

A review on management of fungal diseases associated with *trigonella foenumgraecum* L. (fenugreek)

Sharvan Kumar Kumawat¹, Richa Sharma²

Department of Biotechnology and Allied Sciences, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.

ABSTRACT

The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Fenugreek (*Trigonella foenum-graecum* L.) is a plant belongs from family Fabaceae. Fenugreek is used both as a herb (the leaves) and as a spice (the seed, often called Methi in Urdu/ Hindi/ Nepali). Fenugreek is regarded as the oldest known medicinal plant in recorded history. Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines. Medicinal uses vary from wound-healing to bust enhancement and, from promotion of lactation in weaning mothers, to its use as a sex stimulant or aphrodisiac. Use of organic and inorganic fertilizers, farmyard manure, nitrogen and phosphorus has been found to be effective in increasing fenugreek yield. India leads the world in fenugreek production, producing 70-80% of the global export. Fungal, bacterial, viral and insect mediated diseases are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence is a serious agronomic concern. The two most common fungal diseases infecting fenugreek are *ercospora* leaf spot and powdery mildew. Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract. Fenugreek was, and re-mains, a food and a spice commonly eaten in many parts of the world. Various experimental and human studies have reported that fenugreek is a potential agent to treat diabetes mellitus. Fenugreek plays a key role to prevent the induction and progression of various disorders such as cancer, ulcer, obesity, etc. Moreover, the pleiotropic actions of fenugreek have been evaluated in hypertension, cataract, inflammation, thyroid dysfunction, malaria, endothelial dysfunction, etc. In this review, we have captured recent findings on fenugreek representing the distinct role of fenugreek in treating various disorders elaborating the mechanisms at molecular level.

Key words: Fenugreek, *Foenum-graecum*, National and international status, Traditional uses

INTRODUCTION:

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume crop. The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region (Petropoulos 2002), but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia (Fazli and Hardman 1968; Edison 1995; AAFRD 1998; Petropoulos 2002). India is the leading fenugreek producing country in the world (Edison 1995).

Fenugreek (*Trigonella foenum-graecum*) is a plant belongs from family Fabaceae. Fenugreek is used both as a herb (the leaves) and as a spice (the seed, often called Methi in Urdu/ Hindi/ Nepali). Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust 1986). Fenugreek has been referred to as a medicinal herb both in Indian Ayurvedic and traditional Chinese medicines (Tiran 2003).

Ancient literature, religious scripture, travel records and anecdotes from different continents and from different periods of human history, record a wide variety of medicinal properties associated with fenugreek (Lust 1986). Medicinal uses vary from wound-healing to bust enhancement and, from promotion of lactation in weaning mothers, to its use as a sex stimulant or aphrodisiac (Petropoulos 2002; Tiran 2003). The leaves and sprouts are also eaten as

vegetables. The plant is cultivated worldwide as a semi-arid crop and is a common ingredient in many curries.

India leads the world in fenugreek production, producing 70-80% of the global export (Edison 1995). India is the largest producer of fenugreek in the worldwide where Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states. Rajasthan produces the lion's share of India's production, accounting for over 80% of the nation's total fenugreek yield. Qasuri Methi, more popular for its appetizing fragrance, comes from Qasur, Pakistan, and regions irrigated by the Sutlej River, in the Indian and Pakistani states of Punjab. A major part of fenugreek production in Rajasthan is carried out in Sikar which accounts to 80% of the total output, followed by areas of Nagor, Chittor, Bhilwara and Jhunjhunu. Ideal sowing time for fenugreek crop in Northern India is last week of October to first week of November. In southern parts of the country, fenugreek is grown both as a Kharif crop and as a Rabi crop. Ideal sowing time for kharif crop is second fortnight of June to July-end and ideal sowing time for rabi crop is first fortnight of October. The average yield of *Trigonella foenum-graecum* crop is however low due to lack of superior varieties, scientific crop production technology and vulnerability to diseases like (Leaf spot, color root, Downey mildew) Wilt, Blight and Powdery mildew caused by *Fusarium oxysporum*, *Fusarium. cumini*, *Alternaria burnsii* and *Erysiphe polygoni*, respectively. While efforts are being made to evolve better varieties and agronomical practices through AICRPs and NRCs but efforts to combat these diseases are meager. All of these diseases, cause serious losses in seed yield particularly, if the weather conditions are congenial for disease development. Though the disease has been investigated at field level and laboratory level but not much could be achieved as far as resistance is concerned. Thus with non-availability of resistance source in the germplasm, it is important to undertake studies to develop suitable genotype using biotechnological tools.

Certain difficulties have been encountered for the management of bacterial/fungal diseases in plants because of scanty availability of known and recommended bactericides in the market. In addition, these antibiotics are costly and not fully efficacious in some cases. In fact, during last two decades, problems of resistance against streptomycin have increased as well as some new important bacterial pathogenic strain have

been identified and documented. Consequently, there is an urgent need to search for economic and eco-friendly alternative strategies for the management of fungal diseases. Recently greater emphasis on stable source of resistance and other useful control measures viz., biological, cultural and botanicals have been laid. Besides, various biotechnological approaches have also opened new vistas in the management of fungal diseases.

Until serological techniques were developed, the only reliable methods available for identification of fungi and bacteria were isolation in culture and performing morphological and biochemical tests. Serological techniques allowed rapid presumptive diagnosis of bacterial diseases (Hampton et al., 1990).

National Status of *Trigonella foenum-graecum* L.

Disease management of *Trigonella foenum-graecum* L.:

Use of organic and inorganic fertilizers, farmyard manure, nitrogen and phosphorus have been found to be effective in increasing fenugreek yield (Detoroja et al. 1995; Khiriya and Singh 2003; Yadav and Kumawat 2003). Abd-Ala and Omar (1998) reported that application of wheat straw and fungi (*Sinorhizobium meliloti*, *Trichoderma harzianum*, *Aspergillus niger* and *Chaetium globosum*) promote nodulation, nodule efficiency and fenugreek growth under saline soil conditions. 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* (Subba Rao and Sharma 1968). Abdelgani et al. (1999) has suggested that inoculation of fenugreek with a suitable strain of *Rhizobium* can improve quality and amount of seed generated.

Fungal, bacterial, viral and insect mediated diseases are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence is a serious agronomic concern (AAFRD 1998; Fogg et al. 2000; Jongebloed 2004; Petropoulos 1973, 2002; Prakash and Sharma 2000). The two most common fungal diseases infecting fenugreek are *ercospora* leaf spot and powdery mildew (AAFRD 1998). Prakash and Sharma (2000), Petropoulos (2002) and Jongebloed (2004) have reported powdery mildew on fenugreek, caused by *Erysiphe polygoni*. Additional studies from Australia (Jongebloed

2004) have shown that yield of fenugreek can be seriously affected by blight disease caused by *Cercospora traversiana* and wilt caused by *Fusarium oxysporum* and *Rhizoctonia solani*. In addition, other well known fungal diseases associated with fenugreek are collar rot, leaf spot and pod spot diseases (Petropoulos 2002). Fogg et al. (2000) reported a bacterial leaf spot in fenugreek which was caused by *Pseudomonas syringae* pv. *syringae* in New Jersey, USA. It also has been suggested that the bacterium *Xanthomonas alfalfa* can infect fenugreek (Petropoulos 2002). Petropoulos (2002) reported that Bean Yellow Mosaic Virus, Alfalfa Mosaic Virus, Cow Pea Mosaic Virus, Soybean Mosaic Virus, Pea Mosaic Virus, Potato Virus A and Y, and Clover Vein Mosaic Virus are common viral infections of fenugreek. These viral diseases have been associated with moderate loss of seed and forage yield. Lucy (2004) reported that in Australia insects such as thrips, pod-borers and heliothis can cause serious damage to forage yield in fenugreek. Root rot by the soil borne nematode *Meloidogyne incognita*, which causes the death of immature plants has also been reported from Australia (Jongebloed 2004). Fenugreek is reported to be sensitive to mineral deficiencies (Petropoulos 1973). It has been suggested that yellowing of some fenugreek plants under field conditions is connected to probable mineral deficiencies, in particular to elements like boron, magnesium, manganese or potassium deficiency (Sinskaya 1961). Physiological diseases have been reported to be associated with early death and loss of forage and seed yield in fenugreek (Petropoulos 2002). The crop prefers faintly alkaline soil with a pH range of 8-8.5. Potash has been used to adjust soil pH to increase nutrient uptake of fenugreek (Yadav and Kumawat 2003). The application of organic and inorganic fertilizers, farmyard manures, nitrogen and phosphorus has been found to be effective in increasing fenugreek yield (Detoroja et al. 1995; Khiriya and Singh 2003; Yadav and Kumawat 2003). Lai et al. (2003) reported that optimization of sowing dates and forage cutting maximize fenugreek yield. Fenugreek is a nitrogen fixing legume. Hence the seed needs to be inoculated with an appropriate *Rhizobium* inoculum to optimize this potential (Abdelgani et al. 1999). The most common nodule-forming bacteria associated with fenugreek are the Gram negative, aerobic, non-sporulating, rod shaped bacterium, *Rhizobium meliloti* (Subba Rao and Sharma 1968). Abd-Ala and Omar (1998) reported that application of wheat straw and

certain fungi promotes nodulation, nodule efficiency and growth of the crop under saline soil conditions. Balajee et al. 2005a, 2007) Clinically, identification of unknown *Aspergillus* clinical isolates to species may be important given that different species have variable susceptibilities to multiple antifungal drugs. Thus, knowledge of the species identity may influence the choice of appropriate antifungal therapy. For example, in vitro and in vivo studies have demonstrated that *A. terreus* isolates are largely resistant to the antifungal drug amphotericin B, *A. ustus* isolates appear to be refractory to azoles, and *A. lentulus* and *Petromyces alliaceus* have low in vitro susceptibilities to a wide range of antifungals including amphotericin B, azoles, and echinocandins. Foot rot disease caused by *Rhizoctonia solani* in fenugreek is one of the important diseases resulting in heavy losses in Rohilkhand. Bareilly is one of the chief production centres of crop, facilitating the bulk supply to Uttaranchal and nearby states. The production of fenugreek is hampered by foot-rot disease. The fungus is soil borne and all parts of fenugreek are prone to infection (Singh et al., 2010). Application of fungicides is the only strategy for the management of fungal diseases since many years. Conventional breeding for fungal disease tolerance has not proven to be very effective in identifying a resistance source. Not only the availability of such a simple, safe and fast technique has been of great importance for diversification but also in various field of DNA analysis as evidenced by the review of literature Kumar et al. (2002) reported that plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* were isolated from the rhizosphere of rice and sugarcane among 40 strains that were confirmed as *Pseudomonas fluorescence*, 18 exhibited strong antifungal activities against *Rhizoctonia bataticola* and *Fusarium oxysporum*, mainly through the production of antifungal metabolites.

Fenugreek has strong flavor and aroma. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine. A wide range of uses were found for fenugreek in ancient times. Medicinally it was used for the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract. Fenugreek was, and re-mains, a food and a spice commonly eaten in

many parts of the world. Various experimental and human studies have reported that fenugreek is a potential agent to treat diabetes mellitus. Fenugreek plays a key role to prevent the induction and progression of various disorders such as cancer, ulcer, obesity, etc. Moreover, the pleiotropic actions of fenugreek have been evaluated in hypertension, cataract, inflammation, thyroid dysfunction, malaria, endothelial dysfunction, etc. In this review, we have captured recent findings on fenugreek representing the distinct role of fenugreek in treating various disorders elaborating the mechanisms at molecular level.

Fenugreek is a plant. The seeds are used to make medicine. Fenugreek is used for many conditions, but so far, there isn't enough scientific evidence to determine whether or not it is effective for any of them. Fenugreek is used for digestive problems such as loss of appetite, upset stomach, constipation, and inflammation of the stomach (gastritis). It is also used for conditions that affect heart health such as "hardening of the arteries" (atherosclerosis) and for high blood levels of certain fats including cholesterol and triglycerides. Fenugreek is used for kidney ailments, a vitamin deficiency disease called beriberi, mouth ulcers, boils, bronchitis, infection of the tissues beneath the surface of the skin (cellulitis), tuberculosis, chronic coughs, chapped lips, baldness, cancer, and lowering blood sugar in people with diabetes. Some men use fenugreek for hernia, erectile dysfunction (ED), and other male problems. Women who are breast-feeding sometimes use fenugreek to promote milk flow. Fenugreek is sometimes used as a poultice. That means it is wrapped in cloth, warmed and applied directly to the skin to treat local pain and swelling (inflammation), muscle pain, pain and swelling of lymph nodes (lymphadenitis), pain in the toes (gout), wounds, leg ulcers, and eczema. The taste and odor of fenugreek resembles maple syrup, and it has been used to mask the taste of medicines. In foods, fenugreek is included as an ingredient in spice blends. It is also used as a flavoring agent in imitation maple syrup, foods, beverages, and tobacco. In manufacturing, fenugreek extracts are used in soaps and cosmetics. Fenugreek leaves are eaten in India as a vegetable.

Indian agriculture is facing several challenges in increasing the productivity from its limited resources to meet the needs of the growing human population. Emphasis of present day agriculture is to produce more with lesser land, water and manpower. Apart from

natural calamities like drought, flood etc., biotic stresses like pests, diseases and weeds also influence the crop productivity greatly. The annual losses caused by the pests, diseases and weeds were estimated at Rs. 7000 crores (Jayaraj, 1988) and Rs. 30,000 crores (Mukhopadhyay, 2009). Farmer's income in recent years is decreasing gradually due to increased cost of inputs particularly in plant protection. Presently practiced plant protection measures mainly orient towards chemical control and as a result there is a steady increase in pesticide usage in Indian agriculture. Chemical pesticide application has gone up from 2.2g (a.i)/ha in 1950 to the current level of 650g (a.i)/ha, which shows a 300 fold increase. The excessive dependence on chemical pesticide leads to several ecological and economic problems. To avoid this situation the concept of IPM/BIPM approach should be considered on top priority.

Integration of bio-control with chemical control has high potential for success. Bio-control agents can be employed even with an applied chemical that is fungitoxic to the wild type of the agent. Integration of bio-control agent with fungicides gave significantly higher disease control in several crops (Sugarbeet, tobacco, tomato, potato, lentil and chickpea) than that obtained by the bio-control agents or fungicide alone. Integration of carbendazim, *T. harzianum* and *T. viride* seed treatment resulted in reduced incidence of dry root rot and increased yield of soybean in farmer's field. Since a long time herbal medicines have been used in the management of diseases. Orally, fenugreek is used for lowering blood glucose in people with diabetes, loss of appetite, dyspepsia, gastritis, constipation, atherosclerosis, high serum cholesterol and triglycerides, and for promoting lactation. Fenugreek is used orally for kidney ailments, beriberi, hernia, impotence, and other male problems. Fenugreek is also used orally for fever, mouth ulcers, boils, bronchitis, cellulitis, tuberculosis, chronic coughs, chapped lips, baldness, and cancer. Topically, fenugreek is used as a poultice for local inflammation, myalgia, lymphadenitis, gout, wounds, leg ulcers, and eczema. In foods, fenugreek is included as an ingredient in spice blends. It is also used as a flavoring agent in imitation maple syrup, foods, beverages, and tobacco. In manufacturing, fenugreek extracts are used in soaps and cosmetics. More evidence is needed to rate fenugreek for these uses.

International Status of fenugreek:

Major fenugreek producing countries are Nepal, India, Pakistan, Bangladesh, Argentina, Egypt, France, Spain, Turkey, Morocco and China.

Trigonella foenum graecum – is an annual herb belonging to family Leguminosae found wild and extensively cultivated in many parts in India. Two fairly distinct types of plants are recognised, the dwarf type grown for culinary purposes and the tall type grown for medicinal purposes. The seeds of this plant contain an alkaloid trigonelline and another compound known as choline. These seeds have been reported to be diuretic, anti-tussive and hypoglycaemic in nature. We thought it was worthwhile to see the effect of consumption of different quantities of powdered fenugreek seeds in blood sugar profiles and glycosylated haemoglobin (HbA1c) levels in stable uncontrolled type II diabetics.

The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. Helpful in lowering fever, it is compared to quinine by some authorities. The seeds' soothing effect makes them of value in treating gastritis and gastric ulcers. The seeds freshen bad breath and help restore a dulled sense of taste. The oil in the seeds is used as a skin softener and emollient.

In China, the fenugreek seeds are used as a pessary to treat cervical cancer. In the Middle East and the Balkans, the aerial parts of plant are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. They are also used to ease labour pains. Traditional Chinese herbalists used plant for kidney problems and conditions affecting the male reproductive tract. The seeds also function as a preservative and are added to pickles, chutneys and other similar products. In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages.

Fenugreek is a leguminous herb, commonly cultivated and used as a condiment in India and North African countries. The seeds are yellow in colour, bitter to taste¹¹ and are a rich source of fiber. It contains mucilaginous fiber and total fiber to the extent of 20% and 50% respectively. In addition it also contains trigonelline, an alkaloid known to reduce blood glucose level. Fenugreek seed powder in the diet reduces blood sugar and urine sugar with concomitant improvement in glucose tolerance and diabetic symptoms in both NIDDM and IDDM.

Fenugreek (*Trigonella foenum-graecum*) is a leguminous, annual plant originating in India and North Africa. It is an herbal product with many proposed health benefits found in the diets of various Middle Eastern countries and is now cultivated worldwide. The leaves and seeds of fenugreek are formulated to an extract or powder form for therapeutic application.

Fenugreek has been studied extensively in human and animal models. The effects of fenugreek supplementation on the regulation of insulin and hyperglycemia are well established. Defatted fractions of fenugreek seeds, high in fiber content and containing steroid saponins, lowered blood glucose and plasma glucagon concentrations after eight days of consumption in dogs. Other investigations utilizing human participants have implemented fenugreek supplementation (daily doses of 1 to 25 g/day) to diabetic patients eliciting positive glucose regulation responses. Another study examined the acute and chronic outcomes of a soluble dietary fiber (SDF) prepared from fenugreek seeds administered to type 1 and type 2 diabetic rats.

Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic it is able to bring on a sweat and to help detox the body. This takes place through the pores of the skin. The pungent aroma of fenugreek may be smelt on the skin and in under-arm perspiration. After using the sprouts for a while, this fenugreek body aroma, does not seem to be so apparent, maybe, the sprouts have done a pretty good cleanse. Fenugreek also has the reputation as a lymphatic cleansing herb. The lymphatic system is the vacuum cleaner of the body. It has the vital role to irrigate the cells with nutrients and to remove toxic wastes, dead cells and trapped proteins. The fluid is cleaned through the lymph nodes, before the body's 13 litres of filtered lymph fluid recycles again, via the subclavian vein near the heart. A blocked lymphatic system can mean poor circulation, fluid retention, pain, loss of energy and disease, anywhere in the body. Fenugreek is a practical herb for all mucus conditions of the body, particularly the lungs, by helping to clear congestion. It is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough. Even drinking the water that seeds have soaked in and been rinsed with, helps to soften and dissolve, accumulated and hardened masses of cellular debris. Use fenugreek for head colds, influenza, catarrh, constipation, bronchial complaints, asthma, emphysema,

pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis.

Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorb toxic material and eliminate it. The healing and soothing action creates a protective coating, like a lubricant, over inflamed areas. The slightly bitter properties of the seed are beneficial for digestion. Fenugreek has a powerful demulcent action, as it is rich in mucilage and it can soothe irritated or inflamed tissue. For relief from the agonizing symptoms of irritable bowel syndrome, colitis and diverticulitis, the 'soak-and-rinse water' is drunk and the sprouts blended to a liquid. It has been called the herb for 'every ailment under the sun'. The Fenugreek herb has been known to help reduce fever when taken with lemon and honey, since it nourishes the body during an illness. Some health food stores also sell herbal Fenugreek teas, which can be used instead of the green tea. Fenugreek is often used in many teas and other products that help balance women's hormones and/or enlarge the breasts. Remedy to Ease Child Birth for Pregnant Women: Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. However, pregnant women should only use Fenugreek for inducing labor after consulting with their doctor.

Fenugreek seeds contain hormone precursors that increase milk supply. Some scientists believe it is possible because breasts are modified sweat glands, and fenugreek stimulates sweat production. It has been found that fenugreek can increase a nursing mother's milk supply within 24 to 72 hours after first taking the herb.

An Immunomodulatory effect of fenugreek extract in mice has been investigated. Overall, Fenugreek showed a stimulatory effect on immune functions in mice. As it is used for a variety of medicinal purposes, its immunostimulatory effect, as reported in this study, strengthens the rationale of its use in several Unani and Ayurvedic drugs. For the removal of Kidney Stones, a study was undertaken to investigate the effect of Fenugreek (*Trigonella foenum-graecum*) seed on experimentally-induced kidney stones in rats. Oxalate urolithiasis in male rats was produced by the addition of 3% glycolic acid to their diet. After 4 weeks, highly significant deposition in the kidneys was noticed and changes in water intake and body weight recorded. Daily oral treatment with *T. foenum-graecum* significantly decreased the quantity of calcium oxalate deposited in the kidneys thus supporting its use in Saudi folk medicine.

The seeds of the fenugreek herb possess toxic oils, and other constituents of the fenugreek leaf have been shown to be toxic to bacteria, parasites and fungi. A 2007 issue of Current Science journal noted the antifungal properties of fenugreek. The research attempted to clone the substance defensins which are native to plants such as fenugreek to test their effects in the petri dish. The defensins protect the plant from fungi which was extracted from leaf tissue. As an antiparasitic agent, fenugreek was pitted in a 2008 Oxford Journals article against the malaria-causing organism Plasmodium. In vitro studies found that fenugreek extracts were effective against resistant species of Plasmodium. The 2004 Asia Pacific Journal of Clinical Nutrition article also noted that germination or sprouting of fenugreek seeds increased their antioxidant profile and antimicrobial activity against H-pylori. Finally, a 2006 African Journal of Biotechnology article compared the effectiveness of fenugreek against two common pathogenic bacteria. Fenugreek was found to strongly inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a petri dish.

Animal Studies have clearly demonstrated the cholesterol-lowering activity of fenugreek in animals. In a typical study, fractions of fenugreek seeds were added to the diets of diabetic hypercholesterolemic and normal dogs. The defatted fraction, which contains about 54% fiber and about 5% steroidal saponins, lowered plasma cholesterol, blood glucose, and plasma glucagon levels from pretreatment values in both groups of dogs. The hypocholesterolemic effect has been reproduced in rats. Administration of the fiber-rich fraction of fenugreek to diabetic rats lowered total cholesterol, triglycerides, and low density lipoprotein (LDL). The level of high density lipoprotein (HDL) was increased. In the Clinical study Serum triglycerides were reduced from baseline in patients with newly-diagnosed, mild, type-2 diabetes mellitus who received a hydroalcoholic extract of fenugreek seeds 1 g/day. Total cholesterol and proportions of LDL and HDL fractions were not altered by treatment. A systematic review identified 5 other randomized clinical trials (N = 140) investigating the cholesterol-lowering effects of fenugreek seeds. Reductions (15% to 33%) of serum cholesterol from baseline were reported in all the trials identified. Total serum cholesterol and LDL cholesterol were reduced, while HDL cholesterol remained unchanged.

The galactomannan-rich soluble fiber fraction of fenugreek may be responsible for the antidiabetic activity

of the seeds. An Animal study evaluated the hypoglycemic effects of the seeds in dogs. The defatted fraction of the seeds lowered blood glucose levels, plasma glucagons and somatostatin levels; carbohydrate-induced hyperglycemia also was reduced. Clinical data shows that Glycemic control was improved in a small study of patients

Fenugreek tissue and cell cultures have been used for either plant regeneration or for the production of secondary products such as diosgenin and trigonelline: a saponin and an alkaloid. The first report on the production of spirostane derivatives by *Trigonella* tissue cultures was published by Khanna and Jain and concerned the establishment of static cultures grown on solid Murashige and Skoog (MS) medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). They reported the production of diosgenin, gitogenin and tigogenin along with other sterols. Six-week-old cultures showed a high growth index (GI= final wet weight – initial wet weight / initial wet weight) and the total steroidal content was higher than in the seeds. In other study, the highest diosgenin and tigogenin content was found in 8-week-old calli (0.40 and 0.15%, respectively).

Khanna et al demonstrated that suspension cultures of *Trigonella foenumgraecum* 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. A first pathway is the incorporation of cholesterol and this pathway is predominant when the precursor is added at subculture. The second pathway involves sidechain cleavage before incorporation and takes place when the sterol is added 10 days after subculture. In a similar way, Trisonthi et al. demonstrated that mevalonic acid promotes the synthesis of steroidal sapogenins in fenugreek tissue, particularly in suspension cultures grown on Miller medium (MS). Oncina et al. reported on the production of diosgenin by callus cultures of *Trigonella foenumgraecum* L. Leaf, stem and root calli were established and cultured on different soil growth media (MS, White's basal medium, Gamborg's B5) supplemented with coconut milk, malt extract and NAA.

Traditional uses:

The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia. Helpful in lowering fever, it is compared to quinine by some authorities. The seeds' soothing effect makes them of

value in treating gastritis and gastric ulcers. The seeds freshen bad breath and help restore a dulled sense of taste. The oil in the seeds is used as a skin softener and emollient.

In China, the fenugreek seeds are used as a pessary to treat cervical cancer. In the Middle East and the Balkans, the aerial parts of plant are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. They are also used to ease labour pains. Traditional Chinese herbalists used plant for kidney problems and conditions affecting the male reproductive tract. The seeds also function as a preservative and are added to pickles, chutneys and other similar products. In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages. Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic it is able to bring on a sweat and to help detox the body. This takes place through the pores of the skin. The pungent aroma of fenugreek may be smelt on the skin and in under-arm perspiration. After using the sprouts for a while, this fenugreek body aroma, does not seem to be so apparent, maybe, the sprouts have done a pretty good cleanse. Fenugreek also has the reputation as a lymphatic cleansing herb. The lymphatic system is the vacuum cleaner of the body. It has the vital role to irrigate the cells with nutrients and to remove toxic wastes, dead cells and trapped proteins. The fluid is cleaned through the lymph nodes, before the body's 13 litres of filtered lymph fluid recycles again, via the subclavian vein near the heart. A blocked lymphatic system can mean poor circulation, fluid retention, pain, loss of energy and disease, anywhere in the body. Fenugreek is a practical herb for all mucus conditions of the body, particularly the lungs, by helping to clear congestion. It is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough. Even drinking the water that seeds have soaked in and been rinsed with, helps to soften and dissolve, accumulated and hardened masses of cellular debris. Use fenugreek for head colds, influenza, catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis. Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorb toxic material and eliminate it. The healing and soothing action creates a protective coating,

like a lubricant, over inflamed areas. The slightly bitter properties of the seed are beneficial for digestion. Fenugreek has a powerful demulcent action, as it is rich in mucilage and it can soothe irritated or inflamed tissue. The Fenugreek herb has been known to help reduce fever when taken with lemon and honey, since it nourishes the body during an illness. Some health food stores also sell herbal Fenugreek teas, which can be used instead of the green tea. Fenugreek is often used in many teas and other products that help balance women's hormones and/or enlarge the breasts. Remedy to Ease Child Birth for Pregnant Women: Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. An Immunomodulatory effect of fenugreek extract in mice has been investigated. Overall, Fenugreek showed a stimulatory effect on immune functions in mice. As it is used for a variety of medicinal purposes, its immunostimulatory effect, as reported in this study, strengthens the rationale of its use in several Unani and Ayurvedic drugs. For the removal of Kidney Stones, a study was undertaken to investigate the effect of Fenugreek (*Trigonella foenum-graecum*) seed on experimentally-induced kidney stones in rats. Oxalate urolithiasis in male rats was produced by the addition of 3% glycolic acid to their diet. After 4 weeks, highly significant deposition in the kidneys was noticed and changes in water intake and body weight recorded. Fenugreek has also been shown to exhibit dose-dependent antioxidant properties, preventing lipid peroxidation and other oxidative damage in several *in vitro* models [48-50]. Finally soluble dietary fiber extracted from fenugreek seeds has been shown to blunt serum glucose increase following an oral glucose load associated with decreased intestinal disaccharidase activity and glucose absorption together with increased gastrointestinal motility.

Kuske et al. (1998) observed efficient nonselective methods to obtain DNA from the environment, which are needed for rapid and thorough analysis of introduced microorganisms in environmental samples and for analysis of microbial community diversity in soil. A small-scale procedure to rapidly extract and purify DNA from soils was developed for field use. They found that bead mill homogenization step was effective for DNA extraction and the hot-detergent bead mill procedure was simplified and miniaturized. Dalmastri et al. (1999) studied *Burkholderia cepacia* populations associated with *Zea mays*. The genetic diversity among *B. cepacia* isolates

were analysed by the random amplified polymorphic DNA (RAPD) technique, using the 10-mer primer AP5. The analysis of molecular variance (AMOVA) method was applied to estimate the variance components for the RAPD patterns. Dendrogram showed bacterial population with frequent recombinations and a non-clonal genetic structure. The dendrograms were also in agreement with the AMOVA results. Dunbar et al. (1999) described the levels of bacterial community diversity in four arid soils and compared them by cultivation and 16S rRNA gene cloning. They found the total of 498 phylotypes among the 16S rRNA clones. While, 34 phylotypes occurred among the cultivated isolates. They investigated the phylotype richness, frequency distribution and composition of the 4-culture collection by using the variety of diversity indices. Gelsomino et al. (1999) studied bacterial community structure by using DNA extraction followed by molecular fingerprinting. Total community DNA was extracted and purified by a direct method, which yielded amplifiable DNA of high molecular weight for all soils. A variable region of the 16S rRNA genes was then amplified by PCR with bacterial primers resulting in a mixture of amplicons separable via denaturing gradient gel electrophoresis (DGGE). The DGGE profiles of soil were indicative of dominant of soil bacterial types.

Theron and Cloete (2000) performed molecular techniques for determining structure and function of microbial diversity in natural ecosystem. They observed that molecular approaches based on 16S rRNA sequence analysis allow direct investigation of the community structure, diversity and phylogeny of microorganisms in almost any environment. While quantification of the individual types of microorganisms or entire microbial communities may be addressed by nucleic acid hybridization techniques.

Dunbar et al. (2000) proved the ability of terminal restriction fragment profiles of 16S rRNA genes to provide useful information about the relative diversity of complex microbial communities and TRF analysis is an excellent method for rapidly comparing the relationships between bacterial communities in environmental samples. This method is interchangeable with other molecular techniques and useful for rapid analysis of replicate samples in field-scale studies. Flore et al. (2001) used a combination of multiple techniques to the identification of bacterial strains isolated from clinical and natural environment. All isolates were examined by

means of rec. A based PCR assays and RFLP specific for these genomovars and species. A combination of different molecular techniques including SDS- PAGE of whole cell protein, RFLP of 16S r DNA and rec A genes, and rec. A – Based PCR assay can be very helpful to assess the species and genomvar composition of a natural population of *B. cepacia* complex.

Bridget et al. (2004) suggested that the study of most dwarf soil microbes requires culture-independent techniques. They used sodium pyrophosphate for eluting the DNA and filtered with a 0.45µm pore size filter. Filtrate DNA was extracted and PCR amplified by using universal bacterial and archaeal 16S rDNA primers, cloned, RFLP–screened and sequenced. Dwarf archaea and bacteria were present in the initial filtrate and in the cultures. Grouped dwarf bacteria into four bacterial phyla; Proteobacteria, Firmicutes, Actinobacteria and the TM-7 group. They observed that several sequences showed no close relationship to any microorganisms that have been grown in culture.

Beeja et al. (2004) emphasized on sampling of wild microorganisms leading to the discovery of new species and novel metabolites. Exploring bacterial diversity is typically done amplifying rRNA genes, in particular 16S rRNA genes from DNA samples isolated from a habitat. 16S rRNA genes are considered standard because they are thought to be conserved across vast taxonomic distance.

Yeates et al. (2006) studied various methods for microbial DNA extraction from soil for PCR amplification and observed that amplification of DNA from soil is often inhibited by co-purified contaminations like organic matter especially of humic acids. DNA extracted using sonication was more degraded than for the other methods and the bead beating method performed better in comparison to other methods. This method is more likely to result in effective lyses of all soil organic matter, reduced co-extraction of inhibitors and provides sufficient amount of DNA for PCR amplification.

1. CONCLUSIONS:

Certain difficulties have been encountered for the management of bacterial/fungal diseases in plants because of scanty availability of known and recommended bactericides in the market. In addition, these antibiotics are costly and not fully efficacious in some cases. In fact, during last two decades, problems of resistance against streptomycin have increased as well as some new important bacterial pathogenic strain have

been identified and documented. Consequently, there is an urgent need to search for economic and eco-friendly alternative strategies for the management of fungal diseases. Recently greater emphasis on stable source of resistance and other useful control measures viz., biological, cultural and botanicals have been laid. Besides, various biotechnological approaches have also opened new vistas in the management of fungal diseases. Fungal, bacterial, viral and insect mediated diseases are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence is a serious agronomic concern. The two most common fungal diseases infecting fenugreek are *ercospora* leaf spot and powdery mildew. In addition, other well known fungal diseases associated with fenugreek are collar rot, leaf spot and pod spot diseases.

REFERENCES:

- Dunbar, J.; T. Shannon; B. M. Susan; J. A. Davis and C. R. Kuske (1999). Applied Environment Microbial, 65(4): 1662-1669.
- Hampton, R.O.; E. M. Ball and S. H. De Boer (1990). Serological test for detection of viral and bacterial pathogens. American phytopathological society. St.Paul, Minn
<http://plants.usda.gov/java/profile?symbol=TRFO80>
- Kumar, R.; N. Arasu; V. Thirumalai and P. Gunasekaran (2002). Genotyping of antifungal compounds producing plant growth- promoting rhizobacteria, *Pseudomonas fluorescens*. Current Science, 82(12): 1463-1466.
- Mathur, A.; A. K. Mathur; P. Verma; S. Yadav; M. L. Gupta and M. P. Darokar (2008). Biological hardening and genetic fidelity testing of micro-cloned progeny of *Chlorophytum borivilianum* Sant. et Fernand. African Journal of Biotechnology 7 (8), pp. 1046-1053.
- Pace, N.R. (1996). New perspective on the natural microbial world: molecular microbial ecology. ASM News, 62: 463– 470.
- Pace, N.R. (1997). A molecular view of microbial diversity and the biosphere. Science, 276: 734– 740.
- Pace, N.R. (1999). Microbial ecology and diversity. ASM News, 65: 328– 333.
- Satyanarayana, T.; C. Raghukumar and S. Shivaji (2005). Extremophilic microbes; Diversity and perspectives. Current science, 89 (1): 79-99.
- Sharma, R.; R. Rajan; K.R. Kapardar and A. Grover (2005). 'Unculturable' bacterial diversity: An untapped resource. Current Science, 89(1): 72-77.

11. Singh C.P., Mishra U.S. and Patel V.V. (2010). Disease management of foot-rot disease of fenugreek caused by through late sowing practice in Bareilly, ASIAN J. EXP. BIOL. SCI. SPL. 174-176.
12. Thakuria, D.; N. C. Talaukar; C. Goswami; S. Hazarika; R. C. Boro and, M. R. Khan (2004). Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. Current Science, 86(7): 978-984.
13. V. A. Parthasarathy, K. Kandinnan and V. Srinivasan, ed. Organic Spices. New India Publishing Agencies. pp. 694.
14. Yeates, C.; M. R. Gillings; A. D. Davison; N. Altavilla and D. A. Veal (2006). Methods for microbial DNA extraction from soil for PCR amplification. Biological Procedures Online, 1: 40-47.
15. Kuske, C.R.; K.L. Banton; D.L. Adorada; P.C. Stark; K.K. Hill and P.J. Jackson (1998). Small scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. Applied and Environmental Microbiology, 64(7): 2463-2472.
16. Dalmastrri, C.; L., Chiarini; C. Cantale; A. Bevivino and S. Tabacchioni (1999). Soil type and maize cultivar affect the genetic diversity of maize root associated Burkholderia cepacia populations. Microbial Ecology, 38: 273-284.
17. Gelsomino A.; W. A. C. Keljher; G. Cacco and J. Elas (1999). Assessment of bacterial community structure in soil by Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis. Journal of Microbial Methods, 38(1-2): 1-15.
18. Theron J. and T. E. Cloete (2000). Molecular techniques for determining microbial diversity and community structure in natural environments. Crit Rev. Microbial, 26(1): 37-57.
19. Dunbar, J.; T. Shannon; B. M. Susan; J. A. Davis and C. R. Kuske (1999). Applied Environment Microbial, 65(4): 1662-1669.
20. Flore, A.; S. Laevens; A. Bevivino; C. Dalmastrri; S. Tabacchioni; P. Vandamme and L. Chiarini (2001). Burkholderia cepacia complex: distribution of genomvars among isolates from the maize rhizosphere in Italy. Environ. Microbiol. 3: 137-143.
21. Bridget, A. R. and L. K. Thomas (2004). Department of biology, New Mexico institute of Mining and technology, pp. 801
22. Beeja, O.; L. Aravind and E.V. Koonin (2000). Bacterial rhodospin: Evidence for a new type of phototrophy in the sea. Science 289:1902-1906.
23. Balajee SA, Lindsley MD, Iqbal N, Ito J, Pappas PG, Brandt ME (2007). A nonsporulating clinical isolate identified as *Petromyces alliaceus* (anamorph *Aspergillus alliaceus*) by morphological and sequence based methods. Journal of Clinical Microbiology 45: 2701-2703.
24. Abd-Ala, M.H. and Omar, S.A. 1998. Wheat straw and fungi application increases nodulation, nodule efficiency and growth of fenugreek (*Trigonella foenum-graecum* L.) growth in saline soil. Biol. Fertil. Soil. 26(1):58-65.
25. Abdelgani, M.E., Elsheikh, E.A.E. and Mukhtar, N.O. 1999. The effect of Rhizobium inoculation and chemical fertilization on seed quality of fenugreek. Food Chem. 64(3): 289-293.
26. Alberta Agriculture, Food and Rural Development (AAFRD). 1998. Fenugreek, agri-fax. Agdex. 147/20-5.
27. Alberta Agriculture, Food and Rural Development (AAFRD). 1998. Fenugreek, agri-fax. Agdex. 147/20-5.
28. Deteroja, H.J., Sukhadia N.M. and Malavia, D.D. 1995. Yield and nutrient uptake by fenugreek (*Trigonella foenum-graecum*). Indian J. Agron. 40(1): 160-161.
29. Edison, S. 1995. Spices-Research support to productivity. In N. Ravi (ed.) The Hindu Survey of Indian Agriculture, Kasturi and Sons Ltd., National Press, Madras, Pp.101-105.
30. Fazli, F. R. Y. 1967. Studies in the steroid yielding plant of the genus *Trigonella* PhD diss., University of Nottingham, UK.
31. Fazli, F.R.Y. and Hardman, R. 1968. The spice fenugreek (*Trigonella foenum-graecum* L.). Its commercial varieties of seed as a source of diosgenin. Trop. Sci. 10:66-78.
32. Fogg, M.L., Kobayashi D.Y., Johnston S.A. and Kline, W.L. 2000. Bacterial leaf spot of fenugreek: A new disease in New Jersey caused by *Pseudomonas syringae* pv. *syringae*. Publication No. P-2001-0012-NEA. In Northeastern Division Meeting Abstracts, 1-3 Nov. 2000. Cape Cod, North Falmouth, MA, USA.
34. Jongbloed, M. 2004. Coriander and Fenugreek, p. 229-235. In S. Salvin et al. (ed.) The New Crop Industries Handbook, Rural Industries Research and Development Corporation (RIRDC), Australian Government, Australia.
35. Lucy, M. 2004. Fenugreek [Online] Available at www.dpi.qld.gov.au/field_crops/9050.html [Accessed September 18, 2005].
36. Khiriya, K.D., and Singh, B.P. 2003. Effect of phosphorus and farmyard manure on yield, yield attributes and nitrogen, phosphorus and potassium uptake of fenugreek (*Trigonella foenum-graecum*), Indian J. Agron. 48(1):62-65.

37. Lust, J.B. 1986. The herb book. Bantam Books Inc. New York. Pp. 1-55.
38. Petropoulos, G. A. 1973. Agronomic, genetic and chemical studies of *Trigonella foenum-graecum* L. PhD. Diss. Bath University, England.
39. Petropoulos, G. A. 2002. Fenugreek -The genus *Trigonella*, Pp. 1-127. 1st ed. Taylor and Francis, London and New York.
40. Prakash, S. and Sharma G.S. 2000. Conidial germination of *Erysiphe polygoni* causing powdery mildew of fenugreek. *Indian Phytopathol.* 53(3):318-320.
41. Tiran, D. 2003. The use of fenugreek for breast feeding woman. *Comp. Ther. Nurs. Midwifery.* 9(3): 155-156.
42. Subba Rao, N.S., and Sharma, K.S.B. 1968. Pectin methylesterase of root exudates of legumes in relation to *Rhizobia*. *Plant Soil.* 28(3):407-412.
43. Sinskaya, E. 1961. 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.
44. Tutin, T.G., and Heywood, V.H. 1964. *Flora Europaea.* Vol. I & II. Cambridge University Press, Cambridge.
45. Yadav, G.L. and Kumawat, P.D. 2003. Effect of organic inorganic, fertilizer and *Rhizobium* inoculation of yield and yield attributes of fenugreek (*Trigonella foenum-graecum* L). *Haryana J. Hort. Sci.* 32(1-2): 147-148.
46. Valette G, Sauvaire Y, Baccou JC, Ribes G: Hypocholesterolaemic effect of fenugreek seeds in dogs. *Atherosclerosis* 1984, 50:105-111.
47. Gupta A, Gupta R, Lal B: Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Assoc Physicians India,* 2001, 49:1057-1061.
48. Raghuram TC, Sharma RD, Sivakumar B: Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. *Phytother Res* 1994, 8:83-86.
49. Hannan JM, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YH: Soluble dietary fibre fraction of *Trigonella foenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. *Br J Nutr* 2007, 97:514-521.
50. Talpur N, Echard B, Ingram C, Bagchi D, Preuss H: Effects of a novel formulation of essential oils on glucose-insulin metabolism in diabetic and hypertensive rats: a pilot study. *Diabetes Obes Metab* 2005, 7:193-199.
51. Vijayakumar MV, Singh S, Chhipa RR, Bhat MK: The hypoglycaemic activity of fenugreek seed extract is mediated through the stimulation of an insulin signalling pathway. *Br J Pharmacol* 2005, 146:41-48.
52. Ajabnoor MA, Tilmisany AK: Effect of *Trigonella foenum-graecum* on blood glucose levels in normal and alloxan-diabetic mice. *J Ethnopharmacol* 1988, 22:45-49.
53. Bhatia K, Kaur M, Atif F, Ali M, Rehman H, Rahman S et al. aqueous extract of *trigonella foenum graecum* L. ameliorates additive urotoxicity of buthionine sulfoximine and cyclophosphamide in mice. *Food and Chemical Toxicology.* 2006; 44: 1744-1750.
54. Naidu MM, Shyamala, Naik JP, Sulochanamma G and Srinivas P. Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. *Food science and technology.* 2010; 44(2): 451-456.
55. Sauvaire Y, Girardon P, Baccou JC, Ristèrucci AM. Changes in growth, protein, free amino acids of developing seeds and pods of fenugreek. *Phytochemistry.* 1984; 23(3): 479-486.
56. Ramesh BK, Yogesh, Raghavendra HL, Kantikar SM, Prakash KB. Antidiabetic and histopathological analysis of fenugreek extract on alloxan induced diabetic rats. *International journal of drug development and research.* 2010; 2(2): 356-364.
57. Thaakar SR, Sarawathy GR, Maheswari E, Kumar NS, Hazarathiah T, Sowmya K et al. Inhibition of CCl₄- induced liver fibrosis by *Trigonella foenum-graecum* Linn. *Natural product radiance.* 2007; 6(1): 11-17.
58. Palaniswamy M, Praddep BV, Sathya R, Angayarkanni J. In vitro anti-plasmodial activity of *trigonella foenum-graecum* L. eCAM advance access published. 2008: 1-5.
59. Navayath S, Thiyagarajan D. Aqueous extract of *trigonella foenum graecum* (fenugreek) prevents cypermethrin induced hepatotoxicity and nephrotoxicity. *SAGE journals online.* 2010; 29(4): 311-319. Available from: <http://het.sagepub.com/content/29/4/311.abstract>
60. Laroubi A, Touhami M, Farouk L, Zrara I, Aboufatima R, Benharref A et al. Prophylaxis effect of *trigonella foenum graecum* L. seeds on renal stone formation in rats. *Phytotherapy research.* 2007; 21(10): 921-925.
61. Chauhan G, Sharma M, Varma A, Khanrkwal H. Phytochemical analysis and anti-inflammatory potential of fenugreek. *Medicinal plants- international journal of*

- phytomedicines and related industries. 2010; 2(1). 64. Available from: www.indianjournal.com
62. Ahmad F, Acharya SN, Mir Z, Mir PS. Localization and activity of r RNA genes on fenugreek (*trigonella foenum-graecum* L) chromosomes by fluorescence in situ hybridization and silver staining. SpringerLink. 1998; 98(2): 179-185. Available from: <http://www.springerlink.com/content/t8ux5ah5q6k8t37h/>
63. Green JM, Sharma D, Reddy LJ, Sexena KB, Gupta SC, Jain KC et al. Methodology and the progress in the ICRISAI in pigeonpea breeding programme. In Proc. International workshop on pigeonpeas. ptancheru. 1981. P437-439. Available from: <http://agropedia.iitk.ac.in/openaccess/sites/default/files/RA%2000024.pdf>
64. Mehrafarin A, Ghaderia A, Rezaradeh SH, Naghdi BH, Nourmohammodi G, Zand E. Bioengineering of important secondary metabolites and metabolic pathways in fenugreek (*trigonella foenum graecum* L). Journal of medicinal plants. 2010; 9(35): 1-18. Available from: <http://www.sid.ir/en>
65. Oncina R, delrio JA, Gomez P, Ortuno A. Effect of ethylene on diosgenin accumulation in callus culture of *trigonella foenum-graecum* L. Food chemistry. 2002; 76(4): 475-479.
66. Brain KR, Williams MH. Evidence for an alternative rate from sterol to sapogenin in suspension cultures from *trigonella foenum graecum*. Plant cell report. Springerlink. 1983; 2(1): 7-10.
67. Oluf L, Gamborg, Shyluk JP. The culture of plant cell with ammonium salts as the sole nitrogen source. Plant physiology. 1970; 45(5): 598-600.