Effect of planting date and spraying of humic acid in the growth traits and active compounds of Fenugreek (Trigonella foenum – graecum L)

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Abstract. The study was conducted at the Crops Research Station, College of Agriculture, University of Diyala during the years 2017-2018 to evaluate the effect of planting dates and spraying of humic acid in the growth characteristics and active compounds of Fenugreek (Trigonella foenum – graecum L). The experiment was carried out according to factorial arrangement in a complete randomized blocks design (RCBD) with three replicates and two factors, the first include planting dates (15/10, 1/11, 15/11) and the second is spraying of the humic acid at a concentration of 2 and 4 ml /L. Results revealed that the planting dates had a significant effect in the studied traits, the first date (15/10) recorded the highest rate of plant height (84.89 cm), seeds yield (7.805 g /plant−1), seeds yield /hectare (770.2 kg / h−1), number of branches (9.67 branches / Plant−1), number of leaves (146.6 leaves / plant−1), dry plant weight (20.11 g/plant−1), number of pods (47.33 pods / plant−1), number of seeds (14.44 seeds /pod−1), weight of 100 seeds (1.502 g) and the active compounds such as Choline (170.99 mg/ml−1), Trigonelline (305.73 mg/ml−1), Carpine (95.89 mg/ml−1) and Gentianine (191.11 mg/ml−1). The spraying of humic acid at a concentration of 4 ml / L was superior significantly in all traits such as plant height (74.67 cm), seeds yield (7.220 g / plant−1), seeds yield /hectare (710.0 kg / h−1), number of branches (8.11 branches / plant−1), number of leaves (61.00 leaves / plant−1), dry plant weight (20.44 g/plant−1), number of pods (38.22 pods / plant−1), number of seeds (14.00 seeds / pod−1), Choline (163.61 mg/ml−1), Trigonelline (283.86 mg/ml−1), Carpine (83.92 mg/ml−1) and Gentianine (183.03 mg/ml−1).

1. Introduction

Fenugreek (Trigonella foenum-graecum) is a leguminous herb belongs to the Fabaceae family and it has many nomenclatures in different languages, viz.Hulba (Arabian), Bockshorklee (German), Fenugrec (French), Methi (Hindi), Pazhitnik (Russian), Fieno greco (Italian), Alholva (Spanish), K’u-Tou (China), Koroha (Japanese), and Halba (Malaya). Fenugreek seeds have a bitter taste and have been used before 2500 years for different purposes, as spices in addition to their known medicinal characteristics, while the leaves of Fenugreek are used as vegetables in the diet [1]. Historically, the peoples of Mesopotamia have known many medicinal plants and used them successfully in the treatment of different diseases, such as the fenugreek [2,3], that is using for gout treatment, rheumatism, constipation, cough, asthma, back pain,
sciatica, hemorrhoids, diabetes, anaemia and lose weight. The fenugreek seeds also contain many medical and pharmaceutical compounds such as coumarin, Cholene, Trigonelline and Carpine [4], these compounds are natural chemical substances belong to the glycosides and flavonoids that give the plant pigments. These compounds have water solubility properties in addition to its nutritional value. They are important compounds for treating diabetes, liver disease, eye pressure protection and anemia treatment [5] also contains vitamins such as (A, B1, B2) which is rich in some nutrients, including K, P, Ca, Mg, Fe and Mn [6]. The crop of the fenugreek is cultivated as a green fodder crop or straw in India and the Mediterranean region and palatable by animals [7]. Recently, the organic fertilizers such as humic acids were used in low-concentrations to improve soil properties and plant nutrition, hence accelerating growth and increasing production [8]. The humic acids have a positive effect on nutrient uptake by the plant as it help actively in the absorption of the macro and micro- elements and their transport, especially the micronutrients [9]. Also, the acids of humic inhibit the activity of IAA oxidase enzyme, which increases the activity of IAA auxin in stimulating the plant growth and roots, thus the humic acids improve the capacity of elements in the soil by the correlation with sodium, which helps plant to tolerate toxicity and osmosis negative effects [10]. Jawad [11] revealed that the use of humec in a concentration of 1.5 ml/l increased the rate of plant height, leaf area, number of leaves, yields and number of pods compared to the concentration of 0.5 ml/l liter. As AL-Taei [12] sprayed fenugreek plants with different concentrations of ethephon in different stages of plant growth found that the date of the first spray at the highest concentration has the superior effect in the ratio of oil and protein compared to the date of the third spray. Generally, the planting date is crucial management practice due to the different climatic conditions and adaptation of varieties to cope with these conditions, where the production of the fenugreek is affected by the dates of cultivation. Al-Taei [13] reported that when they used three planting dates for chickpea crop, the earlier date was superior in the growth characteristics pods / plant, weight of pods / plant, plant yield / gm and weight of 100 seed. Al-Bayati [14] observed that the planting date at the beginning of November was superior in the growth traits of broad bean such as the plant height while the planting date in mid-November was superior in seed weight and plant yield. Al-Bayati [15] showed that when they used three dates of cultivation of chickpea, the results indicated that the planting date of 1/11 was significantly superior in the traits of 100 seeds weight and dry plant weight and seed yield. The date of planting 5- November gave the highest rate of cumin height and the number of seeds /pod and the number of pods / plant and the seeds yield and biological yield kg / h [16]. Jevdjovic [17] reported that the early dates of fenugreek plant cultivation gave the highest rate of total germination ratio, the total yield of seeds and active substances. The study aimed to evaluate the effect of planting dates and spraying of humic acid in the growth characteristics and active compounds of Fenugreek (Trigonella foenum – graecum L).

2. Materials and methods
The experiment was carried out in the Field Crops Department, college of Agriculture, University of Diyala during 2017-2018 to study the effect of planting dates and spraying of the humic acid in the growth characteristics and active compounds of Fenugreek crop. A factorial experiment was used with the randomized complete block design (RCBD) in three replicates. Means were compared by using the least significant difference L.S.D at the level of probability of 5% [18].

The experiment included two factors, the first was planting dates (15/10, 1/11, 15/11) meanwhile the second was the spraying of different concentrations of the humic acid (0, 2, 4 ml / L). Soil samples were taken before planting to analyze some of the physical and chemical properties, five random samples were collected at a depth of 0-30 cm, the samples were mixed and exposed to sunlight for 8 hours then grinded and sieving. Physical and chemical analysis (Table 1) was conducted in the soil department laboratories, college of Agriculture, University of Diyala.
Table 1. Some physical and chemical characteristics of the soil before planting.

| Measurements      | Unit of measurement | Field soil |
|-------------------|---------------------|------------|
| PH                |                     | 8.4        |
| Ec                | dS siemens /m       | 2.74       |
| N                 | Mg/Kg               | 34.8       |
| P                 | Mg/Kg               | 13.7       |
| K                 | Mg/Kg               | 509        |
| So₄               | Mg/Kg               | 1.4        |
| Organic matter    | g/Kg                | 18.26      |
| porosity          |                     | 52%        |
| Gypsum            | g/Kg                | 1.34       |
| sand              | g/Kg                | 24%        |
| Clay              | g/Kg                | 22%        |
| Texture           |                     | Mixed clay |

The soil was irrigated until the immersion before tillage, then it was plowed by using Plow, the field was divided into three blocks, each containing 9 treatments, length of treatment 2 m, the distance between the lines 0.25 m, the distance between the treatments 0.5 m and the distance between plants 20 cm, the area of experimental unit 4 m and the number of plants in the experimental unit according to the distance required in the study. Nitrogen fertilization was added in the form of urea before planting at a rate of 90 kg/h. The seeds (Possum variety) were obtained from the local market, seeds germination were tested by placing 100 seeds to each Petri dish with three replicates, adding a little water and recording the number of germinated seeds per day, the germination rate was 90% after 6 days. The seeds were planted according to the dates required in the study, 4-6 seeds were placed in the hole at a depth of 3-4 cm covered with soft soil and irrigated, then matured plants were harvested. The spraying of humic acid was started after one month of planting and for three times during the growing season [19], one spray every two weeks with 2 and 4 ml/L. The experimental measurements were carried out on five plants randomly taken from each experimental unit, which included plant height (cm), number of leaves/plant, number of branches/plant, number of pods/plant, dry plant weight (g), number of seeds/pod, weight of 100 seed (g), seeds yield g/plant, seeds yield kg/h and four active compounds (Choline, Trigonelline, Carpine, Gentianine).

2.1. Active compounds (Choline, Trigonelline, Carpine, Gentianine)

The method of water extraction was applied to obtain the active compounds (Choline, Trigonelline, Carpine, Gentianine) from the dry fenugreek seeds [20,21]. One gram of dry fenugreek seed from each experimental units was grinded and placed in 50 ml of hot water (100 c) for three hours and filtered with filter papers (no1). Filtrated substance containing active compounds was collected and placed in glass tubes measure by high performance liquid chromatography (HPLC), at the Ministry of Science and Technology / Department of Chemistry.

The method is summarized as follows:
1. Takes 1 gram of fenugreek seeds powder.
2. Melt the powder in 10 ml of methanol (40% ) in glass tube.
3. Places the tube in the ultrasound device (KARAL KOLB) for 10 minutes in order to obtain a pure extract.
4. Passes the extract on microfilter disk 0.4 nm to isolate the fibers to obtain a pure extract.
5. Injected 20 micro-liters of samples into (HPLC)
6. Stabilizes the extract in column C-18, which is a small nylon column filled with silica of 5 cm in length.
7- diagnosis and identify quantity and quality of active compounds compared to standard materials separated from column C-18 under the same conditions and imported from Japan Sigma Company.
8- measurement concentration of active compounds was accomplished on the column by using the model concentration equation.

Concentration of sample $\mu g/ml = \frac{\text{area of sample}}{\text{area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$

The applied HPLC conditions for separating active compounds were detailed in table 2 and 3.

**Table 2. HPLC conditions used for the separation of active compounds of Fenugreek**

| Mobile phase | Tetrahydrofuran : deionized water acidified : 0.1% acetic acid (80:20 v/v) |
|-------------|---------------------------------------------------------------------------------|
| Flow speed of mobile phase | 1.0 ml / min |
| Type of detector | Ultra violet (uv) 254 nm |
| Separate Column | Bonda – Pak c 18 |
| Separate temperature | 35 $^\circ$C |

**Table 3. The retention time for standard models of the separated active compounds**

| Compound      | Compound retention time / min |
|---------------|------------------------------|
| Choline       | 2.64                         |
| Trigonelline  | 3.85                         |
| Carpine       | 4.92                         |
| Gentianine    | 6.63                         |

3. Results

The effect of planting dates on all studied traits including plant height (cm), number of leaves/ plant, number of branches/plant, number of pods/plant, dry plant weight, number of seeds/pod, weight of 100 seed, seeds yield g/plant, seeds yield kg/h and active compounds (Choline, Trigonelline, Carpine and Gentianine) was significant (Table 4 and 5). The first date (15/10) was superior and achieved the highest mean of the following characteristics viz. plant height (84.89 cm), number of leaves/ plant (146.6), number of branches/plant (9.67), number of pods/plant (47.33), dry plant weight (20.11 g), number of seeds/pod (14.44), weight of 100 grains (1.50 g), seeds yield g/plant (7.80 g), seeds yield kg/h (770.2 kg/h), and active compounds viz. Trigonelline (305.73 mg/ml), Carpine (95.89 mg/ml) and Gentianine (191.11 mg/ml) as compared with other planting dates.

Whereas the effect of the spraying with humic acid on previously mentioned traits was significant too, thus the concentration of 4 ml / L expressed the highest rate of plant height (74.67 cm), number of leaves/plant (113.1), number of branches/plant (8.11), number of pods/plant (38.22), plant dry weight (20.44 g), number of seeds/pod (14.00), weight of 100 seed (1.71 g), seeds yield g/plant (7.22 g), seeds yield kg/h (710.0 kg/h), and active compounds viz. Choline (163.61 mg/ml), Trigonelline (283.36 mg/ml), Carpine (83.92 mg/ml) and Gentianine (138.03 mg/ml) as compared with the other concentrations of humic acid.

While the interaction-interaction between the cultivation dates and the humic concentrations was significant. The first date (15/10) with the concentration of 4 ml / L recorded the highest mean for the following traits; plant height (96.00 cm), number of leaves/plant (184.7), number of branches/plant (11.33), number of pods/plant (58.67), plant dry weight (27.00 g), number of seeds/pod (18.00), weight of 100 seed (1.81 g), seeds yield g/plant (9.23 g), seeds yield kg/h (906.7 kg/h).
and active compounds viz. Choline (184.20 mg/ml), Trigonelline (341.45 mg/ml), Carpine (113.13 mg/ml) and Gentianine (274.92 mg/ml) as compared with the other interaction-interaction values.

4. Discussion

The first planting date was the best in all previous studied traits due to the suitable temperature for germination and growth in the first date, as well as the relatively long period of vegetative growth spent by these plants (180 days), whereas less plant height was achieved by the last planting date that shortening the life time of plant [22,13]. The first date was superior in number of branches due to the rapid plant growth during the first stages synchronized with ideal temperatures, humidity and light intensity which stimulated the growth of lateral buds and gave long duration of light (180 days) which in turn increasing rate of metabolic processes such as photosynthesis. The increase leaves number was due to appropriate climatic conditions of these dates which led to an increase in the efficiency of photosynthesis and enhancing the roots and formation finally increasing the production of cytokines which have a vital role in increasing the division of cells [15,23,24]. There was a significant effect of planting dates on the active compounds (Choline, Trigonelline, Carpine, Gentianine) due to impact of the cultivation date on the growth and yield traits through the effect on the amount of active ingredients in the plant, also the active substance for any medicinal plant is a secondary product in photosynthesis processes [17,25]. Humic acid has a positive role in the functional processes improving plant growth via a higher rate of cell division and elongation [26]. Furthermore the availability of sufficient nutrients is necessary to synthesize chlorophyll, protein, nucleic acids and increase the plants ability to perform photosynthesis and supplying metabolites necessary for cell division and elongation [27,28]. The organic fertilizer (humic acid) can participate effectively in producing more carbohydrate combined with a higher enzymatic activity at all will positively reflect on plant productivity and quality [29,30]. Spraying organic fertilizer 4 ml/liter caused a significant increase in all studied traits which in turn led to an increase in seed yield per unit area [19]. The proper concentration of humic acid has increased the efficiency of photosynthesis process which led to the accumulation of nutrients in the plant and stimulates the plant to increase the number of branches also has a role in increasing the Cytokinins in promoting the growth of side buds [31,32,33]. The element of humic is a necessary for plant growth and developmental stages, although it does not enter into any cellular components and acts as a co-factor in many biological processes including the synthesis of proteins, nucleic acids and photosynthesis [34,35,36,37]. Dry weight has a key role representing an indicator for photosynthesis efficiency because 90% of the dry weight comes from this process [38]. Humic acid participate effectively in the synthesis and the activity of several enzymes [39]. It has a hormonal effect similar to auxin serving in the production of more flowers nodes [40]. In the same context humic acid has a significant effect in the active compounds especially in response to increased alkaloids that represents the metabolites of amino acids, increasing of the produced alkaloids amount in the fenugreek seeds due to the role of gibberellin in increasing the biological processes for the formation of alkaloids [41,42].

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Table 4. Effect of planting dates and spraying of humic acid in Plant height (cm), Number of leaves/plant$^1$, Number of branches/plant$^1$, Number of pods/plant$^1$, Number of seeds/plant$^1$, Number of seeds/pod$^1$, Weight of 100 seed (g) of Fenugreek plants

| Plant height (cm) | Number of leaves/plant$^1$ | Number of branches/plant$^1$ | Number of pods/plant$^1$ | Number of seeds/plant$^1$ | Number of seeds/pod$^1$ | Weight of 100 seed (g) |
|-------------------|-----------------------------|-------------------------------|--------------------------|---------------------------|-------------------------|------------------------|
Table 5. Effect of planting dates and spraying of humic acid on Seeds yield / plant (g), Seeds yield kg/h and active compounds (Choline, Trigonelline, Carpine, Gentianine) of Fenugreek.

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 6.35 | 7.83 | 9.23 | 7.80 |
| Second date (1/11) | 5.27 | 6.14 | 7.15 | 6.18 |

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 73.67 | 85.00 | 96.00 | 84.89 |
| Second date (1/11) | 51.33 | 62.00 | 72.67 | 62.00 |
| Third date (15/11) | 39.00 | 46.00 | 55.33 | 46.78 |
| Mean | 54.67 | 64.33 | 74.67 |   |

L.S.D 0.05 Planting dates = 2.64, Spraying = 2.64, interaction = 4.57

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 116.0 | 139.0 | 184.7 | 146.6 |
| Second date (1/11) | 75.7 | 85.7 | 93.7 | 85.0 |
| Third date (15/11) | 32.7 | 48.7 | 61.0 | 47.4 |
| Mean | 74.8 | 91.1 | 113.1 |   |

L.S.D 0.05 Planting dates = 6.72, Spraying = 6.72, interaction = 11.65

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 46.00 | 58.67 | 78.22 | 67.33 |
| Second date (1/11) | 35.67 | 46.67 | 58.22 | 47.33 |
| Third date (15/11) | 23.33 | 35.67 | 46.67 | 35.67 |
| Mean | 21.33 | 27.67 | 38.22 |   |

L.S.D 0.05 Planting dates = 2.88, Spraying = 2.88, interaction = 5.00

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 12.00 | 21.33 | 27.00 | 20.11 |
| Second date (1/11) | 9.67 | 17.33 | 20.33 | 15.78 |
| Third date (15/11) | 6.33 | 10.33 | 14.00 | 10.22 |
| Mean | 9.33 | 16.33 | 20.44 |   |

L.S.D 0.05 Planting dates = 1.27, Spraying = 1.27, interaction = 2.21

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 11.67 | 13.67 | 18.00 | 14.44 |
| Second date (1/11) | 9.67 | 12.00 | 13.33 | 11.67 |
| Third date (15/11) | 7.67 | 9.33 | 10.67 | 9.22 |
| Mean | 9.67 | 11.67 | 14.00 |   |

L.S.D 0.05 Planting dates = 1.20, Spraying = 1.20, interaction = 2.08

| Treatments | Control | Spraying 2 ml | Spraying 4 ml | Mean |
|------------|---------|---------------|---------------|------|
| First date (15/10) | 1.15 | 1.54 | 1.81 | 1.50 |
| Second date (1/11) | 1.08 | 1.42 | 1.70 | 1.40 |
| Third date (15/11) | 0.74 | 1.25 | 1.63 | 1.20 |
| Mean | 0.99 | 1.40 | 1.71 |   |

L.S.D 0.05 Planting dates = 0.03, Spraying = 0.03, interaction = 0.06
### Seeds yield kg/h⁻¹

|               | First date (15/10) | Second date (1/11) | Third date (15/11) | Mean | L.S.D 0.05 |
|---------------|--------------------|--------------------|--------------------|------|------------|
|               | 624.0              | 511.0              | 410.0              | 511.7| Planting dates = 114.0, Spraying = 114.0, interaction = 198.0 |
| Mean          | 780.0              | 611.7              | 470.0              | 620.6|            |
|               | 906.7              | 711.7              | 511.7              | 710.0|            |
|               | 770.2              | 611.4              | 463.9              | 700.0|            |

### Choline mg/ml⁻¹

|               | First date (15/10) | Second date (1/11) | Third date (15/11) | Mean | L.S.D 0.05 |
|---------------|--------------------|--------------------|--------------------|------|------------|
|               | 156.48             | 142.88             | 77.43              | 125.59| Planting dates = 20.77, Spraying = 20.77, interaction = 35.97 |
| Measn         | 172.30             | 155.24             | 96.24              | 141.26|            |
|               | 184.20             | 161.26             | 145.37             | 163.61|            |
|               | 170.99             | 153.12             | 106.34             | 198.00|            |

### Trigonelline mg/ml⁻¹

|               | First date (15/10) | Second date (1/11) | Third date (15/11) | Mean | L.S.D 0.05 |
|---------------|--------------------|--------------------|--------------------|------|------------|
|               | 266.86             | 201.10             | 187.15             | 218.37| Planting dates = 21.84, Spraying = 21.84, interaction = 37.83 |
| Mean          | 308.90             | 241.48             | 228.79             | 279.72|            |
|               | 341.45             | 266.86             | 241.79             | 283.36|            |
|               | 305.73             | 236.48             | 219.24             | 237.80|            |

### Carpine mg/ml⁻¹

|               | First date (15/10) | Second date (1/11) | Third date (15/11) | Mean | L.S.D 0.05 |
|---------------|--------------------|--------------------|--------------------|------|------------|
|               | 78.30              | 58.88              | 33.03              | 56.73| Planting dates = 10.57, Spraying = 10.57, interaction = 18.31 |
| Mean          | 96.24              | 62.70              | 48.20              | 69.04|            |
|               | 113.13             | 88.45              | 50.19              | 83.92|            |
|               | 95.89              | 70.01              | 43.80              | 70.41|            |

### Gentianine mg/ml⁻¹

|               | First date (15/10) | Second date (1/11) | Third date (15/11) | Mean | L.S.D 0.05 |
|---------------|--------------------|--------------------|--------------------|------|------------|
|               | 120.19             | 62.44              | 19.08              | 67.23| Planting dates = 13.77, Spraying = 13.77, interaction = 23.84 |
| Mean          | 178.24             | 65.30              | 33.03              | 92.19|            |
|               | 274.92             | 73.78              | 65.39              | 138.03|            |
|               | 191.11             | 67.17              | 39.16              | 118.72|            |

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