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Gibberellic acid improves seed germination and growth of *Vicia faba* plants subjected to clethodim herbicide

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Abstract: Hazardous impacts of clethodim herbicide on seed germination, plant growth parameters, and leaf chlorophyll contents of faba bean cultivars (*Vicia faba* L. cv. Noubria 4 and Giza 843) as well as the benefit role of gibberellin on clethodim treated plants were investigated. Clethodim is one of the postemergence systemic herbicides that inhibits acetyl CoA carboxylase (ACCase). On this, it can indirectly affect the fatty acid synthesis required to build a various new cell membrane of growing tissues. The results showed that clethodim causes a negative effect on seed germination especially at higher concentration used (1.2 mM). At the seedling stage, the shoot and root lengths of both cultivars significantly decreased with increasing clethodim doses compared to the control. Combination treatments of GA_3 + clethodim to seeds increased seed germination and root length compared to that only treated with the clethodim. At the growth stage, fresh and dry weights, and leaf area of both cultivars decreased with increasing clethodim doses, whereas GA_3 treatments before three days of Clethodim treatment enhanced fresh and dry weights, and leaf area of clethodim treated and untreated of both *Vicia faba* cultivars. Leaf pigment contents of both cultivars decreased in response to increasing clethodim doses. GA_3 application (50 µM) to clethodim-treated and untreated plants increased pigment contents compared to those of the corresponding clethodim-treated and control plants. Among photosynthetic pigments, the chlorophyll *a* of both cultivars was much more sensitive to clethodim herbicide. The study suggests that the faba bean is seriously affected by clethodim treatments, especially at the seedling stage. GA_3 spraying improved the seed germination, growth, and pigment contents of faba bean plants exposed to the clethodim stress.

Keywords: Dry weight, antioxidant enzymes, shoot and root length

1. Introduction

Herbicides are used for the management of undesired plants in the areas of agriculture, landscaping, forestry, gardening, and industry [1,2]. In 2020 about 52.5% of the pesticides used globally were herbicides [3]. Herbicides have the ability to inhibit several biological processes such as photosynthesis, cell division, root growth, and the synthesis of proteins, fatty acids, and pigments [4-11]. Clethodim herbicide (5-[2(ethylsulfanyl)propyl]-3-hydroxy-2-propionylcyclohex-2-en-1-one) belongs to the chemical family of the cyclohexanediones (DIMS) and it is a selective and effective herbicide for the post-emergent control of annual and perennial grasses in widespread broadleaf crops including soybean, cotton, tobacco, sugar beet, maize, and peanuts [12,13]. Clethodim herbicide prevents fatty acid biosynthesis through the inhibition of enzyme acetyl CoA carboxylase (ACCase; EC 6.4.1.2) [14].

ACCase-inhibiting herbicides are divided into three chemical families: aryloxyphenoxypropionates (FOPs), cyclohexanodiones (DIMs), and phenylpyrazole (DENs) [15,16]. In 1978, the market saw the first of acetyl-CoA carboxylase (ACCase) inhibitors with the release of diclofop–methyl [17]. Thus, ACCase-inhibiting herbicide has become a critical factor in controlling weeds [18]. Herbicides of this group can cause overproduction of ROS, bind to the carboxylase transferase domain of the protein and block its function, thereby killing plants [19-21]. Gibberellic acid (GA_3) is an endogenous phytohormone involved in several physiological processes in plants such as promoting plant cell elongation and division, stimulating seed germination, and regulating plant flowering [22]. Moreover, it plays a vital role in mitigating the disturbances that caused by abiotic stressors such as drought, salinity, herbicides, and heavy metals in plants by regulating diverse physio-biochemical and molecular processes [22]. GA_3 is active in the ground for extended periods of time and is generally persistent [23]. GA_3 has broad-spectrum effects, which can reduce the phytotoxic effects of a variety of herbicides on various crop species [24-29]. GA_3 protected winter rapeseed seedlings that subjected to salt and heat stresses [30]. It also reduced drought stress responses in tomato, pepper, and mint [31].

The greatest weed reduction and wheat grain yield were obtained from the combination of hormone and herbicide application. It has been reported that addition of 200 µg GA_3 into the leaf sheaths of oats (*Avena sativa*) 2 days before spraying with fluozifop or glyphosate increased the efficacy of both herbicides against weeds [32]. Faba bean (*Vicia faba*) is a potentially useful and adaptable leguminous crop belonging to the Fabaceae family that may be cultivated anywhere in the world with a variety of climates [33,34]. It is the most widely grown crop for human and animal consumption in the Mediterranean region, China, Africa, Europe, and Asia [35]. Faba bean dry seed is a great source of amino acids, protein,

carbohydrates, minerals vitamins, and other bioactive phytochemical compounds [36-40]. This work was to study the alterations in seed germination, growth of faba bean seedlings and leaf chlorophyll content in response to clethodim herbicide and the combination effect of clethodim and GA_3 on faba bean plants.

2. Materials and methods:

2.1. Plant materials and treatments

Seeds of *Vicia faba* (Noubria 4 and Giza 843 cultivars) were provided by the Agriculture Research Centre, Ministry of Agriculture, Shandawil, Sohag, Egypt. Seeds of each cultivar that have the same size were chosen for the experiments of seed germination, seedling and vegetative plant growth.

2.2. For germination experiment

Seeds of *Vicia faba* cultivars were surface sterilized for 10 min in 5% v/v chlorox followed by several washes with distilled water. The sterilized seeds of *Vicia faba* cultivars were distributed into 12 groups in Petri dishes. Seeds were immersed in enough solution containing GA_3, clethodim, and GA_3 + clethodim separately or in combination as well as dist. water at 20°C under dark conditions for 7 days. The groups of seed germination treatments were the following:

- **Group 1:** Control 1, seeds of faba beans were immersed in dist. water.
- **Group 2:** Control 2, seeds of faba beans were immersed in 50 µM GA_3.
- **Group 3:** Control 3, seeds of faba beans were immersed in 100 µM GA_3.
- **Group 4:** Seeds of faba beans were immersed in 0.4 mM of clethodim solution.
- **Group 5:** Seeds of faba beans were immersed in 0.8 mM of clethodim solution.
- **Group 6:** Seeds of faba beans were immersed in 1.2 mM of clethodim solution.
- **Group 7:** Seeds of faba beans were immersed in 50 µM GA_3 + 0.4 mM of clethodim solution.
- **Group 8:** Seeds of faba beans were immersed in 50 µM GA_3 + 0.8 mM of clethodim solution.
- **Group 9:** Seeds of faba beans were immersed in 50 µM GA_3 + 1.2 mM of clethodim solution.
- **Group 10:** Seeds of faba beans were immersed in 100 µM GA_3 + 0.4 mM of clethodim solution.
- **Group 11:** Seeds of faba beans were immersed in 100 µM GA_3 + 0.8 mM of clethodim solution.
- **Group 12:** Seeds of faba beans were immersed in 100 µM GA_3 + 1.2 mM of clethodim solution.

At the end of the experimental period (7 days), the % of seeds germination and seedling shoot, and root lengths were estimated: The results of germination experiment showed that a concentration of 50 µM GA_3 had a more positive effect than a concentration of 100 µM GA_3, therefore, a concentration of 50 µM GA_3 was used in the vegetative growth experiment.

2.3. For growth experiment

Seeds of *Vicia faba* cultivars were sown in a mixture of 2 kg soil (sand and clay (1/1 v/v) in plastic pots. After three weeks from the seed cultivation, plants with similar growth were selected and distributed into eight groups. GA_3 (50 µM) sprayed to plants three days before spraying with clethodim. The identification of the plant groups was distributed as follows:

- **Group 1:** Control 1, plants were sprayed with dist water.
- **Group 2:** Control 2, plants were sprayed with 50 µM GA_3.
- **Group 3:** plants were sprayed with 0.4 mM of clethodim solution.
- **Group 4:** Plants were sprayed with 0.8 mM of clethodim solution.
- **Group 5:** Plants were sprayed with 1.2 mM of clethodim solution.
- **Group 6:** Faba bean plants were sprayed with a solution of 50 µM GA_3 three days before spraying plants with 0.4 mM of clethodim solution.
- **Group 7:** Faba bean plants were sprayed with a solution of 50 µM GA_3 three days before spraying plants with 0.8 mM of clethodim solution.
- **Group 8:** Faba bean plants were sprayed with a solution of 50 µM GA_3 three days before spraying plants with 1.2 mM of clethodim solution.

Growth measurements of shoot length, fresh shoot weight, dry shoot weight, leaves fresh and dry weights and leaf area were estimated after two weeks from the treatments. Plant dry weight was measured after drying the fresh plant material to constant weight at 70 °C for 48 h.

Table 1: Effect of clethodim herbicide, GA_3, and combination of GA_3 + clethodim after one week of treatments on seed germination (%) of *Vicia Faba* leaves (L.cv. Noubria 4 and Giza 843). The values are means of three replicates ± standard deviation (SD).

Treatments	Noubria 4 Seed germination (%)			Giza 843 Seed germination (%)		
	M	±	SD	M	±	SD
Control	100.00	±	0.00	100.00	±	0.00
50 µM GA_3	100.00	±	0.00	100.00	±	0.00
100 µM GA_3	100.00	±	0.00	100.00	±	0.00
0.4 mM Clethodim	100.00	±	0.00	100.00	±	0.00
0.8 mM Clethodim	83.33	±	14.43	91.67	±	14.43
1.2 mM Clethodim	75.00**	±	14.43	58.33**	±	28.86
0.4 mM Cle +50 µM GA_3	100.00	±	0.00	100.00	±	0.00
0.8 mM Cle +50 µM GA_3	91.67	±	14.43	100.00	±	0.00
1.2 mM Cle +50 µM GA_3	83.33	±	14.43	91.67	±	14.43
0.4 mM Cle +100 µM GA_3	91.67	±	14.43	100.00	±	0.00
0.8 mM Cle +100 µM GA_3	91.67	±	14.43	91.67	±	14.43
1.2 mM Cle +100 µM GA_3	83.33	±	14.43	66.67	±	38.18

Statistical significance of differences compared to the control.
*** Significant at $P < 0.05$.** Significant at $P < 0.01$.**

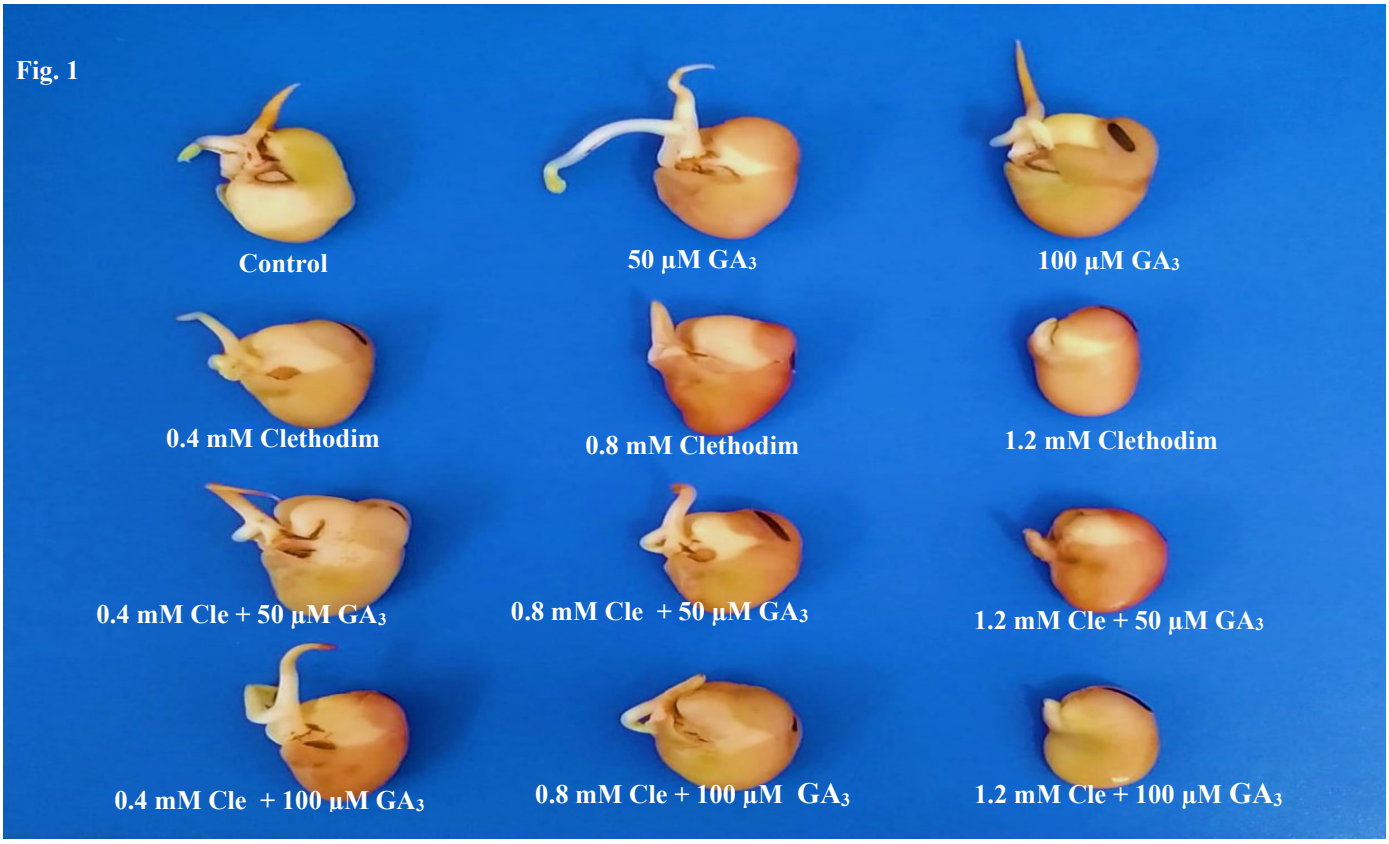

Figure 1: Photograph shows seedlings growth of *Vicia faba* (L.cv. Noubria 4) treated and untreated with GA_3, clethodim and the. interaction of GA_3 + clethodim after one-week treatments

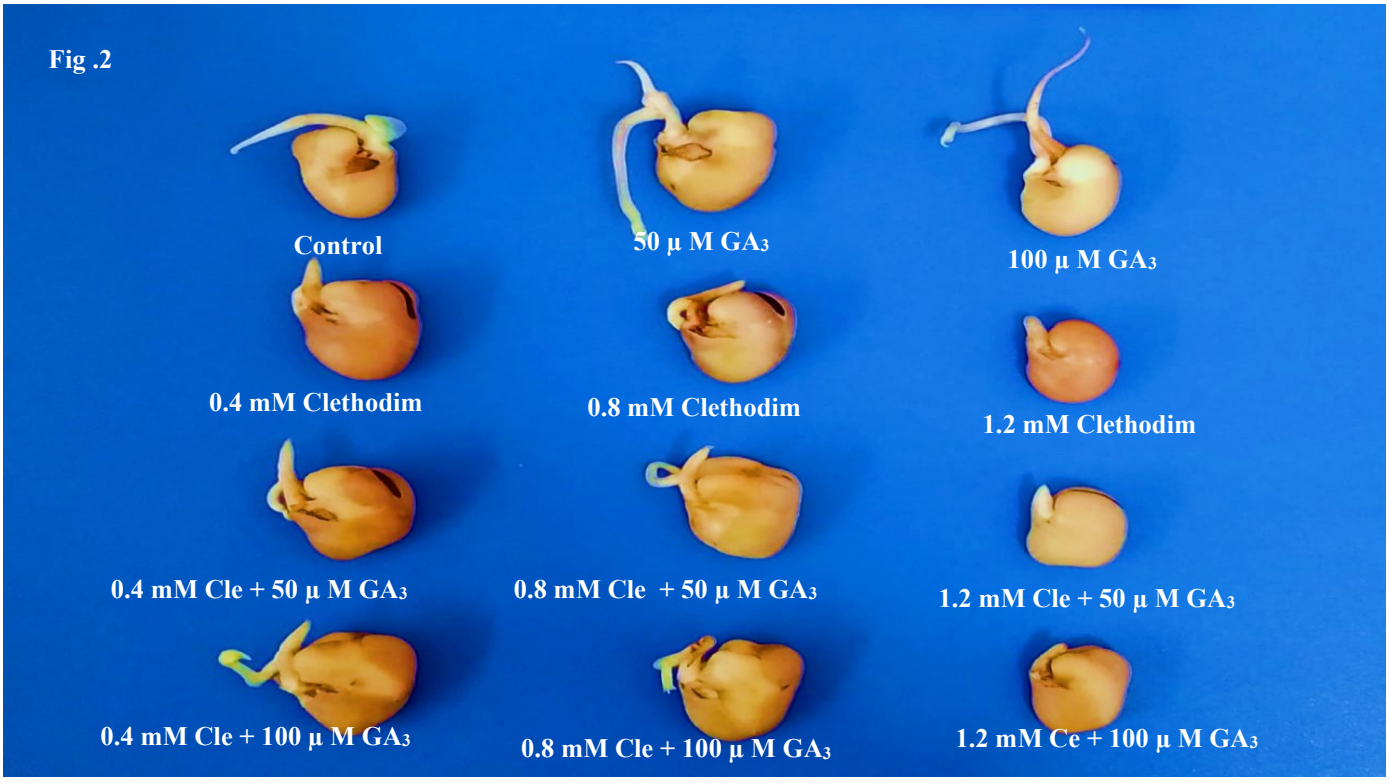

Figure 2: Photograph shows seedlings growth of *Vicia faba* (L.cv. Giza 843) treated and untreated with GA_3, clethodim and the interaction of GA_3 + clethodim after one - week treatments.

2.4. Leaf area

Individual leaf was determined following the procedure of McKee [41]. By measuring the length of leaf blade from its base to the leaf tip (Leaf L) and the width of the leaf at its widest point (Leaf W) and multiplying these values with a correction factor (0.75) as shown in the following equation:

Individual Leaf Area = Leaf L × Leaf W × 0.75

2.5. Estimation of photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids) were measured spectrophotometrically in fresh leaf samples according to Lichtenthaler [42]. Leaf samples (0.1g) were homogenized in acetone (85% v/v). The extract was centrifuged at 4,000 × g for 10 min. at three different wavelengths (663, 645, and 470 nm). The absorbance of the supernatant was measured in relation to a blank of pure 85% aqueous acetone. The pigments contents were calculated according to the following equations and expressed as mg. g^{-1} fresh leaves.

Chlorophyll *a* = $(11.75 \times A_{663} - 2.35 \times A_{645}) \times V / 1000 \times W$

Chlorophyll *b* = $(18.61 \times A_{645} - 3.96 \times A_{663}) \times V / 1000 \times W$

Car. = $[(1000 \times A_{470}) - (2.27 \times \text{Chl } a) - (81.4 \times \text{Chl } b)/227] \times V/1000 \times W$

Where V is the volume of leaf extract (ml), W is the weight of fresh leaf (g).

2.6. Statistical analyses

The obtained data were tested for significance by using the ANOVA test. Means were compared by the least significant difference (LSD) test at levels $P < 0.01$ and $P < 0.05$. All statistical tests were carried out using SPSS (v. 15.0) software.

3. Results and Discussion:

3.1. Germination stage

Herbicides are considered the most widely class of pesticides applied in the agriculture and have been used by farmers to control weeds. The increase in the world's population in the 20th century must be required an increase in food production. In the meantime, about one-third of agricultural products are produced depending on the application of pesticides [43,44]. Our results revealed that the seed germination, shoot and root lengths and pigment contents of faba beans were highly affected by application of clethodim. In contrast, gibberellic acid showed a protective role against clethodim herbicide effect. However, the excessive and unregulated use of herbicides has shown a negative effect on crop plants [45].

Faba beans (*Vicia faba* L.cv. Noubria 4 and Giza 843) were germinated for 7 days (Figs. 1 and 2) in distilled water, GA_3 (50 and 100 µM), and different concentrations of clethodim (0.4, 0.8 and 1.2 mM) herbicide, and in combination of 0.4 mM Cle + 50 µM GA_3, 0.8 mM Cle + 50 µM GA_3, 1.2 mM Cle +50 µM GA_3, 0.4 mM Cle + 100 µM GA_3, 0.8 mM Cle + 100 µM GA_3 and 1.2 mM Cle + 100 µM GA_3.

Seed germinations of both cultivars were totally germinated in water, 50 and 100 GA_3, 0.4 mM Clethodim and 0.4 mM Cle + 50 µM GA_3 (Table 1), while in response to 1.2 mM Clethodim, the seed germination of both cultivars was significantly decreased. GA_3 slightly increased seed germination of 0.8 and 1.2 mM clethodim treatments compared to that only treated with 1.2 mM clethodim. Clethodim treatments significantly decreased seed germination of both cultivars (Table 1). Comparing between faba been cultivars, the seed germination of Giza 843 cultivar was more affected by clethodim treatments than that of Noubria 4. In this respect, at 1.2 mM clethodim, the % of Noubria 4 and Giza 843 seed germinations was 75 and 58.33 compared to the control (100), respectively. The % of seed germination at low dose (0.4mM) clethodim with or without GA_3 was 100 % as those of the control, 50 and 100 µM GA_3. In response to 0.8 mM clethodim with or without GA_3, the seed germination of both cultivars decreased compared to the control except that of Giza 843, which treated with 0.8 mM Cle + 50 µM GA_3 showed 100 % as the control. Generally, GA_3 with or without clethodim treatments enhance seed germination of both cultivars (Table 1, Figs. 1 and 2). In this respect, the seed germination of Giza 843 cultivar was much more sensitive to clethodim than that of Noubria 4. Comparable to other stress, Gibberellic acid (GA_3) enhances seed germination, stem elongation, and flowering of *Solanum melongena* [46,47]. Seedling Shoot and root lengths of the two faba beans cultivars are shown in (Figs. 3-6). Seedling shoot and root lengths of the control of Giza 843 are higher than those of Noubria 4.

Comparing the length between shoots and roots of each cultivar, the roots of two cultivars are taller than their shoots. With clethodim herbicide treatments, the shoot and root lengths of both cultivars were sharply decreased (Figs. 3-6). The lengths of seedling shoot decreased with increasing clethodim doses. Moreover, Shoots are seriously affected than roots in response to clethodim. In this respect, at the lowest dose of clethodim (0.4 mM), seedling shoot and root lengths of Noubria 4 cultivar decreased by 27.50 and 6.22 % in comparison with their controls, respectively. At the highest dose used (1.2 mM), shoot and root lengths decreased by 71.13 and 50.85 %. In the case of Giza 843 seedling, its shoot and root lengths decreased significantly in response to clethodim doses (Figs. 5 and 6). The calculations revealed that 0.4 mM clethodim decreased shoot and root lengths of Giza 843 by 66.02 and 38.1 % respectively, compared to the controls. In this context, in response to 1.2mM clethodim, the reduction in shoot and root lengths of Giza 843 were 84.38 and 55.72%, respectively.

The reduction in shoot lengths is more than that of roots. The results showed that the Giza 843 cultivar is more sensitive to clethodim than Noubria 4. It is obvious that using gibberellic acid with clethodim herbicide alleviated clethodim stress and increased seed germination, shoot and root lengths of faba bean (Table 1; Figs. 3-6). In contrast, GA_3 decreased the activity of antioxidant enzymes in glyphosate treated sorghum seeds through germination [26]. Morphologically, using GA_3 with a concentration of 50 µM is better than 100 µM for protecting plants against clethodim injuries (Figs. 1 and 2).

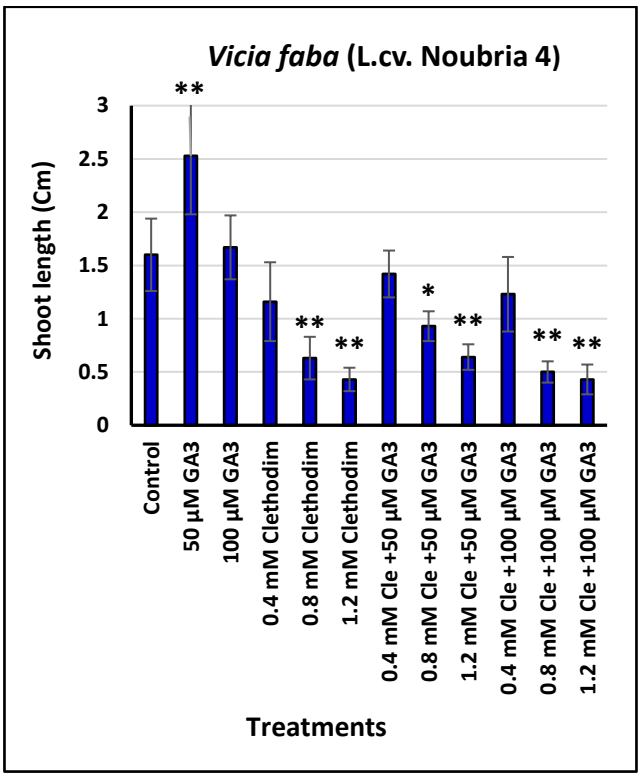

Figure 3: Effect of clethodim herbicide, GA_3, and combination of clethodim + GA_3 after one week of treatments on seedling shoot lengths (Cm) of *Vicia faba* (L.cv. Noubria 4).

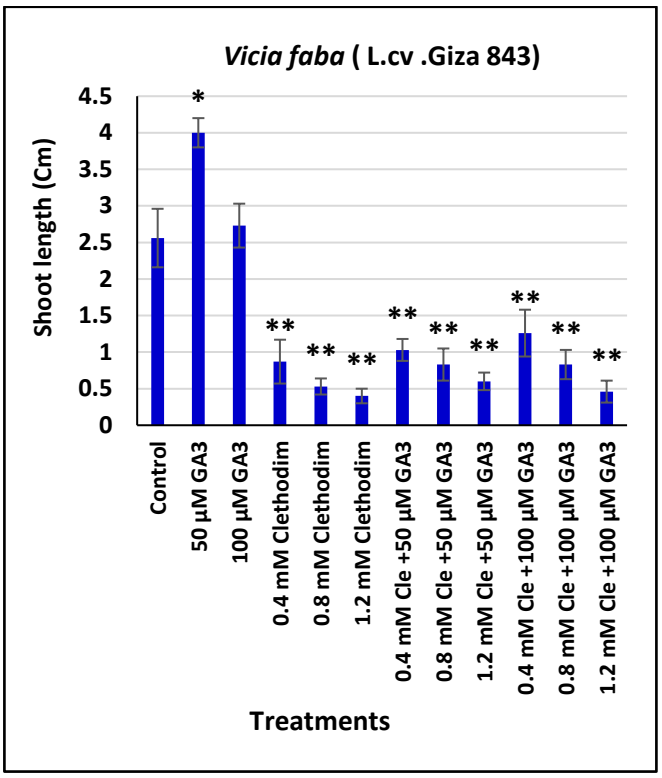

Figure 5: Effect of clethodim herbicide, GA_3, and combination of clethodim + GA_3 after one week of treatments on seedling shoot lengths (Cm) of *Vicia faba* (L.cv. Giza843).

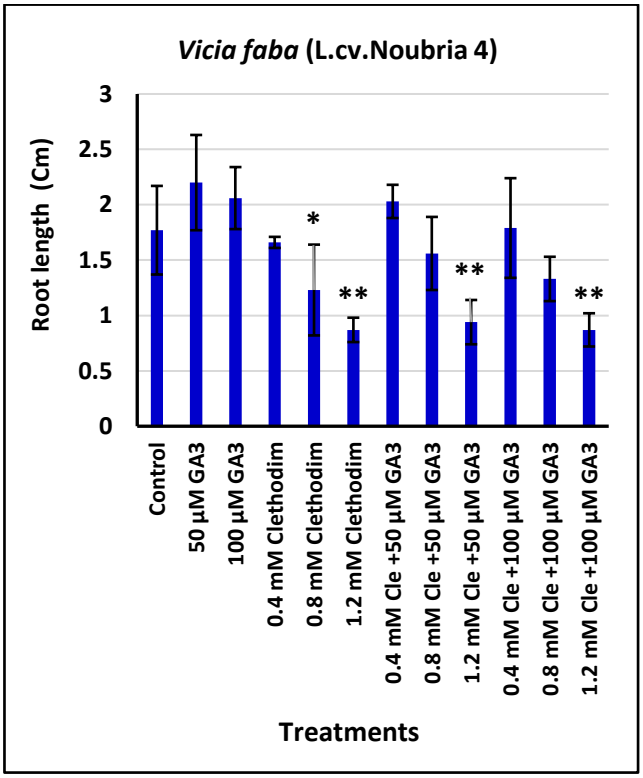

Figure 4: Effect of clethodim herbicide, GA_3, and combination of clethodim + GA_3 after one week of treatments on seedling root lengths (Cm) of *Vicia faba* (L.cv. Noubria 4).

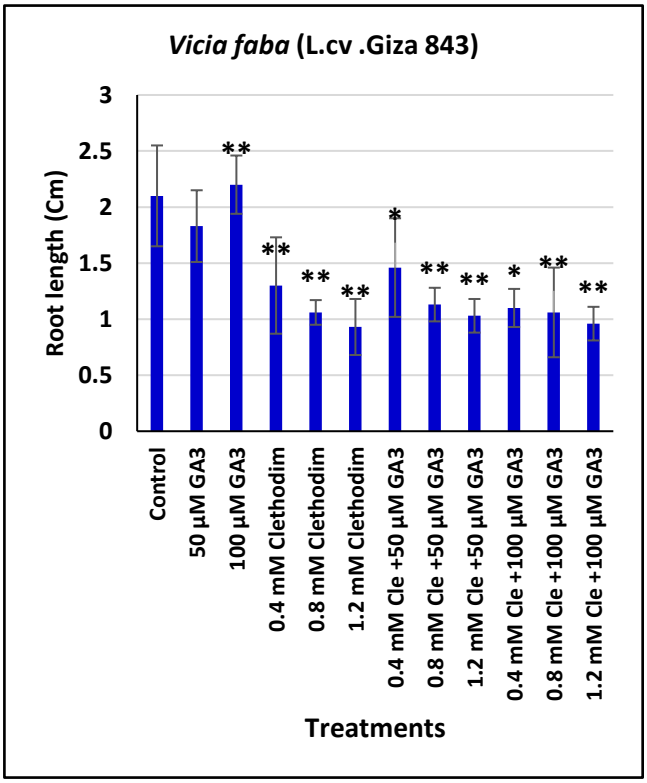

Figure 6: Effect of clethodim herbicide, GA_3, and combination of clethodim + GA_3 after one week of treatments on seedling root lengths (Cm) of *Vicia faba* (L.cv. Giza843).

Fig .7

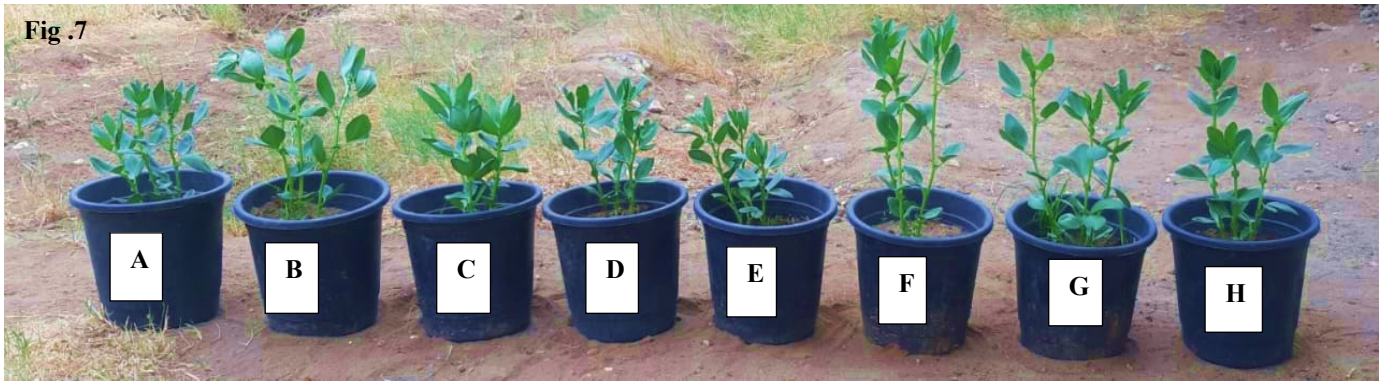

Figure 7: Photograph shows the growth of *Vicia faba* (L.cv. Noubria 4) treated and untreated with GA_3, clethodim and the combination of GA_3 + clethodim at vegetative stage.
A= control, B = 50 µM GA_3, C = 0.4 mM Clethodim, D = 0.8 mM Clethodim, E = 1.2 mM Clethodim, F = 0.4 mM Clethodim + GA_3, G = 0.8 mM Clethodim + GA_3, H =1.2 mM Clethodim+GA_3.

Fig .8

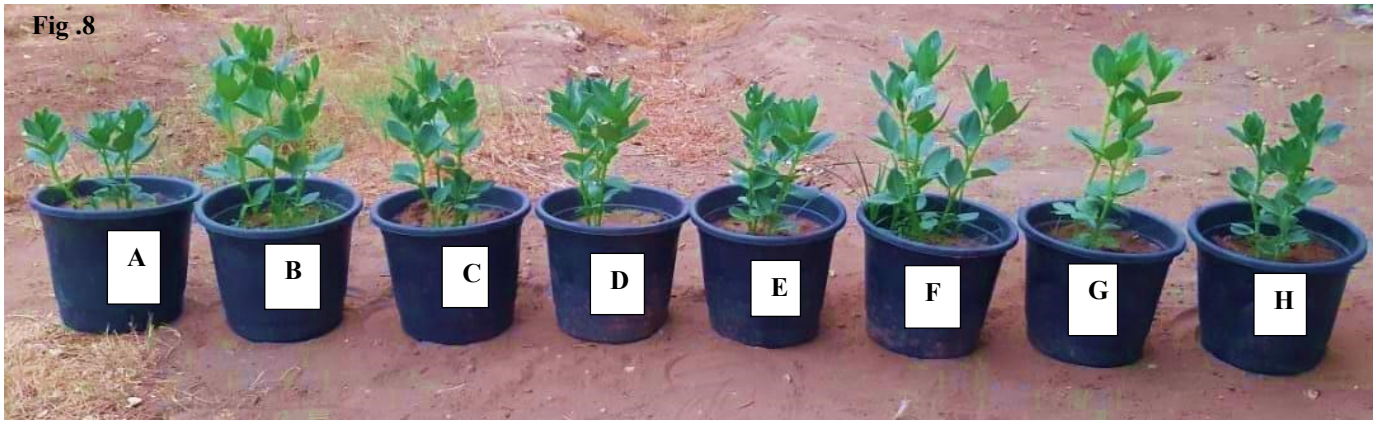

Figure 8: Photograph shows the growth of *Vicia faba* (L.cv. Giza 843) treated and untreated with GA_3 and clethodim and the combination of GA_3 + clethodim at vegetative stage.
A= control, B = 50 µM GA_3, C = 0.4 mM Clethodim, D = 0.8 mM Clethodim, E =1.2 mM Clethodim, F = 0.4 mM Clethodim + GA_3. G = 0.8 mM Clethodim + GA_3. H = 1.2 mM Clethodim + GA_3.

Table 2: Effect of GA_3, clethodim, and combination of clethodim + GA_3 treatments on shoot length, shoot fresh weight and shoot dry weight of *Vicia Faba* (L. cv. Noubria 4). The values are means of three replicates ± standard deviation (SD).

Treatments	Shoot length (cm)		Shoot F. Wt. (g)		Shoot D. Wt. (g)	
	M ± SD	%	M ± SD	%	M ± SD	%
Control	24.33 ± 0.76	100.00	2.14 ± 0.24	100.00	0.20 ± 0.03	100.00
50 µM GA_3	30.83** ± 2.25	126.71	3.26* ± 0.31	152.34	0.33* ± 0.09	165.00
0.4 mM Clethodim	23.13 ± 2.87	95.07	2.50 ± 0.62	116.82	0.24 ± 0.02	120.00
0.8 mM Clethodim	23.43 ± 0.60	96.30	1.75 ± 0.50	81.77	0.17 ± 0.03	85.00
1.2 mM Clethodim	19.16* ± 2.87	78.75	1.59 ± 0.15	74.30	0.17 ± 0.04	85.00
0.4 mM Cle +50 µM GA_3	30.56** ± 1.77	125.60	2.76 ± 0.40	129.13	0.30 ± 0.09	150.00
0.8 mM Cle +50 µM GA_3	28.36 ± 4.75	116.56	2.66 ± 0.48	124.29	0.27 ± 0.05	135.00
1.2 mM Cle +50 µM GA_3	27.00 ± 2.64	110.97	2.38 ± 0.76	111.21	0.22 ± 0.08	110.00

Statistical significance of differences compared to the control.
*** Significant at $P < 0.05$.** Significant at $P < 0.01$.**

Table 3: Effect of clethodim herbicide, GA_3, and combination of GA_3 + clethodim treatments on shoot length, shoot fresh weight, and shoot dry weight of *Vicia Faba* (L.cv. Giza 843). The values are means of three replicates ± standard deviation (SD).

Treatments	Shoot length (cm)		Shoot F. Wt. (g)		Shoot D. Wt. (g)	
	M ± SD	%	M ± SD	%	M ± SD	%
Control	24.30 ± 0.60	100.00	2.55 ± 0.68	100.00	0.25 ± 0.05	100.00
50 µM GA_3	31.90** ± 2.59	131.28	3.36** ± 0.18	131.76	0.36* ± 0.04	144.00
0.4 mM Clethodim	26.43 ± 1.60	108.76	2.63 ± 0.26	103.13	0.27 ± 0.06	108.00
0.8 mM Clethodim	27.53 ± 0.76	113.29	2.60 ± 0.54	101.96	0.25 ± 0.08	100.00
1.2 mM Clethodim	22.56 ± 2.70	92.83	2.33 ± 0.03	91.37	0.24 ± 0.02	96.00
0.4 mM Cle +50 µM GA_3	31.50** ± 2.78	129.63	3.22* ± 0.52	126.27	0.35* ± 0.07	140.00
0.8 mM Cle +50 µM GA_3	28.66* ± 2.75	117.94	2.76 ± 0.18	108.23	0.32 ± 0.03	128.00
1.2mM Cle +50 µM GA_3	23.43 ± 1.98	96.41	2.50 ± 0.45	98.03	0.25 ± 0.04	100.00

Table 4: Effect of clethodim herbicide, GA_3, and Clethodim + GA_3 treatments on fresh and dry weight (g/ plant) of *Vicia Faba* leaves (L.cv. Noubria 4 and Giza 843). The values are means of three replicates ± standard deviation (SD).

Treatments	Noubria 4. F. Wt.	Noubria 4 D. Wt.	Giza 843 F. Wt.	Giza 843 D. Wt.
	M ± SD	M ± SD	M ± SD	M ± SD
Control	3.66 ± 0.66	0.50 ± 0.07	3.94 ± 0.91	0.52 ± 0.05
50 µM GA_3	3.91 ± 0.41	0.56 ± 0.06	4.83 ± 0.44	0.59 ± 0.09
0.4 mM Clethodim	3.86 ± 0.13	0.48 ± 0.05	4.15 ± 0.52	0.55 ± 0.03
0.8 mM Clethodim	3.31 ± 0.77	0.42 ± 0.01	3.92 ± 0.70	0.53 ± 0.07
1.2 mM Clethodim	2.67 ± 0.49	0.39 ± 0.05	3.86 ± 0.86	0.50 ± 0.06
0.4mM Cle +50 µM GA_3	3.88 ± 0.52	0.50 ± 0.02	4.66 ± 0.05	0.59 ± 0.03
0.8mM Cle +50 µM GA_3	3.59 ± 0.49	0.50 ± 0.01	4.06 ± 0.14	0.57 ± 0.11
1.2mM Cle +50 µM GA_3	3.35 ± 0.24	0.47 ± 0.02	3.91 ± 0.92	0.55 ± 0.11

Table 5: Effect of GA_3, clethodim, and combination of GA_3 + clethodim treatments on pigments content of *Vicia Faba* leaves (L. cv. Noubria 4). The values are means of three replicates ± standard deviation (SD).

Treatments	*Chl a*	*Chl b*	*Carotenoids*	*Chl a/b* ratio	Total	%
	M ± SD	M ± SD	M ± SD			
Control	1.14 ± 0.01	0.40 ± 0.09	0.54 ± 0.06	2.97	2.06	100.00
50 µM GA_3	1.26** ± 0.01	0.49** ± 0.01	0.61** ± 0.07	2.55	2.37	115.04
0.4 mM Clethodim	1.05** ± 0.03	0.39 ± 0.05	0.51** ± 0.01	2.69	1.96	95.14
0.8 mM Clethodim	1.04** ± 0.03	0.39 ± 0.01	0.49 ± 0.06	2.62	1.95	94.66
1.2 mM Clethodim	0.95** ± 0.04	0.37 ± 0.02	0.46** ± 0.02	2.54	1.79	86.89
0.4mM Cle +50 µM GA_3	1.16 ± 0.01	0.43 ± 0.04	0.55 ± 0.13	2.67	2.15	104.85
0.8mM Cle +50 µM GA_3	1.15 ± 0.11	0.42 ± 0.03	0.54 ± 0.03	2.60	2.14	103.88
1.2mM Cle +50 µM GA_3	1.05** ± 0.03	0.38 ± 0.02	0.49 ± 0.05	2.85	1.95	94.66

Statistical significance of differences compared to the control.
*** Significant at $P < 0.05$.** Significant at $P < 0.01$.**

Table 6: Effect of clethodim herbicide, GA_3, and combination of GA_3 + clethodim treatments on pigments content of *Vicia Faba* leaves (L.cv. Giza 843). The values are means of three replicates ± standard deviation (SD).

Treatments	*Chl a*	*Chl b*	*Carotenoids*	*Chl a/b* ratio	Total	%
	M ± SD	M ± SD	M ± SD			
Control	1.52 ± 0.09	0.54 ± 0.05	0.66 ± 0.03	2.71	2.73	100.00
50 µM GA_3	1.73** ± 0.05	0.58 ± 0.04	0.73 ± 0.08	3.04	3.04	111.35
0.4 mM Clethodim	1.22** ± 0.08	0.48 ± 0.03	0.57* ± 0.04	2.27	2.27	83.15
0.8 mM Clethodim	1.12** ± 0.06	0.42** ± 0.03	0.52** ± 0.02	2.06	2.06	75.45
1.2 mM Clethodim	1.00** ± 0.03	0.43** ± 0.05	0.53** ± 0.06	1.96	1.96	71.94
0.4mM Cle +50 µM GA_3	1.39** ± 0.07	0.53 ± 0.04	0.64 ± 0.03	2.56	2.56	93.77
0.8mM Cle +50 µM GA_3	1.37** ± 0.03	0.52 ± 0.01	0.63 ± 0.02	2.52	2.52	92.30
1.2mM Cle +50 µM GA_3	1.22** ± 0.05	0.47 ± 0.06	0.58 ± 0.07	2.27	2.27	83.15

Statistical significance of differences compared to the control.
*** Significant at $P < 0.05$.** Significant at $P < 0.01$.**

3.2. Growth parameters

Weeds are competed with the crop for the source of plant growth requirements (water, nutrients and light), causing a reduction in the yield of crops [48,49]. So, herbicides are considered a one of the effective applications to control weeds that grow with crops. However, the excessive application of herbicides can affect all stages of plant growth and development, which leads to yield loss [50]. The high concentrations of herbicide suppress seed germination, reduce shoot and root growth, and disrupt physiological functions and induces oxidative stress [51].

On the other hand, seed germination is controlled by a number of mechanisms and is necessary for the growth and development of the seed embryo [52]. One of these mechanisms is Plant hormones which affect different plant activities including seed dormancy and germination [53]. Our results of fresh and dry weight of leaves, leaves area, shoot length, shoot fresh and dry weights of faba plants decreased significantly in response to clethodim stress treatment, especially at high concentrations compared to control plants (Tables 2-4; Fig. 9). GA_3 (50 µM) spraying enhanced fresh and dry weight of leaves, leaf area, shoot length, shoot fresh and dry weights of faba plants compared to control plants or to that growing under clethodim stress conditions.

In this context, application of 50 µM GA_3 significantly increased shoot length, shoot fresh weight and shoot dry weight of *Vicia faba* (L. cv. Noubria 4) by 26.71, 52.34 and 65%, respectively, compared to the control (Table 2). The shoot length as well as the shoot fresh and dry weights of clethodim doses (0.4 and 0.8mM) more or less, unaffected significantly. While in response to 1.2 mM clethodim, the shoot length significantly decreased by 21.75%. Due to the combination effect of 0.4 mM clethodim + 50 µM GA_3, the shoot length, shoot fresh weight and shoot dry weight of Noubria 4 cultivar significantly increased by 25.60, 29.13 and 50.00%, respectively, compared to those of the control.

Also, the combination of 50 µM GA_3 with 0.8 or 1.2 mM clethodim caused an insignificant increase in the previous parameters (shoot length, shoot fresh and dry weights). The results of shoot length, shoot fresh weight and shoot dry weight of *Vicia faba* (L. cv. Giza 843) are shown in table 3. Spraying of 50 µM GA_3 increased shoot length, shoot fresh weight and shoot dry weight of *Vicia faba* (L. cv. Giza 843) by 31.28, 31.76 and 44.00%, respectively, compared to those of the control (Table 3). In response to 0.4 and 0.8 mM clethodim, shoot length, shoot fresh weight and shoot dry weight slightly increased, while at 1.2 mM clethodim decreased compared to the control. Due to the combinations of 50 µM GA_3 with o.4 and 0.8 mM clethodim, the Shoot length, shoot fresh weight and shoot dry weight of *Vicia faba* (L. cv. Giza 843), in most cases, significantly increased compared to the control. In response to the combination of 50 µM GA_3 + 1.2 mM clethodim, the value of shoot length and shoot fresh weight decreased compared to the control, while the shoot dry weight had the same value of the control (100%).

3.3. Photosynthetic pigments

Generally, the photosynthetic pigment contents (*chl a*, *chl b* and carotenoids) of *Vicia Faba* leaves (L.cv. Giza 843) are higher than those of (L.cv. Noubria 4.). Spraying of 50 µM GA_3 increased *chl a*, *chl b* and carotenoids of both cultivars compared to their control (Tables 5 and 6). Comparing between the two cultivars, the pigments contents (chl *a*, *chl b* and car) of Giza 843 are much more affected by clethodim treatments than those of Noubria 4, especially chlorophyll *a*. In details, the photosynthetic pigment contents (chl *a*, *chl b* and car) of the two cultivars decreased in response to various doses of clethodim herbicide used compared to the control. Photosynthetic pigments decreased with increasing clethodim doses.

Changes in photosynthetic pigment contents are responsible for stress – induced color changes in leaves [54-57], or might be occurred as a response of direct pigment degradation and suppression biosynthesis of Chls and car.

At low dose of clethodim 0.4 mM, Chl *a*, chl *b* and carotenoids of Noubria 4 cultivar decreased by 7.89, 2.5 and 5.56%, respectively. On the other hand, Giza 843, the chl *a*, chl *b* and carotenoids of Giza 843 decreased by 19.74, 11.11 and 13.64% compared to those of the control plants, respectively.

At high dose 1.2 mM of clethodim, the chl *a*, chl *b* and carotenoids decreased significantly by 16.67, 7.5, 14.81%, respectively, but in case of Giza 843 cultivar the chl *a*, chl *b* and carotenoids decreased by 34.21, 20.37 and 19.7% in comparison with that of the control, respectively. Herbicides affect physiological processes of plants, specifically their photosynthetic machinery, changes in leaf color or bleaching due to stress is mostly related to alterations in photosynthetic pigment contents disorganization of chloroplast thylakoids and inhibition of electron transport of Photophosphorylation [54,58]. In this context, clethodim lowered the carotene (Cars) content of leaves. ACCase enzyme is in part responsible for synthesizing precursors of carotene and the phytol group for Chl and thus, inhibition of the ACCase enzyme by clethodim caused disorganization in structure and function of chlorophyll molecules and decrease in pigments contents.

Moreover, carotene (Cars) (known to be important quenchers of highly reactive triplet chlorophyll or singlet oxygen) protect Chls from photodamage [59-61]. External application of GA_3 has been shown to delay the breakdown of proteins and chlorophyll, reduce the amount of malondialdehyde (MDA), and delay plant senescence [62]. Application of GA_3 three days prior clethodim treatment caused an increase of all pigment fractions. leaves pigment contents treated with GA_3 before clethodim application are quite similar to the control plants (Tables 5 and 6). In this respect, Mark et al. [63] reported that GA_3 maintained the metabolism of redox homeostasis inside *Hordeum vulgare* and Himalaya grains.

4. Conclusion

Clethodim herbicide significantly inhibited seed germination and negatively impacted growth parameters and leaf chlorophyll contents of faba bean cultivars. The plant hormone gibberellins are necessary for seed germination and plant growth. Moreover, gibberellin application mitigated the phytotoxic effects of clethodim by increasing seed germination, plant growth and pigment contents. In contrast, the study showed that the faba bean is seriously affected by clethodim treatments, especially at the seedling stage. GA_3 treatment improved the seed germination, growth, and pigment contents of faba bean plants exposed to the clethodim stress. Further research is needed to fully elucidate the mechanisms involved and develop effective strategies to minimize herbicide injury on this economically important crop.

CRediT authorship contribution statement:

Supervision and conceptualization, K.A. Fayez, F. A. Faheed and D. E. M. Radwan; Methodology, investigation and data analysis, A. Edrees; Data duration and writing the manuscript, A. Edrees, K. A. Fayez, F. A. Faheed and D. E. M. Radwn; Revised the manuscript, K. A. Fayez; All authors have read and agreed to the published version of the manuscript.

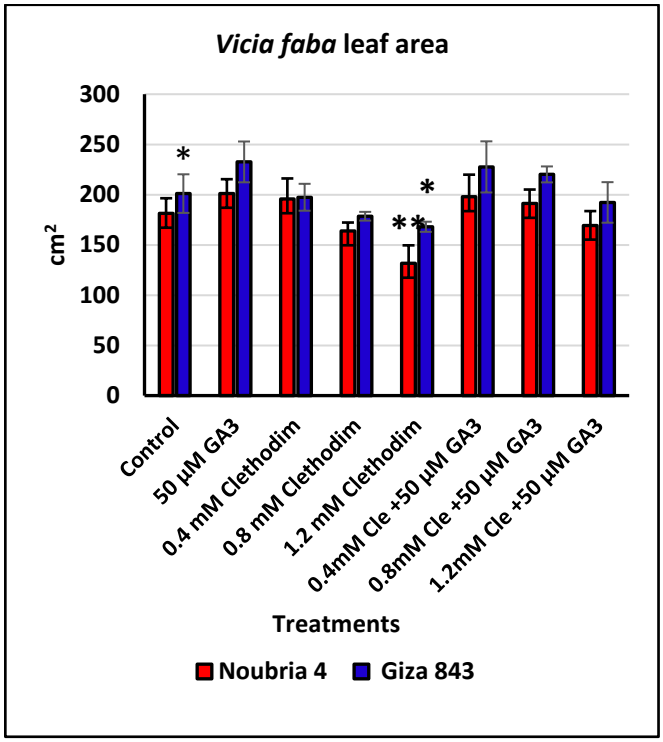

Figure 9: Effect of clethodim herbicide, GA_3, and combination of GA_3 + clethodim treatments on leaf area of *Vicia Faba* leaves (L.cv. Noubria 4 and Giza 843).

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Data availability statement:

The data used to support the findings of this study are available from the corresponding author upon request.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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