

A Review on Biology, Cultivation and Biotechnology of Fenugreek (*Trigonella foenum-graecum* L.) as a Valuable Medicinal Plant and Multipurpose

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.), wild or cultivated, is widely distributed throughout the world and belongs to the Fabaceae family. It is an old medicinal plant and has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects. Recent research has identified fenugreek as a valuable medicinal plant with potential for multipurpose uses and also as a source for preparing raw materials of pharmaceutical industry, especially steroidal hormones. A significant increase in quantity and quality yields through the suitable management of cultivation, breeding and biotechnology practices could make an immediate and important contribution to farm and pharmaceutical industry income. To achieve these goals with regard to sustainable production, we reviewed a summary of biology, cultivation and biotechnology of fenugreek in this paper.

Keywords: *Trigonella foenum-graecum*, Cultivation, Biotechnology, Diosgenin, Trigonelline



Introduction

Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.), making food (stew with rice in Iran, flavor cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, and etc. Fenugreek can be a very useful legume crop for incorporation into short-term rotation and for hay and silage for livestock feed, for fixation of nitrogen in soil and its fertility, and etc [1].

Fenugreek seeds have been known and valued as medicinal material from very early times. Fenugreek as a chemurgic crop has a wide use for industrial purposes. Its seeds are considered to be of commercial interest as a source of a steroid diosgenin, which is of importance to the pharmaceutical industry [2, 3, 4]. Nowadays, fenugreek is widely cultivated as a drug plant. The mucilaginous seeds are reputed to have many medicinal virtues, as a tonic, emollient, carminative, demulcent, diuretic, astringent emmenagogue, expectorant, restorative, aphrodisiac and vermifugal properties and were used to cure mouth ulcers, chapped lips and stomach irritation [5]. In Iranian traditional medicine the seeds are used as tonic and blood sugar lowering [6].

The biological and pharmacological actions of fenugreek are attributed to the variety of its constituents, namely: steroids, N-compounds, polyphenolic substances, volatile constituents, amino acids, etc [4]. Fenugreek seed contains 45-60%

carbohydrates, mainly mucilaginous fiber (galactomannans), 20-30% proteins high in lysine and tryptophan, 5 - 10% fixed oils (lipids), pyridine alkaloids, mainly trigonelline (0.2 - 0.38%), choline (0.5%), gentianine and carpine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin, free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 - 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin), cholesterol and sitosterol, vitamins A, B₁, C and nicotinic acid and 0.015% volatile oils (n-alkanes and sesquiterpenes) [4, 7, 8].

There are some possibilities for increasing the chemical constituents contained in the seed, either during the growing period by using different cultural techniques [9, 10], or during post harvest treatments by different techniques (enzymes, hormones, etc.) of germination with incubation [11], different conditions of incubation and fermentation [12], by storage [11], by the use of tissue and cell culture (static or suspension) [13, 14] and by biological manipulation of yield [4, 11, 15].

When fenugreek grown under modern production techniques, resulted in an increased yielding ability. The yield potential of fenugreek can be defined as the total biomass produced or agricultural important part of the crop. The total biomass is a result of the integration of metabolic reactions in the plant. Consequently, any factor influencing the metabolic activity of the plant at any period of its growth can affect the yield [16]. Metabolic processes of fenugreek plants are greatly governed by both internal, i.e. genetic make up of the plant and external conditions which involve two main factors namely climatic and edaphic environmental factors. The yield

potential of fenugreek could be regulated through alternation of genetical make up and reconstitution of genetical structure through breeding programs and/or by modifications of environment through cultural treatments [16, 17].

Despite fenugreek being multipurpose crop, it has not obtained due importance in our cropping pattern and little research work has been done on agronomic and biotechnologic aspects of fenugreek, especially in Iran. Thus the objectives of this study are to investigate the cultivation and biotechnology aspects of fenugreek.

Species of *Trigonella*

Petropoulos [15] and Basu [18] indicated that older taxonomists like Linnaeus have suggested that as many as 260 species of fenugreek exist. In contrast, about 128 species of fenugreek were reported by Vasil'chenko [19], 97 by Fazli [20] and 70 by Hector [21], Hutchinson [22] and, Rouk and Mangesha [23]. The most medicinal species of the genus *Trigonella* are presented in Table 1. *T. foenum-graecum* (fenugreek) is the only widely cultivated species of the genus *Trigonella* [15].

Names

The species *Trigonella foenum-graecum*, wild or cultivated, is widely distributed throughout the world, as is indicated by the great number of names it possesses with Arabic, Indian (Sanskrit) and European (Greek and Latin) roots [15, 24]. The genetic name,

Trigonella, comes from Latin meaning 'little triangle', in reference to the triangular shape of the small yellowish-white flowers. The species epithet *foenum-graecum* means 'Greek hay' and according to Rosengarten [25] the Romans, who got the plant from Greece where it was a very common crop in ancient times, gave it this name. It is also called 'ox horn' or 'goat horn' because of the two seed pods projecting in opposite directions usually from the nodes of the stem base that resemble ox or goat horns [15].

Important vernacular names of fenugreek are Hhelbah, Hhelbeh, Hulba and Hulabah in Arabic; Shambala in Armenian; K'u-Tou in Chinese; Fenegriek in Dutch; Fenugreek and Fenigrec in English; Abish in Ethiopian; Fenugrec and Senegre in French; Trigoniskos, Tsimeni, Tintelis, Moschositaro, Tili and Tipilina in Greek; Methi in Indian and Pakistani; Fieno Greco in Italian; Koroba in Japanese; Schemlit and Shambalilae in Persian (Irani); Pazhitnik, Pazsitnyik and Grezsezki szeno in Russian; Khul'ba, Ul'ba and Boidana in Uzbekistani [15, 18].

Origin and distribution

Fenugreek, *Trigonella foenum-graecum* L., is an ancient and annual legume crop mainly grown for multiple uses in many parts of the world. Landraces and species of *Trigonella* have been found on the continents of Asia, Europe, Africa and Australia. Fenugreek was also cultivated in parts of Europe, northern Africa, west and south Asia, North and South America and Australia (Fig. 1) [18, 26].

Table 1- A list of the medicinal species of the genus *Trigonella*, from the pharmaceutical point of view [15, 18]

Genus	Species
<i>Trigonella</i>	<i>T. foenum-graecum</i> , <i>T. balansae</i> , <i>T. corniculata</i> , <i>T. maritima</i> , <i>T. spicata</i> , <i>T. occulta</i> , <i>T. polycerata</i> , <i>T. calliceras</i> , <i>T. cretica</i> , <i>T. caerulea</i> , <i>T. lilacina</i> , <i>T. radiata</i> , <i>T. spinosa</i>





Figure 1- Fenugreek cultivation throughout the world [26]

Different authors have widely divergent opinions about the probable ancestry of *T. foenum-graecum*. Vavilov [27] has suggested that fenugreek is native to the Mediterranean region, while De Candolle [28] and Fazli and Hardman [2] proposed an Asian origin for the crop [26]. De Candolle [28] and Fazli and Hardman [2] notice that fenugreek grows wild in Punjab and Kashmir, in the deserts of Mesopotamia and Persia, in Asia Minor and in some countries in Southern Europe such as Greece, Italy and Spain. De Candolle [28] believes that the origin of fenugreek should be Asia rather than Southern Europe, because if a plant of fenugreek nature was indigenous in Southern Europe it would be far more common and not be missing in the insular floras of Sicily, Ischia and the Balearic Isles [15].

Many authors maintain that the direct ancestor of cultivated fenugreek is the wild *T. gladiata* Ste. that differs from *T. foenum-graecum* in respect of the entire aggregate of characters, of which seed tuberculation and the small size of the pods are only the most striking. It is possible that the species *T. foenum-graecum* evolved from *T. gladiata*, which had possibly given rise to some new extinct forms of *T. foenum-graecum* [15, 29].

Botanical perspective

Fenugreek is also known as one of the oldest medicinal plants recognized in recorded

history [26, 30, 31]. Linnaeus has described the species *Trigonella foenum-graecum* first [15, 18, 32]. Fenugreek is an annual dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminaceae (=Fabaceae). A morphological description of the plant is presented by Sinskaya [33], Hutchinson [22], Tutin and Heywod [34], Fazli and Hardman [2], Petropoulos [15, 29], and Basu [18] in Table 2 and Figure 2 [35]. In general, two types of flowering shoots are observed [15, 29]. The common type bears axillary flowers showing an indeterminate growth habit, whereas the less common or so called "blind shoots" have both axillary and terminal flowers, each of which become "tip bearers". Two types of fenugreek flowers also have been described [15, 29]; i.e., cleistogamous (closed) and aneictogamous (open) flowers. However, the majority of fenugreek flowers are cleistogamous; aneictogamous flowers are not common in fenugreek [18].

There are firm indications that there is a linkage of the quantitative character of diosgenin content with the morphological character of the number of pods per node near the top of stem, and that high content of diosgenin is inherited together with the formation of twin pods. So, the phenotype of twin pods in comparison with that of solitary pods is a good index of selection and should



Fig. 2- Fenugreek (*Trigonella foenum-graecum* L.) from source of Koehler's Medicinal Plants images [35]

provide a reliable basis to predict the performance of their progenies for a higher diosgenin content of seed, from very early generations [15, 18, 29].

Fenugreek in Iran

Fenugreek exist wild in the provinces of Esfahan, Ardebil, Lorestan, Fars, Kerman, Balughistan, Khorashan, Semnan, West and Eest Azarbaijan [36]. Almost in all parts of Iran, fenugreek is cultivated as vegetable and spice crop for a long time and its cultivated area is about 400 ha. Annual fenugreek production in Iran is 800 tons and its grain yield is 0.8 t/ha [37]. In one of the experiment, Moradi et al. [38] compared twenty accessions of Iranian fenugreek coming from some provinces of Iran for quantitative and qualitative features (Table 3). These results showed significant variation ($p < 0.01$ and $p < 0.05$) among accessions. The analysis of ploid level of the accessions native to Iran revealed that all the accessions under study were diploid ($2n=16$). The longest chromosome was 7 Microns in Ardestan sample and the shortest chromosome was 2.54 Microns in Borazjan sample. The longest

whole chromosome in Zanjan sample and the shortest whole chromosome in Borazjan sample were measured 48.3 and 26.46 Microns, respectively [38].

Growing period of fenugreek

The time of germination in soil usually varies from 3-10 days. Six to ten days after the fenugreek germination the seedlings produce the first leaf, which is usually simple; there is still no noticeable epicotyl as the first trifoliate leaf is formed after a further 5 – 8 days (Figure 3) [15]. Growth is slower under cooler and wetter conditions, and long periods of these conditions may cause a failure of plants to mature for seed harvest. The growth rate of fenugreek is slow at the beginning of the growing season, and leaf development is temperature-dependent [39]. Dawidar and Fayeze [40] studied the sapogenin make up of the plant at various stages of growth along with the different parts of the seeds and they revealed that the seedlings have the highest diosgenin (and other steroid sapogenin) content compared to all other stages of growth [15].

Table 2 - Morphological characteristics of fenugreek [18]

Morphological characteristics	Description, color and texture	Dimensions
Plant habit	Erect or prostrate, straight or profusely branched	20-130 cm in length
Stem	Circular to slightly quadrangular, greenish, often characterized by pinkish color due to anthocyanin accumulation under field condition	0.5-1 cm in diameter
Leaf	Simple and trifoliate, distinctly petiolate, stipulate; leaf lamina oval or orbicular with an entire margin. The petioles and leaf lamina varies from greenish to pinkish in the field	1.5-4.5 cm × 0.8-1.5 cm
Petiole	Pale green, pubescent, often anthocyanin tinged	Very small; 0.5-1.1 mm
Flower	Yellow when young but white on maturity	1.6 - 2.2 cm
Calyx	Campanulate, pale green, pubescent	6 - 8 mm
Individual sepal	Pale green, pubescent	13 - 19 mm
Corolla	Papilionaceous, white, papery	1.5-1.9 cm
Standard/Vexillum/Banner	White, papery	1.5 - 1.8 cm
Keels/Carina	White, papery	6-10 mm
Wings/Alae	White, papery	4.5 - 5.5 mm
Anther lobes	Bright yellow, rectangular	1 -1.5 mm × 0.4- 0.5 mm
Filament	Hyaline, tubular	1.7 - 1.9 mm
Ovary	Deep green, glaucous	1.8-2.5 mm
Stigma	Pale green, glaucous	1.5 - 2.1 mm
Style	Pale green/hyaline glaucous	0.2 - 0.5 mm
Pollen grain	Oval (70-90 %) to circular, orbicular, ellipsoidal grains (10-30 %). Hyaline; stained pink or red when treated with 0.5 % acetocarmine	0.032- 0.042 mm × 0.025 -0.027 mm
Ratio of terminal to axillary flowers	All flowers yellow when immature and white when matured	Extremely rare, however the ratio varies as 1:8/1:10/ 1:11/1:13
Number of pods per plant and pod dimensions	Pods brownish or yellowish brown with mucronate tips	2-8/plant 9.5-18.6 cm × 0.2-0.4 cm
Seed	Rectangular to oval in shape with deep grooves between the radicle and cotyledon Varies in color from pale brown to golden yellow	10-20/pod 3-5 mm × 2-3 mm



Table 3 - Some Characters of Iranian fenugreek accessions [38]

Characters	Lowest - Highest	Unit
Leaf length	16.9 (Ahvaz) – 37.4 (Shiraz)	mm
Leaf width	11.32 (Kerman) – 18.63 (Shiraz)	mm
Leaf thickness	0.229 (Kerman) – 0.348 (Ahvaz)	mm
Petiole length	22.38 (Borazjan) – 66.21 (Zanjan)	mm
Nodes number to flowering	5.25 (Kermanshah) – 7.67 (Semnan)	number
Leaf number (before flowering)	7 (Ardestan) – 23 (Seesakht)	number
Plant fresh weight	23.9 (Shahr-rey) – 44.41 (Ahvaz)	g
Plant dry weight percent	0.77 (Shahr-rey) – 0.144 (Borojerd)	%
Chlorophyll content/ fresh weight	0.13 (Ardestan) – 0.23 (Kerman)	g/kg
Leaf protein content (per 100 g)	7.97 (Ahvaz) – 14.65 (Ghaenat)	g
Seed protein content (per 100 g)	8.58 (Ahvaz) – 22.27 (Esfahan)	g
Time to flowering	39 (Borazjan) – 76 (Shahr-rey)	days
Total flowering rate	73 (Kakan) – 92 (Seesakht)	number
Flowering time	36 (Ardestan) – 61 (Ghaenat)	days
Pod length	65.2 (Kashan) – 140.08 (Borojerd)	mm
Pod width	3.42 (Kakan) – 4.46 (Borojerd)	mm
Seeds per pod	10 (Kermanshah) – 18 (Borazjan)	number
Total seeds per plant	806 (Kermanshah) – 1573 (Borazjan)	number
1000 seed weight	5.56 (Semnan) – 19.44 (Kermanshah)	g
Sowing to harvest (Growth period)	81 (Borazjan) – 133 (Ghaenat)	days

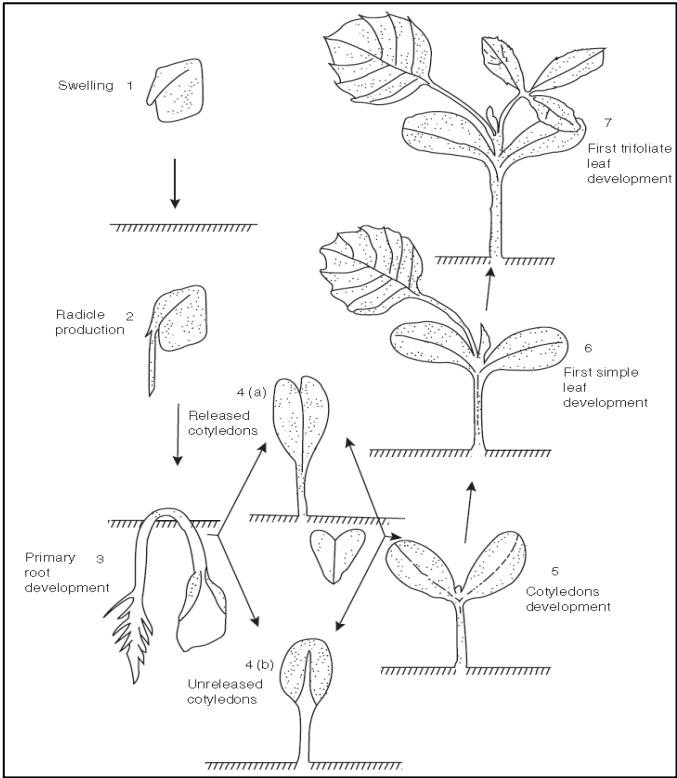


Fig. 3 - The first growth habit of a fenugreek seedling [15]

After the seed germination and the first growth of the seedling, follows the main plant growth, which includes the development of stems, flowers, pods and seeds. The fenugreek has an indeterminate growth habit, which means the growth continues from the terminal and axially buds, while the flowering and formation of pods are both in progress [5, 18]. In the cleistogamous flowers of fenugreek there are four distinguished stages of development; flower bud (first stage), main development (second stage), pollination (third stage), fertilization (fourth stage). The pod of fenugreek also has the following four distinguishing stages of development including, length development (first stage), width development (second stage), germ development (third stage), ripening (fourth stage) [15]. However, fenugreek is botanically a short living (4 – 7 months) annual crop. Sinskaya [33], based on the growing period, morphological characters and habits, classified fenugreek into series, subseries and ecotypes and into five groups: very early (80–85) days, early (80 – 90) days, midearly, late (90 – 100/115) days and very late (120 – 140) days [15].

Ecology

Although the main area cultivated with fenugreek is concentrated in some countries of Asia and Africa, however it has been distributed in many countries throughout the world under different environments. This wide distribution of its cultivation in the world is characteristic of its adaptation to variable climatic conditions and growing environments [15, 29] (Table 4). Duke [5] reports that fenugreek, ranging from cool temperate steppe to wet through tropical very dry forest life zone, is reported to tolerate an annual precipitation of 3.8–15.3 dm and an annual mean temperature of 7.8 – 27.5°C. There are indications of the possible benefit of colder nights on the sapogenin content of the seed [2]. Depending on the geographical source of the seed its sapogenin content, calculated as diosgenin, varied from 0.8–2.2 percent expressed on a moisture free basis [2]. The highest sapogenin content was found in an Ethiopian sample and the lowest in a sample from Palestine [15].

Table 4 - Some ecological factors of fenugreek growth

Factor	Descriptions and reports
Climate	- Temperate climate with mild winters and cool summers [15]
	- Moderate or low rainfall
	- Fairly drought resistant and fairly frost sensitive [41]
	- It can tolerate 10°C of frost [5]
Altitude	- Up to 1300 - 1400 m
	- In Ethiopia to 3000 m
	- But its main zone of distribution in that country is between 2150-2400 m [33]
Soil	- Well-drained loams and generally slightly alkaline Soils are ideal, lime application in some strongly acid condition may be necessary [25]
	- Heavy and wet soils limit fenugreek growth [15, 18]
	- Optimum pH 8 – 8.5 [18]
	- Potash has been used to adjust soil pH to increase nutrients uptake of fenugreek [42]

As a legume crop, it can condition the soil by fixing nitrogen from the atmosphere and can reduce the need for nitrogen fertilizer for subsequent crops. Because fenugreek is a nitrogen-fixing legume, seeds must be inoculated with appropriate *Rhizobium* species for optimal growth [15, 18, 43]. As a dry-land crop, its water requirements are low; use of fenugreek can reduce the cost of irrigation, save water and reduce eutrophication of surface water and limit contamination of ground water source. These properties also make fenugreek a useful legume crop for incorporation into short term rotations [18, 31].

Cultivation practices

Higher yield per hectare will be obtained through superior varieties and better management practices of production. In the old times, a fenugreek yield of 1 ton of seeds per hectare was considered very well, but nowadays yields of more than 2 tons per hectare are being obtained. The large yields of fenugreek are mainly dependent to suitable cropping and agronomic practices. A significant increase in yields through the suitable use of irrigation and adequate levels of soil fertility could make an immediate and important contribution to farm income [15, 29].

Fenugreek is a dry-land crop which responds even to minimal levels of irrigation. Interest in cultivating fenugreek in temperate climates, has increased because of its rain-fed adaptation [26, 31]. Baricevic and Zupancic [44] reported lower diosgenin yield from drought stressed fenugreek cultivars. However, when grown under optimal irrigation regime (35% depletion of available soil water) diosgenin yield increased in comparison to normal irrigated plants, suggesting that the plant does well under minimal irrigation [15, 44].

N, P and K fertilizers had a beneficial effect on the fenugreek seed yield, while N and K improved the quality of fenugreek hay [45]. In pot experiments with fenugreek, it was found that the highest seed yield was obtained from the double N, P and K rates combined with Ca and Mg application. Crops showed the highest requirement for N and K, lesser for Ca and least for P [15, 46]. Kozlowski et al. [9] in pot experiments with fenugreek also found that seed yield was highest when the N–P–K rate was doubled. Mg addition increased the effect of the doubled N rate, but the highest seed yields were obtained when Ca was also added. The addition of Ca alone without Mg had a more positive effect on seed yield than addition of Mg alone. The average mucilage value was highest when Ca and Mg were added at doubled N rates. When Ca and Mg were added at doubled K rates the mucilage value decreased, while doubled K alone yielded the lowest diosgenin concentration. Without Ca and Mg the diosgenin concentration increased most when the N rate was doubled. A negative relation between N uptake and diosgenin content was observed [9, 15]. In order to reduce salt accumulation in the soil, small amounts of fertilizers are applied frequently rather than large quantities at longer intervals [15, 29].

Fenugreek is a nitrogen fixing legume. Hence the seed must be inoculated with an appropriate *Rhizobium* inoculum to optimize its growth potential. The most common nodule-forming bacteria associated with *Trigonella foenum-graecum* L. is the Gram negative, aerobic, non-sporulating, rod shaped bacterium, *Rhizobium meliloti* [18, 31]. Abdelgani et al. [47] has suggested that inoculation of fenugreek with a suitable strain of *Rhizobium* can improve quality and amount of seed generated.



As different varieties of fenugreek are cultivated in different conditions throughout the world, a wide range of seed yields have been reported by various authors. In India seed yields are 500–3320 kg/ha and that yields of 1800 kg/ha were economically viable, while the average seed yield of the last twenty years (1975–95) in India is 1203 kg/ha [15, 48]. Mohamed [10] reported a seed yield of 1595 kg/ha in Egypt, Piper [49] reported 1680 kg/ha in USA, while Talelis [41] estimated the seed yield in Greece as 2465 kg/ha. In Ethiopia, the seed yield for fenugreek was presented as very low, fluctuating between 582 and 608 kg/ha, while in Poland [48] this value was 495–1480 kg/ha and in Germany 1700 – 2100 kg/ha [50].

In England, a seed yield of 3700 kg/ha has been reported from experimental fields [15].

At different stages of growth, a broad range of forage yields has been reported. Piper [49] reports that the yield of fenugreek as fresh matter was estimated to be 13170 kg/ha at Santa Paolo of California and 17400 kg/ha in San Joaquin Valley, while Duke [5] reported that according to the Wealth of India the green forage production of fenugreek is estimated at 9 – 10 t/ha. Purohit and Vyas [51] also reported an average leaf yield of 9000 - 10000 kg/ha.

In summary, agronomic practices of fenugreek and relevant references for additional information reported in Table 5.

Table 5 - Some of fenugreek cultivation practices

Practice	Descriptions and reports
Soil preparation	- Deep plowing and harrowing (two or three ploughing) [51] - When fenugreek follows most cereal crops or soil moisture was limited and low cost, plowing may not be necessary [15, 18].
Sowing depth	1.0-1.5 cm [15], or 2 cm [50] depth
Methods of sowing	Namely broadcasting on the surface of the soil and drilling are applied [15]
Seed quality	It should possess a least 95% seed purity and 80% germination ability [15]
Spacing of sowing	- Rows 45 cm apart and 8 cm within rows [5, 25] - Rows 30-50 cm apart [41, 52] - Rows 30-40 cm apart [48] - Rows 10-30 cm apart, but highest seed yield in 20 cm [10] - Optimum plant density: 158480 plant/ha [15] - Rows 25 cm apart [15, 50, 51] - 22.5 kg/ha for broadcast [5, 25]. When drill seeded, lesser quantities of seed could give satisfactory results. - 40-67 kg/ha [41, 52]
Seed rate	- 17-22 kg/ha for seed production and 35 kg/ha for green manure [49] - 15-20 kg/ha [48] - 25 kg/ha [15, 50, 51] (Estimating that a 1 kg seed of fenugreek contains approximately 35000-53000 seeds [15], but in Iran contains 52000-90000 by my studies).
Hard seed Scarification	- Sulphuric acid for 35-40 min [15, 18] - Soaked in water or GA (at 25, 50 or 100 ppm) for better germination [18, 51]
Time of Sowing	It is generally grown as a winter crop in areas with mild winter and as spring crop in areas with soil that keeps moisture in the summer. However, spring sowings are recommended for all areas with prolonged periods below freezing [15, 41]. - Methods of application are both flooding and spraying [15, 31].
Irrigation	- It is estimated that a water quantity of 200 m ³ /ha every time for sandy soils, and 250 m ³ /ha for heavier soils replicated every fortnight [15]. - Five times for the whole growing period of a fenugreek crop under Indian conditions [15, 45]

Continue Table 5 - Some of fenugreek cultivation practices

Practice	Descriptions and reports
Fertilizer	<p>General recommended basal doses of;</p> <ul style="list-style-type: none"> - Infantile nitrogen about 20 ka/ha at sowing time [15] - N: 60 [53, 54], and 87.5 kg/ha [15, 55] - The main source of N fertilization is nitrate ammonia for acid soils and sulfate ammonia for limey soils [15]. - 10 tones manure, 25 kg N, 25 kg P₂O₅, and 50 kg K₂O per ha [51] - Foliar application of urea (0.25 to 1.25%), 10 to 20 days after germination [51] - The N×P interaction can significantly increase the yield per hectare. The highest seed yield and seed weight can be obtained from plants receiving N: P at 30:60 kg/ha but it reduces the diosgenin content from 0.35% to 0.12% with NP treatment [51]. - P₂O₅: 30 [15, 55], 45-60 [56], 50-60 [43], and 60 kg/ha [57] - K₂O: 40 kg/ha [53] - In Poland, Germany, and Hungary: 20-30 kg N, 60-70 kg P₂O₅, and 80-100 kg K₂O per ha [15] - On sandy loam with a pH value at least 6.5, 80-90 kg/ha K₂O [15] - Hand weeding twice, 15 and 30 days after sowing (critical period), resulted in the highest seed yield [58].
Weed control	<ul style="list-style-type: none"> - Pre-sowing treatments; paraquat, and glyphosate (non-selective herbicides). Pre-emergence treatments; trifluralin, fluchloralin, chlorpropham, and pendimethalin (soil-acting). Post-emergence treatments; bentazon, MCPB, diclofop-methyl, and alloxymid [15, 29].
Rotation	<ul style="list-style-type: none"> - A very good two year rotation crop is fenugreek–wheat, which is widely practiced [50]. The usefulness of fenugreek as a commercial crop is now being recognized and also as a break-crop for cereal areas [15]. - Fenugreek can be successfully grown in conservation tillage systems in rotation with a wide range of crops [26, 31].
Harvesting	<ul style="list-style-type: none"> - In most of the cases, especially under wet conditions, harvesting starts when most of the pods are mature. For spring sowing, ripens usually 3–5 months after planting and for fall sowing, this time exceeded 7 months [15]. - The best harvesting time of fenugreek for green fodder should be when the pods of the base are in the first stage of their development, where the plants are a well formed mass and are very tender [15].

Application of plant growth regulators

Alhadi et al. [59] found that application of gibberellic acid to the seed before sowing causes a slight change in the growth characteristics and physico-biochemical properties of standing fenugreek crops under water deficient conditions. Ortuno et al. [60] reported a considerable increase in diosgenin content in young leaves (20 mg g⁻¹ dry weight) in fenugreek seeds treated with benzylaminopurine. Alagukannan and Vijaykumar [61] reported that application of 2-1-naphthylacetic acid (NAA) to seed increased the number of seeds per pod and, seed size in

fenugreek, while application of maleic hydrazide (MH) and 2,4 dichlorophenoxyacetic acid (2,4-D) on seed increased the seed protein content [18, 31].

Genetic and Breeding

Fenugreek according to Darlington and Wylie [62] has 2n=16 chromosomes, while Joshi and Raghuvanshi [63] have investigated the presence of B-chromosomes. Singh and Singh [64] isolated five double trisomics along with primary trisomics from the progenies of autotriploids, which had 2n+1+1=18 chromosomes.



Fenugreek is grown for multiple uses and breeding programmes need to be concerned with the suitability of the product according to its existing uses, such as high diosgenin content of seed for steroidal industry, high protein content for human and animal feeding, high mucilage (galactomannan) content with appropriate ratio of Man./Gal.¹ for industrial uses and as the case may be for fixed oils, aromatic and spicy substances, as well as pharmaceutical constituents etc [15, 18, 31]. The development of improved varieties of fenugreek with higher diosgenin content in the seed should be obtained at first from the existing populations, cultivated or landraces, using known breeding methods especially those with the induction of mutations [15]. The final goal of a fenugreek breeder is the development of a variety of excellent quality and quantity yield over a wide range of environments [52]. In actual practice three methods namely selection, hybridisation and mutation used separately or in combination, may be involved in the development of an improved variety of fenugreek.

Fenugreek is self-pollinated, but there are opportunities for natural out crossing. The inherent variation in fenugreek is quite immense and so it is grown today in the wide range of climatic conditions of all continents [15, 18]. Allard [65] has suggested that legumes are considered cross-pollinated when more than 10 % of them are "outcrossed". On this basis Petropoulos [15, 29] described fenugreek as a rarely cross pollinated plant as its stigma becomes receptive before the anthers mature. Because of this Petropoulos [15, 29] has suggested that cross

pollination for breeding purposes, can be done in closed flowers of fenugreek at initiation of the second stage of floral development, when the stamens are lower in position than the stigma, i.e. when the anthers are closed but the stigma is receptive to pollination [18].

Consequently selections among world accessions and mutation breeding have been advocated as the best ways to improve the crop [15], and much of the breeding with fenugreek has utilized these two approaches [18, 66]. On the basis it and for example, the first North American fenugreek forage cultivar "Tristar" was released for use in western Canada in 2004 by Agriculture and Agri-Food Canada (AAFC). Tristar fenugreek was developed from a line L3314 (formerly PI-138687), originally collected in 1940 from Iran [18].

A lot of fenugreek mutants have been isolated by the treatment of dry seeds with different chemical mutagens [67, 68], while shoot apices of fenugreek treated by colchicine produced tetraploid plants with promising economic characteristics [69]. The effect of mutagens on tissue cultures of fenugreek with UV-irradiation, ethyl-methanesulphonate (EMS), methyl-methanesulphonate (MMS), and sodium azide (NaN₃) increased steroidal saponin about two- to three-fold [4, 15, 68].

Biotechnology

Fenugreek tissue and cell cultures have been used for either plant regeneration or for the production of secondary products of economic interest. Among these products are diosgenin and trigonelline: a saponin and an alkaloid with therapeutic properties, which are constituents of fenugreek seeds [70, 71, 72]. The demand for fenugreek metabolites, mainly with a higher diosgenin and trigonelline content, prompted more directed tissue culturing efforts [4].

¹ Mannose/Galactose; The ratio of mannose to galactose varies in the different plant genus but the most appropriate for industry applications is 4:1 [15]

The first report on the production of spirostane derivatives by *Trigonella* tissue cultures was published by Khanna and Jain [13] and concerned the establishment of static cultures grown on solid Murashige and Skoog (MS) medium [73] supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) [72]. They reported the production of diosgenin, gitogenin and tigogenin along with other sterols. Six-week-old cultures showed a high growth index ($GI = \frac{\text{final wet weight} - \text{initial wet weight}}{\text{initial wet weight}}$) and the total steroidal content was higher than in the seeds [74]. In other study, the highest diosgenin and tigogenin content was found in 8-week-old calli (0.40 and 0.15%, respectively) [74].

Khanna et al. [75] demonstrated that suspension cultures of *Trigonella foenum-graecum* L. grown on media supplemented with various concentrations of cholesterol produced higher sapogenin contents than those grown on medium without cholesterol. There are alternative pathways from sterol to diosgenin [76]. A first pathway is the incorporation of cholesterol and this pathway is predominant when the precursor is added at subculture. The second pathway involves side-chain cleavage before incorporation and takes place when the sterol is added 10 days after subculture. In a similar way, Trisonthi et al. [14] demonstrated that mevalonic acid promotes the synthesis of steroidal sapogenins in fenugreek tissue, particularly in suspension cultures grown on MS or Miller medium.

More recently, the effects of diniconazole, a triazole-type fungicide with a broad antifungal spectrum, on cell suspension cultures have been studied [70]. This fungicide was added (125 μM) to the fenugreek cell cultures, and, after 21 days, the cell growth decreased by about 20% compared to the control cell growth. Furthermore, diniconazole treatment led to a decrease of about 50% of

total sterol content, causing an inhibition of sterol biosynthesis at the 14- α -demethylation step leading to accumulation of 14- α -methyl Δ^8 -sterols. Oncina et al. [71] reported on the production of diosgenin by callus cultures of *Trigonella foenum-graecum* L. Leaf, stem and root calli were established and cultured on different solid growth media (MS, White's basal medium, Gamborg's B5) [77] supplemented with coconut milk, malt extract and NAA. In all cases, MS medium supplemented with 15% (v/v) coconut milk and $3 \times 10^{-6}\text{M}$ NAA was the most suitable medium for callus growth. Diosgenin levels were higher in leaf calli than in stem and root calli and corresponded to about three to five times the levels observed in the calli from other plant organs. Maximum diosgenin levels were attained after 45 days and reached 2.2 mg/g dry weight in leaf calli, 0.74 mg/g in stem calli and 0.60 mg/g in root calli, which represents 22, 10 and 27% of the levels detected in the corresponding organs of the mother plant at 45 days [60, 74].

The pyridine alkaloid, trigonelline, is widely distributed in the plant kingdom and is known for its hypoglycaemic and hypocholesterolaemic activity [78]. It has been reported to occur in the seeds (0.3-0.4%) of *Trigonella foenum-graecum* L. [79]. Joshi and Handler [80] demonstrated the biosynthesis of trigonelline from nicotinic acid and S-adenosylmethionine. Cell-free extracts of root callus cultures have been reported to catalyze the conversion of nicotinic acid and S-adenosylmethionine to trigonelline in the presence of ATP and MgCl_2 [81].

Callus cultures of *Trigonella foenum-graecum* contained 3 to 4 times more trigonelline than the seeds of this plant and 12 to 13 times more than the roots and shoots. Even higher levels of this alkaloid were produced by suspension cultures. This high

productivity was maintained during successive subculturing of calli and cell suspensions for eight months. Trigonelline accumulated in callus and suspension cultures with aging [4, 15].

Eight-week-old callus tissue cultures of fenugreek, established from seeds on solid Revised Tobacco (RT) medium and supplemented with 1 mg/l 2,4-D, produced 4.5% trigonelline. In the presence of 0.5 and 1.0 mg/l nicotinic acid, trigonelline increased to 5.25 and 5.01% respectively [75]. Comparison of trigonelline contents of seeds, roots, shoots and in vitro cultures was carried out by Radwan and Kokate [82]. The explants were first subjected to shocks with high concentrations of 2, 4-D, IAA, indolepropionic acid (IPA), NAA, GA and kinetin to determine which auxin(s) was suitable for the development of calli. Hormonal shocks for 1 h stimulated significantly the growth of the calli. Whereas 2,4-D, IAA, IPA and NAA (10 mg/l) led to some increases in the trigonelline content, GA (1 mg/l) and kinetin (2 mg/l) did not have pronounced effects. Gamborg's B5 [77] medium, modified by supplementing it with 3 g/l of casein hydrolysate, 2 g/l of yeast extract and 40 g/l of saccharose, was used as growth medium. Four-week-old callus cultures of *Trigonella foenum-graecum* L. produced 15.6 mg/g dry wt. of trigonelline, which represents 3 to 4 times more trigonelline than the seeds and 12 to 18 times more than the roots and shoots of the parent plants. Four- and 6-week-old suspension cultures produced 38.2 and 44.2 mg/g dry wt., respectively, of trigonelline which is more than twice the amount found in the calli. Proportions of 9-12% of the trigonelline were released into the solid medium. In suspension cultures, one third or more of the trigonelline was dissolved in the liquid medium. Therefore, it appears that the biosynthesis of this compound is

favoured by its enhanced removal from the site of its biosynthesis in the cells, into the medium. Cultures grown in the presence of 50 mg/l nicotinic acid contained more trigonelline than those grown in the presence of 1 mg/l only of this substrate. At the same time, the proportion of the alkaloid released into the liquid medium increased from 31%, with a low level of nicotinic acid, to 37%, with a high level of the same compound [74].

In other way, regeneration of shoots has also been achieved from fenugreek protoplasts. Protoplasts were isolated from the root apices of 48-h-imbibed seeds. The first divisions of root fenugreek protoplasts were observed after a 3–4 day culture and subsequent divisions gave cell colonies. However, a culture of these colonies gave only roots [15, 74].

Conclusion

The usefulness of fenugreek as a commercial and chemurgic crop is now being recognized, not only as a break-crop for cereal areas, where it is a very good soil renovator, but also as forage, medicinal plant, source of diosgenin (the most important raw material for the steroid industry) and other constituents such as trigonelline, protein, fixed oils, mucilage, as well as culinary uses for traditional and modern flavoring.

Our knowledge of agronomic and biotechnology practices which could promote high quality and quantity of fenugreek production under Iranian growth conditions is very limited. There is need to develop an efficient agronomic and biotechnological package for assured production in fenugreek. Widespread cultivation of fenugreek requires that we solve the production problem through proper understanding of the agronomic complexities of growing this crop, and select germplasm. Since fenugreek is a self-

pollinated crop, a mutation breeding method can be applied to generate mutants with a determinate growth habit. Irradiation and chemical mutagens can be used to produce point mutations in fenugreek. Selection for improved agronomic properties in the world collections is another possible approach that can help solve the seed yield and quality problem. In conclusion, higher quantity and quality yield will be obtained through better management practices of cultivation, breeding and biotechnology of fenugreek or in combination them. This approach will help scientists to develop, through integrated research management programs, means to

establish optimum levels of fenugreek production and to optimize the yield of active constituents per unit area for a wide range of environmental and other conditions and for specific farming situations.

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References

1. Sadeghzadeh-Ahari D, Kashi AK, Hassandokht MR, Amri A and Alizadeh Kh. Assessment of drought tolerance in Iranian fenugreek landraces. *Journal of Food, Agriculture & Environment*. 2009; 7(3&4): 414 - 419.
2. Fazli FRY, and Hardman R. The spice, fenugreek (*Trigonella foenum-graecum* L.): Its commercial varieties of seed as a source of diosgenin. *Tro. Sci*. 1968; 10: 66-78.
3. Hardman R. Pharmaceutical products from plant steroids. *Trop. Sci*. 1969; 11: 196 – 222.
4. Mehrafarin A, Qaderi A, Rezazadeh Sh, Naghdi Badi H, Noormohammadi Gh, and Zand E. Bioengineering of Important Secondary Metabolites and Metabolic Pathways in Fenugreek (*Trigonella foenum-graecum* L.). *J. of Medicinal Plants* 2010; 9 (35): 1 – 18.
5. Duke AJ. Handbook of Legumes of World Economic Importance, Plenum Press, New York and London. 1986, pp: 345.
6. Hajimehdipoor H, Sadat-Ebrahimi SE, Amanzadeh Y, Izaddoost M, Givi E. Identification and Quantitative Determination of 4-Hydroxyisoleucine in *Trigonella foenum-graecum* L. from Iran. *J. Medicinal Plants* 2010; 9 (6): 29 – 34.
7. Budavari S. The merck index: An encyclopedia of chemicals, drugs, and biologicals, 12th ed. Whitehouse Station, N.J. Merck & Co, Inc. 1996.
8. Newall CA, Anderson LA, and Phillipson JD. Herbal medicines: A guide for healthcare professionals. London: The Pharmaceutical Press. 1996.
9. Kozłowski J, Nowak A, and Krajewska A. Effects of fertilizer rates and ratios on the mucilage value and diosgenin yield of fenugreek. *Herba Polonica*. 1982; 28(3–4): 159 – 70.
10. Mohamed MA. Differences in growth, seed yield and chemical constituents of fenugreek plants (*Trigonella foenum-graecum* L.) due to some agricultural treatments.



Egyptian J. of Agronomy. 1990; 15(1-2): 117 – 23.

11. Hardman R, and Fazli FRY. Methods of screening the genus *Trigonella* for steroidal sapogenins. *Planta Medica*. 1972; 21: 131 – 138.

12. Elujoba AA, Hardman R. Fermentation of powdered fenugreek seeds for increased sapogenin yields. *Fitoterapia* 1985; 56 (6): 368 – 70.

13. Khanna P, Jain SC. Diosgenin, gitogenin and tigogenin from *Trigonella foenum-graecum* tissue cultures. *Lloydia*. 1973; 36: 96 – 8.

14. Trisonthi P, Baccou JC, and Sauvaire Y. Trial to improve production of steroidal sapogenin by fenugreek (*Trigonella foenum-graecum* L.) tissue grown in vitro. *C.R. Seances Acad. Sci., Ser. D*. 1980; 291 (3): 357 – 60.

15. Petropoulos GA. Fenugreek, The genus *Trigonella*. Taylor and Francis, London and New York. 2002, p: 255.

16. Ahmed MA, Ibrahim OM, Elham AB. Effect of bio and mineral phosphorus fertilizer on the growth, productivity and nutritional value of fenugreek (*Trigonella foenum-graecum* L.) in Newly Cultivated Land. *Res. J. Agriculture and Biological Sci.* 2010; 6 (3): 339 – 48.

17. Basu SK, Acharya SN, Bandara MS, Friebel D, and Thomas JE. Effects of genotype and environment on seed and forage yield in fenugreek (*Trigonella foenum-graecum* L.) grown in western Canada. *Australian J. Crop Sci. Southern Cross Journals* 2009; 3 (6): 305 – 14.

18. Basu SK. Seed production technology for fenugreek (*Trigonella foenum-graecum* L.) in the Canadian. Master of Science Thesis.

Department of Biological Sciences University of Lethbridge, Alberta, Canada. 2006, p: 202.

19. Vasil'chenko, I.T. Bericht uber die Arten der Gattung (SSSR.1:10). *Trigonella* Trudy Bot. Inst. Akad. Nauk. 1953; 17: 331 - 3.

20. Fazli, F.R.Y. Studies in steroid-yielding plants of the genus *Trigonella*, PhD Thesis, University of Nottingham, England. 1967.

21. Hector, J.N. Introduction to the Botany of Field Crops (Non cereals), Central News Agency Ltd., Johannesburg. 1936, pp: 1127.

22. Hutchinson J. The Genera of Flowering Plants. Clarendon Press, Oxford. Vol. 1. 1964, pp: 947 – 52.

23. Rouk HF, Mangesha H. Fenugreek (*Trigonella foenum-graecum* L.). Its relationship, geography and economic importance, Exper. Stat. Bull. No. 20, Imper. Ethiopian College of Agric. & Mech. Arts. 1963, pp: 120 – 7.

24. Edison S. Spices – research support to productivity. N. Ravi (ed.), The Hindu Survey of Indian Agriculture, Kasturi & Sons Ltd., National Press, Madras, 1995, pp: 101.

25. Rosengarten F. The Book of Spices, Livingston, Wynnewood, Penns., USA. 1969, 250p.

26. Acharya SN, Thomasl and JE, and Basu SK. Fenugreek: an "old world" crop for the "new world". *Biodiversity (Tropical, Conservancy)*. 2006; 7 (3 & 4): 27 – 30.

27. Vavilov NI. Studies in the origin of cultivated plants. *Inst. Appl. Bot. Plant Breed.* Leningrad. 1926, pp: 289. http://res2.agr.callethbridge/scitech/acharya_e.htm.

28. De Candolle A. Origin of cultivated plants. Hafner, New York. 1964, 468p.

29. Petropoulos GA. Agronomic, genetic and chemical studies of *Trigonella foenum-graecum*

- L. PhD. Diss. Bath University, England. 1973, 145p.
- 30.** Lust JB. The herb book. Bantam Books Inc. New York. 1986, p: 1 - 55.
- 31.** Acharya SN, Thomas JE, and Basu SK. Fenugreek, an alternative crop for semiarid regions of North America. *Crop Sci.* 2008; 48: 841 - 53.
- 32.** Linnaeus C. Species Plantarum, Silvius, Stockholm. 1753, p: 1200.
- 33.** Sinskaya E. Flora of the Cultivated Plants of the U.S.S.R. XIII. In Perennial Leguminous Plants. Part I: Medicago, Sweet clover, Fenugreek. Israel Programme for Scientific Translations, Jerusalem. 1961, pp: 514 – 70.
- 34.** Tutin TG, Heywood VH. Flora Europaea. Vol. I & II. Cambridge University Press, Cambridge. 1964, pp: 452 – 82.
- 35.** Schoepke T. Fenugreek (*Trigonella foenum-graecum* L.) image, source of Koehler's medicinal plants images. <http://pharm1.pharmazie.uni-greifswald.de/allgemei/koehler/koeh-273.jpg>.
- 36.** Rechinger KH. Flora Iranica (*Trigonella foenum-graecum* L.). No: 157/Dezen, Akademische Druck-u, Verlagsanstalt, Austria. 1984, pp: 252.
- 37.** Sadeghzadeh-Ahari D, Hassandokht MR, Kashi AK, Amri A, Alizadeh KH. Genetic variability of some agronomic traits in the Iranian Fenugreek landraces under drought stress and non-stress conditions. *African J. Plant Sci.* 2010; 4 (2): 12 – 20.
- 38.** Moradi P. Study of genetic diversity of Fenugreek (*Trigonella foenum-graecum* L.) native to Iran. Ph.D. Thesis of Horticulture. Department of Horticulture, Science and Research Branch, Islamic Azad University. Tehran, Iran. 2008, 260p.
- 39.** McCormick K, Norton R, and Eagles HA. Fenugreek has a role in south-eastern Australian farming systems. In Proceedings of “Groundbreaking stuff”, 13th. Annual Agrenomy Conference, Perth, Australia. 2006, pp: 639.
- 40.** Dawidar AM, Fayez MBE. Thin-layer chromatographic detection and estimation of steroid sapogenins. *Z. Anal. Chem.* 1972; 259: 283 – 5.
- 41.** Talelis D. Cultivation of Legumes, Agric. College of Athens, Athens (in Greek). 1967, pp: 101 – 5.
- 42.** Yadav GL, Kumawat PD. Effect of organic inorganic, fertilizer and *Rhizobium* inoculation of yield and yield attributes of fenugreek (*Trigonella foenum-graecum* L.). *Haryana J. Hort. Sci.* 2003; 32 (1 - 2): 147 - 8.
- 43.** Basu SK, Acharya SN, Thomas JE. Appilcation of phosphate fertilizer and harvest management for important fenugreek (*Trigonella foenum-graecum* L.) seed and forage yield in a dark brown soil zone of Canada. *KMITL Sci. Tech. J.* 2008; 8 (1): 1 – 7.
- 44.** Baricevic D, Zupancic A. The impact of drought stress and/or nitrogen fertilization in some medicinal plants. *J. Herb. Spi. Med. Plants.* 2002; 9 (2-3): 53 - 64.
- 45.** Pareek SK, Gupta R. Effect of fertilizer application on seed yield and diosgenin content in fenugreek. *Indian J. Agric. Sci.* 1981; 50 (10): 746 – 9.
- 46.** Golez L, Kordana S. Effect of nitrogen, phosphorous and potassium doses, as well as magnesium and calcium fertilisation on a crop yield and uptake of mineral nutrients by *Trigonella foenum-graecum*. *Herba Pol.* 1979; 25 (2): 121 – 31.



47. Abdelgani M.E, Elsheikh EAE, and Mukhtar NO. The effect of Rhizobium inoculation and chemical fertilization on seed quality of fenugreek. *Food Chem.* 1999; 64 (3): 289 - 93.
48. Anonymous. Kozieradka pospolita – Instruction of cultivation (*Trigonella foenum-graecum* L.), Instytut Roslin I Przetworow zielarskich, W. Poznaniu. 1987, p: 94.
49. Piper CV. Forage Plants and Their Cultures, The Macmillan Company, New York. 1947, p: 671.
50. Dachler M, Pelzmann H. Heil- und Gew_rzpflanzen, Anbau-Ernte-Aufbereitung, Österreichischer Agrarverlag, Wien. 1989, p: 244.
51. Purohit SS, Vyas SP. Medicinal plant cultivation: A scientific approach (Including processing and financial guidelines). Agrobios (India). 2004, pp: 539 –42.
52. Bunting ES. Cultivation of fenugreek and some existing its varieties, Univ. of Feed. Lab., Oxford. 1972, p: 101.
53. Billaud C, Adrian J. Fenugreek composition, nutritional value and Physiological properties. *Sci. Aliments* 2001; 21: 3 – 26.
54. Rizk SG. Atmospheric nitrogen fixation by legumes under Egyptian conditions. II. Grain legumes. *J. Microbiol. U.A.R.* 1966; 1 (1): 33 – 45.
55. Chhibba IM, Nayyar VK, and Kanwar JS. Influence of mode and source of applied iron on fenugreek (*Trigonella corniculata* L.) in a typic ustochrept in Punjab, India. *Int. J. Agri. Biol.* 2007; 9 (2): 254 – 6.
56. Bismillah Khan M, Aslam Khan M, Sheikh M. Effect of phosphorus levels on growth and yield of fenugreek (*Trigonella foenum graecum* L.) grown under different spatial arrangements. *Int. J. Agri. Biol.* 2005; 7 (3): 504 – 7.
57. Slinkard AE. Production fenugreek (*Trigonella foenum-graecum* L.) in Saskatchewan. Saskatchewan Ministry of Agriculture. University of Saskatchewan. 2009, p: 4.
58. Mandam AR, Maiti RG. Efficacy of different herbicides for weed control in fenugreek (*Trigonella foenum-graecum* L.). *Environ. Ecol.* 1994; 12: 138 – 42.
59. Alhadi FA, Yaseen BT, Jabr M. Water stress and giberrellic acid effects on growth of fenugreek plants. *Irrig. Sci.* 1999; 18 (4): 185 - 90.
60. Ortuno A, Oncina R, Botia JM, Rio JAD. Distribution and changes of diosgenin during the development of *Trigonella foenum-graecum* plants. Modulation by benzylaminopurine. *Food Chem.* 1998; 63 (1): 51 - 4.
61. Alagukannan G, Vijaykumar M. Effect of plant growth substances on yield attributing parameters, yield and quality in fenugreek (*Trigonella foenumgraecum* L.). *S. Ind. Hort.* 1999; 47 (1-6): 130 - 3.
62. Darlington CD, Wylie AP. Chromosome atlas of flowering plants, George Allen & Unwin Ltd., London. 1945, p: 164.
63. Joshi S, Raghuvanshi SS. Bchromosomes, pollen germination in situ and connected grains in *Trigonella foenumgraecum*. *Beitr. Biol. Pf.I.* 1968; 44 (2): 161 – 6.
64. Singh D, Singh A. Double trisomics in *Trigonella foenum-graecum* L. *Crop Improvement.* 1976; 3 (1 – 2): 25 – 7.
65. Allard RW. Principles of Plant Breeding, J. Hilley and Sons Inc. 1960, London. p: 485.

66. Green JM, Sharma D, Reddy LJ, Saxena KB, Gupta SC, Jain KC, Reddy BVS, and Rao MR. Methodology and the progress in the ICRISAT in Pigeonpea Breeding Programme. In Proc. Intern. Workshop on Pigeonpeas. Ptancheru. 1981, pp: 437 – 49.
67. Auerbach C. Chemicals and their effects. Proc. Symp. on Mutation and Plant Breeding, Cornell, Nov.– Dec. 1961; 25: 585 - 621.
68. Jain SC, Agrawal M. Effect of chemical mutagens on steroidal sapogenin in *Trigonella* species. *Phytochem.* 1987; 26 (8): 2203 – 6.
69. Roy RP, Singh A. Cytomorphological studies of the colchicine-induced tetraploid *Trigonella foenum-graecum*. *Genet. Iber.* 1968; 20 (1 – 2): 37 – 54.
70. Cerdon C, Rahier A, Taton M, Sauvaire Y. Effect of diniconazole on sterol composition of roots and cell suspension cultures of fenugreek. *Phytochem.* 1945; 39: 883 - 93.
71. Oncina R, Botia JM, Del Rio JA, Ortuno A. Bioproduction of diosgenin in callus cultures of *Trigonella Foenum-graecum* L. *Food Chem.* 2000; 70 (4): 489 - 92.
72. Jain SC, Rosenberg H, Stohs SJ. Steroidal constituents of *Trigonella occulta* tissue cultures. *Planta Ned.* 1977; 31: 109 - 11.
73. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 1962; 15: 473 – 97.
74. Christen P. *Trigonella* species: In Vitro Culture and production of Secondary Metabolites. In Nagata T, Ebizuka Y. (Eds.), *Medicinal and aromatic plants* (Vol. 12). Biotechnology in Agriculture and Forestry 51. Springer. 2002, pp: 306 - 48.
75. Khanna P, Jain SC, Bansal R. Effect of cholesterol on growth and production of diosgenin, gitogenin, tigogenin and sterols in suspension cultures. *Indian J. Exp. Biol.* 1975; 13 (2): 211 – 3.
76. Brain KR, Williams MH. Evidence for an alternative route from sterol to sapogenin in suspension cultures from *Trigonella foenumgraecum*. *Plant Cell Rep.* 1983; 2: 7 - 10.
77. Gamborg OL. The effects of amino-acids and ammonium on the growth of plant cells in suspension culture. *Plant Physiol.* 1970; 45: 372 - 5.
78. Iribarren AM, Pomilio AB. components of *Bauhinia candicans*. *J. Nat. Prod.* 1983; 46: 752 - 3.
79. Kuhn A, Gerhard H. The trigonellin and nicotinic acid content of seeds of fenugreek. *Arch. Pharm. U. Ber. Deutsch Pharm. Ges.* 1943; 281: 378 - 9.
80. Joshi JG, Handler P. Biosynthesis of trigonelline. *J. Biol. Chem.* 1960; 235: 2981 - 3.
81. Antony A, Gopinathan KP, Vaidyanathan CS. Biosynthesis of trigonelline in root cultures of Fenugreek (*Trigonella Foenum-graecum* L). *Indian J. Exp. Biol.* 1975; 13: 39 - 41.
82. Radwan SS, Kokate CK. Production of higher levels of trigonellin by cell cultures of *Trigonella foenum - graecum* than by the differentiated plant. *Planta.* 1980; 147: 340 – 4.