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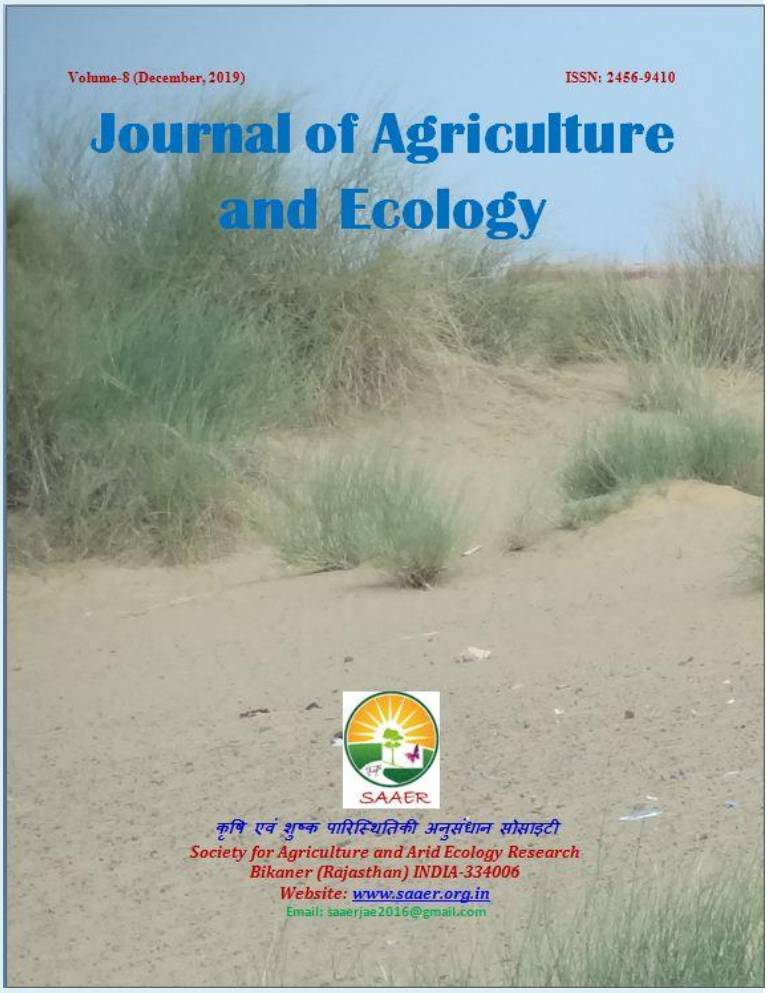


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# Nutritional and nutraceutical properties of different plant parts of fenugreek (*Trigonella foenum-graecum* L.)

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**Abstract**

Studies on influence of different growth stages on physiological and biochemical parameters of fenugreek *(Trigonella foenum-graecum* L.*)* was carried out at HC & RI, TNAU, Coimbatoreduring 2017-18. Seven different plant parts *viz.*, young seedlings (7-10 days after germination), fresh leaf at matured stage (30-40 days after sowing), dry leaf at matured stage (30-40 days after sowing), fresh leaf at matured stage (60-70 days after sowing), dry leaf at matured stage (60-70 days after sowing), dry seed and sprouted seed were tried. The study revealed that, the sprouted seeds and the dried seeds were found to be best with higher quality parameters *viz*., total protein, total carbohydrate, total phenol, total fat, crude fibre, calcium, magnesium and iron contents. On the other hand, the fresh leaves at matured stage (60-70 days after sowing) recorded higher (54.37 mg $100g^{-1}$) ascorbic acid content. The chlorophyll 'a', chlorophyll 'b' and total chlorophyll content in fresh fenugreek leaves ranged from 2.07 to 2.19 mg $g^{-1}$, 1.02 to 1.16 mg $g^{-1}$ and 3.09 to 3.35 mg $g^{-1}$ respectively, while dried leaves had lower total chlorophyll content ranged from 0.983 to 1.18 mg $g^{-1}$.



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## Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an important seed spices, originated from South-Eastern Europe and belongs to Fabaceae family. it is an annual herb, commonly known as methi, diploid in nature with chromosome number 2n =16. Fenugreek is a self pollinated crop. The plant is characterized with trifoliate leaves, white papilionaceous flowers, roots with conspicuous root nodules and hard textured golden yellow seeds. The name fenugreek is derived from the Latin word 'Greek hey' illustrating its classical use as fodder. India is the largest producer of fenugreek, and in spices, third largest spice after coriander and cumin. During the year of 2016-17, in India, fenugreek is cultivated in an area of 2, 10,000 ha with an annual production of 2, 97,000 MT. In Rajasthan state alone, it is

cultivated is an area of 1,57,000 ha with a production of 1,90,360 tons (Spices Board, 2017). Fenugreek is mainly grown as leafy vegetable throughout India and there is an ample scope for its cultivation as seed spice. It is a short duration crop is fitting well in several cropping system. Seed crop requires cool dry climate and takes about three months duration thus fitting well as a *rabi* crop after the harvest of *kharif* main crops like paddy, chillies, cotton and pigeon pea. Fenugreek contains a number of chemical constituents including steroidal sapogenins, 23-26% protein, 6–7% fat and 58% carbohydrates of which about 25% is dietary fiber. Diosgenin component has been found in the oily embryo of fenugreek. Fenugreek is also a rich source of iron, containing 33 mg/100 g dry weight.

Fenugreek leaves contains seven saponins, graecunins compounds of diosgenin, 86.1% moisture, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber, 6% carbohydrates, ascorbic acid (220.97 mg per 100 g of leaves), β-carotene (19 mg per 100 g of leaves), vitamin C (52 mg), thiamine (40 µg), riboflavin (310 µg) and nicotinic acid (800 µg). For better retention of nutrients in fenugreek leaves, should be stored in refrigeration, dried in oven, blanched for a short period of time (5mins) and cooked in pressure cooker. Seeds are rich source of vitamin C, β-carotene, thiamine, riboflavin, nicotinic acid, folic acid, diosgenin, alkaloids (trigonelline, gentianine, carpaine), rhaponticin (polyphenol compound) and proteins. Germinated seeds have several beneficial properties over ungerminated seeds. Germination improves *in vitro* protein digestion, as well as fat absorption capacity, higher phenolic and flavonoid content, reduces blood sugar levels and cholesterol in diabetic patients. Considering all these aspects, the main aim of this study is to estimate the nutritional and nutraceutical properties of different growth stages of fenugreek.

## Materials and Methods

The present investigation was carried out during the Rabi season (Nov- Dec). The seeds of CO-2 variety of fenugreek was raised in the polybags for nutritional and nutraceutical analysis. The study was followed by Completely Randomized Block Design (CRD) with three replications. The experiment was conducted with seven different treatments *viz.*, young seedlings (7-10 days after germination), fresh leaf at matured stage (30-40 days after sowing), dry leaf at matured stage (30-40 days after sowing), fresh leaf at matured stage (60-70 days after sowing), dry leaf at matured stage (60-70 days after sowing), dry seed and sprouted seed. Physiological and biochemical parameters *viz.*, total chlorophyll content, total protein content, total carbohydrate content, total phenol content, total fat, crude fibre content, total polyphenol content, ascorbic acid content, calcium, magnesium and iron contents were analyzed and the following methods were used:

### Total chlorophyll content (mg $g^{-1}$)

The fully matured leaves of fenugreek were collected, weighed and macerated in a homogenizer with 80% acetone. The extract was centrifuged at 4000 rpm for 15 minutes. The supernatant was collected and made up to a known volume. The absorbance of extract was read in a spectronic 20 photoelectric- colorimeter at 645 and 663 nm and expressed in mg $g^{-1}$ leaf tissue (Reuter et al. 1986).

### Total soluble protein content (mg $g^{-1}$)

The Lowry's method of estimating soluble protein as described by (Lowry et al. 1951) was employed in the study.

### Total carbohydrate content (mg $g^{-1}$)

The total carbohydrate content in fenugreek was carried out by Anthrone method (Firestone 1990).

**Total phenol content (mg $g^{-1}$)**

Total phenol content of the leaves was estimated by using the procedure proposed by Malik and Singh (1980). Leaf sample (500 mg) was taken and cut into small leaf bits in five ml of 80% ethanol and kept in the hot water bath for 10 minute and the content was cooled. The leaf sample was macerated along with another 5 ml of 80% ethanol and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and the volume made up to 25 ml with distilled water. To the 1 ml of the supernatant, 2 ml of 20% sodium carbonate and 1 ml of folin's reagent were added. After 10 minutes, the OD was measured at 650nm..

**Fat content (%)**

Fat content of fenugreek was determined by Soxhlet Method (AOAC, 1995). 2-3 g of dried sample was weighed in a thimble and places it in the Soxhlet apparatus. The required volume of solvent (petroleum ether, boiling point of 40-60°C or ethyl ether or hexane) was added and connected to condenser and extract for 16 hours. The thimble was removed and the excess of ether was evaporated from the solvent flask on a hot water bath and dry the flask at 105°C for 30 min. The flask was cooled in desiccators and weighed (g).

$$\text{Fat content (\%)} = \frac{\text{Final weight of beaker - empty weight of beaker}}{\text{Weight of sample}} \times 100$$

**Crude fibre content (%)**

The crude fibre was calculated according to the method of Joslyn (1970) and expressed in percentage (%).

**Polyphenol oxidase activity (min $g^{-1}$)**

Polyphenol oxidase was assayed using the method suggested by Srivastava (1987). Standard reaction mixture contains 1.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.5 ml of enzyme preparation and 0.5 ml of 0.01 M catechol. At the start of the enzyme reaction, the absorbance was set to zero at 494 nm. The changes in the absorbance were recorded at 30 seconds interval and the polyphenol oxidase activity was expressed as changes in the OD of the reaction mixture per min $g^{-1}$ of fresh weight of tissue.

**Ascorbic acid content (mg $100g^{-1}$)**

Ascorbic acid content was determined by Horwitz (1975) method.

**Calcium content (mg $100g^{-1}$)**

Calcium was determined by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute $H_2SO_4$ against standard $KMnO_4$ employing Firestone (1990).

**Magnesium and Iron content (mg $100g^{-1}$)**

To estimate Fe & Mg, digest the mixture with di-acid (nitric acid & perchloric acid in 2: 1ratio) mixture and dilute it to 50 ml with deionized water and directly run on atomic absorption spectrophotometer. The standard was calibrated on AAS and then samples are read. The concentration reading given by AAS was multiplied by the dilution factor used during digestion.

**Statistical analysis**

The mean values of all the laboratory experiments were subjected to statistical analysis. The results of the experiments were statistically analyzed using SPSS (Statistical

Package for the Social Sciences) software developed by Nei 1978.

## Results and Discussion

### Physiological and biochemical parameters

The quality parameters were significantly influenced by all the different treatments of fenugreek. Data on chlorophyll 'a', chlorophyll 'b' and total chlorophyll content, total soluble protein, total carbohydrate, total fat, total crude fibre content, total polyphenol content, ascorbic acid content, calcium, magnesium and iron contents were analyzed and presented in the tables. The chlorophyll 'a' content was higher (2.19 mg $g^{-1}$) in young seedlings ($T_1$) followed by $T_2$ (fresh leaf at 30-40 DAS) with 2.13 mg $g^{-1}$. While in $T_5$ (dry leaf at 60-70 DAS) recorded lower chlorophyll 'a' content of 0.643 mg $g^{-1}$ (Table 1). The chlorophyll 'b' content was higher in young seedling ($T_1$) with 1.16 mg $g^{-1}$. While lowest content was recorded in ($T_5$) dry leaf at 60-70 DAS with 0.216 mg $g^{-1}$. Total chlorophyll content was higher in treatment ($T_1$) young seedling exhibited with 3.35 mg $g^{-1}$, followed by the treatment ($T_2$) fresh leaf at 30-40 days after sowing (3.22 mg $g^{-1}$) while lower total chlorophyll content was registered in treatment ($T_5$) dry leaf at 60-70 days after sowing with 0.983 mg $g^{-1}$, respectively. Chlorophyll is a pigment which is responsible for antioxidant activity. It gets diminished with maturity due to biosynthesis ends up to the stages of growth after which it declines. It was reported that total chlorophyll, chlorophyll 'a', 'b' have been decreased due to enhancement of chlorophyllase enzyme activity which might have resulted in degradation of chloroplast, when the chloroplast degradation occurs (Ramakrishnan et al. 1969). It was also reported that reduction in total chlorophyll content was due to chlorophyll destruction at senescence while cutting was done at second and third time in comparison to first (Drew & Sisworo 1977).

**Table 1.** Effect of different growth stages of fenugreek on chlorophyll 'a', chlorophyll 'b' and total chlorophyll content

| Treatment | Chlorophyll 'a' content (mg $g^{-1}$) | Chlorophyll 'b' content (mg $g^{-1}$) | Total chlorophyll content (mg $g^{-1}$) |
|---|---|---|---|
| $T_1$ (Young seedlings at 7-10 days after germination) | 2.19 | 1.16 | 3.35 |
| $T_2$ (Fresh leaf at matured stage at 30-40 days after sowing) | 2.13 | 1.09 | 3.22 |
| $T_3$ (Dry leaf at matured stage at 30-40 days after sowing) | 0.964 | 0.341 | 1.18 |
| $T_4$ (Fresh leaf at matured stage at 60-70 days after sowing) | 2.07 | 1.02 | 3.09 |
| $T_5$ (Dry leaf at matured stage at 60-70 days after sowing) | 0.643 | 0.216 | 0.983 |
| Mean | 1.60 | 0.765 | 2.36 |
| SEd | 0.037 | 0.014 | 0.052 |
| CD (1%) | 0.116 | 0.045 | 0.164 |
| CD (5%) | 0.081 | 0.032 | 0.115 |

The chlorophyll content increased during development up to 28 days after this duration, the chlorophyll content was decreased (Parekh et al. 1990**)**. The chlorophyll "a" is considered as the main photosynthetic pigment in higher plants, in which this pigment is responsible by the light absorption that promotes the start of photosynthesis process (Taiz & Zeiger 2002). The maintenance of the chlorophyll "b" of the plants was promoted by the increase of the antioxidant enzyme activities as super oxide dismutase and catalase (Gong et al. 2005**)**. The soluble protein content was higher in ($T_7$) sprouted seed (10.98 g $100g^{-1}$) followed by dry seed (10.52 g $100g^{-1}$), while lowest (6.25g $100g^{-1}$) protein content was recorded in ($T_5$) dried leaves at 60-70 DAS (Table 2). Proteins are involved in processes such as catalasing chemical reactions, facilitating membrane transport, intracellular structure and energy generating reactions involving electron transport. Total carbohydrate content was higher in dry seed with 21.24 mg $g^{-1}$ and followed by the sprouted seed which registered 18.07 mg $g^{-1}$. The carbohydrate content was found to be higher in fresh leaves (5.09 to 11.88 mg $g^{-1}$) than the dried leaves. The total phenol content was higher in the form of dry seed and followed by sprouted seed with 4.86 mg $g^{-1}$ and 4.12 mg $g^{-1}$. The phenolic compounds may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content (Syeda et al. 2008). The total fat content was lower in the form of dried leaves at the stage of 30-40 DAS with 0.90 %. In the form of seeds, both dry and sprouted have the higher fat content which recorded 5.57 % and 4.38 % respectively (Table 3). Low fat content in foods enhance storage life due to reduced chance of lipid peroxidation. The result of the total fat obtained from this study compares favorably with the works of Singh et al. (2010) and Berwal et al. (2018). Total crude fibre content was higher in treatment ($T_6$) dry seed (14.67 %), which was followed by ($T_7$) sprouted seed (12.58 %). The lower value of crude fibre was obtained from young seedlings ($T_1$) with 8.43 % respectively. %). In leaves, the fibre content was slightly increased from young seedlings to matured stage. The increase in crude fibre content was probably due to the reason that with advancement of ages, the concentrations of carbohydrate increases. Because during the time of maturity the starch was translocated , amino acid and proteins were synthesized and after this sugar were produced, therefore, the crude fibre content increased during maturity (Singh & Pradhan 1973; Haldhar et al. 2018).

**Table 2.** Effect of different growth stages of fenugreek on soluble protein, total carbohydrate and total phenol content

| Treatment | Soluble protein content (g $100g^{-1}$) | Total carbohydrate content (mg $g^{-1}$) | Total phenol content (mg $g^{-1}$) |
|---|---|---|---|
| $T_1$ (Young seedlings at 7-10 days after germination) | 8.93 | 11.88 | 1.42 |
| $T_2$ (Fresh leaf at matured stage at 30-40 days after sowing) | 8.75 | 6.51 | 2.64 |
| $T_3$ (Dry leaf at matured stage at 30-40 days after sowing) | 6.74 | 5.09 | 1.08 |
| $T_4$ (Fresh leaf at matured stage at 60-70 days after sowing) | 8.27 | 6.49 | 3.49 |

| | | | |
|---|---|---|---|
| $T_5$ (Dry leaf at matured stage at 60-70 days after sowing) | 6.25 | 5.25 | 1.26 |
| $T_6$ (Dry seed) | 10.52 | 21.24 | 4.86 |
| $T_7$ (Sprouted seed) | 10.98 | 18.07 | 4.12 |
| Mean | 8.63 | 10.65 | 2.70 |
| SEd | 0.210 | 0.288 | 0.068 |
| CD (1%) | 0.624 | 0.856 | 0.203 |
| CD (5%) | 0.450 | 0.617 | 0.147 |

**Table 3.** Effect of different growth stages of fenugreek on total fat, total crude fibre and total polyphenol content

| Treatment | Total fat content (%) | Total crude Fiber content (%) | Total polyphenol content (min $g^{-1}$) |
|---|---|---|---|
| $T_1$ (Young seedlings at 7-10 days after germination) | 1.01 | 8.43 | 0.324 |
| $T_2$ (Fresh leaf at matured stage at 30-40 days after sowing) | 1.16 | 10.62 | 1.013 |
| $T_3$ (Dry leaf at matured stage at 30-40 days after sowing) | 0.903 | 8.57 | 0.937 |
| $T_4$ (Fresh leaf at matured stage at 60-70 days after sowing) | 1.24 | 11.47 | 1.245 |
| $T_5$ (Dry leaf at matured stage at 60-70 days after sowing) | 0.918 | 8.92 | 0.987 |
| $T_6$ (Dry seed) | 5.57 | 14.67 | 1.357 |
| $T_7$ (Sprouted seed) | 4.38 | 12.58 | 0.973 |
| Mean | 2.17 | 10.75 | 0.977 |
| SEd | 0.045 | 0.146 | 0.026 |
| CD (1%) | 0.133 | 0.435 | 0.077 |
| CD (5%) | 0.096 | 0.313 | 0.055 |

The highest polyphenol content was observed in the form of dry seed (1.357 min $g^{-1}$). The highest polyphenol content was found in matured leaves at 60-70 DAS. The polyphenolic compounds of fenugreek seeds can be considered cytoprotective during EtOH induced liver damage. Polyphenolic flavonoids have been shown to protect various cell types from oxidative stress-mediated cell injury. Polyphenols are used for the prevention and cure of various diseases, which are mainly associated with free radicals. The antioxidant activity may result from the neutralization of free radical initiating oxidation processes or from the termination of radical chain reactions (Gupta & Singh 2002). Ascorbic acid content was found highest in the form of fresh leaves at matured stage at 60-70 DAS (54.37 mg $100g^{-1}$) followed by fresh leaves at 30-40 DAS 54.26 mg $100g^{-1}$ (Table 4). The difference in ascorbic acid content was probably due to balance of oxidation and reduction of ascorbic dehydro ascorbic acid that leads to determination of vitamin C content of leafy vegetables. (Sahoo & Acharyya, 2005). Fresh leaves at matured stage (60-70 days after sowing) have the higher amount of calcium content with 329.56 mg $100g^{-1}$. The lowest amount was found in sprouted and dry seed which recorded 197.51 mg $100g^{-1}$ and 192.51 mg $100g^{-1}$, respectively. Jones & Lunt, (1967) reported that calcium functions both as a

structural component and as a cofactor of certain enzymes. Calcium has a significant role in nitrogen metabolism. Highest magnesium content was registered in the form of sprouted seed followed by dry seed with the range of 82.57 mg $100g^{-1}$ and 79.26 mg $100g^{-1}$. Magnesium is required for ribosome integrity. This might explain the observation that high amounts of magnesium appear to be associated with young growing tissue containing high protein level. Iron content was higher in the form of sprouted seed (17.27 mg $100g^{-1}$) followed by fresh leaves at 60-70 days after sowing which recorded 16.25 mg $100g^{-1}$. Iron is implicated in the synthesis of chlorophyll in plants and essential for the conversion of coproporphyrinoges to protoporphyrinogent and its activity was highest in young leaves. Punia, (2006) also reported that leafy vegetables are the good source of calcium and iron content.

**Table 4.** Effect of different growth stages of fenugreek on ascorbic acid, calcium, magnesium and iron content

| Treatment | Ascorbic acid content (mg $100g^{-1}$) | Calcium content (mg $100g^{-1}$) | Magnesium content (mg $100g^{-1}$) | Iron content (mg $100g^{-1}$) |
|---|---|---|---|---|
| $T_1$ (Young seedlings at 7-10 days after germination) | 52.02 | 318.26 | 50.32 | 15.03 |
| $T_2$ (Fresh leaf at matured stage at 30-40 days after sowing) | 54.26 | 324.63 | 50.97 | 16.09 |
| $T_3$ (Dry leaf at matured stage at 30-40 days after sowing) | 51.17 | 315.72 | 49.23 | 15.21 |
| $T_4$ (Fresh leaf at matured stage at 60-70 days after sowing) | 54.37 | 329.56 | 52.29 | 16.25 |
| $T_5$ (Dry leaf at matured stage at 60-70 days after sowing) | 52.32 | 317.38 | 50.19 | 15.43 |
| $T_6$ (Dry seed) | 42.96 | 192.51 | 79.26 | 14.49 |
| $T_7$ (Sprouted seed) | 40.07 | 197.48 | 82.57 | 17.27 |
| Mean | 49.61 | 285.07 | 59.26 | 15.68 |
| SEd | 1.262 | 6.168 | 1.529 | 0.357 |
| CD (1%) | 3.756 | 18.36 | 4.55 | 1.06 |
| CD (5%) | 2.706 | 13.23 | 3.279 | 0.765 |

## Conclusion

Over all it can be concluded that the dried and sprouted seed recorded higher amount of soluble protein, carbohydrate, total fat, crude fibre, total polyphenol, magnesium and iron contents. Fresh leaves at 60-70 days after sowing contain higher amount of ascorbic acid and calcium content.

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