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Research article

Effect of Gibberellic Acid and Superphosphate Fertilizer on Medical Compounds and Growth Characters of Fenugreek Plant (*Trigonella foenum-graecum* L.) Local Variaty

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ABSTRACT

The study was carried out using pots (capacity, 4kg soil/pot) in the glasshouse and different concentrations of gibberellic acid (0, 25, 50 and 100) ppm and different levels of superphosphate (0.25, 0.50 and 100) gm/kg:soil/Pot, these equal to (83.33, 166.66 and 333.32) kg/hectar. In some medical compound which effect on Diabetes like coumrin, trigonellin and fenugreeken and some growth characters of fenugreek plant (local variaty) like absolute growth rate, biomass duration, protein percentage, chlorophyll percentage and protein percentage in seeds were used. This factorial experiment (4 × 3) was conducted by using complete randomizing design (C.R.D) with three replication, means were compared using the least significant difference at 0.05 probability level. The results could be summarized that the treatments by gebberllic acid (GA3) alone showed significant increase in absolute growth, biomass, chlorophyll contant, protein percentage and medical compounds. While treatments with superphosphate fertilizer alone showed significant increase only in protein percentage and medical compounds. The interaction between GA3 and phosphate fertilizer showing the best results in all growth characters for all plants in this experiment, specially the treatment with 50 ppm of GA3 with 0.50 gm/pot and 1.0 gm/pot of superphosphate fertilizer gave the highest values of studied compared with control treatment.

Keywords: Fenugreek, Gibberellic Acid, Local Variaty, Superphosphate.

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INTRODUCTION

Plants have been an important source of medicine for thousands of years, even today. The world health organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for medicine plants are also the source of many modern medicine [1]. Fenugreek is considered a rich source of essential component like protein, oils, carbohydrates, minerals and vitamine [2]. Its seed contains medical and secondary compounds like type of glycosides,

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alkaloid, steroidal saponince and mucilage substance [3]. Seventy percent of German doctors are describing the herbal for the treatment of their diseases [4]. Seeds of fenugreek are used in wide range in the medical description and in the medicine manufacture because it contains high quantity and many type of active medical compound which is used in the treatment of many diseases such as diabetes, hypotension, heart diseases treatment, heart attack and decreasing of blood cholesterol level [5, 6].

Gibberellins (GA3) is a type of plant growth substances, which is stimulating cell division and cell elongation and other regulatory function, it has been used as standard in bioassay systems [7]. Previous study [8] was observed that using of superphosphate fertilizer effect on growth of plant and we found good response for plants to phosphorus which fixated nitrogen. Another study found [9] that the adding of superphosphate fertilizer has significantly increased plant root growth and reduced root banding in the soil which effect on increased the absorption of water and material elements. Moreover, other investigators found that the phosphorus element is important and enters the plant cell compounds and is associated with production and the structure of the adenosine tri phosphate (ATP) and Ribonucloic acid (RNA), Deoxyribo nucleic acid (DNA) and some proteins where contributes in respiration, photo synthesis, cell divisions, flowering and seeds production in plants [10].

The aim of this study was to determine the effect of hormone (GA_3) and superphosphate fertilizer to stimulate the growth in fenugreek plant, and increased some chemical compound in the seeds of fenugreek plant which effected on the diabetes by using high-performance liquid chromatography (HPLC).

MATERIALS and METHODS

The study was conducted by using pots in the glass house of biology department during 2014-2015 growth season, by bringing soil from the botanical garden of the department of biological sciences and softed and riddled by sieve diameter of pors (2 mm), and estimate some chemical and physical characteristics (**table 1**) according to the previous methods [11]. Four kg of sifted soil for each pot was taken and used four concentration from gibberellic acid (GA3) which is (0, 25, 50 and 100) ppm was prepared from the main solution according to the low of dilution, and three levels of superphosphate fertilizer which is (0.25, 0.50 and 1.00) gm/pot, which is equal (83.33, 166.66 and333.32) kg/Ha. The experiment was designned according to randomized complete design, experiment factorial with three replicates (3, 3, 4) so that included (36) experimental units, the results statistically analyzed by the statistical analysis system (SAS) by using the least significant difference (L.S.D) at the level of probability (0.05) [12].

Table 1. Physical and chemical properties of the soil used in the experiments

Value	Units	Soil texture
244	$gm.kg^{-1}$	Clay
440	$gm.kg^{-1}$	Silt
316	$gm.kg^{-1}$	Sand
2.7	$ds.m^{-1}$	Ec
7.06	$mg.kg^{-1}$	Ph
4.66	$mg.kg^{-1}$	N
20.0	$mg.kg^{-1}$	P
291.5	$mg.kg^{-1}$	K

Superphosphate fertilizer added before agricultural operation, the fenugreek seeds planted in 20/11/2014 with 14 seeds in each pot. The agricultural operations were conducted like irrigation and remove the bush from time to time, and after three weeks reduced the plants to 10 plants in each pot, and sprayed with gibberellic acid after completion of the fourth leaves, after that studied the first harvest was taken after 50 days from the planting (H1 – D50), the second harvest was taken after 120 days (H2-D120) some morphological characteristics which including:

1. Absolute Growth Rate: (g. g dw/d), plant absolute growth rate is calculated based on [13] equation, which based on shoot dry weight during first and second harvest time:

A.G.R = $\frac{W_2 - W_1}{T_2 - T_1}$

(W, weight and T, time].

2. Biomass Duration: is calculated based on following equation [14]:

Biomass duration (B.M.D) (g. g of dw/Day)

= $\frac{(T_2 - T_1) \times (W_2 + W_1)}{2}$

3. Determination of the nitrogen concentration and protein percentage: To determine the nitrogen, certain weight of the plant sample from shoot and digested according to the previous method. Digestion of the plant sample, after this nitrogen was determined by the micro kjeldahl apparatus [15,16] using the following equation.

[V, volume; N, normality].

%N = $\frac{V_1 \times V_2 \times N_1 \times 14 \times 100}{A \times B \times 1000}$

4. Nitrogen concentration was converted to protein percentage through following equation [17]:

Protein percentage = % N X 6.25

5. Total chlorophyll content (mg/cm)

Leaf chlorophyll content was estimated using the chlorophyll-meter instrument (for chlorophyll measurement), type (SPADA-502 plus. A1RT-206. Japan). Three leaves from each pot were taken and each one was put on the sensitive lens of instrument then the leaf chlorophyll, content was determined.

6. Extracting and separating the most important medical compounds found in the fenugreek plant was also done by using HPLC after extracting the oil from the seeds, these compounds (coumarin, fenugreeken and trigonelline).

Combined with glucose; they causes blood thinning, anti-fungicidal, antitumor and increases the blood flow in the veins, it be toxic when used at high doses for a long time. These substances work hardly against diabetes [18].

RESULT and DISCUSSION

It can be observed that there was significant in the absolute growth rate with increasing in the each of gibberellic acid without superphosphate, and the interaction of gibberellic acid and superphosphate fertilizer (**Table 2**). In the gibberellic acid without superphosphate the high significant increase in absolute growth rate of leaves, means was observed at the level of 100 ppm, which was 0.207. While the superphosphate fertilizer showed no significant increasing in absolute growth rate. The gibberellic acid with superphosphate interaction showed that the increasing in levels at 0.5 gm superphosphat and 100 ppm GA3, which record 0.215 gm/day for absolute growth rate compared with other results. While the minimum result was record for (0 ppm GA3 and 1 gm superphosphate).

It was seen that there was significant increase in biomass duration means with increase in gibberellic acid without sup-

Table 2. Effect of Superphosphate levels and concentrations of gibberellic acid on absolute growth rate (gm/ day)

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	0.18	0.17	0.165	0.172
25	0.18	0.205	0.215	0.20
50	0.19	0.21	0.21	0.203
100	0.20	0.215	0.205	0.207
Mean	0.188	0.20	0.199	

LSD: superphosphate=0.022 NS, gibberelic = 0.027 NS, superphosphate x gibberelic = 0.039*; *P<0.05, NS, Non-significant.

erphosphate, superphosphate fertilizer without gibberellic acid and the interaction of gibberellic acid with superphosphate fertilizer levels (**Table 3**). Treatment with GA3 produced high significant increase in means at 50 ppm which was 572.92 gm/day, comparing with other results. Treatment with superphosphate only produced a significant increase in means can be noticed at level (1.0 gm/pot) which was 545.93 gm/day. Gibberellic acid with superphosphate fertilizer interaction showed that an increase in interaction levels from 0 ppm GA3 and 0.25 gm superphosphate to 50 ppm GA3, and 1.0gm superphosphate led to significant increase in biomass duration values from 449.96 to 606.11 gm/day.

Table 3. Effect of Superphosphate levels and concentrations of gibberelic acid on biomass duration (gm/day).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	449.96	483.81	502.81	478.86
25	517.11	550.55	546.48	538.04
50	540.54	572.11	606.11	572.92
100	514.58	521.40	528.33	521.43
Mean	505.54	531.96	545.93	

LSD: superphosphate = 31.67*, gibberelic = 40.52*, superphosphate x gibberelic = 59.22*; *P<0.05.

Table 4 showed significant increase in chlorophyll content with increase in gibberellic acid and interaction of gibberellic acid with superphosphate fertilizer, but there was no significant increase when treatment with fertilizer only in plants under study. Increasing was observed with treatment by gibberellic acid only; the best result in means was at 50 ppm GA3 which was 51.28 gm/ cm comparing with other concentrations. Interaction of gibberellic acid and superphosphate fertilizer also showed significant increase in interaction levels from 0 ppm

Table 4. Effect of superphosphate levels and concentrations of gibberelic acid on chlorophyll content (gm/cm).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	44.14	44.26	44.97	44.45
25	46.67	48.24	45.89	46.93
50	49.19	52.44	52.21	51.28
100	46.93	42.40	49.22	46.14
Mean	46.73	46.83	48.07	

LSD: superphosphate = 2.94 NS, gibberelic = 3.075*, superphosphate x gibberelic = 4.66*; *P<0.05, NS: Non-significant

GA3 and 0.25 gm superphosphate to 50 ppm GA3 and 0.5 gm and 1.0 gm superphosphate that led to increase in chlorophyll content values from 44.14 to 52.44 and 52.21 gm/cm.

Results in **table 5** presented, there was significant increase in protein percentage in vegetative part of plants, in each of superphosphate fertilizer, and interaction of superphosphate with gibberellic acid, but there no significant increase were present in treatment with gibberellic acid alone, the best increases in protein percentage means present at levels 0.5 and 1.0 gm/pot of superphosphate fertilizer, which were 4.26% and 4.07%, respectively.

While interaction between superphosphate and gibberellic acid at levels 50 ppm GA3 and 0.5 gm/pot fertilizer and 50 ppm GA3 and1.0 gm/pot fertilizer showed the highest increases in protein percentage which were 4.58% and 4.25%, respectively.

Table 5. Effect of Superphosphate levels and Concentrations of Gibberelic acid on concentration of protein for vegetative part (%).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	3.61	4.00	3.88	3.83
25	3.95	4.26	4.09	4.10
50	4.12	4.58	4.25	4.31
100	4.10	4.20	4.05	4.12
Mean	3.94	4.26	4.07	

LSD: superphosphate = 0.208*, gibberelic = 0.336 NS, superphosphate x gibberelic = 0.486*; *P<0.05, NS: Non-significant.

Significant increase was seen in protein percentage of seeds for all treatments and the interaction between treatments. The best results in means were presented at concentration of gibberellic acid 50 ppm and 100 ppm, which were 28.82% and 27.96%, respectively. At levels of superphosphate fertilizer 0.5 and 1.0 gm/pot, which were 25.42% and 25.46%, respectively. While the interaction between gibberellic acid and superphosphate fertilizer showed the highest results at levels 50ppm GA3 and 0.5 gm/pot fertilizer and 50 ppm GA3 and 1.0 gm/pot fertilizer, which were 29.81% and 29.29%, respectively (**Table 6**).

Table 6. Effect of superphosphate levels and concentrations of gibberelic acid on protein of seeds (%).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	18.27	20.69	21.66	20.2
25	22.69	22.81	23.21	22.9
50	27.36	29.81	29.29	28.82
100	27.81	28.39	27.69	27.96
Mean	24.03	25.42	25.46	

LSD: Superphosphate = 2.69*, gibberelic = 4.85*, superphosphate x gibberelic = 6.1*; *P<0.05.

Results of **tables 7,8** and **9** showed significant increase in levels of compounds trigonelline, goumarine, and fenugreken with increase in gibberellic acid only without superphosphate and in interaction between gibberellic acid and superphosphate fertilizer. While there were no significant increase in treatment with superphosphate fertilizer alone. The best results were observed in means shown in tables above; at concentration 50

ppm GA3, which were 6.120, 2.943 and 1.391%, respectively. In **table 9** at concentration 100 ppm GA3 was 1.395% of

Table 7. Effect of superphosphate levels and concentrations of Gibberelic acid on concentration of trigonell (HPLC).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	4.331	4.77	5.437	4.846
25	5.114	6.111	5.913	5.713
50	5.817	6.221	6.321	6.120
100	5.211	6.002	6.110	5.774
Mean	5.118	5.776	5.945	

LSD: Superphosphate = 0.885 NS, gibberelic = 1.09 *, superphosphate x gibberelic=1.73*; *P<0.05, NS: Non-significant.

fenugreken. The highest increase found levels of interaction 50 ppm GA3 and 1.0 gm/pot fertilizer, which were 6.321, 2.992 and 1.441%, respectively and as shown in **table 9** at level 100 ppm GA3 and 1.0 gm/pot fertilizer it was 1.514%.

Table 8. Effect of superphosphate levels and concentrations of gibberelic acid on concentration of coumarin (HPLC).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	2.141	2.413	2.466	2.340
25	2.723	2.614	2.781	2.706
50	2.898	2.939	2.992	2.943
100	2.422	2.333	2.452	2.476
Mean	2.568	2.575	2.672	

LSD: superphosphate = 0.346NS, gibberelic = 0.502*, superphosphate x gibberelic = 0.663*; *P<0.05, NS: Non-significant.

The results of present study due to the active effect of gibberellic acid increase uptaking the nutrients through increasing the permeability of cell membreans observed thus that will be increase the biomass and growth of plants, furthermore, the gibberellic acid necessary for promoting the protein, and chlorophyll bluding in legumes [19]. The phosphorus element in fertilizer has a very effective role in promoting growth of plants is necessary for increases the root and shoot system [20]. These results agree with other previous studies [20,21, 22], which were promoting of interactional effect between superphosphate and gibberellic acid in increasing growth and chemical compounds in different legums like field bean, alfafa and lentil.

Table 9. Effect of superphosphate levels and concentrations of gibberelic acid on concentration of fenugreleen (HPLC).

Concentrations of Gibberelic acid (ppm)	Superphosphate levels (gm.pot)			Mean
	0.25	0.50	1.00	
0	0.143	0.322	0.362	0.276
25	0.841	0.911	0.992	0.915
50	1.312	1.421	1.441	1.391
100	1.311	1.36	1.514	1.395
Mean	0.901	1.003	1.077	

LSD: superphosphate = 0.378NS, gibberelic = 0.512*, superphosphate x gibberelic = 0.832*; *P<0.05, NS: Non-significant.

From current study we concluded that using gibberellic acid (GA3) and superphosphate fertilizer together in breeding have an active role in promoting growth of plants. From the present results we noted that interaction effect of GA3 and superphosphate fertilizer increase growth rates of plants and its better than using GA3 or phosphate fertilizer alone in breeding.

Conflict of interest

The authors declare that they have no conflict of interests.

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