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ARTICLE

Bio stimulation of Nodules formation and growth of common bean by using Fenugreek Aqueous Seed Extract[1]

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Abstract
To reduce the number of chemical compounds a big worldwide effort introducing modern biological methods in crop production. allelopathy is One of the possible solutions that involve both inhibitory and stimulatory biochemical interactions between plants. So, this work aimed to study the effect of aqueous seed extract of fenugreek on growth characters in terms of shoot height (cm), root length, number of leaves/plant as well as plant shoot and root dry weight (g) Photosynthetic pigments. All aqueous seed extract of fenugreek (2, 4, and 6%) showed a significant positive effect on growth parameters, and photosynthetic pigments The results showed that all treatments led to significant increases in Chlorophyll b and total for two bean cultivars (Iraqi 7 and Turkish) when compared to the corresponding controls. maximum significant increases of Chlorophyll b and total of both cultivars by treatment with 6% aqueous seed extract fenugreek, NPK ratio of both bean cultivars. The results revealed that 6% aqueous seed extract of fenugreek caused the maximum significant increases in the most of investigated parameters. Showed Formation of root nodules of bean cultivars (Turkish) by the effect of Concentration at 6% from Fenugreek Aqueous seed extract.

Keywords: Bio Stimulation; Nodules; Formation; Allelopathy.

1. Introduction

In worldwide agriculture, there is an effort to reduce the use of chemical substances in crop production by introducing modern biological and ecological methods. allelopathy is One of the possible solutions, that is been involves both inhibitory and stimulatory biochemical interactions between plants: crops; trees; shrubs; and weeds (El-Khawas, and Shehata, 2005). Allelopathy comprises chemical interactions among plants, Allelopathy means chemical interactions among plants or other organisms such: as algae, fungi, or microorganisms. by releasing chemicals compound from one plant effect in growth and reproduction of other plants in the vicinity. That secreted by volatile, root exudates, or released to the soil during tissue decay, and leaching, which allelopathic interactions, are called allelochemicals.

Allelochemicals can be classified as secondary metabolites; that is produced from the primary metabolic pathways of the plants. that including "phenolic compounds; flavonoids; terpenoids; alkaloids; steroids". Sometimes when a mixture of allelochemical compounds formed a greater allelopathic effect than individual compounds alone. Allelochemicals are selective in action and selective in their responses (Einhellig, 2004). Although most plants produce allelochemicals, relatively few, like fenugreek.

Fenugreek (Trigonella foenum-graecum) is an annual plant from Leguminocae family, leaves and seeds of fenugreek can be used in traditional medicine and as a spice used in food (Abd Elhamid *et al.* 2016). It is a rich source of carotene; calcium; iron; and other vitamins (Sharma *et al.* 1996).

Fenugreek seeds are included have hematinic for that can be used in the normal diet of family, and older persons (Ody, 1993). Fenugreek seeds contain lysine, L-tryptophan-rich proteins, and other chemical compounds: saponins; coumarin; fenugreekine; nicotinic acid; sapogenins; phytic acid; scopoletin, and trigonelline (Bukhari *et al.* 2008), that may be account for effect of Fenugreek in other plants. All fenugreek seeds extracts exhibit antioxidant activity because found of phenolic acids and flavonoids. studies showed ranged of phenolic compounds in fenugreek seeds that (1.35 - 6.85 mg/g), and flavonoids range from (208 - 653 µg/g) according to type of extract (Bukhari *et al.* 2008).

2. Materials and Methods

Experiment was carried out at greenhouse at Biology Department ,Collage of science , Mosul university, Iraq, during winter season of 2020 , To study the effect of aqueous extracts of fenugreek seeds on the germination and growth of two types of bean(Iraqi and Turkish). Experimental design was a complete randomized block design (C.R.B.D) with four replicates. Bean grains were sown on the 20 November pots with dimeter 13.8cm and height 20cm. bean grain were sprayed twice after 15 and 30 days from sowing with aqueous extract of seeds fenugreek.

prepared of seed aqueous extracts: To study the effect of aqueous extracts of fenugreek seeds on the germination and growth of two types of bean(Iraqi and Turkish), the extracts were prepared at concentrations of 2,4,6 %: w/v (2,4,6 g of seed powder) / 100 mL water mixed with Motor stirrer. For 30 minutes, filtered using a Buechner funnel fitted with Whatman No. 1 and collected a clear solution (Abbas and Hussain,2020).

2.1 Data recorded: Plant samples were collected after 60 days from sowing for recording growth characters in terms of shoot height (cm), root length, number of leaves/plant as well as plant shoot and root dry weight (g). Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll) in fresh leaves were determined as the method recommended by Knudson et.al (1977).

Leaf N, P, and K contents as percentages in dry matter of leaves samples were determined as follows: Nitrogen, according to Pregel (1945) (using Micro-Kjeldahl), Phosphorus, according to the method described by Champman and Pratt (1961), Potassium, according to Brown and Lilleland (1945).

2.2 Statistical analysis: Data were statistically analyzed at 5% probability according to Duncan's Multiple Range Test.

3. Results and Discussion

Results in the table (1) show increases in (plant height, root length, shoot dry weight, and root dry weight) in all treatments, with the highest percentage increase in (plant height, root length and shoot dry weight) in the Iraqi cultivar at 2% respectively (35.30, 100, 65.27%) that may be due to that allelochemicals show their effect on the effective concentration(Hussain, 2020a). These compounds either have positive or negative effects, the production of these compounds is mainly a result of the secondary metabolism of plants and microorganisms (bacteria, viruses, and fungi) and these compounds can affect many processes in ecosystems and agroecosystems(Hussain et. al, 2021).

Table 1. Effect of Fenugreek Aqueous Seed Extract in some growth traits of bean cultivar

Cultivar	Concentration%	Plant High	Roots Length	Shoot Dray Wight	Root Dray Wight
Iraqi	Control	27.9cd	22.35d	0.527bc	0.086d
	2%	37.75a	47.25a	0.871a	0.146c
	4%	34.95b	32.55bc	0.647b	0.226b
	6%	29.2c	35.35b	0.44c	0.26a
	Cultivar effect	32.45a	34.37b	0.617ab	0.178b
Turkish	Control	32.6c	30c	0.576b	0.273d
	2%	27.5d	22.5d	0.386c	0.351c
	4%	35.5b	46b	0.801a	0.790a
	6%	40.5a	84a	0.794a	0.590b
	Cultivar effect	34.02ab	45.62a	0.639a	0.501a

Results in Figure (1) showed that treated plants with the different concentrations of fenugreek aqueous seed extract led to significant increases in the Number of leaves of bean cultivars (Iraqi) when compared to the corresponding controls, while that showed a decrease in the number of leaves of bean cultivars (Turkish). The superiority of the Iraqi variety may be due to the strength of vegetative growth of this variety in plant height and number of leaves, which led to giving the opportunity to form the largest number of branches and leaves, the reason for the variation in the effect between the varieties can be due to the genetic variation (Hussain, 2020a)

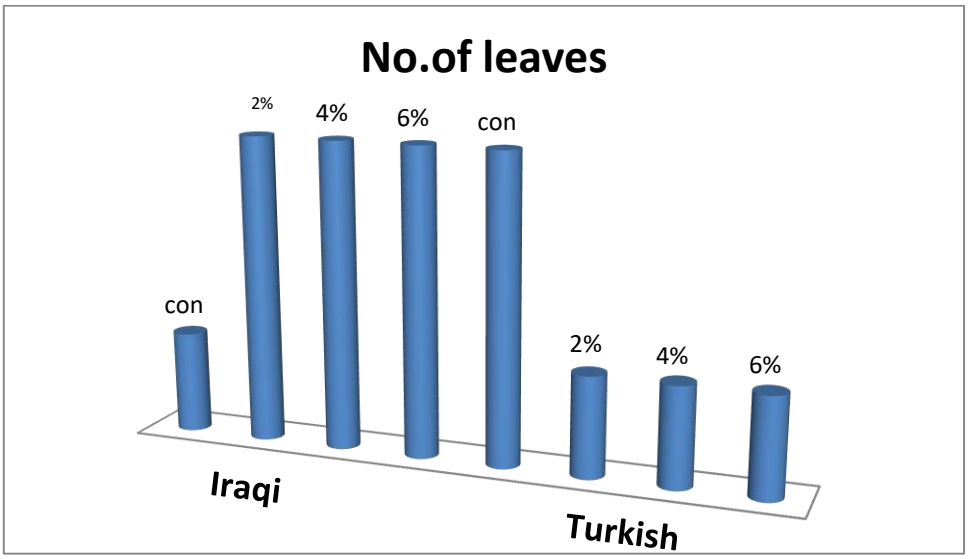

Figure 1. Effect of Fenugreek Aqueous Seed Extract in Number of leaves in been cultivar

A significant difference was registered between treatments. The increasing percentage was 100% for Turkish and 30% for Iraqi Figure (2). This increase was accompanied by an increase in the height of the plant, meaning that the relationship is direct, which shows the increase in the height of the plant with the increase in the number of root branches (Hussain, 2020b). This increase can be due to the direct effect of soil on the roots growing in it, and these soils contain allelopathic compounds released from plant residues and their effect on the nutritional status and the abundance of minerals in the soil (Scavo *et al.*, 2019).

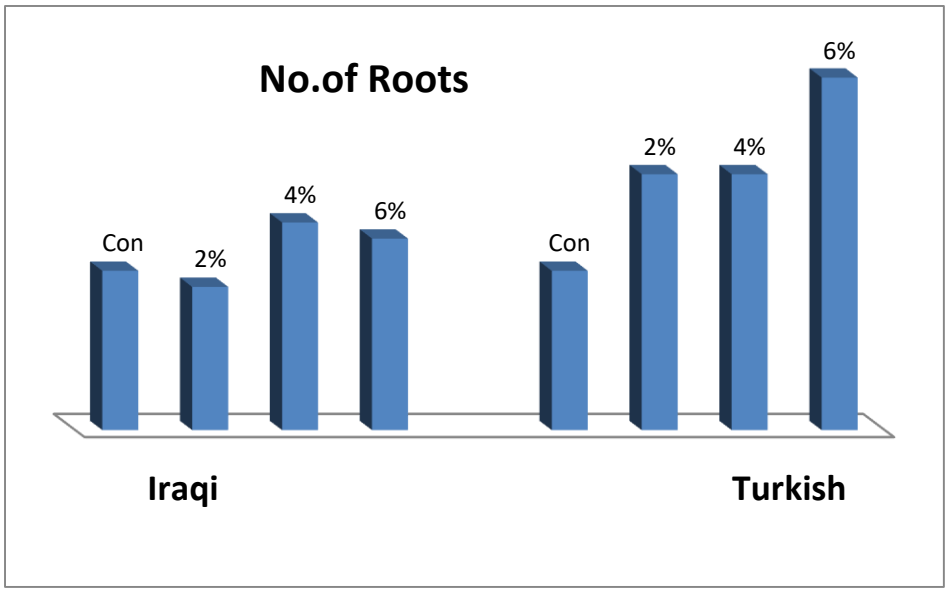

Figure 2. Effect of Fenugreek Aqueous Seed Extract on Number of Roots in been cultivar.

Table (2) showed the response of photosynthetic pigment of Fenugreek Aqueous Seed Extract to different concentrations of aqueous seed extract of fenugreek (2, 4, and 6%). The results showed that all treatments led to significant increases in Chlorophyll b and total for two bean cultivars (Iraqi 7 and Turkish) when compared to the corresponding controls. maximum significant increases of Chlorophyll b and total of both cultivars by treatment with 6% aqueous seed extract fenugreek. These photosynthetic pigments enhancements of both cultivars may be due to the that fenugreek seeds extract important nutrients, such as Fe and Mg elements that positively affect on chlorophyll building and photosynthesis (Abu-Dhahi and Al Younis,1988), the reason for the increase in chlorophyll in the leaves of treated plants may be due to the effect on the process of building chlorophyll or building the enzyme Mg-chattels responsible for including Mg with Proporphrin, or it may be due to the effect on the process of taking the Mg element by the plant, These results are in agreement with those observed by Dawood(2020)that aqueous seed extract of fenugreek at (5, 10 and 15%)led to significant increases in photosynthetic pigments of two wheat cultivars.

Table 2. Effect of Fenugreek Aqueous Seed Extract in Ch a, Ch b and Total Ch.

Cultivar	Concentration%	Cha	Ch b	Total Ch
Iraqi	Control	26.77a	17.15d	47.89d
	2%	25.19b	33.37c	63.39c
	4%	24.75c	36.42b	66.14b
	6%	23.59d	41.612a	70.37a
effect Cultivar		25.07a	32.138b	61.947b
Turkish	Control	23.771a	40.838c	69.739c
	2%	23.569b	39.594d	68.193d
	4%	22.921c	43.57b	71.701b
	6%	21.769d	48.73a	75.909a
Cultivar effect		23.007b	43.183a	71.385c

Data in Table (3) fenugreek indicate that 6% from Iraqi cultivar and 4% from Turkish cultivar increased content of NPK in plants compared with other treatments .In Iraqi cultivar at 6% NPK respectively content 2.25% , 0.6266 and0.61 compared with control 1.47, 0.3093 and0.28 and in Turkish cultivar at 4% NPK in plant as respectively1.96, 0.5076 and 0.62.These observations are consistent with previous reports obtained by Dawood et.al (2012) who mentioned that fenugreek treatment caused increases in the total carbohydrate

content and element content of sunflower leaves, this may be due to genetic and physiological differences, while other treatments showed a reduction in the concentration of elements, and the reason for this reduction may be due to the effect of allelopathic compounds liberated from fenugreek seeds in absorbing those elements from the soil and transporting them into the plant in addition to the direct effect on root growth and poor formation Root hairs, which leads to poor absorption efficiency.

Table 3. Effect of Fenugreek Aqueous Seed Extract in bean plant contain of NPK

Cultivar	Treatment	N٪	P٪	K٪
Iraqi	Control	1.47d	0.3093d	0.28c
	2%	1.86c	0.4680c	0.51b
	4%	2.05b	0.5156b	0.59ab
	6%	2.25a	0.6266a	0.61a
Turkish	Control	0.98d	0.1903c	0.38d
	2%	1.66c	0.4497b	0.45c
	4%	1.96a	0.5076a	0.62a
	6%	1.76b	0.4283b	0.52b

Data in Table (4) clearly indicate that all concentrations treatments significantly increased N, P, and K contents in soil compared with untreated plants Concentration at 4% from Fenugreek Aqueous seed extract has a maximum content of N, P, and K. The increase in N, P and K contents is due to the fact that it is released from plant residues, which are a major source of these elements after the dissolution of internal cellular structures (carbohydrates, proteins, nucleic acids, enzymes), whose molecular structure contains these elements, as well as phenolic compounds such as phenolic acids that are produced from the residues Plants or that are filtered from cells, are considered a source of these elements (El-Saadawi *et al.*, 2007).

Table 4. Effect of Fenugreek Aqueous Seed Extract in Soil contain of NPK

Treatment	N٪	P٪	K٪
Control	0.117%b	14.60d	60d
2%	0.117b	16.864c	74c
4%	0.1568a	27.02a	80a
6%	0.101c	19.73 b	77b

Results in Figure(3) Showed the Formation of root nodules of bean cultivars (Turkish) by the effect of Concentration at 6% from Fenugreek Aqueous seed extract that can be due to the effect of an allelopathic compound released from Fenugreek Aqueous seed, that can be stimulation plant nitrogenase activity in soil or this may be due to the effect of allelopathic compounds that led to a bacterial infection in the roots of the bean plant, which led to the formation of root nodes.

Figure 3. Effect of Fenugreek Aqueous Seed Extract in Root nodules formation in been cultivar.

4. Conclusions

This work aimed to study the effect of aqueous seed extract on fenugreek growth characters in terms of shoot height (cm), root length, several leaves/plant as well as plant shoot and root dry weight (g) Photosynthetic pigments. All aqueous seed extract of fenugreek (2, 4, and 6%) showed a significant positive effect on growth parameters, and photosynthetic pigments The results showed that all treatments led to significant increases in Chlorophyll b and total for two bean cultivars (Iraqi 7 and Turkish) when compared to the corresponding controls.

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