynthesis which results in phenols, flavonoids, and carotenoids biosynthesis as natural antioxidants [23]. Biosynthesis of secondary metabolites (such as phenols, flavonoids, and carotenoids) is the type of compound with the capability to resist oxidative stress through interactions and degradation of free radicals [24]. From the results, it was observed that the IAA2 + GA200 treatment achieved the highest values of total phenol of 16.06 mg g<sup>-1</sup> dry weight, total flavonoid of 10.25 mg g<sup>-1</sup> dry weight, total carotenoid of 2.960 mg g<sup>-1</sup> dry weight, total antioxidant activity of

63 mg g<sup>-1</sup> dry weight, and free radical inhibition efficacy of 67.58%. While the total phenol 16.06 mg g<sup>-1</sup> dry weight, total flavonoid 10.25 mg g<sup>-1</sup> dry weight, total carotenoid 2.960 mg g<sup>-1</sup> dry weight, total antioxidant activity 63 mg g<sup>-1</sup> dry weight, and free radical scavenging efficacy of 67.58% for IAA2 + GA200 treatment; GR0 treatment has the lowest value of total phenol of 10.61 mg g<sup>-1</sup> dry weight, total flavonoid of 6.42 mg g<sup>-1</sup> dry weight, total carotenoid of 2.223 mg g<sup>-1</sup> dry weight, total antioxidant activity of 57.77 mg g<sup>-1</sup> dry weight, and free radical scavenging efficacy of 59.03%. Auxin and gibberellin play an important role in enhancing plant vegetative growth and activating primary and secondary metabolism in plants through activation of cell division and elongation, and increasing carbon flux towards secondary metabolic pathways and increasing photosynthetic efficiency, carbohydrate metabolism, providing energy and building blocks to synthesize phenolic compounds, flavonoids, and carotenoids; therefore, combination of both regulators leads to synergism, resulting in accumulation of secondary compounds which have antioxidant activity, thus enhancing the total antioxidant activity and free radical inhibition efficacy in this treatment [25]. The results show the effectiveness of the interference treatment of P15 × IAA2 + GA200 as it achieved the highest values of total phenol 17.24 mg g<sup>-1</sup> dry weight, total flavonoid 11.33 mg g<sup>-1</sup> dry weight, total carotenoid 3.241 mg g<sup>-1</sup> dry weight, total antioxidant activity 64 mg g<sup>-1</sup> dry weight, and free radical scavenging activity of 68.80%. The results also revealed the lowest values of total phenol 9.34 mg g<sup>-1</sup> dry weight, total flavonoid 5.72 mg g<sup>-1</sup> dry weight, total carotenoid 2.081 mg g<sup>-1</sup> dry weight, total antioxidant activity 56.00 mg g<sup>-1</sup> dry weight, and free radical scavenging activity 56.45%.

**Table 3.** *Effect of Planting Distance and Some Growth Regulators on Antioxidant Activity Parameters of Flax*

| Treats             | TP    | TF    | TC     | AC    | DPPH  |
|--------------------|-------|-------|--------|-------|-------|
| P5                 | 12.19 | 7.46  | 2.400  | 59.61 | 61.69 |
| P15                | 15.17 | 9.55  | 2.784  | 62.44 | 66.07 |
| LSD (0.05)         | 0.108 | 0.026 | 0.0107 | 0.036 | 0.015 |
| GR0                | 10.61 | 6.42  | 2.223  | 57.77 | 59.03 |
| IAA2               | 13.37 | 8.11  | 2.519  | 60.91 | 63.78 |
| GA200              | 14.69 | 9.25  | 2.666  | 61.95 | 65.14 |
| IAA2 + GA200       | 16.06 | 10.25 | 2.960  | 63.45 | 67.58 |
| LSD (0.05)         | 0.153 | 0.037 | 0.0151 | 0.051 | 0.021 |
| P5 × GR0           | 9.34  | 5.72  | 2.081  | 56.00 | 56.45 |
| P5 × IAA2          | 11.67 | 7.05  | 2.372  | 59.54 | 61.21 |
| P5 × GA200         | 12.88 | 7.92  | 2.467  | 60.27 | 62.73 |
| P5 × IAA2 + GA200  | 14.88 | 9.17  | 2.679  | 62.61 | 66.36 |
| P15 × GR0          | 11.88 | 7.12  | 2.366  | 59.54 | 61.61 |
| P15 × IAA2         | 15.06 | 9.18  | 2.667  | 62.28 | 66.34 |
| P15 × GA200        | 16.50 | 10.59 | 2.864  | 63.62 | 67.54 |
| P15 × IAA2 + GA200 | 17.24 | 11.33 | 3.241  | 64.30 | 68.80 |
| LSD (0.05)         | 0.216 | 0.052 | 0.0214 | 0.072 | 0.029 |

TP = Total Phenol (mg Kg<sup>-1</sup>), TF = Total Flavonoids (mg Kg<sup>-1</sup>), TC = Total Carotins (mg Kg<sup>-1</sup>), AC = Antioxidant Capacity (mg Kg<sup>-1</sup>), DPPH = Free Radical Scavenging (%)

#### 4. CONCLUSIONS

Application of 15 cm inter-root spacing during flax farming practice is considered to be the most suitable approach for ensuring an optimum balance of vegetative development, productivity and bioactive compound synthesis. Foliar application of IAA2+GA200 is the most efficient control in terms of positive effects on yield components, oil content and quality properties associated with antioxidant capacity of flax. Moreover, the application of these treatments is clearly connected to stimulation of secondary metabolism and that is confirmed by elevated levels of phenolic compounds, flavonoids and carotenoids, being directly linked to flax resistance to biotic and abiotic stress factors as well as being important from the viewpoint of improving overall health and nutrition value of the crop. In this context, it should be mentioned that the application of growth regulators can be highly recommended for practical purposes as an environmentally safe and easily accessible agricultural input that does not demand additional equipment and advanced expertise from farmers.

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