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Original Contribution

RESPONCES OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) TO EXOGENOUS APPLICATION OF PLANT GROWTH REGULATORS (PGRs)

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ABSTRACT

PURPOSE: Evaluation of growth, phytochemical and morpho-physiological properties in fenugreek (*Trigonella foenum- graecum* L.) under application of plant growth regulators (PGRs). **METHODS:** The experiment was conducted on randomized complete blocks design (RCBD) with 13 treatments and 3 replications. The treatments were consist of control (distilled water application), giberrellic acid (GA_3) and naphthalene acetic acid (NAA) each at 25 and 50 ppm by either a pre-plant soaking, a spraying at 20 days after planting, and a combination of pre-plant soaking plus a spraying at 20 days after planting. **RESULTS:** Application of PGRs through combination of pre-plant soaking plus a spraying significantly increased shoot dry weight, 1000-seeds weight, number of seeds per pod, content of seed trigonelline, leaf area per plant ($p \leq 0.01$), and also, plant height, stem diameter, number of pods per plant, content of seed mucilage, and root, stem, leaf and pod dry weight ($p \leq 0.05$). Of course, application of PGRs had no significant effect on the amount of SPAD value and number of leaves per plant. **CONCLUSIONS:** Application of GA_3 and NAA 50 ppm through combination of pre-plant soaking plus a spraying were effective to obtain maximum phytochemical and morpho-physiological properties in fenugreek.

Key word: *Trigonella foenum- graecum* L. gibberellic acid, naphthalene acetic acid, soaking and spraying application.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop, self-pollinating and dicotyledonous plant belonging to the Leguminacae (Fabaceae) family. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses, making food, roasted grain as coffee-substitute, controlling insects in grain storages, and perfume industries (1).

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Several intrinsic and extrinsic factors affect on growth, development and secondary metabolites biosynthesis and accumulation of medicinal and aromatic plants. Phytohormones and plant growth regulators (PGRs) have been defined as one of the main factors influence plants growth and their primary and secondary metabolites pool (2). The use of PGRs in the field of agriculture has become commercialized in some advanced countries like Europe, USA and Japan. PGRs can be divided into five classes as auxin, gibberellins, cytokinins, abcisic acid, and ethylene. Naphthalene acetic acid (NAA) belongs to synthetic forms of auxins. Auxins play key role in cell elongation, cell division, vascular tissue, differentiation, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering (3).

Giberrellic acid is one of the most important growth stimulating substances which used in agriculture and occurs naturally in many plants. It regulates various important functions such as elongation of stems, creation of proteins and germination of seed plants (4).

This study survey the effect of PGRs on growth performance, yield potential and quality improvement. Since, there is any precise and conclusive information available on the effect of PGRs on various physiological process and productivity potential in fenugreek. Therefore, objective of this research was to elucidate further the effect of PGRs and methods of their application on yield, phytochemical and morpho-physiological characteristics of fenugreek.

MATERIALS AND METHODS

This study was carried out in 2011-2012 at Academic Centre for Education, Culture and Research (ACECR), Institute of Medicinal Plants (56° 35′ N and 50° 58′ E; 1500 m elevation). The soil of experimental farm was a loam-silt containing 0.071% N, 48.9 $mg.kg^{-1}$ P, 33.6 $mg.kg^{-1}$ K, EC 2.71 $ds.m^{-1}$, and pH 8.3. The details of the treatments are mentioned in **Table 1.**

Table 1. *The characterizations of studied treatments*.

Code	Treatments	
	Concentration of PGRs	Application methods
G_2D	GA_3 at 25 ppm	Pre-plant soaking*
G_2S	GA_3 at 25 ppm	Spraying**
G_2DS	GA_3 at 25 ppm	Combination of pre-plant soaking plus spraying
G_5D	GA_3 at 50 ppm	Pre-plant soaking
G_5S	GA_3 at 50 ppm	Spraying
G_5DS	GA_3 at 50 ppm	Combination of pre-plant soaking plus spraying
N_2D	NAA at 25 ppm	Pre-plant soaking
N_2S	NAA at 25 ppm	Spraying
N_2DS	NAA at 25 ppm	Combination of pre-plant soaking plus spraying
N_5D	NAA at 50 ppm	Pre-plant soaking
N_5S	NAA at 50 ppm	Spraying
N_5DS	NAA at 50 ppm	Combination of pre-plant soaking plus spraying
C	Control	Pre-plant soaking and spraying with distilled water

*Pre-plant soaking of seeds was for 8 hours (5).

**Spraying at 20 days after sowing covered the plant until the drops start to fall (5).

Seeds were sown by hand with 25 cm apart in eight lines per plot. Seed rate was 25 $kg.ha^{-1}$ (6). In this research, plot sizes were 3 m in length, 2 m in wide and total plot area was 6 m^2. Before harvesting, two edge rows and 50 cm from each plot heads were discarded as side effects. In order to measure total dry matter, 10 plants were selected randomly from each plot and then were placed in the electric oven of 75℃ until the constant weight was gained. Morphological and physiological characteristics including plant height (cm), stem diameter (mm), 1000-seeds weight (g), number of leaves per plant ($leaves.plant^{-1}$), number of pods per plants ($pods.plant^{-1}$), number of seeds per pod ($seeds.pod^{-1}$), leaf area per plant ($cm^2.plant^{-1}$), SPAD value (SPAD), and root, stem, pod and shoot dry weight ($kg.ha^{-1}$). Phytochemical characteristics including content of seed mucilage (%) and trigonelline ($mg.g^{-1}$ DW) were determined.

Isolation and extraction of mucilage

Fenugreek seeds (200 g) were soaked in distilled water (1.5 L) at room temperature for 1 h and then boiled under stirring condition in a water bath until the slurry was prepared. The solution was cooled and kept in a refrigerator overnight to settle out undissolved materials. The upper clear solution was decanted and centrifuged at 500 rpm for 20 minutes. The supernatant was separated and was concentrated at 60℃ on a water bath to one third of its original volume. The solution was cooled to the room temperature and was poured into thrice the volume of acetone with continuous stirring. The precipitate was washed repeatedly with acetone and dried at 50-60 ℃ under vacuum. The dried material was powderd and kept in a desiccator (7).

Analysis and quantization of trigonelline

For measurement of trigonelline in the seed samples, the modified method of Zheng and Ashihara (8) was applied. The samples were ground with 80% methanol and magnesium oxide (MgO) in a mortar and pestle. After incubation at 60℃ for 30 min, the homogenates were centrifuged and the supernatant was collected. After complete

evaporation of methanol, the methanpl-soluble extracts were dissolved in distilled water. The samples were filtered using a disposable syringe filter unit and the aliquots were used for determination of trigonelline (TG) by HPLC. The analyses of the samples were carried out using a Knauer K2600A liquid chromatography (Germany), equipped with a Nucleosil C18 (150 mm * 4.6 mm I.D, 5 µm) column. A mixture of methanol: water (50:50 v/v) served as the mobile phase and pH of solution adjusted to 5.0 with 50 mM sodium acetate. The elution has been made in an isocratic mode at a flow rate of 1 $mL.min^{-1}$ and the detection made at 268 nm by UV detector from the above mentioned company (9-10). One analysis requires 20 min. The retention time of this alkaloid was 4.4 min. Before carrying out HPLC analysis, we made calibration curve by using different concentration (0.1, 0.3, 0.6, 0.8 and 1.0 $mg.mL^{-1}$) of trigonelline in phase media. Then calibration curve made with trigonelline and the correlations were excellent for trigonelline **(Figure 1).** This process was performed according to United States Pharmacopoeia by cold extraction method as directed for alcohol soluble material, except to use water in place of alcohol (6).

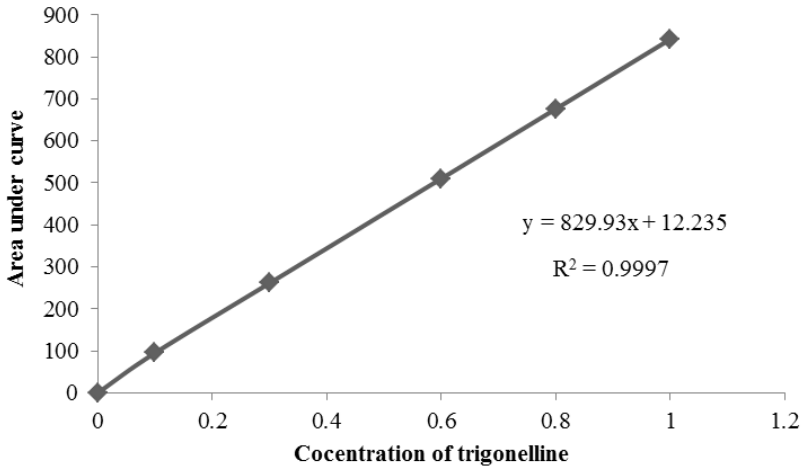

Figure 1. Calibration curve using trigonelline as standard

This study was done on the base of randomized complete block design (RCBD) with 13 treatment and 3 replications. Analysis of variance (ANOVA) was done by SPSS statistical software package version 17. Mean values and significance were determined by "the least significant difference (LSD) mean comparison test".

RESULTS

The present research work was conducted to evaluate the effect of PGRs on growth, phytochemical and morphological characteristics of fenugreek (*Trigonella foenum-graecum* L.). The results indicated that the application of different methods and concentrations of NAA and GA_3 were significantly influenced (at $p \leq 0.01$) on shoot dry weight, 1000-seeds weight, number of seeds per pod, content of seed trigonelline and leaf area per plant. Treatments were also affected (at $p \leq 0.05$) on plant height, stem diameter, number of pods per plant, content of seed mucilage, and root, stem, leaf and pod dry weight. Of course, theirs effects were not significant on SPAD value and number of leaves per plant **(Table 2).**

Mean comparisons showed that the highest plant height, stem diameter, stem, leaf and root dry weight and leaf area per plant were obtained in NAA at 50 ppm (through combination of pre-plant soaking plus a spraying) and the lowest of all were observed in control **(Table 3)**. The highest pod and shoot dry weight, 1000-seeds weight, number of seeds per pod, content of seed trigonelline and mucilage were occurred in GA_3 at 50 ppm (through combination of pre-plant soaking plus a spraying) **(Table 3)**. The highest number of pods per plant was recorded in NAA at 50 ppm and GA_3 at 25 ppm (through combination of pre-plant soaking plus a spraying) and GA_3 at 50 ppm (through spraying, and also combination of pre-plant soaking plus a spraying, separately) and the lowest was observed in control **(Table 3)**.

DISCUSSION

PGRs can be caused changes in the absorption of nutrients and water. The amount of PGRs

application had a substantial role for increasing the plants yield. Herb growth, yield and morphological characteristics were significantly influenced by PGRs and the method of application. The increased herb growth with PGRs application can be due to the stimulation of cell division and elongation while increasing plasticity of cell wall (11).

Spraying of PGRs in other seed spice crops such as gibberellic acid (GA_3) on cumin had been reported to improve growth and seed yield (21). NAA, a synthetic growth regulator has proved its potentiality that in appropriate concentration NAA affects the growth and yield of tomato, bitter gourd and cowpea (12). Bakhsh *et al* (2011) reported that NAA level of 90 $ml.ha^{-1}$ has caused maximum plant height (3). Sedghi *et al* (2010) showed that the soaking seeds in GA_3 and NAA before planting of *Cucurbita pepo* L. and herb spray with NAA subsequently results in enhancement of yield (13). Varna (1990) showed that foliar application of plant growth regulators (NAA and CEPA) on cucumber seedlings increased the yield (14). The positive effect of NAA (synthetic auxin) on rooting can be attributed to the stimulation of division the primer root cells (15-16). The use of growth regulators is considered as one of the way of increasing yield. That has been suggested that germination and establishment of seedlings of various plants improved by seed treatments with PGRs resulted in the emergence of uniform seedling (17). Treatment of saffron corms with different concentrations of gibberellin showed that increase in concentrations of gibberellin improved weight and number of flowers (18). Sharma and Saran (1992) showed that seed priming of *Vigna mungo* with 40 mg L^{-1} GA_3 can increase speed of germination and emergence of seedlings in non-stress conditions (19). Priming sorghum seed with 50 $mg.L^{-1}$ gibberellic acid increased seed yield. The promoting effect of GA_3 on DNA, RNA and protein synthesis and ribose and polyribosome multiplication (20) would contribute towards biomass production of vegetative parts as well as pod dry weight, 1000-seeds weight and their contents.

GA_3 is known to induce an influx of Ca^{2+} into the endoplasmic reticulum of guard cells, thereby initiating a process that leads to increase in stomata activity (22). A less stomata resistance enables an easier exchange of gases (23). With increasing CO_2, photosynthesis was increased and subsequently it improved yield of seeds. In general, the GA_3 treatment may have also strengthened the sink potential of the developing pods and through enhancement of the duration rate of assimilate translocation to these reproductive structures caused the observed increase in pod dry weight and 1000-seeds weight. It is during this critical growth phase that the basic infrastructure of the plant functioning is laid down, the effective dividends of which are reaped when the plant reaches harvest (24- 25). Higa (1972) reported that application of auxin and gibberellin improves the evolutionary process of flowers and increased fruit set in saffron (26). Vijayaraghavan (1999) showed that the grain yield of pearl millet (*Pennisetum glaucum* L.) was increased by the soaking seeds in GA_3 before planting (27).

PGRs have been defined as one of the main factors influence plants growth and their secondary metabolites pool. Phytohormones play a crucial role in the regulation and coordination of plant growth, morphogenesis and metabolism. It is thus postulated that they also will play a role in the biosynthesis of alkaloids. More researches have been devoted to the improvement of alkaloid production in cell suspension cultures (28). In recent researches, PGRs have been shown to improve quality yield in basil, coriander and fenugreek (11). The positive effect of foliar PGRs on alkaloid production might be attributed to the improved overall plant growth and metabolism as revealed in present study. It seems that PGRs might have enhanced the intrinsic genetic potential of the fenugreek plants to produce additional yield with improved quantity of alkaloid through enhanced plant growth, photosynthesis and overall plant metabolism in the present study. The positive effect of foliar PGRs on content of trigonelline and mucilage seeds might be attributed to the improved overall plant growth and metabolism as revealed in present study. GA enhances metabolic activity within pathways leading to accumulation of secondary metabolites, *e.g*. steroids, anthocyanin and essential oil (terpenoid) production (29). Srivastava and Srivastava (2007) reported that total alkaloid contents in leaves, stems, and roots of GA-treated plants (*Catharanthus roseus*) were significantly higher than in untreated plant parts (29). Subroto and Doran (1994) reported that GA_3 had positive effect on improvement of steroidal alkaloids accumulation in the *Solanum aviculare* (30).

Table 2. *Analysis of variance (mean of square) for phytochemical and morpho-physiological characteristics of fenugreek*

S.O.V	df	Plant height	Stem diameter	Root dry weight	Stem dry weight	Leaf dry weight	Pod dry weight	Shoot dry weight	1000-seeds weight
Block	2	0.359	0.0026	2365.471	2626.297	1488.828	14563.364	23481.437	0.049
Treatment	12	11.295 *	0.133 *	5239.862 *	8056.663 *	11109.214 *	48633.305 *	147714.014 **	1.873 **
Error	24	4.946	0.045	2371.584	2804.186	4736.020	17905.567	28939.831	0.481
CV (%)	-	15.57	12.52	18.2	13.91	15.13	12.84	9.06	10.13

*, **, ns shows significant at 5%, 1% and not significant, respectively.

Table 2. *Continued*

S.O.V	df	Number of leaves per plant	Number of pods per plant	Number of seeds per pod	SPAD value	Content of seed mucilage	Content of seed trigonelline	Leaf area per plant
Block	2	1.955	0.100	0.284	8.110	79.241	1.943	0.398
Treatment	12	6.856 ns	0.326 *	1.421 **	81.664 ns	21.250 *	19.471 **	296.793 **
Error	24	3.557	0.139	0.454	59.051	9.133	5.521	41.135
CV (%)	-	18.45	14.25	15.69	17.98	13.92	12.42	9.03

*, **, ns shows significant at 5%, 1% and not significant, respective

Table 3. *Comparison of means* for phytochemical and morpho-physiological traits of fenugreek.*

Treatment	Plant height (cm)	Stem diameter (mm)	Root dry weight ($kg.ha^{-1}$)	Stem dry weight ($kg.ha^{-1}$)	Leaf dry weight ($kg.ha^{-1}$)	Pod dry weight ($kg.ha^{-1}$)	shoot dry weight ($kg.ha^{-1}$)
C	9.940 c	1.147 c	161.690 c	266.710 d	317.697 c	722.533 c	1306.940 d
G_2D	11.99 bc	1.6 b	225.380 bc	373.390 bc	416.717 bc	1040.447 ab	1830.560 bc
G_2DS	14.05 abc	1.74 ab	289.080 ab	360.060 bcd	462.100 ab	1062.127 ab	1884.287 bc
G_2S	14.77 ab	1.69 ab	254.780 ab	373.390 bc	462.100 ab	989.880 b	1825.363 bc
G_5D	13.57 bc	1.68 ab	254.780 ab	373.390 bc	449.723 b	1054.897 ab	1878.013 bc
G_5DS	15.50 bc	1.80 ab	289.077 ab	453.400 ab	519.867 ab	1300.560 a	2273.830 a
G_5S	14.94 ab	1.75 ab	293.980 ab	426.730 abc	462.103 ab	1156.053 ab	2044.890 abc
N_2D	13.25 bc	1.59 b	254.780 ab	373.390 bc	424.967 bc	989.867 b	1788.237 c
N_2DS	15.66 ab	1.76 ab	289.077 ab	373.390 bc	474.483 ab	975.420 b	1823.297 bc
N_2S	14.66 ab	1.69 ab	289.077 ab	373.390 bc	470.357 ab	1062.123 ab	1905.873 bc
N_5D	13.99 abc	1.60 b	254.780 ab	373.390 bc	424.970 bc	1054.903 ab	1853.260 bc
N_5DS	18.02 a	2.08 a	333.177 a	480.070 a	585.883 a	1062.123 ab	2128.083 ab
N_5S	15.38 ab	1.87 ab	289.077 ab	346.720 cd	441.470 bc	1076.577 ab	1864.773 bc

Table 3. *Continued*

Treatment	1000-seeds weight (g)	Number of pods per plant ($pods.plant^{-1}$)	Number of seeds per pod ($seeds.pod^{-1}$)	Content of seed mucilage (%)	Content of seed trigonelline ($mg.g^{-1}$ DW)	Leaf area per plant ($cm^2.plant^{-1}$)
C	5.1 d	2.0 b	3.0 c	16.19 c	13.55 c	48.83 c
G_2D	6.2 cd	2.33 ab	3.77 bc	19.50 bc	17.36 bc	65.93 b
G_2DS	7.4 abc	3.0 a	4.66 ab	23 ab	20.23 ab	72.76 b
G_2S	6.7 abc	2.66 ab	3.99 bc	21.33 abc	18.30 b	72.1 b
G_5D	6.5 bc	2.33 ab	3.77 bc	22.40 ab	17.85 bc	72.1 b
G_5DS	8.0 a	3.0 a	5.44 a	25.80 a	24.44 a	72.13 b
G_5S	7.7 ab	3.0 a	4.77 ab	24.63 ab	20.95 ab	72.53 b
N_2D	6.3 cd	2.33 ab	3.88 bc	18.83 bc	17.59 bc	65.36 b
N_2DS	7.2 abc	2.66 ab	4.44 ab	22.50 ab	18.74 b	72.63 b
N_2S	6.5 bc	2.66 ab	4.33 ab	21.63 abc	18.41 b	67.8 b
N_5D	6.5 bc	2.33 ab	3.77 bc	19.30 bc	17.62 bc	65.1 b
N_5DS	7.7 ab	3.0 a	5.36 a	24.23 ab	20.48 ab	89.66 a
N_5S	7.2 abc	2.66 ab	4.60 ab	22.73 ab	20.53 ab	86.26 a

* Means in each column having at least a same letter are not significantly different.

CONCLUSION

The results obtained from the present experiment showed that fenugreek (*Trigonella foenum-graecum* L.) positively responded to PGRs application. As a conclusion it can be stated that the application of PGRs have an effect on seed yield and active ingredients seeds of fenugreek (*Trigonella foenum- graecum* L.). Related to different purposes of fenugreek cultivation, special treatments can be recommended. So that, if the aim of fenugreek cultivation is to improve vegetative yield, at least three times application of NAA (at 50 ppm) during the growing season is recommended. But if the purpose of planting is achieving the maximum content of secondary metabolites, it is recommended to use GA_3 (at 50 ppm).

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