



Comparative Study between Chemical and Allelochemical Treatments on Faba Bean Vegetation Growth and Broomrape Control



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A FABA BEAN (*Vicia faba* L.) is one of the most significant field crops due to its great nutritional value for humans and animals. However, they were attacked from the roots by parasitic weed plants like broomrape (*Orobanche crenata* F.), which destroys their host due to their rapid development and deficiency in chlorophyll, a necessary component for photosynthesis. This study was carried out during winter seasons 2020/2021 and 2022/2023 at naturally infested field in Qaha Research Station, Plant Protection Research Institute, Qalyubia Governorate, Egypt. The goal of this study was to apply an alternative strategy of crop protection against broomrape weed control by two chemical treatments including indole acetic acid and salicylic acid to enhance systemic defense responses of faba bean plants. Allelochemical treatments by using trap crops as flax and radish with the main plant that stimulate suicidal broomrape germination in their fields. The obtained results of chemical control illustrated that biochemical assay conducted an increase in total chlorophyll, total phenols and peroxidase activity during the two seasons for IAA and SA respectively. Compared with the allelochemical control they conducted, an increase in total chlorophyll, total phenols for flax and radish were observed. Increasing total protein, prolin and peroxidase enzyme for flax during the two seasons were also observed. Furthermore, all treatments could be used as an integrated control strategies to increase faba bean growth vegetation, decrease the number of spikes per m² and spikes dry weight per m² by increasing of broomrape weed control during both seasons.

Keywords: Allelochemical control, Chemical control, Faba bean and Broomrape weed control.

Introduction

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt (Mahmoud and Abd El-Fatah 2020), which is also an important break crop in intensive cereal systems and a source of plant protein (Abou-Khater *et al.*, 2022; Ghareeb *et al.*, 2023). (FAO, 2019) reported that in the last five years, around 113.810 fed. Faba beans were produced in Egypt, with an average yield of 9.2 ardbbs/feddan. To keep up with Egypt's rising need for faba bean, productivity and overall production must be increased. Enhancing crop breeding and agronomy research could help achieve this (FAO, 2019; Ghareeb *et al.*, 2023). All over the world, faba bean (*Vicia faba* L.), a highly nutritious, cool-tolerant legume, are widely grown. France, China, Ethiopia, the United Kingdom and Australia are the top producers of faba bean. The production of various foods enhanced with biomolecules that

have better functioning, nutritional value, and health benefits caused rising interest in the health and nutritional benefits of faba bean in recent years. Rich in lysine-rich proteins, carbohydrates, minerals, vitamins, and a variety of bioactive substances, faba beans are a great source of nutrition. Furthermore, it is a good source of l-3,4-dihydroxyphenylalanine (L-DOPA), a precursor to dopamine that may be used to treat Parkinson's disease (Dhull *et al.*, 2022). Faba bean seeds are sown in the fall or early winter and harvested in the late spring or early summer in regions with Mediterranean climates, which feature moderate winters and dry, warm summers. While winter-hardy cultivars can be sown in the fall, plantings in colder climates are delayed until the spring to prevent frost damage (Mínguez and Rubiales, 2020).

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Broomrapes are a major source of productivity loss in Mediterranean and Middle Eastern farming systems (Rubiales, 2023). The most common and destructive kind of broomrape is called crenate broomrape (*Orobancha crenata* Forsk.). *O. crenata* is not a recent problem in legume farming; in fact, it was first mentioned by ancient Roman writers. Since then, there hasn't been much progress in managing it; unfortunately, the problem is expanding to previously think to be infestation-free areas, including outside the Mediterranean region, to the north of Europe, to the south of Africa, and the east of Asia. This could become worse as a result of climate change (Rubiales, 2020; Negewo *et al.*, 2022). The attributes of broomrape are harmed by its status as an obligatory parasite. Because of how significantly this parasite reduces productivity, farmers can be obliged to give up their farms. Scientists present a significant problem in developing effective management techniques because the majority of contamination and pathogenicity phases occur underground at various stages during the parasite's life cycle, such as during root penetration before attachment to the host or during phloem attachment. Sadly, no control mechanism (agricultural, chemical, mechanical, etc.) has proven successful until now (Darvishzadeh and Khalifani, 2023)

Applying control techniques that are sufficiently selective for controlling these parasitic weeds without damaging crops is just as challenging as using chemical herbicides, which contaminate soil and water, have negative effects on beneficial organisms, and reduce the nutritional value of the crop that is being farmed. Chemicals that are left over after the breakdown of herbicides can occasionally remain extremely hazardous to human health and to the environment. In order to reduce parasitic weeds and manage them in nutritious crops, nearly all of the evaluations focused on research that has not yet been done on significant alternatives to herbicides, such as biological control, natural products, agricultural practices, and cropping systems (El-Dabaa *et al.*, 2022). An alternate crop protection strategy that would successfully protect the environment would be to treat plants with plant growth promoters such as gibberellin (GA3), humic acid (HA) and cytokinen (Fouda, 2017), salicylic acid (SA) or indole acetic acid (IAA) that induced systemic acquired resistance against *Orobancha Crenata*. (Briache *et al.*, 2020; El-Mergawi *et al.*, 2022). In addition many crops, including fenugreek, turnip, flax, and coriander, were employed as catch-or-trap plants to manage broomrape (El-Dabaa *et al.*, 2022; Zeid and Komeil, 2019). Actually, integrated management approaches are the ideal way to use that technique. The aim of this study was to use alternative herbicide techniques to improve plant health and to

study their effect against parasitic weeds (broomrape) under infested field conditions.

Materials and Methods

2.1. Chemical materials

- a. Indole-3-acetic acid 98%, (1*H*-Indol-3-yl)acetic acid), (175.184 g/mol. was supplied by S D Fine Chem Limited)
- b. Salicylic acid (2-hydroxy benzoic acid), 138.12 g/mol. was supplied by Sigma Aldrich Company.

2.2. Plant materials

- a. Faba bean seeds, Nobarria 1 as susceptible genotype and Giza 843 as tolerant genotype (Zakaria *et al.*, 2015; Abd El-Aty *et al.*, 2016) were obtained from field crops research institute-Agricultural Research Center- Giza-Egypt.
- b. Flax (*Linum usitatissimum* L.) were obtained from Fiber crops research institute- Agricultural Research Center- Giza- Egypt.
- c. Radish (*Raphanus sativus* L.) were obtained from Egyptian seeds oil and Chemical Company, El-Korma Cairo- Egypt.

2.3. Field Experimental

In naturally *Orobancha crenata* infested soil at the Qaha Research Station, Plant Protection Research Institute, Qalyubia Governorate, Egypt, the studies were conducted in the winter seasons 2020–2021 and 2022–2023. Four replications of each treatment were conducted in a Randomized complete design for the field studies. The experimental unit consisted of three rows, 0.6 m wide and 3.0 m long, making an area of 5.4 m². Hills were 25 cm apart and contained whole cold stored locally produced faba bean seeds. The seeds of faba beans Nobarria 1 and Giza 843 were sowed in hills on either side of the ridge. Three and six weeks after sowing, hand hoeing was used to control both narrow and broadleaved weeds. During the growing season, irrigation was used to keep the soil nearly at its field capacity. Ammonia nitrate (33%) a fertilizer was added to the field during the experiment. Before planting, the superphosphate fertilizer was also added, and the urea was divided into two equal dosages. After planting, the first dose was taken after 15 days, and the second dose was added 30 days later. All other agricultural practices for faba beans production were carried out as common in this area. After 120 days from sowing the harvesting stage began in both seasons. The weed control treatments were randomly arranged in the plots as follows:

1. Chemical control (plant growth regulators):

Regulators of plant growth, such as salicylic acid (0.1 mM) and indole acetic acid (0.1 mM), were used as presoaking treatments for six hours, after which they were dried outside till the following day for sowing.

2. Allelochemicals control

Every hole was seeded with faba bean with flax, or radish seeds as trapping crops. After three of the plants were thinned, the remaining plants were stepped down, keeping the root system of the plants in the soil.

Tables 1 and 2 show the classification of the soil as well as some of its physical, chemical, and organic matter content (O.M.).

Table (1): Chemical Characteristics of Soil.

Chemical Characteristics of Soil		
	Qalyubia Governorate	Optimum Limit
PH	8.3	7+0.5
*EC (mmhos/cm)	0.42	1_2
CaCO ₃ (%)	2.4	7_10
Soluble Cations (meq/L)		
Ca ⁺⁺	2.8	0.35
Mg ⁺⁺	0.6	0.35
Na ⁺	0.6	0.35
K ⁺	0.2	—
Soluble anions (meq/L)		
CO ₃ ⁻²	—	—
HCO ₃ ⁻	0.4	0.35
Cl ⁻	1.5	0.35
SO ₄ ⁻²	2.3	0.35
Major Elements (ppm)		
N	30	40-60
P	42	15-25
K	480	250
Minor Elements (ppm)		
Fe	11	4_6
Cu	0.19	1_1.5
Zn	3	1.2_1.2
Mn	6.1	1.8_2

*EC: Electrical Conductivity

Table (2): Particle Size Distribution of Soil Samples.

Collected Soil	Clay %	Silt %	Sand %	Textural Class
Qalyubia Governorate	34	8.08	57.92	Sandy Loam

2.4. Determination of leaves photosynthetic pigments (Chlorophyll and carotenoids contents)

After 42 days from planting, fresh leaves were randomly selected from the third node of the main

shoot for each of the various treatments from Faba bean plants and then sent immediately to the lab. for pigment determination. The protocol described by (Hiscox and Israelstam, 1979) was followed.

One test tube containing ten milligrams of leaf tissue fraction received five milliliters of dimethyl sulphoxide (DMSO). Carotenoids and chlorophyll were extracted into the solvent without grinding after being incubated at 65 °C for the entire night. After filtration, 10 milliliters of the extracted liquid was added to a graduated tube with DMSO. Absorbance for carotenoids and chlorophyll was measured with a spectrophotometer at 470, 644 and 662 nm, respectively. Calculating total chlorophyll, chlorophyll a, and chlorophyll b was carried out by the Arnon equation (1949) and (Cañal *et al.*, 1985) was used for the calculation of carotenoids.

Arnon equation:

Chlorophyll a (Chl. a) = 12.7 x O.D 662 – 2.69 x O.D 644 mg/l

Chlorophyll b (Chl. b) = 22.9 x O.D 644 – 4.68 x O.D 662 mg/l

Total Chlorophyll (Chl. a+b) = 20.2 x O.D 644 + 8.02 x O.D 662 mg/l

Cañal equation:

$$\text{Carotenoids (mg /l)} = \frac{A_{470} - 1.28(\text{Chl.a mg/l}) + 56.7(\text{Chl.b mg/l})}{256 \times 0.906}$$

2.5. Biochemical analysis of root samples

After sixty days from germination, the roots of Faba bean plants treated with plant growth regulators and with trapping crops were harvested. The samples were sent directly to the lab for study of their biochemical components (Al-Wakeel *et al.*, 2013; Abdelaal *et al.*, 2022; Briache *et al.*, 2023)

Preparation of root samples

0.1–0.4 g root samples were weighed, stored at -20 °C, and processed in accordance to (Ni *et al.*, 2001). The enzymes from the frozen plant samples were extracted using a cold potassium phosphate buffer (0.1M, pH 7.0) that contained 1% (w/v) polyvinylpyrrolidone and 1% (v/v) Triton X-100. For each sample, two milliliters of the extraction buffer were used. A 1.5 ml of the extract was centrifuged with 10,000 x g of force for 10 minutes at 4°C. The supernatant was frozen immediately to make sure it could be used in the following enzyme activity tests.

a. Determination of Peroxidase activity

The method used to measure peroxidase activity was (Hammerschmidt *et al.*, 1982). A sample cuvette for a spectrophotometer was filled with 1.5 ml of pyrogallol (0.05 M) and 100 µl of enzyme extract. The measurements were zero at 420 nm.

The reaction was initiated by adding 100 µl of 1% hydrogen peroxide to the sample cuvette. The expression for the enzyme activity was the change in absorbance/min/g sample.

b. Determination of Total proteins

Total proteins were determined by the method of (Bradford, 1976). To prepare the protein reagent, 50 milliliters of ethanol 95% were used to dissolve 100 milligrams of Coomassie Brilliant blue G-250. To this solution, 100 ml of phosphoric acid (85% W/V) was added. The diluted solution's maximum volume was one liter. 50µl of the sample solution (or 50µl of serial concentrations ranging from 10 to 100µg of bovine serum albumin) was used to generate the standard curve. The test tube's capacity was reduced to 1 ml by adding phosphate buffer (0.1M, pH 6.6). Five milliliters of the protein reagent were added to the test tube, and then the contents were mixed by vortex. In comparison to a blank made from 1 milliliter of phosphate buffer and 5 milliliters of protein reagent, the absorbance at 595 nm was measured after 2 minutes and before 1 hour.

c. Determination of Amino Acid Proline

The procedure defined in (Sadasivam and Manickam, 1991) was used to estimate proline. Homogenization in two milliliters of 3% aqueous sulfosalicylic acid was used to extract 0.1 g root samples. Following homogenate filtration through Whatman No. 2 filter paper, 2 ml of the filtrate was added to a test tube along with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. Then, it was heated in a boiling water bath for an hour. Finally, placed in an ice bath to stop the reaction. After mixing the reaction mixture for 20 to 30 seconds with 4 milliliters of toluene, toluene layer was removed and allowed to cool to room temperature. The red color intensity was eventually measured at 520 nm. A standard curve was created by making a series of standards using pure proline in the same way.

d. Determination of Total carbohydrate:

The total carbohydrates in the sample's acid extract were measured using phenol-sulphuric acid reaction, which was created by (Dubois *et al.*, 1956). Total carbohydrates were removed in accordance with the guidelines provided by (Crompton and Birt, 1967). Five milliliters of 0.3N HClO₄ were used to homogenize one gram of the material for one minute at 0°C. The homogenate was kept on ice for ten more minutes. It was

centrifuged for three minutes at 2000 rpm. in ice-cold HClO_4 (5 ml) and then redispersed and centrifuged twice more to remove insoluble debris. The acid extract was made by combining three supernatants. One hundred microliter of the acid extract was placed within a colorimetric tube together with 0.5 milliliters of 20% w/v phenol. Next, a rapid addition of 5 ml of concentrated sulfuric acid was made while shaking. After 10 minutes of standing, the tubes were shaken and submerged in a water bath set at 25 to 30 °C for another 10 to 20 minutes. To prepare the blanks, distilled water was used in place of the sugar solution. The unique yellow-orange color's absorbance is measured at 490 nm in respect to a blank. The total carbohydrate formula is equal to μg glucose per gram fresh weight.

e. Determination of Total phenolic content

The extraction procedure followed the guidelines provided by (Kähkönen *et al.*, 1999). Using an electric homogenizer, 20 milliliters of 80% aqueous methanol were extracted from five grams of grounded plant seedlings for five minutes. Following a 10-minute, 3,000 rpm centrifugation of the samples, the mixed extracts were transferred into tiny conical flasks that had been previously weighed. Lower pressure was used to extract the methanol. After the solid residue (crude extract) was weighed, 5 milliliters of hot water were added to dissolve it. To determine the extracts' total phenolic content, (Singelton and Rossi, 1965) modified the Folin-Ciocalteu method. One milliliter of Folin-Ciocalteu reagent and 0.8 milliliter of sodium carbonate (7.5%) were added to 200 μl of plant extracts in test tubes. The tubes were mixed after standing for thirty minutes. The absorption at 760 nm was measured using a blank that was devoid of any material other than the sample. Using a standard gallic acid stander (5 %), the total phenolic content was calculated as mg gallic acid per g dry weight of the original sample (mg GA/g dw).

2.6. Morphological parameters of faba bean steam

Sixty days after planting, measurement of plant height (cm), fresh and dry weight of plants (El-Mergawi *et al.*, 2022)

2.7. Broomrape samples

Four months after planting, evaluation of the effects of different treatments on *O. crenata* infestation was conducted (Briache *et al.*, 2023), five guarded broomrape spikes were selected randomly from

each subplot at harvest so as to determine the number and dry weight (g) of broomrape spikes m^{-2} (Dawood *et al.*, 2022; Kenapar *et al.*, 2023). Broomrape was dried for 48 hrs at 70° C using a forced air oven before its dry weight was measured.

2.8. Statistical analysis

Statistical analysis of all data was carried out using the "SPSS" program by ANOVA one way method.

Results and Discussion

3.1 Chemical control as plant growth regulators control

Table (3) showed the effect of growth regulators used, IAA and SA on pigments contents of faba bean after 45 days from planting during 2020/2021-2022/2023 seasons. In the first season both IAA and SA used revealed greater carotene, chlorophyll a and chlorophyll b content compared to control (0.067, 1.590 and 0.309 mg/g f.w.) for IAA and (0.062, 1.451 and 0.287 mg/g f.w.) for SA, while both of them showed lower carotene, chlorophyll a and chlorophyll b content than Giza 843 that showed (0.071, 1.760 and 0.326 mg/g f.w.). The results appeared clear in the total chlorophyll amount. The total chlorophyll amounts either in case of IAA and SA were higher than that of control (1.899 and 1.738 mg/g f.w.) but were lower than that of Giza 843 (2.086 mg/g f.w.). The least significant difference was 0.43 and 0.35 for chlorophyll a and total chlorophyll content in the first season respectively.

In the second season the obtained results declared that IAA increased the contents of all pigments under study carotene, chlorophyll a and chlorophyll b compared to control (0.083, 0.956 and 0.359 mg/g f.w.), while it showed little lower amounts compared to the Giza 843 that showed (0.094, 1.080 and 0.405 mg/g f.w.). SA affected carotene content by relatively little lower amount than Giza 843. Also, it reduces the amount of chlorophyll b (0.256 mg/g f.w.) than both control and Giza 843 used (0.263 and 0.405 mg/g f.w.), although it showed little higher amount of chlorophyll a (0.721 mg/g f.w.) than control only (0.554 mg/g f.w.) and little decrease than Giza 843 (1.080 mg/g f.w.). Giza 843 showed the highest total chlorophyll amount (1.486 mg/g f.w.), followed by IAA (1.316 mg/g f.w.), SA (0.978 mg/g f.w.) compared to control that showed the lowest total chlorophyll content. The least significant difference was 0.02, 0.36, 0.10 and 0.34 for carotene, chlorophyll a, b and total chlorophyll content in the second season respectively, according to (Zakaria *et al.*, 2015; El-Awadi, 2017; karamany *et al.*, 2019; Briache *et al.*, 2020).

Table 3: Effect of IAA and SA treatments on pigments content of faba bean plant (*Vicia Faba L.*) after 45 days from planting during seasons 2020/2021- 2022/2023.

Treatment	2020/2021				2022/2023			
	Carotene (mg/g f.w.)	Chlorophyll a (mg/g f.w.)	Chlorophyll b (mg/g f.w.)	Total Chlorophyll (mg/g f.w.)	Carotene (mg/g f.w.)	Chlorophyll a (mg/g f.w.)	Chlorophyll b (mg/g f.w.)	Total Chlorophyll (mg/g f.w.)
Control	0.061 ^a ± 0.008	1.326 ^b ± 0.234	0.278 ^a ± 0.034	1.604 ^b ± 0.249	0.062 ^b ± 0.009	0.554 ^c ± 0.116	0.263 ^b ± 0.039	0.818 ^b ± 0.154
Giza 843	0.071 ^a ± 0.011	1.760 ^a ± 0.127	0.326 ^a ± 0.045	2.086 ^a ± 0.140	0.094 ^a ± 0.007	1.080 ^a ± 0.166	0.405 ^a ± 0.024	1.486 ^a ± 0.147
IAA	0.067 ^a ± 0.010	1.590 ^{ab} ± 0.167	0.309 ^a ± 0.040	1.899 ^{ab} ± 0.177	0.083 ^a ± 0.002	0.956 ^{ab} ± 0.129	0.359 ^a ± 0.006	1.316 ^a ± 0.125
SA	0.062 ^a ± 0.007	1.451 ^{ab} ± 0.113	0.287 ^a ± 0.030	1.738 ^{ab} ± 0.135	0.059 ^b ± 0.007	0.721 ^{bc} ± 0.113	0.256 ^b ± 0.032	0.978 ^b ± 0.140
LSD	_____	0.43	_____	0.35	0.02	0.36	0.1	0.34

Values are presented as mean ± SD of three replications. Data in column followed by different letters are significantly different at the 0.05 levels by Duncan's test.

The effect of growth regulators on chemical parameter of faba bean fresh roots were studied during two successive seasons 2020/2021 and 2022/2023 after sixty days from planting. Table (4) showed the effect of IAA and SA on total protein by mg/g root fresh weight. During 2020/2021 IAA and SA showed relatively the same total protein content as control in the first season, but during the second season of treatment IAA and SA showed higher total protein content in roots fresh weight (4.37 and 9.07 mg/g f.w.) compared to control (4.00 mg/g f.w.) respectively. Giza 843 showed the highest total protein content (6.927 and 12.93) compared by either plant growth regulators (2.11 and 4.37 mg/g f.w.) for IAA and (2.28 and 9.07 mg/g f.w.) for SA or control (2.43 and 4.00 mg/g f.w.) during the two seasons of respectively. The least significant difference was 4.5 and 3.87 during both seasons respectively.

For proline determined in µg/g root fresh weight, SA showed higher amounts during both seasons of study (10.93 and 17.67 µg/g f.w.) compared to control (9.83 and 13.67 µg/g f.w.) during 2020/2021 and 2022/2023 respectively. As in case of total protein, Giza 843 showed the highest proline content compared to control (17.07 and 22.33 µg/g f.w.) and other treatments under study during both seasons of the study respectively, these results agreed with (Al-wakeel *et al.*, 2013) because proline is one of the metabolic products that frequently accumulate in response to variety of environmental stresses. Their accumulation could

be attributed to decreasing the osmotic potential of host root to restrict the movement of water to broomrape. Also, proline can be considered one of important factors involved in plant defense mechanisms by formation of rigid cross-links between cellulose of secondary cell wall and hydroxyproline-rich glycoproteins. The least significant difference was 2.8 and 4.67 during both seasons respectively.

For peroxidase enzyme activity (POX), Giza 843 showed also the highest enzyme content (15.33 and 18.27) compared by the plant growth regulators (9.28 and 13.27) for IAA and (9.64 and 17.07) for SA or control (9.73 and 11.97) during both seasons of study respectively. The results obtained showed clearly that salicylic acid showed remarkable effect on peroxidase enzyme activity especially in the second season compared to control. The least significant difference was 5.6 and 3.8 during both seasons respectively. The results that agreed also with (Katoch *et al.*, 2005; Al-wakeel *et al.*, 2013) because systemic acquired resistance (SAR), which is induced by IAA and SA and is an endogenous plant growth regulator that regulates multiple physiological functions under biotic and abiotic stress, activates a variety of defense compounds, including phenolic acid, coumarins, flavonoids, and lignin. Furthermore, increased peroxidase activity results in hypersensitivity reaction, phenolic cross-linking with glycoproteins, and lignification of the cell wall.

Table 4: Effect of IAA and SA treatments on some chemical parameters of faba bean fresh roots (*Vicia Faba L.*) after 60 days from planting during seasons 2020/2021- 2022/2023.

Treatment	Total Protein (mg/g f.w.)		Proline (µg/g f. w.)		Peroxidase (ΔO.D./min/g f. w.)	
	2020/2021	2022/2023	2020/2021	2022/2023	2020/2021	2022/2023
Control	2.427 ^b ± 0.27	4.00 ^c ± 0.10	9.833 ^b ± 0.65	13.67 ^b ± 0.76	9.73 ^b ± 0.45	11.97 ^b ± 0.42
Giza 843	6.927 ^a ± 0.99	12.93 ^a ± 1.12	17.067 ^a ± 1.01	22.33 ^a ± 4.04	15.33 ^a ± 1.04	18.27 ^a ± 0.64
IAA	2.113 ^b ± 0.19	4.37 ^c ± 0.45	7.00 ^c ± 0.85	14.00 ^b ± 1.00	9.277 ^b ± 0.21	13.27 ^b ± 0.38
SA	2.280 ^b ± 0.19	9.07 ^b ± 0.60	10.933 ^b ± 0.61	17.67 ^b ± 1.53	9.643 ^b ± 0.32	17.07 ^a ± 1.90
LSD	4.5	3.87	2.8	4.67	5.6	3.8

Table (5) shows the effect of plant growth regulators on total phenols and total carbohydrates of faba beans after two months from planting during the two studied seasons of 2020/2021 and 2022/2023. For total phenols, Giza 843 showed the highest total phenols amount (1.98 and 2.17 mg/g) followed by SA (1.76 and 1.85 mg/g) followed by IAA (1.45 and 1.74 mg/g) compared to control (1.30 and 1.43 mg/g) during two seasons respectively relatively the same results were obtained by (Zakaria *et al.*, 2015; Dawood, 2017).

For total carbohydrates in the first season, the highest amount was obtained for Giza 843 (180 and 185 mg/g) compared to control (95.17 and 95.33 mg/g), while SA and IAA showed the least amount during two seasons respectively. The least significant difference was 0.31 and 0.42 for total phenols and 11.6 and 7.4 for total carbohydrates during both seasons respectively.

Table 5: Effect of IAA and SA treatments on total phenols and total carbohydrates in faba bean roots (*Vicia Faba L.*) after 60 days from planting during seasons 2020/2021-2022/2023.

Treatment	Total Phenols (mg/g)		Total Carbohydrates(mg/g)	
	2020/2021	2022/2023	2020/2021	2022/2023
Control	1.30 ^b ± 0.06	1.43 ^c ± 0.13	95.17 ^b ± 1.04	95.33 ^b ± 1.53
Giza 843	1.98 ^a ± 0.18	2.17 ^a ± 0.35	180.00 ^a ± 1.00	185.00 ^a ± 5.29
IAA	1.45 ^b ± 0.18	1.74 ^{bc} ± 0.14	67.00 ^d ± 2.00	76.17 ^d ± 2.75
SA	1.76 ^a ± 0.17	1.85 ^{ab} ± 0.05	83.57 ^c ± 4.14	87.93 ^c ± 2.08
LSD	0.31	0.42	11.6	7.4

Table (6) shows the effect of IAA, SA and Giza 843 on the height of faba beans after 60 days from planting. In both seasons under study, Giza 843 showed the highest height followed by IAA treatment, followed by SA treatment compared to control. Their values in the first season were (68.70, 49.30 and 47.10 cm/plant) and (65.40, 49 and 50 cm/plant) in the second season respectively. The effect of plant growth regulators on fresh and dry weight of faba beans stems after 60 days from planting was displayed in (Table 6). In the first season 2020/2021, for fresh weight showed no significance between all treatments, Giza 843 gave the highest weight (0.752 kg/3plants), followed by

control (0.745 kg/3plants), followed by IAA (0.735 kg/3plants) and SA (0.719 kg/3plants). For dry weight IAA showed the highest value (0.533 kg/3plants) followed by Giza 843 (0.528 kg/3plants), SA (0.523 kg/3plants) and control that revealed the least amount (0.519 kg/3plants). For both fresh and dry weights in the first season, all values obtained were very close around the value obtained for control according to (Briache *et al.*, 2020)

In the second season 2022/2023, relatively the same order was obtained for dry weight in the second season as for fresh weight, Giza 843 gave the highest value followed by SA treatment

followed by IAA treatment compared to control and their respective values were (0.160, 0.134 and 0.127 kg/3plants) respectively. The least significant

noticed was 19.40 and 4.00 for height of plants after two months from planting and 0.014 and 0.03 for dry weight during both seasons.

Table 6: Effect of IAA and SA treatments on height and weight of faba bean plant (*Vicia Faba L.*) after two months from planting during seasons 2020/2021- 2022/2023.

Treatment	Height after 2months (cm/plant)		F.W. (kg/3plants)		D.W. (kg/3plants)	
	2020/2021	2022/2023	2020/2021	2022/2023	2020/2021	2022/2023
Control	46.70 ^b ± 5.03	46.30 ^c ± 3.56	0.745 ^a ± 0.037	0.398 ^b ± 0.021	0.519 ^b ± 0.006	0.085 ^c ± 0.001
Giza 843	68.70 ^a ± 6.87	65.40 ^a ± 4.48	0.752 ^a ± 0.026	0.591 ^a ± 0.025	0.528 ^{ab} ± 0.004	0.160 ^a ± 0.015
IAA	49.30 ^b ± 4.97	49.00 ^{bc} ± 3.92	0.735 ^a ± 0.004	0.541 ^a ± 0.023	0.533 ^a ± 0.005	0.127 ^b ± 0.005
SA	47.10 ^b ± 5.13	50.30 ^b ± 3.09	0.719 ^a ± 0.046	0.556 ^a ± 0.041	0.523 ^{ab} ± 0.008	0.134 ^b ± 0.019
LSD	19.40	4.00	—	0.14	0.014	0.03

Table (7) showed the effect of IAA and SA on the number of broomrape spikes per ², dry weight of broomrape spikes per m² and the percentage of weed control during 2020-2021/2022-2023 seasons. The first season, for the number of broomrape spikes per ², the control showed the highest amount (3.00 broomrape spikes/m²) than all other treatments SA, IAA and Giza 843, their respective values were (2.22, 1.61 and 0.99 broomrape spikes/m²). For the dry weight of broomrape spikes per m², the control also showed the highest value (36.48 g) followed IAA followed by SA and Giza 843 showed the least value, their respective values were (31.48, 27.78 and 15.43 g). Consequently, the percentage of weed control showed that Giza 843 has the highest value followed by IAA followed by SA and control that showed low degree of weed control, their respective percent were (67, 47 and 27%).

In the second season, for the number of broomrape spikes per m², treatments showed relatively

decreasing order from IAA, SA and Giza 843 compared to control, their respective values were (2, 1.67 and 1 broomrape spikes/m²). The same order was obtained for the dry weight of the broomrape spikes /m², the IAA, SA and Giza 843 showed the least amount compared to control, their amount were (39.81, 28.70 and 19 g) respectively. The obtained results from the number of broomrape spikes/m² and their dry weight was reflected on the percentage of weed control as Giza 843 showed the highest weed control as compared to susceptible genotypes (control) (Briache *et al.*, 2019) followed by salicylic acid and then indole acetic acid that showed the least percentage of weed control, their respective percent were (71, 45 and 40 %). The least significant differences obtained were 1.42 and 12.35 in the first season, 1.33 and 9.26 in the second season for number of broomrape spikes/m² and dry weight of broomrape spikes/ m² respectively (Briache *et al.*, 2020).

Table 7: Effect of IAA and SA on Broomrape density in faba bean yield (*Vicia Faba L.*) after 120 days from planting during seasons 2020-2021/ 2022-2023.

Treatments	2020/2021			2022/2023		
	No. broomrape spikes/ m ²	Dry Weight of broomrape spikes/ m ² (g)	%Weed Control	No. broomrape spikes/ m ²	Dry Weight of broomrape spikes/ m ² (g)	%Weed Control
Control	3.02 ^a ± 0.95	36.48 ^a ± 5.49	0	3.33 ^a ± 0.58	41.79 ^a ± 3.29	0
Giza 843	0.99 ^b ± 0.75	15.43 ^b ± 8.09	67	1.00 ^b ± 1.00	19.00 ^c ± 7.58	71
IAA	1.61 ^b ± 0.39	31.48 ^a ± 6.83	47	2.00 ^b ± 0.00	39.81 ^a ± 0.93	40
SA	2.22 ^{ab} ± 0.73	27.78 ^a ± 1.70	27	1.67 ^b ± 0.58	28.70 ^b ± 0.93	45
LSD	1.42	12.35		1.33	9.26	

3.2. Allelochemicals control

Trapping crops with radish and flax treatments after six weeks from planting gave the same carotene content (0.077 mg/g f.w.) compared to control and Giza 843, but in case of chlorophyll a, flax treatment showed the highest value (1.78 mg/g f.w.) followed by Giza 843 (1.76 mg/g f.w.) followed by radish treatment (1.66 mg/g f.w.) compared to control. For chlorophyll b, flax and radish treatment showed the highest value (0.35 mg/g f.w.) followed by Giza 843 (0.33 mg/g f.w.) compared to control (0.28 mg/g f.w.). The results that were reflexed on the amount of the total chlorophyll, the flax treatment showed the highest value (2.14 mg/g f.w.) followed by Giza 843 (2.09 mg/g f.w.) followed by radish treatment (2.01 mg/g f.w.) compared to control (1.60 mg/g f.w.) in the first season 2020/2021.

In the second season 2022/2023, Giza 843 revealed the highest carotene content (0.094 mg/g f.w.) followed by radish treatment (0.066 mg/g f.w.) and flax treatment (0.055 mg/g f.w.) that showed the least carotene content compared to control (0.062

mg/g f.w.). Furthermore Giza 843 showed the highest chlorophyll a content (1.080 mg/g f.w.) followed by flax treatment (0.707) followed by radish treatment (0.639 mg/g f.w.) compared to control (0.554 mg/g f.w.). For chlorophyll b the descending order was Giza 843 and radish treatment, and their respective values were (0.405 and 0.283 mg/g f.w.), but flax treatment that showed the least value (0.240 mg/g f.w.) compared to control (0.263 mg/g f.w.). Giza 843 contained the highest chlorophyll a and b values, it gave the highest total chlorophyll value, followed by flax treatment, radish treatment compared to control, their respective values were (1.49, 0.947 and 0.922 mg/g f.w.) (Table 8). The least significant differences obtained was 0.02, 0.43, 0.07 and 0.41 for carotene, chlorophyll a, chlorophyll b and total chlorophyll, 0.03, 0.37, 0.12 and 0.54 during two seasons respectively. These results were reported by (Mabrouk *et al.*, 2016; Al-Hilfy *et al.*, 2017; Madany *et al.*, 2020; Abdel-Wahab and Abdel-Wahab, 2021) and agree also with (Elsakhawy *et al.*, 2020) who showed that broomrape infestation significantly decreased leaf chlorophylls a and b of faba bean plant.

Table 8: Effect of trapping crops on pigments content of faba bean plant (*Vicia Faba L.*) after 45 days from planting during seasons 2020/2021-2022/2023.

Treatment	2020/2021				2022/2023			
	Carotene (mg/g f.w.)	Chlorophyll a (mg/g f.w.)	Chlorophyll b (mg/g f.w.)	Total Chlorophyll (mg/g f.w.)	Carotene (mg/g f.w.)	Chlorophyll a (mg/g f.w.)	Chlorophyll b (mg/g f.w.)	Total Chlorophyll (mg/g f.w.)
Control	0.061 ^b ± 0.008	1.326 ^b ± 0.234	0.278 ^b ± 0.034	1.604 ^b ± 0.249	0.062 ^b ± 0.009	0.554 ^b ± 0.116	0.263 ^b ± 0.039	0.818 ^b ± 0.154
Giza 843	0.071 ^{ab} ± 0.011	1.760 ^a ± 0.127	0.326 ^{ab} ± 0.045	2.086 ^a ± 0.140	0.094 ^a ± 0.007	1.080 ^a ± 0.166	0.405 ^a ± 0.024	1.486 ^a ± 0.147
Faba bean +Flax	0.077 ^a ± 0.005	1.785 ^a ± 0.114	0.354 ^a ± 0.022	2.139 ^a ± 0.132	0.055 ^b ± 0.006	0.707 ^b ± 0.171	0.240 ^b ± 0.026	0.947 ^b ± 0.195
Faba bean +Radish	0.077 ^a ± 0.006	1.662 ^{ab} ± 0.240	0.352 ^a ± 0.028	2.013 ^a ± 0.267	0.066 ^b ± 0.007	0.639 ^b ± 0.073	0.283 ^b ± 0.029	0.922 ^b ± 0.101
LSD	0.02	0.43	0.07	0.41	0.03	0.37	0.12	0.54

Table (9) showed the effect of trapping crops on the chemical parameters of faba bean fresh roots after sixty days from planting. For total protein in the first season, Giza 843 afforded the highest total protein value (6.93 mg/g f.w.) followed by flax treatment (2.56 mg/g f.w.) compared to control (2.43 mg/g f.w.) but radish treatment (2.05 mg/g f.w.) showed the least value. Relatively the same results were obtained in the second season 2022/2023 Giza 843 gave the highest total protein value followed by radish and flax treatment compared to control, their respective value were (12.93, 5.60 and 5.20 mg/g f.w.).

For proline, in the first season flax treatment showed the highest amount (18.90 µg/g f.w.) followed by Giza 843 (17.07 µg/g f.w.) compared to control and radish treatment that relatively showed very close value as control. The same results were nearly obtained in the second season, as the flax treatment showed the highest proline content (29.00 µg/g f.w.) followed by radish treatment (28.50 µg/g f.w.), Giza 843 (22.33 µg/g f.w.) compared to control.

For peroxidase enzyme, all treatments used showed higher amounts compared to control, in the first season 2020/2021, Giza 843 revealed the highest

amount (15.33) followed by flax treatment (10.97) compared to control and radish treatment that relatively showed the same value. In the second season also all treatments gave higher peroxidase amounts than control, their respective values were (20.63, 18.27 and 18.07) for radish, Giza 843 and

flax compared to control respectively. The least significant differences obtained was 4.37, 1.83 and 1.23 for total protein, proline and peroxidase in the first season and 1.6, 6.17 and 6.1 in the second season respectively.

Table 9: Effect of trapping crops on some chemical parameters of faba bean fresh roots (*Vicia Faba L.*) after 60 days from planting during seasons 2020/2021-2022/2023.

Treatment	Total Protein (mg/g f.w.)		Proline (μ g/g f. w.)		Peroxidase (Δ O.D./min/g f. w.)	
	2020/2021	2022/2023	2020/2021	2022/2023	2020/2021	2022/2023
Control	2.427 ^b \pm 0.27	4.00 ^c \pm 0.10	9.83 ^c \pm 0.65	13.67 ^c \pm 0.76	9.73 ^c \pm 0.45	11.97 ^b \pm 0.42
Giza 843	6.927 ^a \pm 0.99	12.93 ^a \pm 1.12	17.07 ^b \pm 1.01	22.33 ^b \pm 4.04	15.33 ^a \pm 1.04	18.27 ^a \pm 0.64
Faba Bean + Flax	2.557 ^b \pm 0.20	5.20 ^{bc} \pm 0.60	18.90 ^a \pm 1.15	29.00 ^a \pm 2.65	10.97 ^b \pm 0.47	18.07 ^a \pm 1.68
Faba Bean + Radish	2.05 ^b \pm 0.18	5.60 ^b \pm 0.66	9.10 ^c \pm 0.36	28.50 ^a \pm 1.50	9.41 ^c \pm 0.25	20.63 ^a \pm 2.07
LSD	4.37	1.6	1.83	6.17	1.23	6.1

Trapping crops showed very marked effect on total phenols and total carbohydrates of faba beans fresh roots after 60 days from planting (Table 10). For total phenols in both seasons faba bean with flax treatment afforded the highest total phenols amount followed by Giza 843 followed by faba bean with radish treatment compared to control, their values were (2.18, 1.98 and 1.75 mg/g) and (2.34, 2.17 and 1.73 mg/g) for both seasons respectively.

For total carbohydrates, in the first season Giza 843 displayed the highest total carbohydrates value

followed by faba beans with radish treatment followed by faba beans with flax treatment and control that showed the least amount, their respective values were (180, 97.97 and 97.33 mg/g). In the second season, Giza 843 also gave the highest total carbohydrates value (185 mg/g) followed by faba beans with flax treatment (105 mg/g) followed faba beans with radish treatment (102.33 mg/g) compared to control. The least significant differences obtained was 0.43 and 0.61 for total phenols and 82.03 and 9.67 for total carbohydrates during the two seasons of study respectively.

Table 10: Effect of trapping crops on total phenols and total carbohydrates of faba bean fresh roots (*Vicia Faba L.*) after 60 days from planting during seasons 2020/2021-2022/2023.

Treatment	Total Phenols (mg/g)		Total Carbohydrates(mg/g)	
	2020/2021	2022/2023	2020/2021	2022/2023
Control	1.30 ^c \pm 0.06	1.43 ^c \pm 0.13	95.17 ^b \pm 1.04	95.33 ^c \pm 1.53
Giza 843	1.98 ^{ab} \pm 0.18	2.17 ^{ab} \pm 0.35	180.00 ^a \pm 1.00	185.00 ^a \pm 5.29
Faba Bean +Flax	2.18 ^a \pm 0.20	2.34 ^a \pm 0.21	97.33 ^b \pm 6.66	105.00 ^b \pm 6.08
Faba Bean +Radish	1.75 ^b \pm 0.14	1.73 ^{bc} \pm 0.19	97.97 ^b \pm 1.45	102.33 ^{bc} \pm 2.08
LSD	0.43	0.61	82.03	9.67

Table (11) revealed the effect of trapping crops on the height of faba beans plants after 60 days from planting. In both seasons Giza 843 showed the highest height followed by faba bean with radish treatment followed by faba bean with flax treatment

compared to control. In the first season their respective values were (68.70, 55.00 and 48.40 cm/plant) and in the second season (65.40, 56.10 and 55.50 cm/plant). In both seasons, all treatments used showed remarkable results compared to

control (Abdel-Wahab and Abdel-Wahab, 2021). The same table showed the effect of all treatments on fresh and dry weight of faba bean, the descending order of all treatment was relatively the same in both seasons. Giza 843 showed the highest fresh weight (0.752 and 0.591 kg/3plants) compared to control. While faba beans with flax treatment (0.694 and 0.340 kg/3plants) followed by faba bean with radish treatment (0.669 and 0.188 kg/3plants) that showed the least amount compared to control.

For dry weight in the first season, faba bean with radish treatment displayed the highest weight

followed by faba bean with flax treatment and Giza 843 at the same value compared to control that showed the least value. Their respective values were (0.538, 0.529 and 0.528 kg/3plants). In the second season, Giza 843 showed the highest weight compared to control followed by faba bean with flax treatment and faba bean with radish treatment that showed the least weight, their amounts were (0.160, 0.079 and 0.038 kg/3plants) respectively. The least significant differences obtained was 6.60, 0.05 and 0.01 for height, fresh and dry weight in the first season, 9.20, 0.06 and 0.04 in the second season respectively.

Table 11: Effect of trapping crops on height and weight of faba bean fresh roots (*Vicia Faba L.*) after 60 days from planting during seasons 2020/2021- 2022/2023.

Treatment	Height after 2months (cm/plant)		F.W. (kg/3plants)		D.W. (kg/3plants)	
	2020/2021	2022/2023	2020/2021	2022/2023	2020/2021	2022/2023
Control	46.70 ^c ± 5.03	46.30 ^c ± 3.56	0.745 ^a ± 0.037	0.398 ^b ± 0.021	0.519 ^c ± 0.006	0.085 ^b ± 0.001
Giza 843	68.70 ^a ± 6.87	65.40 ^a ± 4.48	0.752 ^a ± 0.026	0.591 ^a ± 0.025	0.528 ^b ± 0.004	0.160 ^a ± 0.015
Faba Bean + Flax	48.40 ^c ± 5.04	55.50 ^b ± 3.72	0.694 ^b ± 0.019	0.340 ^c ± 0.014	0.529 ^b ± 0.003	0.079 ^b ± 0.020
Faba Bean + Radish	55.00 ^b ± 7.97	56.10 ^b ± 3.48	0.669 ^b ± 0.008	0.188 ^d ± 0.012	0.538 ^a ± 0.003	0.038 ^c ± 0.004
LSD	6.60	9.20	0.05	0.06	0.01	0.04

Table (12) showed the effect of trapping crops on the number of broomrape spikes per m², dry weight of broomrape spikes per m² and the percentage of weed control in both seasons under study. In the first season, for the number of broomrape spikes per m², Giza 843 and faba bean with radish showed the least value that related with the percentages of weed control (67%), followed by faba bean with flax that gave (63%) weed control and then the control that showed no percentage of inhibition at all. For dry weight of broomrape spikes per m², faba bean with flax followed by faba bean with radish and Giza 843 that showed the least values compared to control, their values respectively were (20.37, 19.75 and 15.43 g).

In the second season, for the number of broomrape spikes per m² that related with percentages of weed control, Giza 843 showed the highest percent (71%) followed by faba bean with flax (51%) followed by faba bean with radish (44%) and then control that showed no inhibition at all. For dry weight of broomrape spikes per m², the control showed the highest value followed by faba bean with flax followed by faba bean with radish and Giza 843

that showed the least values compared to control, their respective values were (32.83, 30.43 and 19.00 g). The least significant differences obtained was 1.91 and 1.67 for the number of broomrape spikes per m² and 16.11 and 10.97 for the dry weight of broomrape spikes per m² during two seasons under study respectively, the same results were previously reported by (Abu-Shall and Ragheb, 2014; Aksoy *et al.*, 2016; Mabrouk *et al.*, 2016; Abbes *et al.*, 2019; Briache *et al.*, 2019; Madany *et al.*, 2020; Abdel-Wahab and Abdel-Wahab, 2021) because, in the faba bean rhizosphere, flax increased the concentration of ascorbic acid in the soil while radish increased the concentration of citric, maleic, and salicylic acid; these actions decreased the infestation of broomrape and prevented the degradation of chlorophyll pigment. The allelopathic chemical found in flax root exudates hurts broomrape. A higher salicylic acid concentration caused the faba bean's chlorophyll content to rise. It also caused the host root's polyamine and fatty acid degradation, which prevented the material from being transferred to the parasite.

Table 12: Effect of trapping crops on broomrape density in faba bean yield (*Vicia Faba* L.) after 120 days from planting during 2020-2021/ 2022-2023.

Treatments	2020/2021			2022/2023		
	No. broomrape spikes/ m ²	Dry Weight of broomrape spikes/ m ² (g)	%Weed Control	No. broomrape spikes/ m ²	Dry Weight of broomrape spikes/ m ² (g)	%Weed Control
Control	3.02 ^a ± 0.95	36.48 ^a ± 5.49	0	3.33 ^a ± 0.58	41.79 ^a ± 3.29	0
Giza 843	0.99 ^b ± 0.75	15.43 ^b ± 8.09	67	1.00 ^b ± 1.00	19.00 ^c ± 7.58	71
Faba Bean + Flax	1.11 ^b ± 0.19	20.37 ^b ± 1.85	63	1.33 ^b ± 0.58	32.83 ^{ab} ± 5.37	51
Faba Bean + Radish	0.99 ^b ± 0.57	19.75 ^b ± 6.82	67	1.67 ^b ± 0.58	30.43 ^b ± 4.00	44
LSD	1.91	16.11		1.67	10.97	

Conclusions

The study concluded that presoaking of faba bean seeds with plant growth regulators, such as IAA and SA, promotes the faba bean plant's natural defensive systems by increasing antioxidant enzyme activity, resulting in the development of systemic acquired resistance (SAR) against broomrape. Additionally, using radish and flax as a trap crop for integrated broomrape management reduces the soil seed bank of *Crenata* broomrape, preventing chemical treatments from having a negative impact on the ecological system.

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