HISTOPATHOLOGY, BIOCHEMISTRY AND CONTROL OF FUNGAL DISEASES IN (Trigonella foenum- graecum L.) IN KOTA DISTRICT OF RAJASTHAN

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A THESIS

Submitted for Award of

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Of

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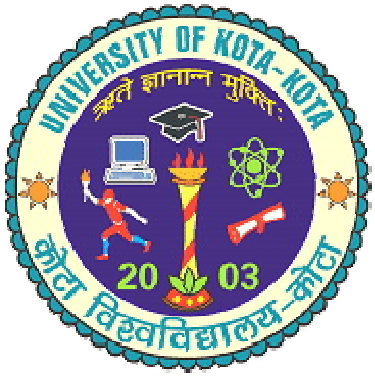
In

BOTANY



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CERTIFICATE

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(1) Thesis entitled "**HISTOPATHOLOGY, BIOCHEMISTRY AND CONTROL OF FUNGAL DISEASES IN (*Trigonella foenum- graecum* L.) IN KOTA DISTRICT OF RAJASTHAN"** submitted by Upma Singh is an original piece of research work carried out by the candidate under my supervision.

(2) Literary presentation is satisfactory and the thesis is in a form suitable for publication.

(3) Work evinces the capacity of the candidate for critical examination and independent judgment.

(4) Candidate has put in at least 200 days of attendance every year.

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FEW WORDS OF GRATITUDE

While putting my emotions on paper, I find myself extremely short of words. If there had been no footsteps for me to follow, no inspiration for me to go and no blessing hand over me, this work and indeed my research period career would not have seen the light of the day.

Language becomes a poor medium of expression when it comes to pen down my deep sense of gratitude and admiration I possess for my esteemed teacher and honorable guide

Dr. Indu Rani Sharma

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For her inspiring supervision, constructive criticism and constant encouragement, without which the present work, would never have seen the light of the day.

I consider myself extremely fortunate to have worked under such a dynamic, scholastic and caring person.

Whatever is worth is all attributable to her, errors are mine.

Upma Singh

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Place : *Upma Singh*

Date

DECLARATION

I hereby declare that the work incorporated in the present thesis entitled "**HISTOPATHOLOGY, BIOCHEMISTRY AND CONTROL OF FUNGAL DISEASES IN (*Trigonella foenum - graecum* L.) IN KOTA DISTRICT OF RAJASTHAN"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of a degree or a diploma.

(Upma Singh)

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INTRODUCTION

From time immemorial, spices have played a vital role in world trade due to their varied properties and applications. We primarily depend upon spices for flavor and fragrance as well as for color as a preservative and for its inherent medicine qualities, although about 107 spices are recorded from which only a dozen are important black pepper, cardamom, ginger, turmeric, large cardamom, cumin, coriander, fennel, fenugreek, chilies, saffron and celery. Among all these seed spices are consumed highly in every part of world. Among these seed spices fenugreek plays an important role due to its extensive pharmacological properties. Though Fenugreek is native to the Mediterranean region of Europe, it extends to central Asia and North Africa as well from Central Asia; India is the largest producer consumer and exporter of seed spices. The global demand estimated for seed crop is 1, 50,000 MT of which India contributes 83,550 MT annually accounting for 55.7 Percent of the total world trade (Malhotra and Vashishtha 2007). Needed semiarid type of climatic condition for spice cultivation is fulfilled in India in Rajasthan and Gujarat is known as “Bowl of Indian spices” of India. Both the states produce 80% of total production of spices in which 60% is of Rajasthan share. All these seed spices are exported to different parts of the World like United States, Japan and Netherland etc. Around 20 types of spices are grown in different states of India from which Fenugreek is most important with its enormous medicinal properties.

In recent decades increasing attention has been paid in utilization and consumption of natural and traditional products (foods, flavors, colors, perfumes, phytotherapeutics etc.) because modern scientific knowledge and technologies have revealed that many chemical products of synthetic origin of this kind are responsible for a lot of new hazards and disorders for human beings.

The plant species of the genus Trigonella and especially that of T. foenum graccum L. (fenugreek) is a good example which has been used traditionally to cover such human needs. Fenugreek is cultivated all over the world and mainly in

India and the Mediterranean countries as cash and good renovator of soil crop and a multipurpose legume is used as forage, food , spice, perfume, insect repellant, dye, herbal medicine etc.

HISTORY, ORIGIN AND DISTRIBUTION OF FENUGREEK SPECIES

Plants of the genus Trigonella and particularly of the cultivated species T.foenum gracum L. were known and used for different purposes in ancient times, especially in Greece and Egypt (Rouk and Mangesha, 1963). In North Africa it has been cultivated around the Saharan Oasis since very early times (Duke, 1986). Historically, fenugreek is one of the oldest known medicinal plants and even Hippocrates thought highly of it (Lust, 1986; Schanenburg And Paris, 1990). Fenugreek was first introduced into Chinese medicine in the Sung dynasty. AD 1057 (Jones, 1989).The herb has long been a favourite of the Arab physicians (Stuart, 1986). As a fodder plant, it is said to be the Hedysarum of Theophrastus and Discorides (Leyel, 1987). Fenugreek was introduced into Central Europe at the start of the Ninth Century (Schanenburg And Paris, 1990).

Sinskaya (1961) reports that direct wild ancestor of cultivated fenugreek belonging to the species Trigonella foenum graccum L. has not been exactly determined and the existence of these wild forms is problematic. It is possible that the species T. foenum graccum evolved from T.gladiata which had possibly given rise to some new extinct forms of T. foenum graccum L. Fenugreek is an ancient crop plant De Candolle (1964) and Fazli and Hardman (1968) 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 Greek, Italy and Spain. De Candolle 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 for more common to those leguminous floras. Vavilov (1926, 1951) suggested that fenugreek is native to the

Mediterranean region of the “Old World” while Dangi et al (2004) proposed that T. caerulca and T. foenum graccum originated in Turkey.

Saraswat (1984) recovered carbonized fenugreek seed from a Rohira village in the Sangrur district of Punjab, India indicating its use in trade by people of Harappan civilization as far as back as 2000-1700 B.C. Fenugreek is also known as one of the oldest medicinal plants recognized is recorded history (Lust, 1986). Indigenous species have been reported (Allen and Allen 1981, Ivimey crook 1968, Petropolous 2002, Phitos and Damboldt 1985, Polunin1988) on the continents of Asia (6 species), Europe (5 species), Africa (1 species), and Australia (1 species).

The exact number of species of fenugreek also has been debated Petropolous (2002) indicated that older taxonomists like Linnaeus have suggested that as many as 260 species of fenugreek exist. In context, about 128 species of fenugreek were reported by Vasil’chenko (1953), 97 by Fazli (1967) and 70 by Hector (1936), Hutchinson (1964), and Rouk and Mangesha (1963), A total of 18 different species of fenugreek (Trigonella) currently are recognized in the the primary literature.

Table 1: - Common species of the Trigonella genus is according to Petropolous (2002).

Genus	**Species**
Trigonella	- T. foenum graecum, T. angwina, T.arabia, T.caerulea, T. corniculata, T.cariensis, T.rigida, T. suavissima, T.torulosa, T.spinosa, T.polycerata, T.radiata, T.platycarpus, T.hamosa, T.cretica, T.occulta, T.arcuta and T.striata

Botanical perspective of fenugreek

Fenugreek is an annual dicotyledonous plant belonging to the subfamily Papilionaceae, family Leguminocae (The Fabaceae) with trifoliate leaves, branched stems, white flowers, roots bearing nodules and golden yellow seeds. In general, two types of flowering shoots are observed. The common ones bear axillary flowers showing an intermediate growth habit, whereas so called “blind shoots” have both axillary and terminal flowers, becoming “tip bearers” for seedpods. Both cleistogamous (closed) and aniestogamous (open) flowers have been described (Petropolous, 1973) but the vast majority of fenugreek flowers are closed or cleistogamous. Allard and Darligton and Wylie (1945) have classified Trigonella species as self and cross pollinated based on observation of open and closed flowers. Stems 20 -130 cm long, straight, rarely ascending, branching rarely simple, sparsely pubescent, usually hollow, anthocynin tinged at base or all the way up, rarely completely green. First leaf simple, sometimes weak trifoliate, oval or orbicular with entire margin and a long petiole. Stipules fairly large, covered with soft hairs. Flowers in leaf axils, mostly twin, more rarely solitary. Calyx 6-8 mm, soft hairy with teeth as long as the tube, half as long as the corolla. Corolla 13-19 cm long pale yellow (while at the ending period of flowering), keel obtuse. Pods with beak, 10-18 cm long and 3.5 x 5 cm broad, curved rarely straight, with transient hairs. Before ripening the pod is green or reddish colored, whipe ripe light straw or brown containing 10-20 seeds.

Seeds vary from rectangular to round in outline with a deep groove between the radical and cotlydons, the length is 3.5-6 mm and the width 2.5-4 mm, light grayish brown, olive green or cinnamon coloured, with a pronounced radical that is half the length of the cotlydons. The minute hilum lies obscured with a deep notch, odour characteristics chromosome number 2n=16.

Cultivation

Although fenugreek is a native of the Mediterranean region of Europe, it extends to central Asia and North Africa as well. This wide distribution of its cultivation in the world is characteristics of its adaptation to variable climatic condition and growing environments. Fenugreek is suitable for areas with moderate or low rainfall having drought resistant and fairly frost sensitive. Rosengarten (1969) states that fenugreek is grown best in well drained loams, Duke (1986) reports also that fenugreek grows fairly well on gravely or sandy soils and it is fairly tolerant to salt.

Furry (1950) describes five cultivated species of the genus Trigonella as ; T. foenum-graecum, T.caerulea, T.polycerata, T.monspeliaca and T. suavissiima, while in flora European (Ivimey-crook 1968) only two species to be reported; T. foenum-graecum and T.caerulea. At the present time Fenugreek is an important cash crop in India. It is mainly cultivated in Rajasthan, Gujrat and Madhya Pradesh and to the limited extent in Andhra Pradesh, Tamil Nadu, Haryana, Maharastra and Punjab covering an area of 53,596 millions ha with an annual production of 64102 MT. India exported 9050 MT of fenugreek valued Rs. 1787.5 lakhs (Anonymous, 2007).

Rajasthan is considered as “fenugreek bowl” of the country and contributes about 90 percent to the country’s production. In Rajasthan, it is mainly grown in Sikar, Chittorgarh, Nagaur and Kota districts covering an area of 40,495 million ha and produces 47,228 MT with 1166 Kg/ha productivity (Anonymous, 2007).

Medicinal and Nutraceutical Properties of Fenugreek

Species of the genus Trigonella and particularly fenugreek are well known for their pungent aromatic, high nutritive and multi-therapeutical properties and serve culinary medicinal and industrial purposes. The biological and pharmaceutical actions of fenugreek are attributed to the variety of its constituents

including steroids (diosgenin), alkaloids (trigonelline) flavonoids (luteolin), coumarins, amino acids (hydroxyisoleucine), mucilage (galactomannan), volatile constituents, fixed oils and other substances.

Fenugreek is one of the oldest known medicinal plants from ancient times and even Hippocrates thought highly of it. Fenugreek seeds which are described in the Greek and Latin pharmacopocisas are said to have anti-diabetic activity and hypocholestrolaemic effects and have been reported to possess a curative gastric anti-ulcer action and anti-fertility and anti-nonconceptive effects. So many of its actions as remedy have been confirmed and the mechanisms of their activity are being studied.

Besides all these medicinal properties fenugreek have some functional food aspects. Mansour and El-Adawy (1994) recommended that fenugreek seed be added to foods like ground meat and baked goods not only as a nutritional supplement but also as a potential functional food. Fenugreek fibers, psyllium husk and wheat bran could be used as dietary supplements to increase roughage in the human diet (Al-Khalidi et al, 1999). Use of corn bread mixed with a small amount of fenugreek 3% or with wheat flour (30%) is used as a staple food in Egypt (Galal, 2001). In India, fenugreek flower blended rice bran was used to improve the physical and sensory properties of bread and cookies, while improving their qualities (Sharma and Chouhan, 2002).

Fenugreek has been differently named in different parts of India, in different languages. Vernacular names of fenugreek are as follows:-

Hindi – Methi

Sanskrit – Medhika

Telugu - Menthulu

Tamil – Venthyam

NUTRITIONAL STATUS

Fenugreek has nutritional value per hundred grams as follows – Energy – (323 Kilo calorie).

Carbohydrate 58.35 g

Protein 23 g

Total fat 6.41g

Cholesterol 0 mg

Dietary fiber 24.6 g

Vitamin A 60 u

Vitamin C 3 mg

Niacin 1.640 mg

Riboflavin 0.366 mg

Thiamin 0.322 mg

Sodium 67 mg

Potassium 770 mg

Calcium 176 mg

Magnesium 191 mg

Zinc 2.50 mg

Iron 33.35 mg

Cobalt 1.110 mg

Phosphorus 296 mg

[Source- United States Department of Agriculture (USDA) Nutrient Database]

Ecological, Agronomic and cultural aspects of the crop

Fenugreek is a specialty crop in India that can benefit production in many ways (Acharya et al, 2004). As a legume crop, it can conditions the soil by fixing nitrogen from the atmosphere and can reduce the need for nitrogen fertilizers for subsequent crops. As a dryland crop its water requirements are low; use of fenugreek can reduce the cost of irrigation, save water and reduce eutrophication of surface waters and limit contamination of ground water sources (Basu et al, 2004).

Agronomic research conducted in different agro-climatic zones in India suggests that optimum productivity can be obtained when fenugreek rows are spaced 20-30 cm apart and planted in early October or November (Korla and Saini, 2003, Gill et al, 2001, Baswana and Pandita 1989, Bhatt 1988).

Agronomic practices known to maximize fenugreek yield involve time to forage cutting (Lal et al, 2003) and use of minimum level of irrigation (Moyer et al 2003, Kumar et al 2000, Ram and Verma 2000, Sheoran et al 2000, Bhatt 1993, Mir et al, 1993). Well drained loam by the crop. Potash has been successfully used to adjust soil pH to increase nutrient uptake by fenugreek (Yadav and Kumawat, 2003) Heavy and wet soils limited fenugreek growth in other parts of the world (Petropolous, 1973). Because fenugreek is a nitrogen fixing legume, seeds must be inoculated with appropriate Rhizobium species for optimal growth. The most common nodule forming bacteria associated with Trigonella foenum graecum L. is the Gram negative, aerobic, non-sporulating bacillus Rhizobium melliloti (Subba Rao and Sharma, 1968). Abdelgani et al (1999) suggested that inoculation of fenugreek with a suitable strain of Rhizobium can improve seed yield and quality. Use of organic and inorganic fertilizers (N and P) as well as farm as farmyard manure has been effective in increasing fenugreek yield (Khiriya and Singh, 2003, Yadav and Kumawat, 2003, Detoroja et al, 1995. Weeds can be difficult to control in fenugreek production. Moyer et al (2003) evaluated weed management in irrigated fenugreek grown fields in rotation with other annual crops. Fenugreek

can grow on marginal lands and may be useful for reclamation of land distributed by industrial activity (Acharya et al, 2006).

All the above data shows that fenugreek can serve as an efficient tool in the process of reclamation and has effective agronomic characters.

Abiotic and Biotic Threats to the Plant

Fungal, Bacterial, viral and insect mediated disease are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence, are a serious agronomic concern (Jougebloed 2004, Fogg et al, 2000, Prakash and Sharma 2000, AAFRD, 1998).

The fenugreek is being widely cultivated in Kota, Rajasthan and its adjoining areas at commercial level. It suffers from several diseases caused by various plant pathogens. Aphids and Mites attacks the crop after flowering occurs in crop and causes floral damage. Among these pathogens, the fungal pathogens are more dominant on this crop and have been found to be very destructive. The major diseases occurring on fenugreek are powdery mildew, damping off, collar rot, leaf spots and rust.

The three most common fungal diseases infecting fenugreek are Cercospora leaf spot, powdery mildew by Erysiphae polygoni and downy mildew by Perenospora trigonallae (AAFRD, 1998). Powdery mildew on fenugreek, caused by Erysiphae polygoni can reduce crop yield (Jongebloed, 2004, Prakash and Sharma, 2000) and has the potential to affect biomass and seed yield in crops under moist agro-climatic condition. In Kota district these disease occur in later season of crop at the time of flowering and causes high damage. Downy mildew caused by Perenospora trigonallae usually occurs in December-January. It covers the whole under surface of leaves and causes leaf fall.

Leaf spot disease is spreaded when it is too cold and dry caused by Cercospora traversiana with characteristics dark irregular spots on leaves and stems and causes a great loss to yield. Some other pathogens like Alternaria

alternate and Helminthosporium also causes disease complexity at the end of the season and the damping off type loss happens to the crop. Many scientists suggested so many weedicides as well as fungicides like Endosulphan (35 ec) Mancozeb (0.25 gm/litre), Karathane L.C. and many more to reduce these pathogens but by using some biocontrolling agents is a good solution to protect the crop and the environment. Some phytoextracts like Aegle leaf extract, Annona leaf extract, Withania and Ocimum aqu. Extracts as well as methanol extracts are thought to be more effective towards these pathogens. They not only help to prevent these fungal diseases but also help to improve quality of crop.

Aim to the Study

Due to environmental and health concerns there has been a growth in the sale of organic spices. Fenugreek has commercial, agricultural and environmental potential. So the study includes the survey of total fungal pathogens which attacks the crop, their effects on the plant and most importantly their control by using fungicides but special concern to bio-controlling agents.

Our Knowledge regarding these aspects is very meager and inadequate therefore to understand the mechanism and development of different diseases and for the use of possible economical control measures for the diseases present plan has been proposed with following objectives :-

1. Survey –

    a) To know the occurance of fungal diseases of Trigonella foenum graecum in different tehsils of the district.
    b) To knows the occurrence of most prevalent disease in the Study area & their effect on Trigonella production.
    c) Protective /curative measures being used at present by the Farmers.

d) Analysis of soil from the rhizosphere of healthy and infected Plants.

2. Ecological study –

a) Survey of weeds associated with fenugreek crop which act as an alternative host.

b) Study of edaphic factors total nitrogen (N) content, amount of available Phosphorus (P) and amount of available Potassium (K) in the soil.

3. Host parasite interaction –

a) Symptoms and etiological studies of different fungal diseases in Trigonella.

b) Morphological , Histopathological , Biochemical changes in healthy and infected plant parts of Trigonella.

4. Control experiments –

a) Antifungal activity test of some botanicals against isolated pathogen.

b) In vitro experiment for testing fungicidal property of aqueous and methanol extracts of Aegle marmelos, Withania somnifera, Ocimum sanctum, Anona squamosa.

INTRODUCTION

In recent decades increasing attention has been paid in utilization and consumption of natural and traditional products (foods, flavours, colours, perfumes, phytotherapeutics etc.) because modern scientific knowledge and technologies have revealed that many chemical products of synthetic origin of this kind are responsible for a lot of new hazards and disorders for human beings.

The plant species of the genus Trigonella and especially that of T. foenum gracum L. (fenugreek) is a good example, which has been tradionally to come such human needs. Fenugreek is cultivated all over the world and mainly in india and Mediterranian countries as chenurgic, Cash and good renovator of soil crop and as a multi-purpose legume is used as forage, food, species, perfume, insect repellent, dye, herbal medicine etc.

Fenugreek has been reported as a cultivated crop in Portugal, Spain, United Kingdom, Germany, Australia, Switzerland, Greece, Turkey, Egypt, Sudan, Ethopia, Kenya, Tanzania, Israel, Lebanon, Morocco, Tunisia, India, Pakistan, China, Japan, Russia, Argentina and the United States of America (Rouk and Mangesha, 1963) (Fazli and Hardmann 1968, Rosengarten 1969).

At the present time fenugreek is an important cash crop in India. The Total cultivation area of fenugreek in India an average for 1975-95 was 34,534 ha with a production of 41,530 tons and exports of 4203 tons that is domestic use accounts for 90 percent of the production (Anonymous 1996).

Till 2000, the total area sown to fenugreek nationally has varied between 35000 and 45000 ha production about 1.25 t ha-1.

Year	Area	Production
2005-06	33398	38990
2006-07	44984	55780

Fenugreek locally known as "Meethi" is attacked by various disease which causes severe losses. Fungal, bacterial, viral and insect mediated diseases are reported to be associated with considerable lowering of forage and seed yield in fenugreek and hence are of serious agronomic concern (Jongebloed, 2004, Fogg et

al 200, Prakash and Sharma 2000, AAFRN 1998). Some workers also have reported physiological disease due to mineral deficiency that is associated with lowering of forage yield in fenugreek (Sinskaya 1961).

Fungal diseases on fenugreek crop and yield losses due to these diseases are equally common in Kota district, Rajasthan as in the other fenugreek growing areas of the world and India.

In the present study area though the fungal diseases have become very severe now-a-days but not much work has been carried out on the diseases of fenugreek. With this view present investigation was carried out. The survey study included:-

1. Occurrence of different fungal pathogens on fenugreek in different localities of the study area.
2. To know the occurrence of most prevalent disease in the study area.
3. Study and comparison of various growth parameters of both healthy and infected plants of fenugreek.
4. Feedback from farmers regarding the disease management strategies already used by them.

Therefore, this study is undertaken with the purpose of defining situation of fenugreek diseases, their effects and control measures in the fenugreek fields of Kota districts. An understanding of the disease situation is essential for the successful implementation of disease management strategies.

REVIEW OF LITERATURE

Fungal, bacterial, viral and insect mediated diseases are reported to associate with considerable lowering of seed yield and quality of crops, are of serious agronomic concern. Although generally fenugreek is little subjected to pest and fungal disease (Sinskaya 1961) a number of investigations have reported the appearance in fenugreek crop of some pest, fungal, bacterial and viral disease. Fungal infections decrease the net yield (48% loss) per year in Indian concern (SKN college of agriculture – Jobner - Jaipur 1995). Various diseases on Trigonella has been noticed from Ethopia (Rouk & Mangesha, 1963), UK (Anonymous, 1970), Morroco (Petropolous, 1973), Bulgaria (A.P. Margina & J. De.gryuter, 1996), Australia (Max Jongebloed), Pakistan (M.Mushtaq, M.A. Haq. and M.H. Hashmi, 1998) and also from India.

Fenugreek is attacked by a number of diseases viz. Downey mildew (*Perenospora trigonella* Granmann) Powdery mildew (*Erysiphae polygoni* D.C and *Leveilula taurica* (lev.) Arnand rust (*Uromyces anthyllidis* (Grev.) Schroet, leafspot (*Cercospora traversiana* Sacc.) collar rot *(Rhizoctonia solani*) and root rot (*Alternaria alternata* (Fr.) Kersiler).

George Manicas and Petropolous reported 28 diseases in T. foenum graecum caused by all pathogens (fungi, bacteria and virus) from almost all parts of the world in India 27 species of fungi have been isolated from fenugreek seed (Prabha and Bohra 1999). A national Survey Study (2010) revealed that disease like leaf spot, root rot, Downy mildew, Powdery mildew, collar rot, pod spot were found widely prevalent across different states with different intensities in India. (AAFRD, 1998)

Powdery Mildew

Zimmer (1984) reported powdery mildew of fenugreek (probably Erysiphae polygoni) from Canada. Powdery mildew on fenugreek caused by E. polygoni can seriously reduce crop yield (Prakash and Sharma 2000), (Jongebloed

2004) Powdery mildew caused by Erysiphae Polygoni D.C and leviella taunsica (lev.) Arnand has been observed to appear in different parts of India.

Ahmed 1970, Saxena and Ahmed 1981 suggested that powdery mildew of fenugreek caused by Leveillula taurica is one of the most serious disease in central India.

Mehra 2006, reported powdery mildew caused by Erysiphae polygoni D.C. is economically important disease in different parts of India particularly in Rajasthan, Gujrat and Haryana.

Maiti and Agrawal investigated Erysiphae polygoni as an obligate parasite which penetrates through the leistothecia present in debries of the previous crop.

Collar Rot

Hiremath et al revealed that fenugreek suffers extensively with foot rot and damping off of disease caused by R. solani in some areas of India (1976). Petropolous (2002).

Datta and Chatterjee (2004) studied this disease in different parts of the world.

Cercospora leaf Spot

Voros, Nagy in 1972 cook 1978 and khare et al 1981, Ryhy 1989 Surveyed Seperately across the world and suggested this disease most common in Australia eastern European countries South and North America and in North East and India.

Several researchers have suggested that C. traversiana is the only species of the Cercospora infecting Fenugreek (Cook 1978, Ryhy 1989).

Cunmington in 2005 studied Symptoms of leaf spot in plant.

Bretag 2006 suggested traditional control methods to control primary infections by the disease efficiently.

Powdery Mildew

Petropolous, 2002 Basn et al 2006 revealed this disease affecting both biomass and yield.

Prakash and Saharan 2000, Basu et al 2006 found Powdery mildew most commonly in hot and humid tropical and subtropical areas and well as in temperate to sub temperate regions.

Downy mildew

Downy mildew has previously been reported from Algeria, India, Pakistan, UK and USA (Rooney Lathan et al 2009).

Downy mildew caused by Perenospora trigonallae Gaumann. was first reported from Bombay by Uppal et al in 1935 and later from Punjab by Thind in 1942.

Only few references are available on Perenospora trigonellae. Hence the Work done on other crops affected from Downey mildew pathogens along with fenugreek under similar agroclimatic conditions have been reviewed and presented.

Lakra reported in 2002, 2003 that disease becomes severe in February under Haryana Conditions.

Spring black stem & leaf spot

Disease was first reported in 2004 from Rupanyup in the Winmera region of Victoria, Australia.

Morgan – Jones & K.B. Burch identified the fungal as Phoma pinodilla. It has been also isolated from seeds of fenugreek.

STUDY AREA

The word is derived from two Latin words Sur = over and Video = to see, meaning thereby "a general view", "an inspection" or "collection of data for mapping".

The present surveyed area is situated in Rajasthan State. Rajasthan is the largest state of India and is located in the northernwest part. It is situated between 23º3’ to 30º12’ North altitude and 69º3’ East to 78º17’ East longitude covering an area of 3,42,274 square kilometers. This accounts for eleven percent of the total area of the country. It is mostly a dry sandy desert but also has fertile plains, plateau, forest and hills rising as high as 1200 meters (4000 feets) above sea level, with Mount Abu as the highest point between the Himalaya and the Nilgiris.

The Maximum length of the state is 869 Km from west to east and about 826 Km from North to South. It represents the shape of a Rhombus.

Presently Study mainly represent the root knot incidence of south eastern Rajasthan contituty the Hadoti specially Kota and adjoining area. Kota and its adjoining area is predominantly an agricultural region with an agrarian economy and are rich to grow fenugreek crop.

Kota region is mainly composed of low hills and discretely distributed plateau area with shallow plains, Situated on the banks of Chambal River.

District Kota forms South-Eastern part of Rajasthan state. It has between 24º33’ to 25 º 51’ north altitudes and 75º37’ to 76º34’ east longitude. It is bounded on the north with district Sawai Madhopur and north east Madhya Pradesh state, in east newly created district Baran and north west district Bundi in west south district Jhalawar. The district is located in the South- East of Rajasthan state, its shape is something like a cross. The land Slopes gently from South to North and is drained by the river Chambal and its tributaries. The Mukundra range of Vindhyachal Hills, which is 145 km long. District has a dry climate. Cold season lasts for about three and half months from November to the end of February when the temperature falls upto 3ºc. The period from April to the end of June constitutes

the hot season when the maximum temperature shoots upto 46ºC. The monsoon season starts in the middle of July.

Rajasthan is considered as "Fenugreek bowl" of the country and contributes about 90 percent to the country's production. In Rajasthan it is mainly grown in Sikar, Chittorgarh, Nagaur and Kota districts covering an area of 40,495 million ha and produces 47,228 MT wit 1166 kg/ha productivity (Anonymous, 2007)

Since the climatic conditions are congenial for the growth and spread of disease fungal flora a various crop in the region, therefore present area has been chosen for the present investigation.

MATERIAL AND METHODS

Different localities of Kota district were surveyed with the objective to know crop with fungal pathogens associated with fenugreek crop with special reference to occurrence, Identification and different disease intensities.

In the present investigation an intensive survey of fungal pathogens associated with Trigonella foenum- graecum L. was conducted at various localities viz. Morak, Bundi Road, Borkhed, Sogaria, Bhadana & Chandresal covering the major fenugreek growing areas of Tehsils of Kota district during Rabi season of 2010 and 2011 from (November to February) The Survey covered the full cropping period i.e. from nursery beds to harvest stage in the surveyed year.

Plant Sampling Technique and Storage of Samples

Each surveyed locality of fenugreek growing areas was divided into different zones of approximately the same area by growing. In each field five fenugreek fields were selected randomly. In each field ten sampling units of 3×3 feet area were laid. A total of 50 plant samples (healthy and Infected) were selected from 10 sampling units (5 plants from each unit), kept in sterilized plastic bags and brought to the laboratory. Plant parts of each sample spot were closely examined for the presence of the disease. On the basis of morphological symptoms healthy an infected plants were separated. Plant samples were washed thoroughly under tap water the plants were kept in refrigerator at 4ºC temperature for further processing.

Identification of Fungal disease on Fenugreek

For the identification of fungal pathogens causing various diseases on Trigonella foenum gracum the plant samples were collected from fenugreek fields of the above said localities of the study area. The fungal pathogens from the field collected fenugreek plant sample were identified on the basis of different morphological symptoms and using standard journals, manuals and keys. (Subramanium 1956 and Barnett 1972). Detailed morphological studies of both

host and fungal pathogens were made for the identification of different diseases, the plant samples were keenly observed using hand lenses and disease symptoms were assessed on the basis of shape, size and colour of the spots on leaf, stem and seed of the plant. Microscopic preparations i.e. stained leaf sections , mycellia and spores from infected plant parts were made and observed under the light microscope for the identification of different pathogenic fungi for this purpose infected plant parts were taken thoroughly washed and finely sectioned. The sectioned pieces were stained with cotton blue and mounted in lactophenol and then observed under microscope for further conformation of the pathogens on fenugreek.

Percent disease incidence

Disease rating and percent disease intensity (PDI) were calculated as per method suggested by James (1971) and Mayee and Datar (1986) with slight modification.

Disease Rating	Description
0	Free from disease
1	1 to 10 % of leaves area infected
2	10.1 to 25 % of leaves area infected
3	25.1 to 50 % of leaves area infected
4	50.1 to 75 % of leaves area infected
5	More than 75 % of leaves area infected

Percent Disease Intensity

$$= \frac{Sum\ of\ individual\ leaf\ disease\ rating}{Number\ of\ leaves\ examined \times Maximum\ disease\ rating} \times 100$$

Assessment and comparison of growth Parameters of Healthy and Infected Fenugreek plants

For this purpose susceptible RMT-1 cultivar of Fenugreek (Trigonella foenum graecum) was selected for the study. The surface sterilized seeds of above

cultivars were sown and seedlings were than transplanted in 30 cm earthen pots containing sterile soil with 3:1 ratio. Seeds were sown at a depth of about 1cm and 15 seedlings were kept in each pot. At 20 days after transplantation plants were artificially inoculated with conidia collected from sporulating diseased plants growing in neighbouring field in previous season

The plants were assessed and compared for growth parameter at maturity. Various growth parameters viz. shoot and root length (cm) shoot and root fresh and dry weight (g) number of leaves, leaf area and yield in terms of seed weight (gm) per plant were studied and compared in both healthy and diseased plant samples.

Detailed natural symptoms of different diseases were studied at laboratory and field .The observations were started from the month of sowing (November 2009) and continued up to harvesting. Fresh weight (Shoot and root) were taken immediately after bloter drying. For dry weight the fenugreek plant parts were dried in oven at 40^{o}c for seventy two hours. Number of leaves and pods were counted visually and noted. Leaf area was calculated using graph paper, plant length (Shoot & Root), leaf size pod length were measured with with the help of measuring scale and seeds were weighed by using electronic balance.

An inventory was also made during the study period to record the curative measures already used by the farmers at present for the management of different diseases in the study area by interacting and asking questions with the farmers at different time intervals of growing season of fenugreek crop.

OBSERVATIONS

During survey period a total no. of 800 fenugreek plant samples were collected from the sixteen different localities of Kota district, Rajasthan during growing period of fenugreek (Table 2, plates 2-5).

During survey it was observed that many fungal diseases were spread around the surveyed area causing considerable yield losses to the crop. Disease occurrence arising from natural infection in the fields varied in different fenugreek growing areas in the district. The prevalance and distribution of fenugreek diseases in the surveyed area are summarized in Table-2 data presented in the Table-2 revealed that mainly four fungal germs were found to be dominantely associated with fenugreek, viz. Powdery mildew (Erysiphae polygoni) Cercospora traversiana (leaf spot), Perenospora trigonallae (Downy mildew), Alternaria blight (Alternaria alternata) Plate-5.

Survey study also revealed that in the terminating period of crop symptoms of disease complex becomes visible. The crop was infected by various fungal pathogens causing great loss at variable extent. Variety RMT-1 has been found to the highly susceptible to above said fungal infections.

Symptoms of Disease (Plate-5,6)

Symptoms on the basis of which different diseases were identified were as follows:-

Leaf Spot disease (*Cercospora traversiana*)

In leaf spot of fenugreek the identifying symptoms were confined to leaves, stems, and petioles. Morphological observation revealed that symptoms caused by Cercospora first appeared on the older parts of the plant after 3-4 weeks of planting. Initially on the upper surface of the leaves plate areas were observed. After 5 days pale areas were turned into blacking brown leaf spot with almost circular and less diffused margins. Mycellium was intercellular, hyaline, branched. Conidiophores were 1-2 septate and geniculate, they emerged out by rupturing the

epidermis in the form of tufts. Conidia were obclavate or cylindrical 40 conidia collected from leaf. Conidia leaved scars after falling. Measurements of lesions were made in distilled water to which straight to curved, indistinctly multiseptate, base turennate, tip subacute to subobtuse. The Systematic position of fungus is as follows:-

A small amount of lactophenol & cotton blue was added conidia varied in length from 48.1 µm- 162.8 µm with the mean of 95.4 µm.

K - Fungi

P - Ascomycotina

C - Dothideomycetes

Q - Capnodials

F - Mycosphaerellacea

G - Cercospora

Species - traversiana

Powdery Mildew (*Erysiphae polygoni*) (Figure-1)

Disease characterized by the presence of white floury patches on green parts of the plants including both sides of leaves. The mycellium of the fungus was entirely superficially, sending haustoria into the epidermal cells. It was generally 4 - 6.5µm, in diameter, branched, septate forming a tangled mass. Conidiophores arise vertically from the upper surface of the mycelial growth. They were erect, stout, measuring 106.5 - 166.5 µm in length. Conidiophores bear several conidia in chain. Conidia were unicellular, hyaline and elliptical; barrel shaped to cylindrical and varies greatly in size measuring 29.0-45.0 µm (Majority 34.0 - 35.0µm). The number of pod per plant and number of seeds per pods were highly reduced.

The taxonomic position of the pathogen Erysiphae polygoni DC. is as follows :-

K - Fungi

P - Ascomycota

C - Leotiomycetes

O - Erysiphales

F - Erysiphaceae

G - Erysiphae

S - polygoni

Downy Mildew (*Perenospora trigonallae*) (Figure-2)

In Downy mildew disease, leaflets exhibited yellow patches on the upper surface and grayish growth on correspondingly lower surface were the main symptoms. Infection becomes severe at flowering and pod formation grayish cottony growth consisted conidiphores which were dichotomously branched and at the tip conidia were present. Oospores were formed towards maturity and were persistant oogonial wall thick epispore deep brown irregular with projections thick and typically spongy, circular pale whitish grey, smooth, oospores 30 - 35 µm in diameter. The disease evidently perennates through oospores. The taxonomic status of pathogens Perenospora trigonellae De bary is as follows:-

K - Chromaleolata

P - Heterokontophyta

C - Oomycetes

O - Perenosporales

G - Perenospora

S - trigonallae

Leaf blight *(Alternaria alternata*) (Figure-3)

Symptoms of disease first appeared on lower, shaded leaves and consisted of small round, yellow-brown or black spots, with concentric rings. Spots slowly enlarged and joined together upto 3 cm in diameter. Infected areas drop out and formed holes in the leaves with drop off. Mycellium was intercellular, conidiophores emerged through stomata conidia were breaked, muriform, dark brown in colour with both septa. The disease becomes more prevelant at maturity of crop. The Systematic position of pathogen is as follows:-

F - Fungi

P - Ascomycota

C - Dothichomycetes

O - Pleosporales

F - Pleosporaceae

G - Alternaria

S - alternata

The survey was conducted to assess the incidence of different fungal pathogens on the fenugreek crop at 16 localities of Kota district. The frequencies of fungal diseases in all the surveyed area highly varied from one area to another (Table -3).

During the study period the curative measures applied by the farmers for the management of different fungal pathogens were chemical as well as some cultural practices. Farmers variously used dry ash as soil drenching for pre treatment and in some localities sowing of plants with antagonastic effect against fungal pathogens such as garlic, ginger on the periphery of field were also observed (Plate-7).

Thus it is need of hour to use some innovative, cheap and ecofriendly control methods to increase the quality and quantity of crop.

RESULT AND DISCUSSION

The present investigation was aimed to find out the occurrence and distribution of fungal diseases of fenugreek in all the sixteen localities of Kota district during Rabi seasons of the years 2010 and 2011 (Table - 2 and 3). The observations from the Table -2, 3 revealed fungal diseases like leaf spot, powdery mildew, downy mildew, leaf blight are fairly present in the areas and in the prevalence order of leaf spot> powdery mildew> downy mildew> leaf blight. The occurrence of the above said diseases in the study area may be due to the favourable climatic conditions, amount of rainfall in the area moderate temperature, relative high humidity and suitable soil conditions. The results obtained in the present work coincided with the findings of many previous workers sus as Chattopadhyay and Maiti (1990), Saxena (1985), Mehra (2006), who suggested that above said climatic conditions were much favourable for the occurrence of fungal diseases of fenugreek crop. The above findings were also in accordance with a national level survey conducted in India (Spice Board India 2010).

Prevalence of fungal pathogens on fenugreek was maximum in the Jhalkhera, Gangaycha, Borkheda, Chechat, Tirat localities. The results indicated that incidence of diseases in different agro-climatic regions ranged from 63.33% (Ladpura Tehsil) maximum for leaf spot disease 24% and 33 % for Powdery mildew and Leaf blight respectively. Downy mildew incidence was maximum (23%) in Pipalda tehsil of district.

Common Occurence of all the pathogens in different localities may be due to susceptibility of the most commonly grown variety of fenugreek RMT-1 in the study area. As the Table-3 reveales the incidence and severity of various diseases of fenugreek, the leaf spot disease caused by Cercospora traversiana was found to be more prominent in almost all the studied localities and less studied in the region, hence further research proceedings were mainly emphasized and conducted on leaf spot disease of fenugreek crop to assess various incidences and control measures against the pathogen.

INTRODUCTION

Soil is a product of the influence of climate relief (elevation, orientation and slope of terrain, organisms and its parent materials (Original minerals) interacting over time. Soil acts as an engineering medium a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality a modifier of atmospheric composition and a medium for plant growth. It is the biologically active porous medium that has developed in the upper most layer of the earth's crust. Soil is one of the principal substrata of life on earth serving as a reservoir of water and nutrients. Soil is used in agriculture where it serves as the anchor and primary nutrient base for plants.

Soil quality is the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries to sustain plant and animal productivity maintain or enhance water and air quality support human health and habitation. The sustainable productivity of a soil mainly depends upon its ability to supply essential nutrients to the growing plants.

India "Land of Spices" is the world's largest producer, consumer and exporter of spices. Every spice is grown in different parts of the country depending upon the climate of the particular place. The different states of country, some of which are widely separated have different soils and climatic conditions but fenugreek is grown in almost all the states especially in well drained loamy soils with irrigation.

The soils of Rajasthan vary from desert sand to heavy clay with all intermediate stages like sandy loam, loam and clay loam. Loam and clay are more prevalent on the eastern, north eastern and south eastern areas of the state. These types of soils have more potential from the agricultural point of view. The present study area lies in the south eastern areas of Rajasthan.

Plant nutrients play specific and important role in growth and development of a plant. Adequate mineral fertilization is considered to be one of the most important

pre-requisites in this respect, fenugreek being a legume crop does not require much nitrogen for its growth. In intensive agriculture cultivation of high yielding varieties of crop has led to heavy withdrawn of nutrients from the soil during last few years and fertilizer use remained much below as compared to removal.

Most of the soils of Rajasthan state are low in organic matter and nitrogen content and medium in phosphorous supply. Plant diseases are a major limiting factor in agricultural production. Mineral nutrition has an important role in disease control. Essential plant nutrients influence the health of the plants and their susceptibility to disease. In other words plants suffering a nutrient stress will be more susceptible to disease, while adequate crop nutrition makes plants more tolerant or resistant to disease.

Different pathogens have different infection mechanism. Fungi penetrate surface cells by passing between or through the cell. Mineral nutrition also affects the production of antifungal compounds in plants. This nutrition of plants has a substantial impact on pre-disposition of plants to be attacked or affected by pest and disease. The implementation of nutrition to improve resistance and tolerance of plants lags behinds its potential.

Soil analysis is a set of various chemical processes that determine the amount of available plants nutrients in the soil, but also the chemical physical and biological soil properties important for plant nutrition or soil health, knowledge of soil in regard to its potential use, distribution and optimum use of land and with a view to understanding the effect of soil parameters on the fungal disease incidences on Trigonella foenum graecum L. a study was taken up in fields of Kota district, Rajasthan in India. The objectives of the study were to improve the knowledge of soils and soil mycoflora associated with fenugreek crop and identifying the soil nutrients influencing the different fungal infections in fenugreek crop.

REVIEW OF LITERATURE

Soil Nutrient status

Oliver and Barbar, 1966 recommended that three mechanisms root interception; mass flow and diffusion govern the rate of supply of nutrients from the soil to the plant root.

Rosengarten (1969) states that fenugreek is grown best well drained loams soil which was also supported by Piper, (1977).

Bunting (1972) reports that heavy and wet soils are unsuitable for cultivation of fenugreek and mentions as an optimum pH 8 - 8.5.

Rhykerd and Overdahl (1975) suggested that a pH value between 7.5 and 8.5 appears ideal for maximum fenugreek production; the optimum pH for a fenugreek crop may vary considerably depending upon soil characteristics such as texture, organic matter and line in the subsoil.

Petropolous, 1973 reported that fenugreek is also sensitive to mineral deficiencies particularly of boron (B), magnesium (Mg), manganese (Mn) and potassium (K).

Devrajan and Oblisami (1995) recommended efficient irrigation as it significantly increased the available N, P, K, Ca, Mg and micronutrients contents, soil pH, EC and organic matter content in soil.

Talwar and Patel (1962) found fenugreek plant to be rich in Ca which promotes the root development of fenugreek and is essential for nodulation and N fixation.

Moloard and Hardman, 1980 suggested that high Ca and high N increased the demand of B.

Kansal and Pahva (1979) reported that fenugreek plants were found to be rich in Mg.

Rathore and Manohar (1989) reported that in India early sowing in the fall (15 October) gave a higher yield than late sowing (14 November).

Nitrogen

Chibnall (1922 and 1924) concluded that Nitrogen plays pivot role in the synthesis of Chlorophyll as well as in amino acids which are considered as a building units of protein and thus growth of plant.

Rahman (1987) found that all growth characters viz. plant height, number of branches and leaf area plant^{-1} in fenugreek increased with increasing levels of N from 0 to 60 Kg ha^{-1}.

Pradhan et al (1995) reported that application of N at 40 Kg ha^{-1} in Soyabean crop significantly increased the plant growth parameters and yield over the control.

Baboo and Sharma (1995) reported significant increase in yield parameters and yield of fenugreek due to application of 25 Kg N ha^{-1} with rhizobium seed inoculation.

Choudhary (1999) recoreded higher number of pods plant^{-1} pod length, seed pod^{-1} , test weight and seed yield of fenugreek with application of 20 Kg ha^{-1} over control.

Salin (2002) conducted an experiment to show the effect of nitrogen on growth, biomass, succulence and chemical composition of rice plants and on the host insect interaction.

Mundy (2008) conducted an experiment to conclude the direct and indirect effect of nitrogen fertilization on bunch rot incidence in wine in field.

Phosphorus

Phosphorous are relatively immobile in soil and the depth of its penetration appears to be related to the rate of P application and to soil texture.

Sundra Rao (1973) suggested that areas where legumes are traditionally grown without phosphorous, shows poor nodulation and low yields.

Maliwal and Gupta (1988) observed that fenugreek fertilized with 60 Kg P_2O_5 ha^{-1} had significantly greater plant height and yield attributes in seed and straw.

Patel et al (1991) observed that fenugreek crop with 60 Kg P_2O_5 ha^{-1} gave higher biomass production of root – shoot with greater nodulation.

Bhati (1993), Banafar et al (1995), Detroja et al (1995) observed 40 – 60 Kg P_2O_5 ha^{-1} amount to improve all the growth parameters of fenugreek crop.

Dayanand et al (1998) worked on loamy soil of Jobner and noticed profound improvements in different growth parameters of fenugreek.

Reddy and Swami (2000), Ram and Verma (2000), Meena (2002) investigated significant increase in growth parameters of fenugreek under various application of P.

Tiwari (2002) stated that repeated application of Phosphorous fertilizer delayed the onset and lessens the severity of all disease of Barley.

Kiraly (1976) and Hiber (1980) noticed that application of phosphorous reduced Pythium root rot infection in cereal crops.

Potassium

Disease occurance may be encouraged by an imbalance between N and K. Good Pottasium fertility is associated with strong cell walls that enhance disease resistance and the ability of crop to maintain firm, healthy stalks.

Pottasium fertilization reduces disease infection, increase yields and enhances the quality of crops (Broadley et al, 2004).

Grewal and Singh (1980), Egilla et al 2005, Cakmak (2005) suggested that Pottasium deficiency generally decreases tolerance of plant to abiotic stress like frost, drought and salt.

Perronould (1990) reported on decrease in powdery mildew on barley due to K availability.

Prabhu et al (2007) suggested that application of potassium to deficient soils usually increases plant resistance to disease.

Cook et al (1993), Mam et al (2004) recorded that foliar application of potassium chloride controlled Blumeria graminis and Septoria tritici on wheat in field studies.

Kattiwell et al (2000) suggested the mechanism of potassium application on pathogen.

Mondal et al (2001) found a negative correlation between K content in Soyabean and Sesame with disease incidence and a positive correlation with their respective yield.

pH

K. Narisawa et al (2005) examined the effect of soil moisture and pH on effectiveness of fungal endophyte Heteroconium chaetospira of clubroot.

Davies et al (1993) revealed that soil pH affects pathogen development in the soil since soil is a natural reserviour of innoculum.

Waterer D. 2002 evaluated the influence of soil pH yields and gradeout due to tuber damage caused by common scab (Streptomyces scrabies) over multiple cropping seasons for potato.

Electrical Conductivity

Carvalho et al (2009) studied the relation between water content, organizational level of seed cellular membranes and quality of leachates as the theoretic base of Electric conductivity.

Effects of seed pathogens on EC results were observed by Panizzi et al.

Colete et al 2004 studied the electrical conductivity and Soyabean seedling emergence.

Mastura et al (2011) considered soil apparent electric conductivity (EC) as one of the most common and frequently used measurement to determine field- soil variability, especially for precision farming.

MATERIALS AND METHODS

The present study was conducted to determine the physiochemical status of soil and its influence on fungal diseases on fenugreek in growing areas of Kota district of Rajasthan. The study area includes sixteen localities viz. Chandresal, Jhalkhera, Gangaycha, Borabas, Mandana, Morak, Nayagaon (Tehsil-Ladpura), Borkheda, Sogaria, Bhadana (Tehsil-Digod) Chechat, Nemana, Panda (Tehsil-Ramganjmandi), Arjunpura, Tirat (Tehsil-Pipalda) and Sankhera (Tehsil-Sangod) covering the major fenugreek growing areas of the tehsils of Kota district.For soil analysis, soil samples were collected from fenugreek fields with the help of soil corer to a depth of 0-15 cm. Four to six pits were dug for each sample .From each pit sample was collected at a depth 0-31cm and gathered together to form a composite sample.These samples were dried, grounded with wooden motto and passed through 2 mm sieve. After then the samples were packed in polythene bags for laboratory investigations.The soil samples were analyzed by standard analytical methods for soil pH, Electrical conductivity (EC), and availability of total Nitrogen (N), Phosphorous (P) and available Potassium (K) contents in soil samples.Physiochemical analysis of soil was done in laboratory by following standard methods.

Soil pH (Mc Lean 1982) –

Soil pH is a measure of hydronium ion (H_30^+ or H^+) activity in a soil suspension. This property influences many aspects of crop production soil chemistry, activity and diversity of microbial population and activity of certain pathogens.

Procedure

Soil pH was measured potentiometrically in slurry using an electronic pH meter. This was calibrated. 5 gram of soil sample was mixed with 5 ml distilled water and stirred vigorously for 5 seconds and let stood for 10 minutes. Electrodes were placed in mixture (slurry) and pH was read immediately.

Soil Conductivity – (Richards 1969)

Electrical conductivity (EC) is a measure of ionic transport in a solution between cathode and anode. Electrical conductivity is a normally considered to be a measurement of dissolved salts in a solution. EC of soil is based on the measurement of EC in soil solution extract from a saturated soil paste.

Procedure

5 gram soil sample was taken and to this 5 ml distilled water was added. The mixture was stirred and saturated. At saturated the soil paste glisted as it reflected light. After this the solution was allowed to stand for at least one hour and saturation level was rechecked. Saturated paste was transferred to filter funnel. Extract thus obtained, was used to measure conductivity using calibrated conductivity meter in microsiemens/cm (µ S/cm) at 25 ºC.

Organic Carbon – (Walkey-Black Method 1934)

The Carbon present in the soil is oxidized to carbon dioxide by heating the soil to at least 900ºC in a flow of oxygen, containing gas that is a free from CO_2. The amount of CO_2 released is then measured by titrimetry.

Reagents-

1. 1 N $K_2Cr_2O_7$: 49.04 gm of potassium dichromate per litre of solution.
2. 0.5 N ferrous ammonium sulphate: 198 gm salt per litre of solution.
3. Diphenylamine indicator: 0.5 gm of diphenylamine in a miture of 20ml water and 100 ml of concentrated sulphuric acid.
4. Concentrated Sulphuric acid.
5. Orthophoshoric Sulphuric Acid.
6. Sodium Fluoride (Naf).

Procedure

Soil sample (1 gm) was taken in a 500 ml conical flask followed by the addition of 10 ml of 1 N $K_2Cr_2O_7$. The flasks were stirred for mixing the soil and reagent.

Added 20 ml of H_2SO_4 and the flask was allowed to stand undisturbed for 30 minutes after which 200ml of distilled water was added.

To the mixture. 10 ml of Orthophosphoric acid, 0.5gm of Sodium Fluoride (Naf) and 1 ml diphenylamine indicator was added.

The content was ultimately titrated with freshly prepared 0.5 ml ferrous ammonium sulphate till end point is observed from from blue-violet to green. A blank was also run without soil sample.

$$\text{Calculation – OC (\%)} = \frac{10\ (B-T)\times 0.003\times 100}{B\times \text{Wt.of soil (gm)}}$$

Where, B is the volume of ferrous ammonium sulphate solution required for soil sample titration.

Total Nitrogen

Total nitrogen was estimated as per the Kjeldahl method given by Piper (1960).

Reagents

1. Concentrated H_2SO_4
2. 0.02 N H_2SO_4
3. Sulphuric – Salicylic acid: 1gm salicylic acid mixed with 30 ml sulphuric acid.
4. Sodium thiosulphate
5. 4% boric acid

6. Mixed indicator 0.066gm of methyl red and 0.099gm of bromocresol green dissolved in 100ml of ethyl alcohol.

7. 50% NaOH

8. Digestion mixture : 10 gm HgO, 5gm $CuSO_4$ and 100 gm K_2SO_4 (2 : 1 : 20)

Procedure

Soil sample of 5gm was mixed thoroughly with sulphuric salicylic acid followed by 5gm of sodium thiosulphate. Heating was carried out for 5 minutes followed by cooling and addition of 10 gm digestion mixture. The contents were mixed well in Kjeldahl flask

The flask was kept in the digestion chamber at 100ºC for two hours.

The colour change was monitored from dark brown to greenish white after which the contents were cooled and 300 ml of distil water was added.

20 ml of the digested sample, 15-20 ml NaOH and glass beds were added to the distillation flasks through the open end of the condenser attachment and stopered. The distillate was collected through a receiver tube in a beaker containing 15 ml boric acid and 2 drops of mixed indicator till the point colour changes from pink to green.

The distillate was titrated against 0.02N H_2SO_4 untill the end point colour changed from green to pink.

Calculation - Total N% = $$\frac{(T-B) \times \text{Normality of } H2SO4 \times 1.4 \times 300}{Weight\ of\ Sample}$$

Where T, is the titre value for sample and B is for blank.

Phosphorus

Reagents

1. 0.5M $NaHCO_3$ extracting solution: 84 gram of sodium bicarbonate ($NaHCO_3$) was added in distilled water and the volume was made up to 2L. The pH was adjusted to 8.5 with 1M or 1N NaOH (Sodium hydroxide).

2. Reagent A: 12.0g of ammonium molybdate in 250ml distilled water and 0.2908 gm of antimony potassium tartarate in 100ml distilled water was added to 1000ml of 2.5 M H_2SO_4, mixed thoroughly and volume made up to 2L with distilled water.

3. Reagent B (Freshly prepared): 1.058gm of ascorbic acid in 200ml of reagent A and mixed.

4. Stock standard P solution (50ppm): 0.2917gm KH_2PO_4 dissolved in water to a final volume of 1L.

5. Working standard P solution (1 ppm): 20ml of (50ppm P) solution diluted to 1L.

Procedure

Soil sample (2.5gram) was placed in a 100ml Erlenmeyer flask followed by the addition of 50ml extracting solution.

The solution was kept on a shaker for 30 minutes and filtered through wattman No. 42 filter paper.

10 ml aliquot of the filtrate was transferred to a 100ml beaker followed by addition of 1ml of 2.5 M H_2SO_4. 15.% ml of distilled water, 18 ml of Reagent B and another 15.5 ml of distilled water.

A blank was prepared as above. For the standard curve: 0, 2, 5, 10, 15 and 20ml of standard solution was placed in 50ml volumetric flasks separately. 1.0 ml of

extracting solution, 1.0ml of 1.5M H_2SO_4, 8ml Reagent B was added and the final volume was made up to 50 ml. The P concentration of these solutions was 0.04, 0.1, 0.2, 0.3 and 0.4 ppm respectively. After 10 minutes, the P concentration was read at 882 nm.

Calculation: P in sample (mg kg^{-1}) =

P in extract (mg^{1-1}) × 20 (the standard sample to solution ratio)

Potassium (Toth and Prince 1949)

Potassium present in the soil is calculated with the help of flame photometer.

Reagent: 25 ml of ammonium acetate

Procedure

5 gram of soil sample was mixed with 25ml of ammonium acetate. The mixture was shaken for 5 minutes and then filtered. The filtrate was used for estimation of potassium. A blank was prepared with ammonium acetate. O D of filtrate was observed at 786 nm.

Calculation

K (kg/ha) = 10 A

Where A = content of K in a sample as read from the standard curve

OBSERVATIONS

In the present investigation soil samples collected and analyzed from 16 different localities viz. Chandresal, Jhalkhera, Gangaycha, Borabas, Mandana, Morak, Nayagaon (Tehsil- Ladpura), Borkheda, Sogariam, Bhadana (Tehsil- Digod), Chechat, Nemana, Panda (Tehsil- Ramganjmandi), Arjunpura, Tirat (Tehsil- Pipalda), Sankhera (Tehsil- Sangod). Covering the major fenugreek growing areas of the Tehsils of Kota district, during Rabi season of 2010-11 (November to March). Results are presented in Table-5.

Total nitrogen content (%) of soil samples collected from fenugreek fields varied from 0.36% to 0.88%. Most of the samples ranged from 0.05 - 0.1%, which results in rich content of nitrogen in soil samples.

Available Phosphorous (P) Kg/ha content of soil samples of fenugreek fields of Kota district, ranged from 0.26% Kg/ha to 0.66 % Kg/ha.

Available Pottasium (K) content of soil samples varied from 349 Kg/ha to 492 Kg/ha. The maximum value was recorded from Chandresal locality and minimum value was recorded from Sogariya locality.

The pH of the soil samples observed from 16 surveyed localities varied from 7.1 to 8.6. The maximum pH recorded from Nemana locality and minimum pH recorded from Sankhera locality.

Maximum and minimum value of organic carbon (%) was recorded as 0.85 % to 0.34 % from Borabas and Sankhera locality respectively.

The highest PDI% was recorded from Jhalkhera and lowest from Bhadana was 50.67% and 13.67% respectively.

RESULT AND DISCUSSION

Soil types found in Kota district were mainly Deep black clayey (43.15 %), deep brown clayey (22.37%) and deep brown loamy (12.41%) from the above mentioned soil types sandy loam soils with good drainage are the most suitable for fenugreek crop in the area.

To understand the soil quality and nutrient status of soil and also correlation with leaf spot disease in the area physiochemical analysis of soil samples from fenugreek fields of 16 localities in Kota district were examined. Different physiochemical soil properties like total N, available P and K, pH, EC and Organic carbon were examined.

Nitrogen

The Nitrogen content of healthy fenugreek plants is at least 2.5 percent and it is a basic constituent of the substances that are essential for protein synthesis. It is a constituent of chlorophyll and cytochrome enzymes, many vitamins and alkaloids.

Soil samples from fenugreek fields resulted in high range of nitrogen content. The total nitrogen content varied from 0.036% to 0.087% which was from Borkheda and Bhadana locality respectively.

The nitrogen disease hypothesis states that plant growth at high nitrogen (N) availability may result in increased plant susceptibility to pathogen as a result of increased foliar nitrogen concentrations (Mitchell et al 2003).

Dordas (2003) suggests that high N availability may favour obligate parasites such as bitrophic fungal plant pathogens while reducing disease severity of facultative parasites.

Thomas (1943) showed that increasing rate of N fertilizer increased the severity of disease in carrot.

Soteros 1979, Vintal et al (1999) suggested that a high fertility treatment causes a delay in Alternaria leaf blight disease development in leaves of carrot. Ali and Roy (1981), Rotem (1994) also accompanied the similar results.

Yon et al (2008) revealed that nitrogen and light sources are two of the most important environmental cues that affect Cercosporin biosynthesis.

Phosphorus

The phosphorous content of a fenugreek plant is usually in the range of around 0.25% it is participates in many vital life process as the most important compound containing P such as nucleic acids, phospholipids, coenzymes etc. Alkaline and Calcerious soils favour the solubility of P.

Available phosphorous content in soil samples were ranged from 0.26Kg/ha. The soil samples studied were in common alkaline which binds the P with other earthy ions. Detoroja et al (1996) investigated an adequate supply of N and P and Organic manures to fenugreek provide an efficient source to sink relationship leading to higher productivity. Increase availability of phosphorous owing to its application in soil improves nutrient availability enhances root nodulation and creates congenial environment for plant rhizophere to increase physiological growth parameters (Mehta et al 2012 and Peiman Zandi et al 2011). An adequate phosphorous supply is important for crop growth and in turn may help to reduce disease (S. Parthasarthy).

Potassium

Potassium is an essential for plants for the performance of multiple plant enzymes functions, metabolic pattern, synthesis of proteins, starches, cellulose. Adequate potassium increase phenol concentrations which play a critical role in plant resistance (Sarwar, 2012), Velazhahan, R. Ramabadran (1992) Potassium affects the plant shape, size, color and measurements attributes to healthy produce.

It is mean that potassium increases epidermal cell wall thickness or disease escape as a result of vigorous crop growth. Kimbrough (1971) Meille (2004) investigated the disease in leaf area of fenugreek due to insufficient level of potassium.

Foliar applied potassium chloride had controlled Blumeria gramanis and suptoria tritici on wheat in field studies due to Osmotic effects on the fungal pathogens disrupting pathogen development. Prabhu et al suggested that K application decreased the incidence of disease mostly. The use of K best increases the yield and the nutrient qualities with special effects on the soil content. (Salgus 1939). Rhykerd and Overdahl (1975) suggested that a concentration of 2 percent or higher is necessary for maximum yield and longetivity of fenugreek crop. In the present investigation the amount of potassium in soil samples was found to range between 349 Kg/ha to 492 Kg/ha. In the locality Jhalkhera where percent disease incidence of leaf spot was highest. The soil paottasium value was 389 Kg/ha low as compared to other localities.

pH

Soil pH is a measure of acidity and alkality in soils, it is considered a master variable in soils as it controls many chemical processes. The examination of plant growth alterations in reponse to pH variation is important in terms of tagging removable sources of restrained crop production and prospectively to access tolerance to acid soils (Waisel et al 2002, Fageria et al 2005). In the present study soil pH was found to be neutral to alkaline in the range of 7.1 to 8.6.

The pH of 7.1 was recorded from Sankhera locality having PDI of 35.14% while pH of value 8.6 was resulted from Nemana with 39.21 PDI% from the above investigations it may be suggested that increase in value of pH shows decrease in PDI in the studied localities. Several researchers studied the soil pH and relating to the establishment of fungal pathogens and their effects in crop plants. (Baath 2003, Rajapaksha 2000, Sactre 2003).

Bernett et al , Norma et al 1981, Marmoet et al 1982, Takamaya et al 1983 revealed that carbon, nitrogen sources, light and pH are the most factors affecting the biosynthesis in species of Cercospora.

Electric Conductivity

It is a measurement that correlates with soil properties that affect crop productivity, including soil texture, drainage conditions, and organic matter level, and salinity and subsoil characteristics. Present investigation represents the value of EC ranged from 220 µs/cm to 440µs/cm in studied localities. The minimum value was recorded from Jhalkhera having PDI of 50.67% and maximum value of 440Us/cm was recorded from Bhadana with PDI of 13.67%, salinity affects plants and microbes via two primary mechanisms osmotic effect and specific ion effects Oren (1999), Chabra (1996).

Organic Carbon

Organic matter affects both the chemical and physical properties of the soil and its overall health. The value of OC ranged from 0.34 to 0.85% Krishnan et al (2009) observed the quantification of organic carbon with respect to soil quality and productivity.

Plants supplied with all necessary nutrients in a balanced manner are more resistant to pests and diseases. The entire essential nutrient can affect disease severity (Huber and Graham 1999). Hence a study on soil nutrition balance is essential to guage the appropriate managements of plant disease especially Cercospora leaf spot in the area. Although disease resistance in genetically controlled, it is considerably influenced by environmental factors that can be easily controlled in agricultural systems, the effects of which can be substantial.

INTRODUCTION

Weeds have surely been with us since the advent of settled agriculture some 10,000 years ago. Weeds are usually defined in the negative; they are plants that are not wanted. Here, any plants that might suppress the corn or otherwise limit its productivity are unwanted and therefore weeds. Information on weeds responses to soil fertility levels is needed to aid development of fertilizer management strategies as components of integrated weed management programs.

The importance characteristics, positive and negative impacts and future role of weeds as an integral part of the natural and agroecosystem are evaluated and discussed. Interference between plants in nature and the importance of differentiating between competition and allelopathy are interpreted weed species with inhibitory action against cultivated crops other weed species and plant pathogens as well as self inhibitory (autopathic) species are reviewed.

The agrotechnical factors which determine plants growth and yielding involve plant protection against weeds and pathogen control. After sowing fenugreek, seeds emerge quickly but plants grow relatively slowly in comparison with many other legumes as result of which fenugreek plants do not complete effectively with the spring weeds. Therefore, mechanical and/or chemical weed control is crucial.

The weed problem in fenugreek is very serious due to its slow growing habits particularly during initial stages; it is highly infested with weeds which drastically reduce the seed yield. Mali and Suvalka (1987) reported that weeds were found hurdle in fenugreek production, mechanical removal of weeds is laborious time consuming and costly.

Weeds exploit disturbed sites, these are areas that have reduced competition exposed mineral soils and high light and nutrient levels. If weeds are present before the disturbance or weed seeds arrive after disturbance weeds will commonly increase on disturbed areas. It is difficult to work with weeds of

anytime without some admiration, however gruding for their persistence and irrepressibility. In spite of all the attacks made on then by traditional and modern methods, They not only survive but remain ready to invade cultivated land if defences are only temporary lowered, there influence on agriculture system is fast. Though fenugreek is enormously cultivated in Kota district of Rajasthan, no concern attempt has so far been made to record the occurrence and distribution of weeds in fenugreek fields of Kota district. Studies on weeds of the fenugreek fields of this part of Rajasthan still remain largely unexplored. So few attempts have been carried out to explore there diversity in Kota district of Rajasthan.

Therefore, a systematic study on the distribution pattern of weeds associated with fenugreek fields was taken to prepare a detailed account of weeds accompanying fenugreek fields in Kota district of Rajasthan.

REVIEW OF LITERATURE

Mali and Suwalka, (1987) investigated that weeds in fenugreek leads to serve as high as 91.4 percent.

Inderjit –Dakshini, (1991), Leela (1981) obtained allelopathic effect of Impernata cylindrica L. and Argemone maxicana L. which have the potential to reduce fenugreek germination and growth.

Jat and Shaktawat, (2001) Lal et al (2003) identified that sowing period and biofertilizer besides contributing in enhancing yields may also leads to invasion of weed flora which ultimately affects fenugreek yields.

Gupta (2001) reported that weeds usually absorb mineral nutrients faster than many of crop plants and accumulate than in their tissues relatively in large amount.

Verma et al (2002) reported maximum seed yield of fenugreek under weed free treatment which was 40.5 percent higher than that of weekly check.

Singh and Singh (1989) revealed that unrestricted weed growth reduced the seed and oil yields of Bulgarian coriander by 40.3 and 37.0 % respectively.

Jadhao et al (1998) reported that the most common weeds in the experimental fields of fenugreek crop (in order to frequency) were Parthenium hysterophorous, Anagallis arvensis, Cynodondactylon, Physalis minima and Dinebra retroflexa.

Singh et al (2002) obtained the highest seed and biological yield (14.62 q/ha) of coriander was obtained in the weed free treatment.

Petropolous (2002) suggested that perennial species like Convolvulus arvensis and Cynodon dactylon can create a very strong competition with fenugreek.

Nandekar et al (2004) studied the weed flora of the experimental field of fenugreek in order of dominance viz. Parthenium hysterophorous (40%), Chenopodium album (22%), Angalis arvensis (20%), Cynodom dactylon (7.5%),

Dinebra retroflora (4.2%), Physalis minima (3.5%) and Sanchus oleraceus (0.02%).

Patel et al (2005) reported that the major weeds in the fenugreek field were Chenopodium album, Chenopodium murale, Melilotus indica, Cyperus rotundas and Asphodelus tenuifolius.

Kamboj et al (2005) suggested that the herbicides significantly reduced weed density and dry matter.

MATERIALS AND METHODS

The study area encompassed at total 16 sites viz. (1) Chandresal (2) Jhalkhera (3) Gangaycha (4) Borabas (5) Mandana (6) Morak (7) Nayagaon (8) Borkheda (9) Sogaria (10) Bhadana (11) Chechat (12) Nemana (13) Panda (14) Arjunpura (15) Tirat (16) Sankhera covering the major Fenugreek growing areas of Tehsils of kota district.

In fenugreek growing region of Kota district Rajasthan India. Temperature and soil samples were recorded between 16 º To 30 º pH ranged from 6.8 - 7.6 (Collection, Preservation and Identification of Samples.)

Samples were collected from sixteen different sites of Kota district. The samplings were done from the quadrates of fenugreek fields. The taxonomic enumeration was performed with fresh materials in the laboratory. The plant samples were preserved by making herbarium sheet first sterilizing them with 2% $Hgcl_2$. Detail studies were made by examining the presence of soil mycoflora of plants and fungal infections under a compound microscope with Nikon E200 photo micrographic attachment. The fungal infections were identified based on their morphological feature and anatomical structure following the monograph.

OBSERVATION

An extensive study was made to find out the occurrence and abundance of fungal pathogens infecting trigonella on different weeds associated with the crop in different study sites of Kota district Rajasthan India. Table -6 indicates the presence of diverse weed genera in sixteen different fenugreek fields in kota district viz (1) Chandresal (2) Jhalkhera (3) Gangaycha (4) Borabas (5) Mandana (6) Morak (7) Nayagaon (8) Borkheda (9) Sogaria (10) Bhadana (11) Chechat (12) Nemana (13) Panda (14) Arjunpura (15) Tirat (16) Sankhera. Maximum occurance of weed genera was recorded from Borkheda (8) Sogaria (9) with Melilotus and chenopodium species specially. Infection of Cercospora sps. was abundant on Melilotus album as well as Tagetus sps. and Spinacia oleracea.

Chenopodium sps. shows symptoms of Blight caused Alternaria species.

List of weeds associated with fenugreek crop of 16 different study sites of Kota district with fungal infections reported on them (Plate - 8,9).

(1). *Spergula arvensis*

Bandhania

Family: Caryophyllaceae

It is annual herb spreading by seed. It is mostly growing between, around and through other weeds. It does not show any fungal infections with fenugreek crop except nutrition uptake. Sowing date and microbial inoculation had no significant influence on density of Spergula arvensis.

(2). *Melilotus indica* (L.)

Sevji methi

Family: Fabaceae

It is an annual or biennial herb with yellow flowers. It extensively grows with fenugreek crop and affects also white powdery mass was observed on the leaves and petiole of the weed which indicates that it plays a role of an alternative host

for powdery mildew. At maturation of the weed as well as the crop symptoms of blight in matured leaves was examined in laboratory. Sowing date of the crop shows significant influence on weed interference in nutritional uptake.

(3). *Anagallis arvensis* (L.)

Krishnaneel

Family: Primilaceae

It is low growing annual plant also considered as a weed as an indicator of light soils which spreads well in clayey soils. In experimental sites around Kota district weed plant shows blight symptoms at later stages of the crop development matured leaves shows dark brown colored patches which was later examined as Alternaria sps.

(4). *Chenopodium album* (L.)

Bathua

Family: Amaranthaceae

It is a fast growing annual weedy plant one of the most robust and competitive weed, capable of producing crop looses with association of the weed plant and fenugreek crop. Some symptoms of Ascochyta blight were observed at the sexual stage of weed. Similar symptoms were present on the fenugreek plants with brownish mass on lower leaves.

(5). *Chenopodium murale* (L.)

Khartua

Family: Amaranthaceae

It is an annual herb and weed of fields and roadsides. At the later stages of the crop the plant showed white powdery appearance on the lower surface of leaves and stems. This fungal infection was observed mainly on the weed plant located at the borders of the fields.

(6). *Convolnulus arvensis* (L.)

Hiran Khuri

Family: Convolvulaceae

It is a prostrate or climbing herbaceous perennial weed. Leaves of plant were infected by Rhizoctomia sps. with whitish patches. Roots also show the disease symptoms. Blight symptoms were also present on the lower leaves of the weedy plant.

(7). *Cynodon dactylon* (L.)

Doob

Family: Poaceae

It is fast growing grassy weed associated with almost all crops. Initial stages of the crop when irrigated weed shows water soaked, stunted, withered symptoms. As the temperature fall weed is characterized by a orange/brown colored patches with borders. Grassy weed also shows symptoms of Rhizoctonia by becoming light green in color, then yellow at last degenerate into brown discolored area.

Above mentioned weeds with symptoms of some fungal infestation on them also includes some other crop plants which also accompanies the fenugreek crop in some of the studied sites.

Tagetus indica (L.)

When fenugreek crop was grown in small fields as a vegetable crop with other vegetables and flowering plants such as Tagetus sps and Crysanthamum (Plate-9). Some different fungal infections were also observed from both crop plant and the other plant. Tagetus sps. which grows with fenugreek or on the borders of the field shows dark black, olivaceous leaf spot sometimes spot on stem and inflorescence was observed which was cultured on PDA and identified as Cercospora tageticola Ell. & Ev.

RESULTS AND DISCUSSION

Understanding, abundance and distribution of weed species within the landscape of an agrosystem is an important goal for weed science. Abundance is a measure of the number or frequency of individuals in an area. Distribution is a measure of geographical range of weed species.

Although fenugreek, as a crop grows and reaches maturity in a relatively short period (4-5 months), it is initially slow growing and vulnerable to weed interference particularly during the seed germination and seedling establishment phases. Weeds interfere with them for available nutrients and moisture and restricting available space (Zimdhal, 1980). Weed competition in fenugreek, therefore can be very strong if there is a heavy infestation by early germination annuals or in the presence of highly competitive and fast growing perennials.

Similar results were obtained in field trials in India for the critical crop- weed competition period over the first 30 days after sowing of fenugreek (Tripathi and Govindra, 1993).

The present study concludes that mostly the weed flora associated with the crop of Kota district plays an alternative hosts for different mycoflora which also causes infestation in fenugreek.

Weed flora like chenopodium sps., Melilotus sps., Spergula arvensis, Anagallis arvensis, Convolvulus sps. and Cynodon dactylon were reported from 16 different study sites with more or less frequency. There effect was clearly noticed in the form of fungal infection nutritional uptake and crop yield loss. Growth parameters of crop were highly influenced with the association of weed plants.

The dominant weeds Spergula arvensis, Melilotus indica and Chenopodium sps. together constituted 56.09 percent of total weed density at harvest. Higher density of Spergula and Cynodon might be due to the fact of their quick germination vegetative propagation and survival capacity.

Soil borne mycoflora affects all the flora more or less Similarly in the above investigation fungal pathogens like Rhizoctonia sps., Ashochyta sps., Cercospora sps., Alternaria sps. were reported on different weeds morphologically.

The percent study was undertaken to evaluate the diversity of weed flora in fenugreek fields of Kota district during growing season of Rabi. Weeds are one of the major components of the nutritional loss in the fenugreek fields as well as alternative primary host for different mycoflora.

Finally, it might be concluded that the documentation on weeds may enhance the understanding of the nutrient status of the field and might be applied for sustainable agricultural practices by reducing fungal infections and chemical practices to avoid the fungal pathogens of crop plants that might reduces the chances of further incidence. (Agavin et al, 2007)

INTRODUCTION

Plant diseases caused by fungi are major potential threats to the yield in both organic and conventional crop production. The plant pathogenic fungi are one of the major causes of crop loses. The infection processes they exhibit are typified by infected host plant cells, remaining alive for several days (Sarah et al, 2001).

To colonize plants, fungal microorganisms have evolved strategies to invade plant tissue, to optimize growth in the plant and to propagate. A fungus capable of activity penetrating plants and of producing a toxin that affects a fundamental biochemical process has the potential to be a universal phytopathogen successful pathogens in turn need to neutralize the plant resistance strategy and so on.

Successful infection of a host plant by a pathogen involve the movement of the pathogen towards the host, attachment of the pathogen to the plant surface, penetration of host by the pathogen, and the proliferation of the pathogen inside the host immediately following entrance (Huang, 1986) knowledge of host - parasite relationship between economic plants and micro-organisms is fundamental to plant pathology (Barnett and Binder, 1973). Host parasite interaction due to infection is helpful to understand the shift in host metabolism.

Fenugreek crop is infested by several fungal pathogens which limits the production and quality of the crop. Diseases are characterized by definite changes in host plant morphology and anatomy. Though fenugreek crop shows several disease symptoms but leaf spot, powdery mildew and downy mildew causes visual and remarkable changes in both morphology and anatomy of the plants. The histopathological studies may contribute and elucidate the relationship between the host plant and different fungal pathogens.

Pathological studies were conducted by morphological and anatomical methodology to know the effect and severity of different fungal disease on fenugreek plant. The objective of the present work was to investigate the morphological and anatomical changes pursuing in the fenugreek plant as a result

of infection by various fungal pathogens. The present study was done to know the process of infection, invasion and histopathological changes brought about by various pathogens in the host cultivar.

REVIEW OF LITERATURE

Pederson, (1989) found that oospore of Perenospora manshhurica developed in infected soyabean leaf tissue simultaneously with the onset of chlorosis, they developed easier.

Nelson et al (1970) reviewed the general phenomenon that occur in many of the plants infected with wilt pathogen and stated that the knowledge of the host tissues affected by a plant pathogen can be useful in determining the amount of injury of host tissue.

Keen, (1986) reviewed the strategies pursued by fungal pathogens in this process vary in different types of interactions with their hosts.

Huang, (1986) while studying bacterial penetration into the plants observed that successful infection of a host plant by a pathogen involved movement of pathogen towards the host, attachment of the pathogen to a plant surface, penetration of the host by pathogen and the proliferation of the pathogen inside the host immediately following entrance.

Parker et al (1988) investigated a specific heptaglucan elicitor from mycelia walls of the fungus active in soyabeen and other leguminous species but not in parsley.

Lakra, (1989) observed oospore formation on fenugreek leaves infected with Perenospora trigonellae.

Vijay kumar, (1990) investigated the histological changes in groundnut leaves due to infection by Cercospora arachidicola and observed hypertrophy in leaf.

Kunkliker and Padaganus, (1991) reported first symptoms of powdery mildew on green gram.

Kolattukudy et al (1995) concluded that cutinase may also be involved in penetration processes.

Chie Kawamura et al (1997) stated that the appresorium pigmentation in M. grisea was known to be essential for host penetration.

Sarah et al (1999) studied the various stages of the infection processes of Colletotricum species.

Prakash and Saharan, (2000) studied the conidial germination of Erysiphe polygoni infecting fenugreek leaves.

Hiran and Upper, (2000) studied that entry into plant tissue was likely a critical first in the establishment of foliar infection. And to gain access to the intercellular spaces and internal leaf tissue, pathogens must cross the surface cuticle and epidermis.

Madhavi et al (2005) studied the histological characteristics in six wild Helianthus species and in cultivated sunflower following infection with Alternaria helianthi.

Basu et al (2006) reported vigorous growth of Erysiphe polygoni under moist and warm growing conditions.

Sunkad and Kulkarni, (2008) studied the structural changes due to infection of pathogen under various histopathological parameters in six groundnut varieties infected by Puccinia arachnids.

Usha Suyal and Sharma, (2011) studied the process of infection, invasion and histopathological changes in chickpea plant cv H-208 infected by grey mould pathogen Botrytis cinerea.

Vierbhadraswami et al (2011) studied the host parasite interaction between Cephalosporium acermonium and maize (Zea Mays) seedling in Belgam district Karnataka. The microscopic observation revealed the presence of pathogen mycelium at both inter and intra cellular root tissue of host plant.

Mohamed et al (2012) revealed that squash leaves were greatly affected as a result of infection by squash leaf curled virus (Sq LCV) and reported decrease in leaf blade thickness, and in both spongy and palisade tissues.

The present study is based on the morphological and histopathological changes in fenugreek plant brought about by different pathogens with special concern to Cercospora sps. This study might help in the better understanding of fenugreek pathology due to various diseases, especially leaf spot disease.

MATERIALS AND METHODS

Identification, Isolotion, Purification and Culture of *Cercospora travensiana* and its pathogenicity affecting fenugreek.

The leaf spot infected Trigonella foenum graecum L. plants were collected from the fields of different localities. Both the healthy and infected plants were carried to the laboratory for investigation of morphological and histopathological studies. The infected leaves of collected plants were taken and cut into small pieces (0.5-1.0 cm.) and the surface sterilized with 2% sodium hypochlorite for 2 minutes. These cut pieces were then washed with sterilized water and placed on different carrot dextrose agar slants supplemented with fenugreek powder media plates under sterilized condition. To isolate the causal agent, these plates were incubated at 20 ± 2^{o}c for 15 days for the growth and development of pathogen. Culturing was repeated till pure culture was obtained. The identification of the pathogen was confirmed by observing the isolates on the culture media plates under the microscope. Identification was carried out with the help of taxonomic keys, manuals and available literatures (Subramanium, 1956 and Barnett, 1972). The slants and petri plates of purified culures were stored at 5^{o}c for further use.

The different media used in the studies were follows:

Non-synthetic media

1. Carrot dextrose agar
2. Potato dextrose agar
3. Oat meal agar

Synthetic media

1. Asthana and Hawker's agar
2. Czapeck's (Dox) agar
3. Rechards's agar
4. Sabouraud's agar

The composition and preparation of the above mentioned synthetic and nonsynthetic media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawsworth et al. (1983). The compositions of media are given below.

1. Asthana and Hawker's agar

Glucose	5.00 g
Potassium nitrate	3.50 g
Potassium dihydrogen Phosphate	1.70 g
Magnesium sulphate	0.75 g
Agar-agar	20.00 g
Distilled water	1000 ml (volume to make up)

Agar –agar was melted in 500 ml of distilled water. All the ingredients were dissoloved in 500 ml of distilled water. Both the solutions were mixed thoroughly are sterilized at 1.1 kg cm^{-2} pressure for 20 minutes and preserved for further use.

2. Carrot dextrose agar

Peeled carrot	200 g
Dextrose	20 g
Agar-agar	20 g
Distilled water	1000 ml (volume to make up)

The 200 g of peeled carrot were cut into small pieces and boiled in distilled water and the extract was cooled by filtering through muslin cloth. Dextrose 20 g and agar 20 g of each were dissolved in carrot extract and the final volume was make up to 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min and preserved for further use.

3. Czapeck's (Dox) agar (CA)

Sucrose ($C_6H_{12}O_6$)	30.00 g
Sodium nitrate $NaNO_3$	20.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.50 g
Potassium chloride (KCl)	0.50 g
Ferric chloride	0.01 g
Agar-agar	20.00 g
Distilled water	1000 ml (volume to make up)

All the chemical ingredients excluding agar were dissolved in 300 ml water and agar-agar was melted separately in 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml by using distilled water and was sterilized at 1.1 kg cm^{-2} pressure for 20 min and preserved for further use.

4. Oat meal agar (OMA)

Oat flakes	30 g
Agar-agar	20 g
Distilled water	1000 ml (volume to make up)

Oat flakes boiled in 500 ml distilled water for 30 minutes and filtered through muslin cloth. Agar was melted in 500 ml distilled water separately. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml and was sterilized at 1.1 kg cm^{-2} pressure for 20 min and preserved for further use.

5. Potato dextrose agar (PDA)

Peeled potato	200 g
Dextrose	20 g
Agar-agar	20 g

Distilled water	1000 ml (volume to make up)

200 g peeled potatoes were cut into small pieces and boiled in distilled water and the extract was cooled by filtering through muslin cloth. Dextrose 20 g and agar 20 g of each were dissolved in potato extract and the final volume was make up to 1000 ml distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min and preserved for further use.

6. Richard's agar

Sucrose	20 g
Potassium dihydrogen phosphate (KH_2PO_4)	5 g
Potassium nitrate (KNO_3)	10 g
Magnesium sulphate ($MgSO_4.2H_2O$)	2.5 g
Ferric Chloride ($FeCl_3.6H_2O$)	1 g
Agar-agar	20 g
Distilled water	1000ml (volume to make up)

All the ingredients except potassium dihydrogen phosphate were dissolved in 450 ml distilled water. Agar melted in 500 ml distilled water was mixed with the above solution. Potassium dihydrogen phosphate was dissolved separately in 50 ml water and mixed together at the time of pouring to plates and sterilized at 1.1 kg cm^{-2} pressure for 20 min and preserved for further use.

7. Sabouraud's agar (SA)

Dextrose	40 g
Peptone	10 g
Agar-agar	20 g
Distilled water	1000 ml (volume to make up)

All the ingredients were dissolved one by one in 400 ml distilled water agar was dissolved separately in 500 ml distilled water and mixed with the above solution and the volume was made up to one litre before sterilization.

Proving Pathogenicity:

Pathogenicity test was conducted on susceptible fenugreek. Seedlings were raised in pots under glass house condition. Thirty days old seedlings were sprayed with the suspension containing mycelia bits of fungus prepared in sterilized distilled water. Such inoculated plants were covered with polythene bags and kept in dark condition for 12 hrs and then transferred to a growth chamber, which was maintained at 25ºc with 100 percent relative humidity. The pots were removed from the growth chamber after 48 hours and kept in glass house. The observations were taken for the appearance and development of symptoms suitable controls were maintained by spraying the plants only with distilled water.

In order to find out whether there was any difference in pattern of colonization of leaf tissues by the pathogens, inoculated leaves of fenugreek were studied and compared with un-inoculated fenugreek plant leaves. Thin and fine sections of leaves were cut to study the difference in anatomical structure of healthy and infected leaves. The section of both the healthy and infected leaves were cut and passed in 30%, 50%, 70%, 90%, 95%, and absolute alcohol series for dehydration, then stained with Safranin in case of healthy leaf. The infected leaf was stained with both Safranin and cotton blue. The leaves were then mounted in anhydrous glycerol for histological examination.

OBSERVATION

Cercospora leaf spot is consider to be one of the most serious threats to fenugreek, To further understand the nature of the disease and its effects studies of morphological symptoms, histopathological changes and biology of pathogen in the leaf tissues of the diseased plant were carried out and compared with healthy fenugreek plant.

Morphology of Healthy fenugreek plant

Fenugreek Stem

The stem of fenugreek is erect, hollow, straight, rarely ascending, anthocyanin tingled at the base or all the way up, rarely completely green. Single layered epidermis covered with thin cuticle 3-5 layers of parenchymatous cortial cells are present in transverse section of stem Endarch vascular bundles with small parenchymatous pith and a single layered endodermis is visible in stem of fenugreek.

Fenugreek Leaf

Leaves are arranged in an alternate array throughout the plant. First leaf simple, sometimes weak trifoliate, oval or orbicular with entire margin and long petiole stipules are large, covered with soft hair. Leaf petiole thickened at the top, soft sparse hairs on underside. Anatomical section of trigonella leaf showed upper epidermis and lower epidermis. Mesophyll was differentiated into pallisade and spongy parenchyma. Spongy parenchyma cells were arranged in 6 rows and contained many intercellular spaces. Trichomes were present.

Fenugreek Pods

The fruit structure in fenugreek is pod. Fenugreek pods are long, erect, pointed, sickle shaped and slinder. They have a sharp beak 2-3 mm in length at the end. The pods are green in colour during growth of the plant but, turn yellow to yellowish brown at maturity. Pods emerge from nodes as solitary pod or twin pod.

Each pod carries approximately 10-20 seeds each, each pod bearing branch can produce about 2-3 pods.

Fenugreek Seeds

Fenugreek seeds are yellow to golden yellow in colour when mature although some varieties can produce mature seeds. Which are green or yellow green in colour. The seeds have a rectangular, squre or irregular rhomboidal shape with grooves situated between the radicle and the cotyledons. Seeds are surrounded by a seed coat which is separated from the embryo by a dark translucent endosperm. A single layer of living tissue known as the aleurone layer lies between the seed coat and the endosperm.

Morphology of Infected Fenugreek Plants (Plate-11).

Observation of infected parts revealed that leaf spot disease possessed a very prominent and remarkable symptom on the above ground parts of the plants. The symptoms appeared in the form of lesions on almost all the parts including leaves, stem, pod and seeds.

Fenugreek Leaves

The symptoms or lesions that appeared on the leaf were sharply defined with most lesions surrounded by a characteristics yellow halo. Newly formed lesions were circular, sunken, bleached in colour with narrow (1-2 mm) chlorotic halos on the surface of the leaves. Severely infected plants were found to have only a few leaves situated towards the apex of the plant or no leaves at all.

Fenugreek Stem

The stem of fenugreek plant at the junction of the petiole shows discoloured shrunken areas as the severity of disease increases.In severely infected plants the main stem becomes yellow and secondary branches were found to dry up.

Fenugreek Pods

Infected areas of the pods were characterized by loss of colour with most seriously infected areas were found to go seriously deformation of pods as shrunken or twisted.

Fenugreek Seeds

Symptoms of leaf spot on seeds consisted of discoloured areas on seed surface with shrunken regions.

Histopathological Study of fenugreek plant (Plate-12)

Both healthy and Cercospora travensiana infected fenugreek plants were studied to compare the histological changes in the leaf tissues brought by the pathogen in the plants of comparative stages.

Anatomy of Healthy leaf

The micrograph illustrated in plate -12 showed the typical anatomy of a fenugreek leaf. The leaf internal structure was consisted of three basic tissues as seen in plate-11. The three basic tissues were epidermis, mesophyll tissue and vascular bundles.

The upper (adaxial) and lower (abaxial) epidermis cells formed the boundary of the leaf blades as a continous covering of the leaf as observed in cross section. Epidermis was protected by a layer of cuticle. Mesophyll was differentiating into pallisade and spongy parenchymatous cells having large intercellular spaces. These cells were filled with densely packed chloroplasts along with the other cell organelles. The midrib was composed of single vascular bundle. The phloem was composed of sieve tubes, companion cells and parenchyma. Xylem was made up of rows of bluntly angular vessels interspersed with parenchyma. The anatomy of healthy fenugreek leaf is characterized by epidermis, parenchyma cells and usually by large intercellular spaces in photosynthetic areas.

Anatomy of diseased leaf (Plate-11)

The affected tissue was classified into :

Disintegrated tissues

The disintegrated tissues displayed a greyish brown color in the centre of the spots, and in the section which was seen clearly withered and desiccated. There was complete loss of matter inside of the cells and part of the membrane was lost. The remaining cells appeared to be pressed from above and below, and their thickness was reduced and looked dried out. Frequently light brown hyphae were observed in the disintegrated tissues. In the disintegrated areas the formation of pathogenic conidiophores were observed. Conidiophores were 3-5 bunched together coming out from stomata in dense fasicules.

Necrotic tissues

The necrotic section was the part surrounding the outer edge of the disintegrated section. Here the coloration of the infected tissue was dark. The contents of the cells were completely lost. Closely situated spots coalesced and formed large circular or irregular necrotic patches. The pathogenic hyphae were seen not only in the assimilatory tissue but also in the epidermis, sometimes in the vascular bundle. The semi-necrotic bordered area was distinct. The deterioration of diseased tissue was very complex.

Pathogen the pathogen responsible for the occurrence of leaf spot disease was Cercospora travensiana Linn. a member of Ascomycetes. The fungus was studied microscopically. The vegetative body was consisted of thread like structures called hyphae that were aggregated to form fungal branches called as the mycellia. The mycellium appeared as the cottony growth on decaying plant tissue. Hyphae were septate, mostly uninucleate and branched. The conidiophores develop in fascicles of 3-12 conidiophores per fascicle with a length of up to 17.6 -28.8 µm and width ranging from 1.78 - 3.01 µm. The conidia are hyaline, acicular, straight or slightly curved apex rounded, base truncale and multicellular. Conidiophores are dark,

paler towards the tip, unbranched, rarely geniculate and rarely septate.Conidia ranged from 2.3 to 2.8 µm in length and 1.2 to 1.9 µm in width.

The infection Process (Plate-13)

The development of a disease epidermis on plants depends on several factors relating to the host, the pathogen and the environment and the complex interaction of these factors. Infection of the fenugreek plant commenced with the deposition of conidia on fenugreek plant leaf surface. On contact with the plant, stomatal penetration and vegetative growth occurs. After penetrating through the epidermal layer, the fungus occupied the spaces into the intercellular spaces of mesophyll tissues and colonized the leaf tissues inter and intracellularly. The infection of leaf tissues were resulted as external symptoms of leaf spot on leaf tissues within 3 to 8 days. The symptoms were appeared first on older leaves and later on younger leaves.

Disease Cycle and Epidemiology (Plate-13)

Conidia disseminates by rain splash and wind. After landing on the leaf surface, the spores germinated due to availability of leaf moisture. The fungal spores germinate and enter through natural openings of leaves when conditions are optimal. Once spores infect a plant it will take 5 to 21 days for symptoms to appear. The fungus has the ability to overwinter in plant debris that remains in the field after harvest. The cycle of disease was repeated many times during growing season. The number of spots and spores that were produced on each leaf were influenced by many the amount of fertilizer and the susceptibility of the host. Lesion size was increased significantly on mature leaves, where sporulation becomes evident, giving the lesions a whitish velvet like appearance. High severity of leaf spot infection resulted in yellowing of leaves withering and the tap root system becomes completely girdled with sharp lesions. The infected seeds served as the source of primary inoculum.

RESULTS AND DISCUSSION

Morphology and anatomical changes occurred in the fenugreek plant caused by fungal infection interfered in the growth progression of plant and accounted for the difference in the growth manner in infected plant as compared to healthy plant.

Due to infection of Cercospora travensiana in fenugreek plant considerable changes in the morphological symptoms of plant occurred and observed. The typical leaf spot symptoms were observed in the infected plants. The initial leaf spot symptoms appeared as circular, sunken lesions bleached in colour, with narrow (1-2 mm) chlorotic halos on the surface of the leaves. These lesions expanded rapidly as the infection progressed by producing necrotic areas. Sharply defined infected areas were surrounded by a characteristic yellowish halo. Lesion size was increased significantly on mature leaves, where sporulation was evident, and lesions appeared as velvety whitish. Stem and pods were also infected with shrunken and twisted areas. Severly infected plants were reported with only a few leaves situated towards the apex of the plant. The symptoms observed were in accordance with the description of (Zimmer, 1984) and Khare et al (1981).

The effect of leaf spot disease caused by Cercospora travensiana was visualized in the internal tissues of the leaf when observed microscopically. Many alterations in the normal anatomical structures of leaf tissues had been observed. In the lesion region cells in the vicinity appeared brownish in color and cell walls get partially collapsed. There was loss of matter inside the cells and parts of the membrane were lost. In the areas of active invasion of the pathogen in the host cells the chloroplasts disappeared protoplasm died and cells partially collapsed. The conidiophores formed from a plexus of the mycellium in the intercellular spaces were emerged out and produced leaf spots.

Several structural changes had been noticed in the fenugreek plant grown under diseased condition. Pathogen caused extensive damage to leaf tissues by destroying epidermal and mesophyll cells most probably by degrading cell wall. The results of the present study were in corroboration with many previous

workers. Such as Cook (1978), Ryley (1989), Khare et al 1981, Petropolus 2002, Acharya et al 2007, Bobem et al 1999.

The leaf spot pathogen completed its life cycle by producing asexual cycle spores called conidia. These were asexual fruiting structures that contained spores, which were rain splashed or dispersed by wind and when spores landed on leaves germinated by a specialized infection structure. At this point the fungus developed hyphae within the leaf and formed the familiar spots. The fungal hyphae then undergo asexual sporulation again.The morphology and shapes of different structures of the fungus reported in this study also found to be in aggrement with former studies (Ryley 1989, Zimmer 1984, Leppick 1960)

Survival of the pathogen through soil and seed was favoured by overwintering inocula in plant debris. Germination of conidia was favoured by high relative humidity and high temperature (Agrios, 1997).These climatic conditions are the characteristics of the present study area in the Rabi season resulting in heavy disease incidence in fenugreek crop in the area.

Aiming to understand how diseases interact in the field, it was further concluded that increased focus should be placed on considering the dynamics of plant growth along the epidemiological development. The present observations and information will be of value in future research designated to manipulate and optimize these values while developing high yielding spot field resistant varieties and practical control recommendations.

INTRODUCTION

Fenugreek as a spice crop has important medical and nutraceutical properties also used as a forage crop in some countries. India consumes domestically 90 percent of its own production (Edison, 1995).

Plant disease adversely affects the quality of plant by inducing higher level of undesirable constituents in plant. Leaf spot is a major destructive foliar disease of fenugreek in growing area; interfere with the biochemical process of healthy plant. Changes in normal physiological condition of plant due to disease affect the yield of plant growth in conspicuous characteristics of a living being. Reduction in growth and photosynthetic activity are among the conspicuous effects of disease. Foliar disease like leaf spot can influence photosynthesis (hence growth) through affecting total leaf area itself.

Although structural barriers may provide a plant with various levels of defense against the attacking pathogen, Chemical defense mechanism attributes a lot to host plant. The effects caused by pathogens on plants are almost entirely the result of biochemical reactions taking place between substance secreted by the pathogen and those in, or produced by, the plant.

Plant disease adversely affects the quality of plant by inducing higher level of undesirable constituents in plants (Isawa, 1985). The disease responses impact on whole plant physiology, disease photosynthesis, increase respiration, impair growth, interfere biochemical processes and reduce yield.

Present study is aimed to investigate the effect of leaf spot pathogen on endogenous level of chlorophyll contents, Carbohydrate, Protein, Amino acid, Proline and Phenol in fenugreek plant in response to Cercospora leaf spot.

The objective of the present research work is to calculate quality and quantity of various metabolites in fenugreek plant under all the growth stages.

REVIEW OF LITERATURE

In order to know the biochemical changes brought about in host plant due to invasion of pathogen, extensive investigations have been carried out.

Harvell and Tollrian (1999) concluded that plants evolved induced defense when stress situation increases.

Wink (1999) believed that most of the secondary metabolites involved in plant chemical defense system have co-existed with their attackers.

Plant resist pathogen infection through physical and chemical defenses that may be due to the application of some biotic and abiotic induces (Van loon 1983, Kessman et al 1990, kuc 1995, Biswas et al 2003).

The Quality of Vegetables significantly differs at different growth stages as reported by Rajan et al (2007).

Abnormal pattern of translocation of organic and inorganic materials are commonly found in plant infected with virus or biotrophic parasite Mirocha and Zaki, 1966.

Chlorophyll Content

Pathogen of leaf spots, blight etc causes destruction of leaf tissues or defoliation which reduces photosynthetic surface of the host plant. The overall chlorophyll content of leaves in many fungal diseases is reduced. Chloroplasts from infected leaves were less efficient in carrying out the Hill reaction than chloroplasts from healthy leaves.

Wang (1961) reported that in bean rust healthy parts are photosynthetically active and starch accumulation is greater than infected parts by Vromyces phaseoli.

Schipper and Mirocha (1969) showed the depletion of starch in rusted leaf of bean due to reduction in the amount of chlorophyll per chloroplast.

Gordan and Duniway (1982) reported the reduced photosynthesis of powdery mildew infected sugar beet.

Sziraki et al (1984) studied the increased net chlorophyll concentrations in Phaseolus vulgaris on infection of Uromyces phaseoli.

Scholes 1985 and Farrar 1985 investigated progressive disease in chlorophylls, carotenoids, chloroplast volume on bluebell leaves affected with Uromyces muscari.

Gupta et al (1987) reported the presence of lower chlorophyll content in susceptible varieties of sesamum affected by Alternaria serami as compared to resistant ones at all the stages of crop growth.

Bruggmann and Schnitzles (2001) reported reduction in total leaf chlorophyll and net assimilatory rate by 50 percent in oak infected with powdery mildew.

Reduction in chlorophyll content of fenugreek infected by powdery mildew (Ramavtar et al 2002) and of cucumber leaves after powdery mildew infection (Shenxi et al 2003) had been studied.

Carbohydrate

Recent advances in our knowledge of carbohydrates storage and metabolism in vascular plants should lead to a more sophisticated view of the carbon nutrition of pathogenic fungi. The pathogen which disturbs photosynthetic activity either by more injury to the photosynthetic organ or by directly affecting metabolic activity certainly brings about changes in carbohydrate content of plants.

Ahmed et al (1985) studied the uptake of sugar by intercellular brown rust hyphae as an important source of carbohydrate for the mycellium in barley leaves.

Thines et al (2000), Both et al (2005) revealed that the initiation and development of early infection structures are supported by the mobilization of lipids and carbohydrates.

Proteins

Changes in nucleic acid and protein metabolism are often some of the earliest physiological effects induced in host plants by fungal pathogen after invasion. The most general effects reported are changes in normal protein turn over in infected leaves.

Klein (1952) suggested that the greatest increases in proteins and soluble nitrogen became apparent in tumour tissues late in their development as the time of cell volume increase.

Bhattacharya et al (1965) studied the changes in Nucleic acids and proteins in wheat leaf nuclei during rust infection.

Uritani (1976) reviewed the changes in protein metabolism in plant tissue during the initiation of pathogenic infection and course of disease development.

Nandgopal (1995) reported a marked significant decrease of protein in wheat infected by Exherohilum hawaiiensis.

Kalappanavar and Hiremath (2000) reported that the resistant Sorghum genotype possessed higher content of protein as compared to those of susceptible genotypes.

Amino Acids

Amino acids are biologically important organic compounds performing critical roles in processes such as neurotransmitter transport and biosynthesis. An amino acid contains both a carboxylic group and an amino group. They occur in plants both in Free State and as basic units of protein and other metabolites (Khokhani et al 2012) Amino acids are important nutrients that are transferred from the host plant to the pathogen and can function as nitrogen source for the fungus.

Patel and Walker (1963) studied the amino acids and amide pool of the bean plant susceptible and resistant to the haloblight bacterium Pseudomonas phascolicola.

Singh and Shukla (1987) concluded that reduction in amino acids concentration of infected tissues may be due to their utilization by the pathogens or due to their utilization in the synthesis of protein during host-parasite interactions.

Berger et al (2004) noticed a reduction in total free amino acid level in host tissue due to Botrytis cinerea infection in tomato leaves.

Reddy et al (2005) revealed that Fusarium solani infection greatly influenced the total proteins and free amino acids of turmeric roots as compared to the corresponding healthy tissues.

Rampitsch et al (2006) reported the decrease of certain amino acids due to utilization by the pathogen or utilized by the host plant for the defense mechanism in Sesanum orientale affected by Alternaria sesani.

El- Khallal (2007) reported that Fusarium oxysporium infected tomato plants showed significant decrease in levels of free amino acids and soluble protein.

Hong et al (2008), Navajothy et al (2011), Zhang et al (2011) studied that arginine induces disease resistance via its effects on nitric oxide biosynthesis and defensive enzyme activity.

Proline

Proline is a major component of structural protein or as osmoprotectant capable of mitigating the impacts of drought, salt, temperature and pathogenic stress in plants. It is generally believed that the increase in proline content following stress injury is beneficial for the plant cell (Mostajeran et al 2009).

Hare PD et al (1997) studied the metabolic implications of stress induced proline accumulations in plants.

Yoshiba et al (1997) reviewed the regulation of levels of proline as an osmolyte in plants under water stress.

Fabro G, Pavet V et al (2004) reported proline accumulation and gene activation are induced plant-pathogen incompatible interactions.

Haudecoeus et al (2009) observed proline accumulation in susceptible species of Nicotina Tobacum (tobacco) to Agrobacterium tumefacians.

At the biochemical level several workers have studied the accumulation of proline in tissues infected with viral (Mohanty and Shridhar 1982, Radwan et al 2007) and bacterial (Meon et al 1978, Fabro, 2004) pathogens.

Phenol

Phenolic compounds distributed in almost all plants and subject a number of chemical, biological, agricultural and medical studies. Two main phenolic compounds which present in plants are hydroxybenzoic and hydroxycinnamic acids. The phenolic substances produced in plant tissues in response to infection, induced synthesis of protein and enzymes seems to play a role in disease resistance. They could be an important part of plants defense system against pests and diseases (Wuyts et al 2006)

The activity of many phenol oxidizing enzymes is higher within the infected tissue of resistant varieties than in infected susceptible ones or in healthy.

Dufrenoy (1936) observed that when the progress of some pathogen is checked, phenolic compounds mostly tannins of the garlic group develop in abundance.

Johnson and Sachaal (1952) reported that chlorogenic acid is partly responsible for the resistance of potato tubers to scab by stimulating protective cambium activity.

Sproston (1957) reported that Balsam (Impatiens balsamina) leaves were free from fungal disease due to abundance of phenolic substances or glycosides.

Hare (1966) noted the accumulation of aromatic substances such as polyphenols, phenolic glucosides, flavonoids, anthocyanins and coumarin derivatives around infected plant tissues and also in tissues adjacent to wounds.

Chakravarty and Shrivastava (1967) have attributed the resistance of carrot roots to Pythium aphanidermatum to an unidentified pre-formed phenolic substance.

Patil and Kulkarni (1977) reported that the healthy leaves of sunflower contained more phenols than the leaves infected with Puccinia helianthi Schw.

Sharma et al (1992) studied biochemical relationship in resistant and susceptible cultivars of maize with Fusarium leaf blight disease.

Shiv Kumar and Sharma (2003) studied increased total phenol content in maize leaf inoculated with Rhizoctomia solani.

Plant Health status is affected by several fungal infections morphologically as well as biochemically.

MATERIALS AND METHODS

All the physiological and biochemical experiment were at P.G. Department lab of Govt. College, Kota. Effect of leaf spot disease caused by Cercospora sps. On fenugreek was estimated in terms of physiological and biochemical estimations. For this purpose susceptible RMT-1 Cultivars were selected for study. The surface sterilized seeds of above cultivars were sown and seedlings were then transplanted in pots containing sterile soil under green house conditions. At 10 days after transplantation, 60 fenugreek plants were inoculated with spore suspension of Trigonella foenum graecum at the concentration of 10^6 conidia per ml. 60 plants were kept healthy (uninoculated) that served as healthy control. The plants were tested for the physiological and biochemical estimations after every 30, 60, 90 days of transplantation and at maturity (120 days) in both healthy and infected situations.

Estimation of chlorophyll content (Arnon, 1949)

One gram of each healthy and leaf spot infected fenugreek fresh leaf were crushed and homogenized with eighty percent chilled acetone and a pinch of magnesium carbonate powder in a mortar and pestle separately. From both, clear supernatant was decanted in a test tube and residue was subjected to grinding repeatedly with acetone, until it became together and centrifuged at two thousand rpm for ten minutes. The supernatants were decanted and the optical density of both was measured at 663nm, 652nm and 645nm on spectrophotometer. The reaction mixture except the plant was taken as blank. The content of different pigments of both healthy and infected plants was determined by using following formulae:-

$$\text{chl a} = (12.7A663 - 2.69\ A645)\ \frac{V \times W}{1000}$$

$$\text{chl b} = (22.9A645 - 4.68\ A663)\ \frac{V \times W}{1000}$$

Total chl = (20.2A645 - 8.02 A663) $\frac{V \times W}{1000}$

Where

V = Volume of extract

W = Weight of tissue

A = Absorbance or optical density of chlorophyll at respective wavelengths.

Biochemical Estimation:

Biochemical estimations such as quantification of carbohydrate, protein, amino acids, proline and phenols were done in both healthy and Trigonella infected plants collected from pot trials. Biochemical estimations were performed by following standard methods. Fresh plant extracts of aerial parts of both healthy and Cercospora infected fenugreek plants were collected from pots after every thirty, sixty, ninety days and at the maturity (120 days) and processed further for estimation of different metabolites.

Estimation of Carbohydrate (Anthrone Method 1946)

One gram of each, healthy and leaf spot infected fenugreek fresh aerial parts were crushed and homogenized in distil water. Clear supernatant was decanted in test tube and centrifuged at two thousand rpm for ten minutes. To this extract 4 ml of freshly prepared anthrone reagent was added. This was placed in boiling water bath for ten minutes. It was then cooled at room temperature. Absorbance of samples was taken at 620 nm in a spectrophotometer. A blank containing all reagents minus plant extract was prepared. Amount of carbohydrate in the samples were calculated using standard curve prepared with glucose.

Estimation of protein (By Lowry Method 1951)

Healthy and fungal infected Trigonella fresh aerial parts were taken as sample. One gram each of the sample material was homogenized with distil water in a mortar and pestle properly. Crushed samples were centrifuged at two thousand

rpm for ten minutes separately. To the sample 2 ml of freshly prepared alkaline copper sulphate reagent was added. Solution was mixed well and incubated at room temperature for 10 minute. After incubation 0.2 ml of freshly prepared folin-ciocalteau reagent was added into reaction mixture. The prepared mixture was incubated at room temperature for thirty minutes. After that optical density was taken at 660 nm in spectrophotometer. Reaction mixture minus sample was considered as blank. Amount of protein was calculated from standard curve prepared with albumin.

Estimation of Amino acid (Moore and Stein 1984)

Fenugreek plant aerial parts of both healthy and leaf spot infected plants were taken as simple. One gram of each sample was crushed in 5 ml of 80 % ethanol. It was then boiled on water bath to extract all the content in solution. Extract was cooled and centrifuged as two thousand rpm for ten minutes. One ml of supernatant was taken for procedure. To the sample a drop of methyl red indicator was added and then neutralized with 0.1 N NaOH. After neutralization 1 ml of freshly prepared ninhydrin reagent was added. Solution was mixed well and a glass stopper was placed at the test tube. It was then boiled in water bath for 20 minute till purple colour got stable. Then OD was taken at 570nm in spectrophotometer. Amino acid was calculated from standard curve prepared with leucine.

Estimation of Proline (Bates et al 1973)

Healthy and leaf spot diseases plant aerial parts were taken as sample. One gram of plant leaf was crushed in 10 ml 3% sulphosaliycylic acid. It was centrifuged at two thousand rpm for ten minutes and clear supernatant was used. To the 2ml of leaf extract, 2ml of glacial acetic acid and 2 ml of freshly prepared acid ninhydrin was added. Contents were mixed well and heated in boiling water bath at 100ºC for one hour. Brick red colour was developed. After cooling, 4ml of toluene was added. Contents were stirred well. A toluene layer gets separated. Its OD was taken at 520nm in spectrophotometer. All the reaction mixture except the plant

sample was taken as blank. Calculation was done using standard curve prepared with D-proline.

Estimation of total Phenol (Bray and Thrope 1954)

Healthy and leaf spot infected fenugreek plant aerial parts were taken as samples. One gram of each material was homogenized with 10ml of 80% alcohol in a mortar and pestle. Sample was centrifuged at two thousand rpm for ten minutes. The supernatant was collected and used for total phenols estimation. To the 1 ml of extract folin-ciocalteau reagent (1ml) was added followed by 2ml of Na_2CO_3. The reaction tubes were shaken vigorously and heated for 1 minute in water bath and cooled under running tap water. The reaction mixture was diluted to 25ml, with distilled water. Absorbance was recorded at 650nm in spectrophotometer. Phenol content in the sample was calculated using standard curve prepared with catechol.

OBSERVATIONS

The investigation of both the physiological parameters and Biochemical constituents, were analysed in both healthy and infected fenugreek plants and results are presented in the Table – 7, 8, (Figure 4-11).

Chlorophyll Content

Photosynthetic pigment concentrations (Chlorophyll a and b, Carotenoids) typically decrease in response to stress or disease. During pathogenesis various metabolic processes of the host are known to undergo deviations from the normal cours, one such process is photosynthesis, Hence, rate of chlorophyll loss in the fenugreek plant as influenced by the development of leaf spot disease was studied. The chlorophyll 'a' chlorophyll 'b' and total chlorophyll content at different growth stages are present in Table-7

From the observations in the present investigation revealed that the values of chlorophyll a was 0.718mg/g at 30 days, 0.798 mg/g at 60 days, 1.141 mg/g at 90 days and 1.172 mg/g at 120 days in healthy plants as shown in Table -7. The values of chlorophyll a in infected leaves were 0.513 mg/g, 0.526 mg/g, 0.732 mg/g, 0.893 mg/g at 30, 60, 90 and 120 days of growth stage (Fig-4).

Values of chlorophyll b also followed the same termed as was noticed in the case of chlorophyll a. The values of chlorophyll b in healthy plant leaves was calculated to be 0.573 mg/g, 0.757 mg/g, 0.819 mg/g and 0.910 mg/g and in infected fenugreek plant was 0.348 mg/g, 0.420 mg/g, 0.475 mg/g amd 0.784 mg/g at 30, 60, 90 and 120 days respectively (Fig-5).

Total chlorophyll also showed the same trend as it was cumulative effect of chlorophyll 'a' and 'b'. The value observed were 1.431 mg/g, 1.771 mg/g, 2.143 mg/g and 2.709 mg/g in healthy plant and 1.007 mg/g, 1.230 mg/g, 1.230 mg/g, 1.281 mg/g and 1.466 mg/g in diseased fenugreek plant at 30, 60, 90 and 120 days of growth (Fig-6). The results are shown in Table-7. The healthy plants recorded

higher amount of chlorophyll a, chlorophyll b and Total chlorophyll than diseased leaves at every growth stage.

Quantitative estimations of biochemicals constituents' viz. Carbohydrates, proteins, Amino acid, Proline and Phenols were carried out in present investigation. The values of above all constituents were recorded at 30, 60, 90 days and at maturity of crop age in both heathy and Cercospora travensiana infected plants. (Table-8)

Carbohydrate

The present study revealed the variations in Carbohydrate content between healthy and infected fenugreek leaves. Calculated values of carbohydrates were 282.0 mg/g, 369.1 mg/g, 481.0 mg/g and 591.0 mg/g in healthy plants and 212 mg/g, 259.0 mg/g, 383.0 mg/g and 485.0 mg/g in infected fenugreek plants at 30, 60, 90 days at at maturity (120days) respectively. In diseased leaves their amount decreased as compared to healthy plants (Fig-7).

Protein

For appropriate growth and development proteins are the basic structural units in plants. In the present investigation protein content was estimated. The results are presented in Table-8-. The study revealed that healthy leaves contained higher amount of protein. The values of protein in healthy plants were recorded as 16.21 mg/g, 16.43 mg/g, 17.2 mg/g and 17.8 mg/g whereas in diseased plants protein content was found to be 14.20 mg/g, 14.43 mg/g, 15.4 mg/g and 15.90 mg/g at 30, 60, 90 days and at maturity (120 days) in both the cases (Fig-9).

Amino Acid

The study of amino acid content in both healthy and infected fenugreek plant was carried out. The amount of amino acid content in healthy leaves was recorded as 3.55mg/g, 4.64mg/g, 4.89 mg/g and 5.22 mg/g whereas in diseased plants was

2.69 mg/g, 3.25mg/g, 3.8 mg/g and 4.18 mg/g at 30, 60, 90 days and at maturity (120 days) respectively as shown in Table -8,(Fig-8).

Proline

The present investigation revealed the amount of proline in both healthy and Cercospora traversiana infected fenugreek plants. The amount of proline content was 0.51 mg/g, 0.59 mg/g, 0.62 mg/g and 0.64 mg/g in healthy plants and 0.68 mg/g, 0.72 mg/g, 0.87 mg/g and 0.92 mg/g in diseased plants at 30, 60, 90 days and at maturity (120 days) respectively. In diseased leaves their amount increased at all the growth stages as shown in Table – 8, (Fig-10).

Phenol

The values of total phenol content at different growth stages of the crop in healthy and diseased fenugreek plants are depicted in Table-8. The values calculated were 2.32 mg/g, 3.19 mg/g, 3.78 mg/g and 3.98 mg/g in healthy plants and 3.48 mg/g, 3.92 mg/g, 4.52 mg/g and 4.78 mg/g in infected plants at 30, 60, 90 days and 120 days (at maturity) (Fig-11).

RESULTS AND DISCUSSION

Chlorophyll Contents

Above said results on chlorophyll-a content indicated that the amount of chlorophyll-a, chlorophyll-b and total chlorophyll was higher in healthy plant over the Cercospora traversiana infected plant (Table-7). The fall observed in chlorophyll “a” values from 33.36 percent to 27.05 percent due to diseased condition of plant. The reduction percent in chlorophyll “b” was from 39.31 percent to 33.42 percent in infected plant over the healthy one. Overall chlorophyll content shows the same trend. Decrease in the amount of total chlorophyll from 41.39 percent to 31.86 percent in infected plant over healthy plant was concluded.

In the present investigation the decrease in amount of chlorophyll a, chlorophyll b and total chlorophyll contents were found as a result of fungal infection. During pathogenesis, various metabolic processes of the host are known to undergo deviation from the normal course. Destruction of chloroplasts is a common feature of diseased tissue/necrotic tisuues in infected plants. Chlorophyll is the primary pigment involved photosynthesis and is an indicator of vigour of the plant (Bhat 1997). These changes may be partially or completely accounted by reduction in chlorophyll content. Results of the present study were similar to the findings of many workers such as Bhat et al (1997) in leaf spot of groundnut and Padma et al (1989) in Mours alba infected by Myrothecium mori, Siddaramaiah and Hedge in mulberry leaves affected with Cercospora. Berova et al (2007) observed significant pigment loss in Phaseolus Vulgaris due to fungal infestation. Lobato et al (2009) also observed reduction in chlorophyll in Phaseolus vulgaris infected by Colletotricum lindemuthianum. Radwan et al (2008) suggested in same trend that amount of total chlorophyll in diseased plants significantly decreased by two factors, mainly by loss in leaf photosynthesis area promoting less light absorption and chloroplast disorders during pathogen infection.

The result are conformity with the findings of Scarpari et al (2005) in case of Theobroma cacoa plants affected by pathogen Crinipellis perniciosa, Akhkha et al 2003 in Hordium vulgare affected by Blumeria graminis.

Carbohydrates

Carbohydrates are compounds produced during photosynthesis they have two main purposes First, they provide building blocks for plants structural components, Secondly carbohydrates are molecules that deliver energy for plant growth. These are the Basic molecules which plays an important role during plant pathogen interactions. Vidyashekaran (1974).

In the the present investigation there was decrease in carbohydrate content of fenugreek plant in infected condition over healthy situation.

The present decrease in carbohydrate content infected fenugreek plant over healthy fenugreek plant ranged from 29.82 percent to 20.37 percent at 30, 60, 90 and 120 days of plant growth. The mean decrease in carbohydrate content in infected plant was 23.23 percent over healthy ones. The above results corroborated the findings of Berger S. et al (2004) in infected plants of tomato with Botrytis unerea.

Kumar and Kulkarni et al 2000 found lower levels of total sugar, non reducing sugars and starch from fungal infected leaves compared with those of healthy leaves in various other crop species. The amount of total sugar, were found to vary between 2.74 – 3.02, 0.59 – 0.71, and 2.06 – 2.31 % in healthy leaves and 1.42 – 1.46, 0.34 – 0.38 and 1.07 – 1.08 % in decreased leaves respectively by Abul K Tang et al (2006).

P. M. Holligan et al (1973) assigned a major role of sugars from healthy leaves of Tussilago farfaro and leaves infected by Puccinia poarum. This reduction in sugar content was probably due to the increase in respiration of infected plant, (Shaw & Samborski 1956, Daly et al 1961). Kosuge (1978) also found the similar results in leaves of senecio infected by Puccinia due to biosynthetic intermediates for the

growth and sporulation of the pathogen. Decrease in the rate of oxidation process and a significant disruption in the process of carbohydrate synthesis was reported in powdery mildew disease (Chanturiya, 1968) The breakdown of carbohydrate in diseased plant in normal plant is higher due to utilization by pathogen in the process of invasion and development (Jaypal and Mahadevan, 1968). The pathogen which disturbs the rate of photosynthesis in leaves is the reason for the lower yield of plants infected by fungus (Jesus Junior et al 2001).

Protein

In the present investigation amount of protein content was higher in healthy plant than in the Cercospora infected fenugreek plant at all the growth rates. The decrease in protein content ranged from 10.46 percent to 12.39 percent with a mean value of 11.34 in infected plant over healthy plant.

As the pathological behavior of fungi and their effects on the physiology and biochemistry of the host varies from pathogen to another and the degree of infection.

The results are in agreement with the findings of Thomas J and Mathew K L (2014) that due to Asterina lawsoniae protein in the infected leaves decreased as compared to healthy leaves of Lawsonia inermis. Gopalkrishnan (2009) observed significant decrease in protein content of rice infected with leaf leaf sheath pathogen from 41.67 percent to 33.33. The above investigated results are in corroboration with Malli et al (2000) in Moth bean, Chandra & Bhatt (1998).

Amino Acids

Amino acids are important biologically as if they play an important in nutrition, growth and nitrogen metabolism.

In the present investigation there was significant reduction in the amount of amino acid in diseased plant over healthy plant. The reduction in terms of percentage

ranged from 19.92 percent to 29.95 percent. The mean reduction in value of amino acid content was recorded to be 24.09 percent.

A noticeable reduction in acidic amino acid was observed in infected leaves of Senecio and Cakile with addition to basic amino acids (Ariginine & Lysine) H.S. Aldesuquy & Z.A.M Baka (1999) Kim & Rohringer 1969 found that in rusted wheat leaves many free amino acids diminish due to formation of Uredospores. The decrease in amino acid may be due to utilization by the plant as well as fungus or due to enzymatic degradation (Narain and Addy 1970). Degraded concentration of amino acids in sugar beet plants was induced by vious yellow (J.M. Fibe 1961). Due to cystobiolic ammonium ion deficiency depletion of amino acid was observed by Himer et al (2006).

Proline

It is well known that proline accumulation in plants during adaptation to various types of environmental stress. Also free proline is produced and accumulated in response to biostress (Ghasempour and Khianian 2002, Maleki 2003). The quantative analysis of proline was investigated in present study. There was a signicicant increase in the amount of proline in diseased plant over the healthy plant which was reported as 18.05 percent to 30.43 percent with a mean value of 25.55 percent.

The result was agreement with the results observed under Cyst nematodes infection of sugar beet plant.

Proline accumulation is a common metabolic response of higher plants to various stresses. (Taylor 1996, Rhodes et al 1999, Yan et al 2000, Wilfred 2005) Amino acid prolines have been particularly considered to contribute to osmotic adjustment in endophyte infected grasses. (Malinowski and Belesky (2000), Kavikishole et al (2005)). It was suggested that the proline content of tomato leaves was a suitable marker for stress induced by both abiotic and biotic factors

(Grote and Claussen 2001) few investigations have been studied under conditions of pathogen attack.

Phenols

Phenolic compounds are widely distributed in higher plants and fungi. Defence strategies of plants against pathogens are several inducing the production of antifungal chemicals which are either performed or induced following infection (Grayer and Kokubun 2001) Phenolsare produced and accumulate at a faster rate after infection (Picinelli et al 1995, Treutter, 2005).

The increase in phenolic content was ranging from 16.37 percent to 33.33 percent in infected plants to healthy plants at all the growth stages. The results were in accordance with the findings of Mayr et al 1995, Chandramohan et al 1967, Mahadevan 1965 in apple scab, in Amaranthus tricolor resistant to Alternaria sps. And in resistance towards cladosporium cucumerinum Kosuge (1969) also found the similar increase in total phemnol during the early stages of infection in tomato by nematode. Walter (1992) and Sharma and Singh 2003 reported that ferulic acid and phenolic compounds accumulate in response to fungal attack for strengthening of cell wall. Identification of individual phenolic acids of sorghum vulgare after interactions with Sclerotium rolfsi was recorded by S Maurya, Rashmi Singh et al (2007). The germinating uredospores of Puccinia graminis release phenols and the resistance of barley plant to Erysiphae graminis also results in release of the phenolic substance which accumulates around haustoria and inhibits further development of fungus (Mehrotra 1994).

Recently it is confirmed by several findings that plants have a variety of inducible defenses in the presence of pathogens. Structural and chemical defense mechanisms are the two defensive approaches made by plants to survive. Analysis of physiological and biochemical constituents in healthy and infected fenugreek plant was carried out at different growth stages to understand their role in health status of fenugreek plant. All the above comparative studies on physiological, biochemical changes during pathogenesis of infected plant as compared to healthy

plant helped in understanding the nature mechanism of resistance and will help to understand some aspects of biological control mechanism to protect the crop plant.

INTRODUCTION

The constant growth of the world's population requires substantial resources for the production of food. One of the greatest challenges of the world is to produce enough food for the growing population. Production as well as protection of food commodities is necessary to nourish the ever growing population. The situation is particularly critical in developing countries, where the rate of net food production is slowing down in relation to population growth. A major proportion of the yearly production of food commodities of world is destroyed by various pest, including bacteria, fungi, viruses, insects, rodents, nematodes etc. losses at times are so severe as to the world lead to famine in large areas of the world that are densely population. Different approaches are used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticulture practices, growers often rely heavily or chemical fertilizers and pesticides, however the effect as well as fear mongering by some opponents of pesticides and agrochemicals has led to considerable change in people's attitude.today there are strict regulations on chemical pesticides use, consequently some researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases (Pal 2006). Among these alternatives safe ecofriendly biological control are reffered Also to reduce the economic and aesthetic damage caused by plant diseases. Recently following strategies are going through :-

(i). Biological control to antagonize plant pathogen (Spadaro and Gultino, 2004)

(ii). The utilization of non-selection fungicides which are non-toxic and biodegradable (Tripathi and Dubey, 2004).

(iii). Inducing plant defense mechanism by applying natural products produced by plants or animals with antifungal properties (Tripathi and Dubey, 2004).

The indiscriminate application of synthetic chemicals as antimicrobials has contributed greatly to the management of losses caused by fungi, but these chemicals have led to their residual toxicity carcinogenicity, hormonal imbalances

(Kenzevi and Serdar, 2008), (Pandey 2003, Kumar et al 2007). Recently in different parts of the world, attention has been drawn towards the alternative control methods by plant products as botanicals. Because of Non-phytotoxicity, nature of host metabolism, plant products posses the potential to be of value in pest management. (Mishra and Dubey, 1994). Products of some pestidial plants have received global attention for the protection of several food commodities because of their antimicrobial properties (Kumar et al 2007) and their natural origin, comparatively biodegradable and non-residual nature (Beye, 1978).

In many Fenugreek growing areas infectious and non infectious diseases are becoming an important production constraint because of their ability to cause variation in crop yield and quality (Basu et al 2006). Many, major and minor diseases of fenugreek are increasing in importance that can cause a reduction in productivity and crop lossess. (Mc Cormick and Holloway, 1999). The most important disease of fenugreek are caused by plant pathogenic fungi. Common fungal diseases infecting fenugreek are Cercospora leaf spot and Powdery mildew caused by Erysiphae polygoni can seriously reduce crop yield and affects biomass under most agroclimatic conditions (Prakash and Sharma 2000, Jongebloed 2004). In India 27 species of fungi have been isolated from fenugreek seed (Prabha and Bohra, 1999), Acharya et al (2007) suggested some biological control agents which are environment friendly and socially acceptable. Resistant cultivars have been used effectively to control diseases in many crops. Therefore the main objective of the present study was to access the impact of some botanicals such as Annona squamosa, Aegle marmelos, Withania somnifera and Ocimum sanctum etc. which are locally available and are non hazardous to the crop.

Ecofriendly substances as control agents

A Brief Introduction

Annona

Annona Squqmosa Linn. one of the most important medicinal plants, commonly called as " Custard Apple" is a well known plant of family Annonaceae. A. squqmosa is a tree that grows up to 3-8m with broad irregularly spreading branches of light brown bark having thin leaves that occur singly. Aggregate fruit is pendulous on a thickened stalk is present. The plant has been reported to possess a wide variety of pharmacological activities and is used in traditional applications. There is a presence of valuable annonaceous acetogenins in various parts of the plant, which are traditionally used for the treatment of many ailments. The fruits of Annona are haematimic, cooling, sedative, stimulant, expectorant and annonaine an alkaloid which is Hypoglycemic and and antidiabetic in nature. Some flavonoids like Aporphine, glycoside and squamoline are also found in leaves. The presence of glycosides, sapemine, tannins, flavonoids, phenols make the plant posses antioxidant and antifungal properties hepatoprotective, cytotoxicativity, genotoxicity, antitumour, antilice etc.

Withania somnifera

Withania somnifera known as ashwagandha, Indian ginseng, poison gooseberry or winter cherry is plant of Solanaceae. The species is a short, tender, perennial shrub, tomentose branches dull green leaves, flower small, green and ball shaped. It is used as a herb in Ayurvedic medicine. W.somnifera has broad spectrum bioactive nature. The ethnopharmacological properties of the plant include adaptogenic, antisedative, anti-conversion activities also used in treating various neurological disorders, geriatric disabilities, arthritis, stress and behavior related problems. Higher amounts of ascorbic acid, anthocyanin and polyphenols contents are the main source for antimicrobial activies (Scalbot A. 1991). Leaves of the plant contain 12 withanolides, 5 alkaloids (yield 0.09%) many free amino acids

chlorogenic acids, glycosides, condensed tannins and flavonoids. (Khare, 2007) Roots possessed heterogenous alkaloids leaf extracts of W. somnifera have maximum antibacterial and antifungal activities by suppressing the growth of all microbes. (Premlata et al 2012).

Aegle marmelose

Aegle marmelos, commonly known as bael, golden apple, stone apple belongs to family Rutaceae. The tree is considered to be sacred by Hindus. It is a midsized, slender aromatic armed, gum bearing tree growing up to 8 meters tall with foliate leaf. Bael fruit are used in the treatment of chronic diarrohea, dysentery and peptic ulcers as a laxative and to recuperate from respiratory affections in various folk medicines. The fruit posses many ethnomedicinal uses as antioxidant, antibacterial, antiviral, antidiarrheal, gastroprotective, hepatoprotective, antidiabetic, cardiaoprotective and radioprotective effects.leaves of this plant have number of alkaloids and coumarins. The essential oil from the leaves of Bael tree has power to antifungal against animal and human fungi (Jain 1977). It reduces the spore germination by interfering Ca^{+2} dipicolinic acid pathway and lowers the vegetative fungal body inside the host. (Rama et al 1997).

Tulsi (Ocimum sanctum)

Ocimum also known as holy basil or tulsi is an aromatic plant in the family Lamiaceae. It is an erect, branched subshrub; simple green or purple, strongly scented leaves with a decussate phyllotaxy. It is widely known across the Indian subcontinebnt as a medicinal plant extensively used in Ayurveda. O. sanctum hub contains 0.6 % essential oils, linolenic acids (48.50%), linoleic acid (21.81%) (Angeis et al 1996), phenolic compounds, flavonoids, (Sukari et al 1995). O. sanctum showed moderate resistivity of Collectotricum falcatum. (L. prince). Ocimum sanctum extracts and essential oils particularly eugenol have been known to be highly effective against a Plethora of plant pathogens.

Tricoderma sp.

Trichoderma species belogs to a class of free living fungi beneficial to plants that is common in the rhizospore. They have been widely studied for their capacity to produce antibiotics, parasite other fungi and complete with deleterious plant microorganisms (Harman et al 2004). Trichoderma enhanced of plant growth has been known for many years and can occur in axenic systems or in soils (Chang et al 1986, Adams et al 2007). Trichoderma species promote plant growth and development. Several strains of Trichoderma have been developed as biocontrol agents against fungal diseases of plant. Various mechanisms include antibiosis, parasitism include host- plant resistance and competition. Different strains of Trichoderma control every pathogenic fungus for which control has been sought. Trichoderma strains for biological control have extensive lists of susceptibilities or resistance to a range of pesticides. Trichoderma species are totally non-parasitic to man, animals and plants. Being biological, they do not leave any harmful chemical residue behind. It can be applied into the soil during any stage of growth in the presence of disease; applications will have a marked effect on reducing the infection rate.

Varietal Screening

Fungal disease development on host plant is a step by step phenomenon. Control of the disease is based on cultural management, the use of chemicals, genetic resistance. The resistance is usually based on a qualitative response. Partial resistance interfers with one or more of the steps of disease cycle resulting in showing disease progress or reducing pathogen multiplication (Parlevilet, 1979). Evaluation of resistance depends on several crucial factors including the choice of appropriate isolates for screening, screening methodology and the source of resistance. Screening techniques are very diverse mostly aiming at selecting resistant lines through the use of semi quantitative disease scales applied at the whole plant level or the canopy level. Recent identification of molecular markers tightly linked to resistance genes has greatly enhanced breeding programs by

making marker assisted selection possible and allowing the development of varieties with multiple disease resistance.

REVIEW OF LITERATURE

Plant disease need to be controlled to maintain the quality and abundance of food, feed and fiber produced by growers around the world. A variety of biological controls are available for use for an advanced survey of the nature and practice of biocontrol to suppress the plant diseases. Plant extracts have been used successful to control disease in plants and tuber crops (Amadioha and Obi 1999). Okigbo and Emoghene (2004) Swarnalata and Neelkantha Reddy, 2009.

Annona

Nene and Thapliyal (1979) studied the efficacy of leaf extracts of Annona squamosa by poisoned food technique.

Biological control of plant disease is safe and sustainable (Cook and Baker 1982, Spadaro and Cullino 2005, Sobowale et al 2008).

Recio (1989) studied the effect of methanolic extract of Annona squamosa on gram positive and gram negative bacteria.

Swami and Mukadam (2006) revealed that the extracts of Annona squamosa inhibiting Alternaria solani, Curvularia lunata, Fusarium oxysporium affecting green gram.

Ql Dang, Wkkin (2011) studied the efficiency of seed methanol extract on Phytopthora infestana and Puccinia recondite and nematodes also.

Ghangaonkar (2007) suggested that the extracts of Annona squamosa retarded the maximum growth of Botrytis cinera.

Kitherian Sahayaraj, R Namasivayam studied the the methanol extracts of Annona seeds over unit of cowpea.

N kalidindi, 2015 reviewed that methanol and chloroform extract of Anona plant were much more effective against Aspergillus niger, Fusarium solani, Alternaria alternata etc.

G. Darwin studied the percent inhibition of some phytopathogenic fungi by leaf extracts of seta phal (Annona) at 10 % in stem rot of groundnut.

Withania

The antifungal activity exhibited by the plants might be attributed to the presence of either single or synergetic effect of more than one compound. W. somnifera is found to be potent antifungal plants.

Mudgal et al (1996) observed that aqueous leaf extracts of Allium sativum, Datura alba and Withania somnifera inhibited the growth of Alternaria alternate.

A.P Ramteke, S.R. Patil The antifungal activity of W. somnifera root extract (at. 0.5, 1.0, 2.0, and 2.5 g) was studied against F.solani using clorimazole (1%).

Selitrennikoff (2001) stated that both aqueous and organic extracts of plant seeds completely inhibited the growth of Aspergillus flavus and Aspergillus niger.

Ye et al (2001) studied the activity of protease inhibitor in W.somnifera extracts which plays an important role in protection of plant tissues from pest and pathogens.

Deepa and Gowda (2002) identified an antifungal glycoprotein from W.somnifera which was tested against agronomically important pathogens.

Arora et al (2004) suggested that crude extracts of W.somnifera was effective on human pathogen bacteria.

Goel et al (2005) suggested that W. somnifera contains inhibitory withaniloids and glycol-withaniloids that are known to be highly effective against Meloidogyne javanica.

Ocimum

Abraham and Prakasan (2000) reported that at 10% concentration leaf extract of Azadiracta indica, Ocimum sanctum and Vitex negundo proved inhibitory against Geotricum conidium and Cladosporium oxysporium.

Gupta SK, J Prakash 2002 revealed the validation of claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant.

B ansod and Rai 2008, Anuadi et al 2010 reported that leaves of Ocimum sanctum show strong antifungal activities against the Aspergillus niger and Fusarium miniliforme.

A Kumar, R Shukla et al 2010 studied the chemical composition, antifungal and antiaflatoxigenic activies of Ocimum sanctum L. with special reference to its essential oil and its safety assessment as plant based antimicrobial.

A Khan, A Ahmed, Xess I et al 2010 tested the antifungal activity of essential oil Ocimum sanctum.

B Prakash, R Shukla et al 2011 tested the efficacy of chemically characterized Ocimum gratissinum L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin.

L. Prince et al (2011) reported that chloroform and ethanol extract of Ocimum sanctum shows moderate effect against Colletotricum falcatum.

Sowjanaya and Manohara et al 2012 tested the antifungal properties of aqueous extracts of Azadiracta indica, Lawsonia inermis, Allium sativum, Murraya koenigii and Ocimum sanctum at two different concentrations (5% and 10%) against the Keratinophilic fungus Microsporium gypseum.

DP Ray, S Shrivastava et al 2012 investigated the antifungal activity of the essential oil of Ocimum sanctum L. against plant pathogens like Rhizoctonia solani and Fusarium oxysporium.

R. Singh reviewed the antifungal efficacy of volatile oil constituents and acyl derivatives.

Aegle

P Singh, A Kumar et al 2009 tested an essential oil of Aegle marmelos as a safe plant based antimicrobial against post harvest microbial infestations and aflatoxin contamination of food commodities.

B.B. Mishra et al 2009 evaluated an antifungal anthroquinone from seeds of Aegle marmelos.

Vasant P. Pawar et al 2010 tested the efficacy of some plant extracts and suggested that 10% aqueous leaf extract of Parthenium hystrophorous, Azadiracta indica, Adhatoda vasica and Aegle marmelos retarded the growth of Alternaria alternate, Aspergillus flavus, and Curvularia lunata.

R. Sivaraj, A Balakrishnan et al (2011) reported the antifungal activity of Aegle marmelos, Ruta graveoleus, Optunia dellini, Euphorbia royleena.

Trichoderma sp.

D.K. Bell et al 1982 studied the antagonistic activities of Trichoderma isolates primarily T. harzianum against isolates of Sclerotium rolfsii, Phytophore parasitica, Pythium sps.

Weindling 1932 demostrated the antagonistic nature of fungal species from the genus Trichoderma.

Papavizas et al 1985, Dubey et al 2007, studied the mycoparasite ability of Trichoderma species against some economically important aerial and soil borne plant pathogens.

Elad et al (1980), Ashrafizadeh et al (2005), Dubey et al (2007) made numerous attempts to use Trichoderma sps on soil borne pathogens such as Sclerotinia, Fusarium, Pythium and Rhizoctonia species.

Demnis and Webster 1971 reported that Trichoderma sps are known to produce a number of antibiotics and some cell wall degrading enzymes.

Spiegel and Chet (1998) evaluated Trichoderma sps as a biocontrol agent against soil borne fungi and plant parasitic nematodes in Israel. They observed improved plant growth of nematode infected plants and decrease in root galling index exposed to T. Harzianum preparation.

Rangaswamy et al 2000 reported that Trichoderma viride stone and in combination with either neem or Castor cake was most effective in inhibiting root knot nematode, M. inognita infecting tomato.

Harman (2000) reported that Trichoderma sps presence in the rhizosphere of plants induce systemic resistance against pathogen.

Gao et al (2002) et al suggested that among the various species of Trichoderma, T. harzianum is considered to be the most effective biocontrol agents.

Vinale et al 2006 studied the antagoinistic effect of Trichoderma harzianum strains with the pathogen Rhizoctonia solani.

Woo and Lorito 2007 believed that Trichoderma secretes hydrolic enzymes at a constitutive level and detects the presence of another fungus by sensing the molecules released from the host by enzymatic degradation.

Screening

Brunet et al (1973) showed effective alternative method of screening white beans (Phaseolus vulgaris L.) for anthracnose (Colletotricum lindemuthianum) resistance using excused leaves of leaflet.

Iqbal et al (1992) reported that out of 87 genotypes of Vigna radiate tested in the field under artificial inoculated conditions only 4 genotypes were highly resistant to C.canescens, under 12 were moderately resistant, 16 intermediate and the remaining were susceptible.

Bashir et al (1993) screened 20 Vigna radiate genotypes against Cercospora canescens under field conditions and found NCM-10 was resistant and remaining genotypes were susceptible.

Katna et al (2001) evaluated forty one genotypes of urdbean (Vigna mungo) for their resistance to Cercospora leaf spot caused by Cercospora canescens and C. cruenta systems in a field experiment during the Kharif season and none of the genotypes were resistant or highly resistant to the disease under both cropping systems.

Raje and Rao (2002) evaluated two hundred germplasm lines along with six commercial varieties of mungbean against Cercospora leaf spot of which only thirty five genotypes were found resistant.

Daisy Bhandari et al (2003) screened 250 stocks of blackgram against Cercospora leaf spot of which 30 genotypes showed varied levels of resistance.

Cantonwin et al (2006) evaluated the effect of integrated management schedule against early leaf spot intensity decreased with increased fungicide applications, use of resistant cultivars and strip tillage.

Twizeyimana et al (2007) evaluated fourteen soyabean accessions and breeding lines for resistance to soyabean rust caused by the fungal Phakopsora pachyrhizi. They observed that inoculation of detached leaves with 1×10^{6} spores/ml resulted in a significantly higher total number of pustules and spores per unit leaf area than inoculations with lower spore concentrations.

MATERIALS AND METHODS

The agro climatic conditions of Kota are most favorable for production of fenugreek crop and other vegetables also. Several diseases has been frequently associated with fenugreek among these leaf spot caused by Cercospora traversiana losses the crop seriously. Therefore it is necessary to develop suitable technology for the management of Cercospora leaf spot in fenugreek. To fulfill this objective during present investigation, experiments were carried out to test the efficacy of some plant extracts (Annona, Aegle, Ocimum, Withania) and bioagents Trichoderma sps.. The experiments were conducted at Govt. P.G. College of Kota, Kota with the temperature range between 22ºc to 40ºc during the course of investigation.

General Procedure: In Vitro Experiments

Glassware and Cleaning

Glassware used for the experiments were of Borosil for eg. Petri plate, test tube, conical flask, beaker, measuring cylinder, pipette, cavity block, borer etc. were cleaned thoroughly and finally rinsed with distilled water. After this all glasswares were placed in potassium dichromate ($K_2Cr_2O_7$), 6ml of Conc. H_2SO_4 in 1000 ml of water for overnight and rinsed in tap water followed by dried in hot air over before use.

Sterilization

All the glassware were sterilized in an autoclave at 1.1 Kg/cm^2 pressure for 15 minutes and then kept in hot air oven at 550ºc for one hour. The solid and liquid media were sterilized at 1.1 Kg/cm^2 pressure for 15 minutes. Isolation and cultural studies were conducted under aseptic conditions in the laminar air flow cabinet. The working surface of laminar air flow was sterilized by swabbing with 70 percent ethanol.

Collection, Isolation of the Pathogen

Infected plants were collected from fenugreek fields of Kota district, Rajasthan as the source of inoculum. The plant parts were examined under microscope to confirm the presence of respective pathogen. The infected leaves showing typical symptoms of the disease were used for the isolation of pathogen. The infected parts were surface sterilized with 1% Sodium hypochloride solution for 30 seconds and washed serially three times in sterilized distillation water to remove the traces of Sodium hypochlorite if any and then transferred to sterilized petri plates containing potato dextrose agar (PDA).

Hyphal tip Isolation

This method was followed for maintaining pure culture, since this fungus is known to be heterokaryotic in nature. Hyphal tip isolation was done on water agar plates. Dilute spore suspension (8-10 spores/ml) was prepared in sterile distilled water. One ml of such suspension was spread uniformly on two percent water agar plates and the excess of which was aseptically drained. Single spore was than marked under the microscopic field with ink on the glass surface of the plate and it was allowed to germinate. Such plates were incubated at 27± 1ºc and periodically observed for germination of spores under the microscope. Hyphae was traced and marked with the ink, and then tip of hyphae was transferred to PDA slants with the help of cork bores under aseptic conditions and incubated at temperature at 27± 1ºc for 10 days. Later, mycellial bits of fungus were placed in the culture of petri plates containing potato dextrose agar medium and incubated at 27± 1ºc for 10 days. No saltation or sectoring was observed in the culture and it was concluded that, it was a pure culture of fungus. Such was used for further studies.

Maintenance of the culture

The fungal pathogen was sub cultured on PDA slants and allowed to grow at 25ºc for 20 days and such slants were preserved in refrigerator at 4ºc and sub cultured once in 30 days.

Growth studies on different solid media

The variation in cultural characters of Cercospora traversiana was studied on different synthetic and non synthetic solid media and the best media for the fungus growth was identified.

The growth characters of the fungus were studied on seven different solid media. All the media were sterilized at 121ºC, 15 pounds pressure for 15 min. Twenty ml of each of the medium was poured into 90 mm diameter petri dishes. Such plates were inoculated with 5 mm disc of fungal growth and incubated at 25ºC. Each treatment was replicated thrice; colony diameter was recorded by averaging the linear growth of the colony in two directions for each plate after 20th day of inoculation. The fungal colony color, surface elevation and sporulation were also recorded at the end of incubation period.

The different media used in the studies were follows:

Non Synthetic media	Synthetic media
1. Carrot dextrose agar	4. Asthana and Hawker's agar
2. Potato dextrose agar	5. Czapeck's (Dox) agar
3. Oat meal agar	6. Richard's agar
	7. Sabouraud's agar

In Vitro evaluations of Bio-agents against *Cercospora traversiana*

Bio-agents obtained indigenously as well other viz. Trichoderma viride, T. harzianum were evaluated for their efficacy under in vitro using dual culture technique against Cercospora traversiana fungus.

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelia disc of test fungus was inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist the bacterium was streaked at middle of the petriplates and two mycelial disc of the fungus were placed at opposite end. The

plates were incubated at 27±1ºC and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen is control plate was also recorded. The percent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincet (1947).

Trichoderma viride	Gene Bank (MTCC), Chandigarh, India
Trichoderma harzianum	

In Vitro evaluation of Botanicals

Plant based pesticides which are relatively economical, safe and non hazardous can be used successfully against the plant pathogenic fungi. In the present study following plant extracts were selected.

S.No.	Botanical Name	Common Name	Family	Parts Used
1.	Annona squamosa	Custard apple	Annonaceae	Leaf
2.	Aegle marmelose	Bael/Stone apple	Rutaceae	Leaf
3.	Withania somnifera	Ashwagandha	Solanceae	Leaf
4.	Ocimum sanctum	Tulsi/Holy Basil	Lamiaeceae	Leaf

Preparation of cold aqueous extract

Fresh plant material were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally thus obtained extract was used as stock solution.

To study the antifungal mechanism of plant extract the poisoned food technique was used (Nene and Thapliyal, 1973). Ten, fifteen and twenty ml of stock solution

was mixed with 90, 85, 80 ml of sterilized molten PDA medium respectively so as to get 10, 15 and 20 percent concentration. Controls were maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27ºc temperature and radial growth was taken when maximum growth was observed in control plate.

The efficacy of plant products or botanicals was expressed as percent inhibition of radial growth over the control which was calculated by using the following formula (Vincent 1947).

$$I = \frac{C-T}{C} \times 100$$

I = Percent inhibition

C = Partial growth in control

T = Partial growth in treatment

In Vivo management of leaf spot of fenugreek by Botanicals

A pot experiment was conducted in the Dept. Of Botany, Govt. College, Kota to find out the best treatment for control of leaf spot disease of fenugreek caused by Cercospora traversiana. An adequate amount of soil necessary for experiment containing two part soil, one part farm yard manure and one part sand was thoroughly mixed and passed through an ordinary course sieve (2 meshes). The mixture was then steam sterilized in an autoclave for two hours and was left in cage house for at least 15 days for maturation. The fenugreek plants were grown in earthern pots which were washed, cleaned and disinfected before use. After filling the pots 2-3 seedlings per pot were transplanted. The pots were watered daily and plants were maintained flooded. The effective botanicals evaluated in invitro studies were further evaluated in under pot culture. Each treatment was replicated thrice. The mycelia bits were smeared on the leaf surface to create the disease. After initiation of symptoms the Botanicals like Annona squamosa, Aegle marmelose, Withania somnifera, Ocimum sanctum were sprayed individually to

respective sets of p[ots. Fenugreek plants smeared with mycelia bits without botanical treatment served as control.

The treatments were as follows:

C = Control

T_1 = Annona squamosa @ 20%

T_2 = Aegle marmelose @ 20%

T_3 = Withania somnifera @ 20%

T_4 = Ocimum sanctum @ 20%

I = Inoculated

T_1+I = inoculated + Anona extract

T_2+I = inoculated + Aegle extract

T_3+I = inoculated + Withania extract

T_4+I = inoculated + Ocimum extract

Observations were recorded on severity of disease by using 0-5 scale and percent disease index was calculated by using the formula given by (Wheeler, 1969).

$$\text{PDI} = \frac{Sum\ of\ Individual\ disease\ rating}{Number\ of\ leaves\ observed \times Maximum\ disease\ grade} \times 100$$

In Vivo management of leaf spot disease of fenugreek by biorationals

A pot experiment was conducted in the Dept. Of Botany, Govt. College, Kota to find out the best treatment for control of leaf spot of fenugreek caused by Cercospora traversiana. The fenugreek plants were grown in earthern pots. The eefective bioagents evaluated in in vitro studies were further evaluated under pot culture with three replicates. Mycellium bits were smeared on the leaf surface for artificial inoculation of plants. After initiation of symptoms the bioagents like Trichoderma sps. were sprayed individually to respective set of pots. Observations were recorded on severity of disease by using 0-5 scale and PDI was calculated by using the formula given by Wheeler (1969).

The treatments were as follows:

C = Control

T_5 = Trichoderma viride

T_6 = Trichoderma harzianum

I = Inoculated

T_5 + I = T. viride + inoculated

T_6 + I = T. harzianum + inoculated

Screening

Five genotypes were screened against Cercospora leaf spot of fenugreek in vitro by using detached leaf technique to identify the resistance sources using 0 to 9 scales (Mayr and Datar, 1986).

Screening was done by using detached leaf technique for detaching resistance to Cercospora traversiana in fenugreek leaves from healthy fenugreek plants were excised, surface sterilized for 30 sec. in 0.05 percent $NaOCl_3$ solution and inoculated with 0.5 mm of 2 day old Cercospora traversiana culture growth on carrot dextrose agar. Inoculated leaves were placed in petri plates containing one sterile filter paper and 10 ml of sterile distilled water. Petri dishes were sealed with parafilm and incubated in a growth chamber at 27ºC with constant fluorescent light.

Number of lesions per leaf and percentage of leaf area infected were recorded daily for six days after inoculation Genotype ranking for lesion development and percentage of infected leaf area were similar to know ranking of the same genotype for field resistance.

Available fenugreek genotypes were screened for Cercospora leaf spot disease. The different genotype were screened in the laboratory conditions to identify the resistant source by detached leaf technique using 0 to 9 scale (Mayr and Datar, 1986) as reported below.

Scale	Description	Category
0	No leaf showing any symptoms	Immune
1	1% or less symptoms	Resistant
3	1-10 % symptoms	Moderately resistant
5	11-20 % symptoms	Moderately susceptible
7	21-50 % symptoms	Susceptible
9	50% symptoms	Highly susceptible

OBSERVATIONS

Experiment was carried out for the management of Cercospora traversiana infecting fenugreek plant causing leaf spot disease of fenugreek through plant extracts such as Annona, Aegle, Withania and Ocimum in In-vitro conditions. Further efficacy of plant extracts was tested under pot trials (Table 9-11), (Fig 12-20) and (Plate-15-19).

Two biocontrol agents such as Trichoderma viridae and Trichoderma harzianum were tested against Cercospora traversiana. Data pertaining to the effect of various plant extracts under pot trials on plant growth characters on both healthy and infected plants were recorded and statistically analysed to interprete the experimental findings from pot trials.

(A) In vitro Evaluation of botanicals

As plant extracts are cost effective means of management, an efforts was made to know the efficacy of different plant extracts against C. traversiana. This was carried out by adopting the poisoned food technique as described in Material and Methods. The results are furnished in Table-9, Fig-12, Plate-16.

Four botanicals were evaluated against C. traversiana. The results revealed that the effect of plant extracts on the fungal growth was significant. Among botanicals Annona squamosa (64.4%). was found effective in inhibiting mycelia growth. Ocimum sanctum (22.93%) and Withania somnifera (18.4%) were next best treatments followed by Aegle marmelosa (17.8%).

The plant extracts at 20 percent were significantly superior over 10 percent. Annona squamosa (64.4%) at 20 percent was the best treatment and significantly superior to all other treatments. Next best was Ocimum sanctum (44.96). At 10 percent concentration Ocimum sanctum (22.93) was least effective in inhibiting mycelia growth.

(a) In vitro evaluation of Biocontrol agents

Two biocontrol agents viz. Trichoderma viride and Trichoderma harzianum were evaluated against C. traversiana and the results are presented in Table-10, Fig-13 and Plate-16.

The results revealed that all the antagonists significantly reduced the growth of C. traversiana. After measuring the colony diameter of C. traversiana, it was noticed that maximum reduction in colony growth was observed in Trichoderma viridae (73.00%) which was significantly superior to Trichoderma harzianum (71.33%).

(B) In vivo management of leaf spot disease of fenugreek by botanicals

A pot experiment was conducted to evaluate the efficacy of four plant extracts viz. Annona, Aegle, Withania and Ocimum sps. as pre sowing seed treatment and foliar spray against leaf spot of fenugreek as explained earlier. The data are presented in (Table-11), (Fig 14-20) and (Plate-18).

(1) Management through various plant extracts

Observations on plant growth characters, including yield in terms of seed weight per plant and percent disease incidence were recorded and presented in Table-11.

Plant Growth Characters: Results revealed that all the treatments enhanced plant growth characters including yield in terms of seed weight per plant and reduced percent disease incidence.

Shoot Length: The maximum shoot length 53.6 cm was recorded with seed treatment and foliar spray of Annona squamosa (T_1) followed by 52.3 cm in T_4 (Ocimum extract), 52.1 cm in T_3 (Withania extract) and then 50.2 cm in T_2 (Aegle extract). In untreated healthy control the shoot length was recorded to be 40.00 cm. In treated plus inoculated fenugreek plants maximum shoot length was observed in T_1+ I followed by T_4 + I and T_3+ I and then T_2+I as 52.3 cm, 51.2 cm, 51.1 cm and 49.9 cm respectively as compared to only inoculated diseased plant (I) 32.8 cm (Fig-14).

Root Length: Among different treatments the maximum root length was observed in T_1 followed by T_4, then in T_3 and T_2 as 15.3 cm, 15.00 cm, 14.8 cm and 14.2 cm as compared to control (12.5 cm). In treated plus inoculated plants maximum root length was 14.3 cm in T_1+I, then 14.1 cm in T_4+I, then 13.6 cm in T_3+I and 13.2 cm in T_2+I, However the minimum root length 8.9 cm was observed in I inoculated diseased plant (Fig-15).

Fresh Weight: Fenugreek plant treated with T_1 showed significant higher fresh weight 19.92 g followed by 19.09 g in T_4 and 18.98 g in T_3 and 18.01 g in T_2 treatment. In healthy control it was recorded as 16.2 g. The fresh weight of treated plus inoculated plants were observed as 19.90 g, 18.97 g, 18.32 g, 17.89 g in T_1+I, T_4+I, T_3+I and T_2+I respectively as compared to only inoculated (diseased) plant (I) as 12.9 g (Fig-16).

Dry Weight: Data on dry weight of plant observed was 5.27 gm as highest weight in T_1 followed by 4.81 g in T_4, 4.02 g in T_3 and 4.00 g in T_2 over healthy control (3.71 g). The dry weight of T_1+I, T_4+I, T_3+I and T_2+I was 5.01 g, 4.32 g, 4.00 g and 3.92 g as compared to only inoculated (diseased) plants 2.21 g (Fig-17).

Number of Leaves: The number of leaves per plant of fenugreek was almost same in all the treatments and observed was 40 except in the inoculated diseased plants (I) where it was recorded to be 28.

Leaf Area: Among the different treatment maximum leaf area was observed in T_1 followed by T_4, T_3 and T_2 as, 12.69 cm^2, 12.61 cm^2, 12.45 cm^2 and 12.41 cm^2 as compared to healthy control 12.35 cm^2. In treated plus inoculated plants maximum leaf area was observed in T_1+I, followed by T_4+I, T_3+I and T_2+I as 12.32 cm^2, 12.31 cm^2, 12.29 cm^2 and 12.26 cm^2 respectively as compared to the inoculated diseased plant, Where the leaf area was observed as 10.26 cm^2 (Fig-18).

Seed Weight: The yield of plant in terms of seed weight was recorded. The maximum seed weight of 182.2 g was recorded with pre sowing seed treatment and foliar treatment of fenugreek plant with Annona extract (T_1) followed by

181.7 g at T_4 , 181.4 g at T_3 and 180.6 g at T_2 as compared to healthy control where seed weight was observed at 177.6 g. In treated plus inoculated plants maximum seed weight was observed in T_1+I followed by T_4+I then T_3+I and T_2+I as 180.0 g, 179.2 g, 179 g, 178.3 g respectively. The maximum seed weight 152.2 g was observed in only inoculated (diseased) plant (I) (Fig-19).

Percent disease Incidence (PDI) : Among the different treatments minimum PDI of 3.36% was observed in T_1+I, followed by 5.89% in T_4+I, 5.92% in T_3+I and 6.23% in T_2+I as compared to only inoculated (diseased) plants (I) where the highest PDI value that is 35.32% was observed (Fig-20).

(2) Management through Biorationals

Observations on plant growth character, including yield in terms of seed weight per plant and percent disease incidence were recorded and presented in Table-12, (Fig 21-27).

Shoot Length : In healthy control the shoot length of 45.3 cm was recorded with minimum shoot length of 36.2 cm in inoculated showed higher value of 47.4 in T_5+I followed by 46.7 cm in T_6+I treatment with Trichoderma species (Fig-21).

Root Length: Among both the treatments on inoculated plants T_5 showed significant higher value of 15.13 cm followed by 15.01 cm in T_6. However treatment inoculated plants showed root length of 15.03 cm in T_5+I and 14.69 cm in T_6+I as compared to inoculated (diseased) plant (I) 8.69 cm (Fig-22).

Fresh Weight: Fenugreek plant treated with T_5 showed significant higher fresh weight 17.93 g followed by 17.41 gm in T_6 treatment. In healthy control it was recorded as 16.24 g. The fresh weight of treated plus inoculated diseased were observed as 17.64 g in T_5+I followed by 17.21g in T_6+I as compared to only inoculated (diseased) plant (I) as 14.31 g (Fig-23).

Dry Weight: Data on dry weight of plant observed was 4.25 g as highest weight in T_5 , followed by 4.11 g in T_6 treatment over healthy control 3.73 g. The dry

weight of T_5+I and T_6+I was 4.13 g and 3.92 g as compared to only diseased plant (I) 2.71 g (Fig-24).

Number of leaves: The number of leaves per plant of fenugreek plant was almost same in all the treatments and observed was 52 in control, whereas 41 in inoculated diseased (I) plant.

Leaf Area: Among the different treatments maximum leaf area was observed in T_5 followed by T_6 as 12.39 cm^2 and 12.28 cm^2 as compared to healthy control 12.22 cm^2. In treated plus inoculated plants maximum leaf area was observed in T_5+I, followed by T_6+I as 12.32 cm^2 and 12.26 cm^2 respectively as compared to the only diseased plant (I) where the leaf area was observed as 9.61 cm^2 (Fig-25).

Seed weight: The yield of plant in terms of seed weight was recorded. The maximum seed weight was recorded 182.5 gm was recorded with pre sowing seed treatment of fenugreek plant with Trichoderma viride followed by 181.9 g in T_6 treatment as compared to control where the seed weight was observed as 177.6 g. In treated and inoculated plants higher seed weight was observed in T_5+I, followed by T_6+I as 180.6 g and 179.3 respectively. The minimum seed weight 153.3 g was observed in only inoculated plant I (Fig-26).

Percent Disease Incidence: According to the number of lesions on plant parts percent disease incidence was recorded with all treatments over inoculated and control. The Lower PDI 5.92 % was recorded with the pre sowing treatment of fenugreek plant with Trichoderma viride, followed by 6.23% at T_6+I, as compared to inoculated plant (I) where the highest PDI value that is 34.21% was observed (Fig-27).

(3) **Screening for Resistance:** five fenugreek genotypes were screened against C.traversiana during crop season as described in “Material and Methods” and the results are presented in the Table 13, Plate - 20, 21.

Disease reaction	Name of genotype
Immune	
Resistant	RMT-305
Moderately resistant	Hisar sonali, RMT-143
Moderately susceptible	RMT-303
Susceptible	RMT-1
Highly susceptible	

It was observed theat among the five genotypes evaluated, RMT-305 was found to be resistant, Hisar sonali and RMT-143 were moderately resistant whereas RMT-303 was found to be moderately susceptible. RMT-1 the highest growing variety was found to be susceptible towards the leaf spot pathogen.

RESULTS AND DISCUSSION

Overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The practical use of natural compounds as control agents is receiving increased attention due to their nontoxicity and biodegradability (Mason, 1996). The use of ecofriendly methods has been taken a new dimension in controlling certain plant disease. The uses of plant - derived products as disease control agents have been studied since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Lee et al 2007).

The present study revealed the antagonistic property of various plant extracts like Annona, Aegle, Withania and Ocimum sps. against C.traversiana. As Botanicals degrade more rapidly than most chemical pesticide and therefore are considered to be ecofriendly and less likely to kill beneficial pests than synthetic pesticides with longer environmental retention. Some efforts were made to know the efficacy of different control agents against C.traversiana. The results revealed that the effect of these control agents on the growth of fungus C.traversiana was significant.

Different evaluation made on various plant extracts on percent inhibition of C.traversiana growth in culture efficacy of phytoextracts and biorationals under pot trials are presented in (Table 9-12), (Fig 12-27) and (Plate 17-19).

Management through Botanicals against Leaf spot pathogen

A significant reduction in mycelial growth of C.traversiana pathogen causing leaf spot disease of fenugreek was observed when treated with methanoic extract of Annona leaves. The mycelial growyh was reduced by 25.4% and 64.4% at 10% and 20% concentrations of Annona methanolic extract as compared to control. The inhibition rates increased twice with double concentrations with Ghangaonkar

(2007) who found that the extract of Annona squamosa was inhibitory for the growth of Alternaria porri, Aspergillus niger, Fusarium oxysporium and Cladosporium allii. Bautista- Banos S.M. et al (2000) also investigated complete inhibition of mycellial growth and sporulation of Rhizopus stolonifer with leaf and bark extracts of Annona squamosa. Studies conducted by Swami and Mukadam (2000) had also demonstrated the inhibitory action of extract of Annona squamosa on Alternaria solani, Curvularia lunata and Fusarium oxysoporium. The present rexults are also in corroboration with G. Darwin, 2013 who observed that 10% plant extract of Annona squamosa reduced the mycelial growth of Sclerotium rolfsii upto 62.04%. The marked reduction in the mycelial growth of pathogen with annona leaf extract may be related to the glycosides, saponins, tannins, flavonoids, phenols present in it (N. Kalidindi, 2015).

The result of the present experiment showed that there was marked reduction in the mycelial growth of C. traversiana when treated with Ocimum santum extract under on vitro condition. The mycelial growth was reduced by 22.93% and 44.96% at 10% and 20% concentration. The inhibition rates increased with increasing concentration of the control agent. The results regarding regarding the efficacy of Ocimum sanctum are in accordance with A.C. Amadioha (2008) who studied both aqueous and alcoholic extracts of O. sanctum on Colletotricum lindermuthianum on cowpea, T.K. Mahapatra and S.N. Tewari (1994) also investigated ethanoic extract of O. sanctum against pathogens causing collar rot and yellow rot diseases of groundnut. Similarly fungicidial property of Ocimum sanctum against Fusarium oxysporium and Rhizoctonia solani was examined by Shivpuri et al (1997).

The data on the inhibition percentage of mycelial growth of C. traversiana revealed that at 10% and 20% concentration of Withania leaf extract the growth inhibitory percentage 18.4% and 20.33% was recorded. The results revealed that the inhibition rates increased with increasing concentrations of the control agent. Above said results are in conformation with Mughal et al (1996) who observed that aqueous leaf extract of Withania somnifera with other plant extracts inhibited

the growth of Alternaria alternata, A. brassicola and Myrothecium rosidum. Z. S. Khan and S. Nasxen (2010) investigated the protein fractions of Lawsonia inermis and Withania somnifera on the mycelial growth of Bipolaris oryzae and Collectotricum lindenmuthianum and results four to five times more percent inhibition through protein fractions of plants. C. Venkateshwar et al (2014) observed alcoholic root and leaves extracts of Withania somnifera inhibiting growth of Fusarium oxysporium and Colletotricum capsici. The oil of Withania Somnifera with other was found to be moderately active against Aspergillus niger and A. funigants by Sunita Bansod and Mahendra Rai (2008). The antifungal activity of Withania can be attributed to the fact that Withanolides and Withania the main phytochemical constituents although volatile soluble with ethanol and water thereby maintaining it antifungal property (K. Bone 1996, M. Elaska 1990). Similarly K.G. Girish et al (2006) revealed potent antimicrobial activity of Withania somnifera glycoprotein against phytopathogenic fungi.

The results presented in Table-9 indicated that inhibition of mycelial growth of C. traversiana obtained with Aegle marmelos plant extract at 10% was recorded 17.8% and at 20% it was recorded as 19.96%. The percent inhibtion was minimum as compared to other treatments at both the concentrations. Aegle marmelos contains varied classes of compounds like Cowmarins (Marmelosin, Marmesin), alkaloids (Aeglin) etc. which has enormous effect with antifungal action also (S. Dhankar et al, 2011). Experimental results were in corroboration with G. Shiv, S. Gharwal (2015) who screened organic extracts Aegle marmelos against Candida albicans. The results are in harmony with Saroj Kothari, Vaibhav mishra et al 2011 whoresulted the antifungal activity of Aegle marmelos against Candida sps. and Aspergillus flavis. They also depicted that organic plant extract from leaves showed variable broad spectrum antimicrobial activies, Pitre S and Shrivastava S.K. (1987) also demonstrated the antifungal activity of ethanoic root extract of A. marmelos against Aspergillus fumigants and Trichphyton mentagrophytes.

From the Table-9 it was very clear that all the treatments reduced fungus growth in culture. On comparing the overall results it can be concluded that Annona

squamosa methanoic plant extract showed the maximum inhibition 64.4% at 20% concentration of mycelial growth. It was followed by Ocimum sanctum extract at 10% concentration (22.93%) and at 20% concentration (44.96%). The inhibition in mycelial growth varied from 19.96% to 64.4% in different control agents. The efficacy was in order of Annona squamosa > Ocimum sanctum > Withania somnifera > Aegle marmelosa extract.

Management through Bioagents against leaf spot pathogen

G. E. Harman (2006) has studied several strains of Trichoderma as biocontrol agents against fungal diseases of plants. Many species in this genus are characterized as opportunistic avirulent plant symbionts. The result of the present experiment showed that there was marked reduction in the mycelial growth of C. traversian when treated with Trichoderma species at 20% concentration. The mycelial growth was reduced by 73.00% in T. viride followed by 71.33% in T.harzianum treated. The results regarding the efficacy of Trichoderma species are in accordance with Faheem Amin et al (2010) who studied the ability of Trichoderma strains against Rhizoctomia solani (isolates from tomato), Sclerotium rolfsii (Causing collar rot of tomato) and Sclerotimia sclerotium (Causing web blight of beans) and found maximum inhibition of mycelial growth upto 71.41 percent in T. viride. Similar results were investigated by Y. Elad et al (1980) against Sclerotium rolfsii and Rhizoctonia solani by Trichoderma harzianum where significant disease reduction of 20% was found. Clandia et al (1997) also studied the inhibitory effects of T. Viride and T. harzianum against Fusarium moniliforme and Aspergillus flavus. The inhibitory action of trichoderma species can be attributed to the fact that they produce inhibitory volatile compounds, antibiotics and extracellular enzymes. Fungitoxic action of Trichoderma sps. on pathogenic Pythium sps. was first reported by Allen and hanseler (1934).

Evaluation of control agents against C. traversiana in pot experiment

Results of pot trials indicated that at different concentration all the botanicals were significantly effective in controlling the leaf spot disease incidence and resulted in improving the growth of host plant (Fenugreek).

The results regarding the efficacy of various plant extracts indicated that maximum shoot length, root length, fresh weight, dry weight, leaf area & seed weight per plant were obtained by T_1 treatment followed by T_4 and T_3 than T_2 which were recorded in Table-11. In treated plus inoculated plants the treatment T_1 + I was the most effective followed T_4 +I and T_3 + I and T_2 + I (Table-11). All the growth characters recorded were maximum for Annona extract and minimum for Aegle extract. The results were in corroboration with Chaterjee et al (1995) who studied the methanoic extracts of some medicinal plants and this Biological activies.

An attempt was made in present study to observe the efficacy of different phytoextracts at different concentration Annona squamosa, Aegle marmelosa, Withania somnifera and Ocimum sanctum on the fungal culture of C. traversiana causing leaf spot of fenugreek. All the four ecofriendly control measures as well as bioagents Trichoderma viride and T. harzianum showed antifungal activity against the pathogen. Studies on different control agents under pot trials also revealed that Annona extracts and T. viride were more effective followed by other treatments. At all the treatments plant growth characters showed significant increase with reduction in percent disease incidence (PDI) and increased yield.

The study will able to provide us the efficient disease management of Fenugreek crop by using control agents which are very cost effective and ecofriendly in developing sustainable management.

Screening

Use of resistant cultivars is the best method for the plant disease control. In the present study, an attempt was made to locate the source of reisitance in fenugreek cultivars against leaf spot pathogen, Cercospora traversiana. In the present study, five variety namely RMT-1, RMT-305, RMT-303, RMT-143 and Hisar sonali were studied and results indicated that RMT-1 extremely cultivated variety was found to be susceptible to the leaf spot pathogen and other diseases also (Plate-22). RMT-303 cultivar was moderately susceptible followed with moderately resistant varities Hisar sonali, RMT- 143. The most resistant variety was recorded as RMT-305. Similar findings were attributed by Rajib Prasad and Acharya et al (2014) who studied 20 selected accessions of T. foenum graecum against C. traversiana and depicted L3717 and PI138687 as resistant and L3698 and F86 as moderately resistant towards the pathogen. Tivoli et al (2006) concluded that use of disease resistant crop cultivars is regarded as an economical and durable method of controlling fungal diseases. Above said results were in regarding to the data of Spice board of India and MPUAT, Udaipur (Methi – Importance and Production).

The results showed that all above treatments of different botanicals, bioagents and screening of accessions can be used as an alternative source in controlling diseases on host plant. The results of the present research work will be useful for detecting effective ecofriendly measures to manage leaf spot and other diseases in Fenugreek crop. The knowledge enlisted during the present investigation will well-head for further research work on biology, epidemiology and management of the disease and others.

Future line of work

The present investigation has answered many questions and provided new informations but also arised new ideas on fungal diseases of fenugreek which may be helpful to invent new strategies in improving the health of plant.

1. Detailed studies on other plant parasitic fungi associated with fenugreek crop are required.
2. Intensive survey for fungal diseases of fenugreek all over the country for assessing the effects of pathogens and yield quality should be developed.
2. Botanicals used in invitro studies should be applied under field conditions and evaluate their efficacy.
3. More resistant varieties should be developed and introduced to farmers to get better yield of crop.

CONCLUSION

Studies on Histopathology, Biochemistry and control of fungal diseases in Fenugreek were carried out and the results obtained in the research were concluded as below:-

1. Fungal diseases of fenugreek were observed in 16 different localities of Kota district. Mainly four fungal diseases especially Cercospora leaf spot was found to be more prominent on the fenugreek crop. The occurrence of fungal diseases of fenugreek in the study area may be due to the favourable climatic conditions, amount of rainfall, moderate temperature, relative high humidity and suitable soil conditions. The extensive survey reports that highest PDI % in Ladpura tehsil for all the studied diseases is supported by the fact that this area is more abundant to fenugreek crop with heavy crop cultivation and favourable edaphic factors in the area for establishment of various fungus.
2. The physiochemical analysis of soil with special reference to available nitrogen content, amount of phosphorous, potassium and pH, EC, OC concluded that the soil is favourable for growth of host plant and pathogens. Excess amount of nitrogen content guage the establishment of fungal flora with the crop plants.
3. The weed flora associated with fenugreek crop in the study areas results in uptake of nutrients and affects the growth and yield of crop. Weeds associated with the crop concluded that some of the fungal pathogens also survive on weeds as an alternative host. Intercropping of some plants like Tagetus and Crysanthamum also shows symptoms of Cercospora leaf spot infection which concludes the presence of pathogen species in and around the growing areas.
4. From the host parasite interaction study it can be concluded that during penetration and pathogenicity remarkable morphological and anatomical changes occur in the crop by the pathogen for their successful establishment, resulting in great loss of plant growth and yield. Heavy

losses were reported from the leaf spot disease of fenugreek as it decreases the number of leaves and leaf area per plant and inhibits the metabolism physiologically.

5. Comparitive biochemical investigation of diseased and healthy plants in concern to levels of chlorophyll, carbohydrate, protein, proline, amino acids and phenol during pathogenesis also accounted many alterations in their values. It has helped in understanding the biochemical nature of pathogen and mechanism of resistance by host plant.
6. Management of disease through different plant extracts Annona squamosa, Aegle marmelose, Withania somnifera, Ocimum sanctum in addition to bioagents Trichoderma species were found to be effective on the fungal culture of C. traversiana. From the present investigation it may be concluded that such botanicals and bioagents can be used as an ecofriendly source of management and to gain better yield of fenugreek crop without ant hazardous effect on nature .Screening of different varieties for resistance towards the leaf spot pathogen also accounts as a better method of managing the disease and improving the quality of crop. The knowledge gained during the present investigation will be a source of futher research work on the biology of the pathogen and management of various diseases.

INTRODUCTION

Fenugreek is one of the oldest known medicinal plants which are used for various purposes and cultivated almost all around the world. From the world production of fenugreek it can be estimated that more than half is produced in India mainly in Rajasthan, Gujarat, Madhya Pradesh and to a limited extent in Andhra Pradesh, Tamil nadu, Haryana, Maharashtra and Punjab. Rajasthan is considered as "Fenugreek bowl" of the country and contributes 90 percent to the country's production. It is commonly cultivated in Kota district of Rajasthan. Fenugreek has many neutraceutical and medicinal properties and used as an ingredient in several ayurvedic medicines. Fungal pathogens are more dominant on this crop and found to be very destructive. The present research was undertaken on various fungal diseases on fenugreek crop, disease incidence, most prevalent disease with histopathological and biochemical changes, host parasite interaction, weed association with crop and biocontroling the disease.

SURVEY

Different localities of Kota district were surveyed with the objectives to know different fungal pathogens associated with fenugreek crop to know the occurrence and disease incidence and more prominent infestation .in the present investigation an intensive survey of the fungal pathogens was conducted at various localities viz.Chandresal, Jhalkhera ,Gangaycha, Borabas, Mandana ,Morak, Nayagaon (Tehsil-Ladpura), Borkheda, Sogaria ,Bhadana (Tehsil-Digod) Chechat, Nemana , Panda (Tehsil-Ramganjmandi) Arjunpura , Tirat (Tehsil–Pipalda) Sankhera (Tehsil-Sangod) covering the major fenugreek growing areas of tehsils of Kota district during Rabi season of 2010-2011 (from Novenber to March).Total 800 plants were studied to study the prevalence and distribution of fenugreek diseases in the surveyed area .Mainly four fungal genera were found to be dominantly associated with fenugreek crop viz. Erysiphae polygoni (powdery mildew), Perenospora trigonallae (Downy mildew), Leaf spot (Cercospora traversiana) and Blight (Alternaria alternata).

Powdery mildew disease was reported from 8 surveyed localities viz. Jhalkhera, Gangaycha, Nayagaon (Tehsil-Ladpura), Borkheda (Tehsil-Digod), Chechat, Nemana, Panda (Tehsil-Ramganjmandi), Tirat (Tehsil-Pipalda).

Downy mildew disease prevailed in 8 surveyed localities viz. Chandresal, Gangaycha (Tehsil-Ladpura), Borkheda, Sogaria (Tehsil Digod), Nimana, Panda (Tehsil Ramganjmandi) Arjunpura, Tirat (Tehsil Pipalda).

Report of Occurrence of leaf blight disease during survey was observed from 7 localities viz. Chandresal, Jhalkhera, Nayagaon (Tehsil-Ladpura), Bhadana (Tehsil-Digod), Chechat (Tehsil-Ramganjmandi), Arjunpura (Tehsil–Pipalda), Sankhera (Tehsil-Sangod).

The fungus Cercospora traversiana was more prominent and highly pathogenic in 11 surveyed localities causing severe losses to the crop. The prevalence order of fungal diseases was leaf spot > powdery mildew > downy mildew > leaf blight. Maximum disease incidence was recorded 63.33% from Ladpura Tehsil and minimum 23 % from Pipalda Tehsil of the district. The survey study enabled us to locate the prone spots for disease in the study area and gave us clear morphological characters of fungal pathogen and helped to developed ecofriendly measures using botanicals and bioagents to control the disease.

SOIL ANALYSIS

Soil acts as an emerging medium, habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality and a medium for plant growth. the present study was conducted to determine the physiochemical status of soil and its influence on fungal diseases especially leaf spot caused by Cercospora traversiana in fenugreek growing areas of Kota district of Rajasthan. Physiochemical analysis for soil pH, EC, OC (organic carbon) and availability of total nitrogen (N), Available Phosphorous (P), Available Potassium (K) contents were done in laboratory by following standard methods. Major soil types in Kota district were deep black clayey (43.15%), Deep brown clayey (23.37%) and deep

brown loamy (12.41%).In the present study soil pH was found to be neutral to alkaline in the range of 7.1 to 8.6, the value of EC ranged between 220 µs/cm to 440 µs/cm, value of OC ranged from 0.34% to 0.85%, the total nitrogen contained varied from 0.036 % to 0.086 %, Available phosphorous contained were ranged from 0.26 Kg/ha to 0.66 Kg/ha and the amount of potassium was found of range between 349 Kg/ha to 492 Kg/ha. The amount of available nutrients in soil was correlated with the occurence and prevalence of various diseases in the area. Soil analysis can be a great help for farmers for applying appropriate amount of fertilizers and improved disease management of plant disease especially leaf spot of fenugreek.

ANALYSIS OF WEED FLORA

The importance, characteristics, positive and negative impacts and future role of weeds as an integral part of the natural and agroecosystem were evaluated and discussed. A systematic study on the distributional pattern of weeds in the fenugreek fields of surveyed area was under taken to prepare a detail account of weed- plant interaction and diversity of fenugreek fields in Kota of Rajasthan. Samples were collected in 16 different sites randomly; taxonomic enumeration was performed with fresh materials in the laboratory. For detail studies herbarium sheets were prepared and examined. A total number of 7 weed species of 6 families viz. Chenopodium sps., Melilotus sps., Spergula arvensis, Anagallis arvensis, Convolvulus sps. and Cynodon dactylon from Amaranthceae, Fabaceae, Caryophyllaceae, Primulaceae, Convovulaceae, Poaceae families were recorded respectively. Weeds played an important role in nutritional loss as well as alternative/ primary host for different pathogens. Intercropped species such as Tagetus, Crysanthemum also showed symptoms of leaf spot caused by Cercospora sps.

HOST PARASITE INTERACTION

The objectives of the present work was to investigate the morphological and anatomical changes occurring in the fenugreek plant specially due to infection of

leaf spot pathogen Cercospora traversiana for this purpose both external symptoms and histopathological changes in the leaf structure of diseased plant was carried out and compared with the healthy plant and healthy leaf structure. The study of morphological symptoms was carried out in pot trials at 15 days of the growth of the pathogen. Thin and fine sections of the leaves were cut to study the difference in anatomical structure of healthy and infected leaves. The sections of the both the healthy and infected leaves were cut and passed in alcohol series for dehydration, then stained with both saffranin and cotton blue. The leaves were then mounted in anhydrous glycerol for histological examination. The isolation of fungus from the surface of the inoculated leaves, further confirmed the pathogen infection according to Koch's Postulates.

Due to infection of C. traversiana in fenugreek plant considerable changes in the morphological symptoms of plant occurred and observed. Several structural changes had been noticed in disease fenugreek plant with disintegrated tissues and necrotic tissues. Pathogen caused extensive damage to leaf tissues by destroying epidermal and mesophyll cells and degrad in cell wall. The present observation and result concluded that increased focus should be placed on considering the dynamics of plant growth and developing high yielding spot resistant varieties and practical control measures.

Biochemical Analysis

The present investigation resulted that leaf spot disease is major destructive foliar disease of fenugreek in growing areas which interfere with the physiological and biochemical processes of healthy plants. Present study was emphasized on monitoring the changes in endogenous levels of chlorophyll contents, quantity of carbohydrate, protein, Amino acids, proline and phenol in fenugreek plant in response to leaf spot disease under all the growth stages.

The surface sterilized seeds of most common, locally grown cultivar RMT-1 of fenugreek were sown and seedlings were then transplanted in pots containing sterile soil under green house condition. The plants were tested for biochemical

estimation after every 30, 60, 90 and 120 days (at maturity) in both healthy and infected situations using standard methods. The data on chlorophyll content indicated the amount of chlorophyll a, chlorophyll b and total chlorophyll was higher in healthy plant over the C. traversiana infected plant. The fall in chlorophyll a value was from 33.36% to 27.05% due to diseased condition of plant. The reduction percent in chlorophyll “b” was from 39.31 percent to 33.42 percent in infected plant over the healthy one. Overall chlorophyll content shows the same trend. Decrease in the amount of total chlorophyll from 41.39 percent to 31.86 percent in infected plant over healthy plant.

In the present investigation there was decrease in carbohydrate of infected fenugreek plant over healthy at 30, 60, 90 and 120 days ranged from 29.82 % to 20.37%. The amount of protein content was higher in healthy plant then the leaf spot infected plant at different growth stages. The decrease in protein content ranged from 10.46 percent to 12.39 percent with a mean value of 11.34 percent in infected plant over healthy plant. A significant reduction in terms of percentage ranged from 19.92 percent 29.95 percent in the amount of amino acids in diseased plant over healthy plant was observed. Considering diseased condition of plant as important biotic stress, quantitative analysis of proline was investigated. The percent increase in proline content in diseased plant over healthy plant ranged between 18.05% to 30.43% with the mean value of 25.55%. Defence strategies of plant against pathogens results in accumulation of phenols. The quantity of phenol in diseased plant was significantly higher than that of healthy plant which ranged from 16.37 percent to 33.33 percent in infected plants to healthy plants at all the growth stages.

All the above comparative studies on biochemical changes during pathogenesis of infected plant helped in understanding the nature, mechanism of resistance which will help to develop some disease resistant genotypes to protect the crop plant.

MANAGEMENT OF DISEASE

The ultimate aim of the present research has been the development of ecofriendly control strategies to reduce disease intensity, dependency on synthetic fungicides and developing resistant cultivar for sustainable management of plant diseases. This study was focused on locally available plant extract viz. Annona, Aegle, Withania, Ocimum sps. against pathogen affecting fenugreek crop. Experiment was carried out with the bioagents Trichoderma species as another source of alternative control measure. All the botanicals and bioagent were evaluated were under in vitro conditions as well as in vivo. Data pertaining to the effect of plant extract and bioagents on plant growth characters viz. shoot root length (cm), fresh and dry plant weight (g), number of leaves, leaf area, yield in terms of seed weight per plant (g) and disease incidence were recorded and statistically analysed to correlate the experimental findings from pot trials. Screening of 5 cultivars of fenugreek were also evaluated for the resistance towards leaf spot pathogen by inoculating healthy leaves with the spore suspension of C. traversiana. Development of diseased lesions on leaves were in result to there resistant and susceptible behavior.

Management through Botanicals against Leaf spot pathogen (In vitro)

A significant reduction in mycelial growth of C.traversiana pathogen causing leaf spot disease of fenugreek was observed when treated with methanoic extract of Annona leaves.The mycelial growyh was reduced by 25.4% and 64.4% at 10% and 20% concentrations of Annona methanolic extract as compared to control. The result of the present experiment showed that there was marked reduction in the mycelial growth of C. traversiana when treated with Ocimum santum extract under on vitro condition. The mycelial growth was reduced by 22.93% and 44.96% at 10% and 20% concentration. The inhibition rates increased with increasing concentration of the control agent. The data on the inhibition percentage of mycelial growth of C. traversiana revealed that at 10% and 20% concentration of Withania leaf extract the growth inhibitory percentage 18.4% and 20.33% was recorded. The results revealed that the inhibition rates increased with increasing

concentrations of the control agent. The results presented in Table-9 indicated that inhibition of mycelial growth of C. traversiana obtained with Aegle marmelos plant extract at 10% was recorded 17.8% and at 20% it was recorded as 19.96%. The percent inhibtion was minimum as compared to other treatments at both the concentrations. All the treatments reduced fungus growth in culture. On comparing the overall results it was observed that Annona squamosa methanoic plant extract showed the maximum inhibition 64.4% at 20% concentration of mycelial growth. It was followed by Ocimum sanctum extract at 10% concentration (22.93%) and at 20% concentration (44.96%). The inhibition in mycelial growth varied from 19.96% to 64.4% in different control agents. The efficacy was in order of Annona squamosa > Ocimum sanctum > Withania somnifera > Aegle marmelosa extract.

Management through Bioagents against leaf spot pathogen (In vitro)

The result of the present experiment showed that there was marked reduction in the mycelial growth of C. traversiana when treated with Trichoderma species at 20% concentration. The mycelial growth was reduced by 73.00% in T. viride followed by 71.33% in T.harzianum treated.

Effect of control agents against C. traversiana in pot experiment

Results of pot trials indicated that at different concentration all the botanicals were significantly effective in controlling the leaf spot disease incidence and resulted in improving the growth of host plant (Fenugreek).

The results regarding the efficacy of various plant extracts indicated that maximum shoot length, root length, fresh weight, dry weight, leaf area & seed weight per plant were obtained by T_1 treatment followed by T_4 and T_3 than T_2. In treated plus inoculated plants the treatment T_1 + I was the most effective followed T_4 +I and T_3 + I and T_2 + I. All the growth characters recorded were maximum for Annona extract and minimum for Aegle extract. An attempt was made in present study to observe the efficacy of different phytoextracts at different concentration

Annona squamosa, Aegle marmelosa, Withania somnifera and Ocimum sanctum on the fungal culture of C. traversiana causing leaf spot of fenugreek. All the four ecofriendly control measures as well as bioagents Trichoderma viride and T. harzianum showed antifungal activity against the pathogen. Studies on different control agents under pot trials also revealed that Annona extracts and T. viride were more effective followed by other treatments. At all the treatments plant growth characters showed significant increase with reduction in percent disease incidence (PDI) and increased yield.

The study will able to provide us the efficient disease management of Fenugreek crop by using control agents which are very cost effective and ecofriendly in developing sustainable management.

In vivo management of leaf spot disease of fenugreek by botanicals

A pot experiment was conducted to evaluate the efficacy of four plant extracts viz. Annona, Aegle, Withania and Ocimum sps. as pre sowing seed treatment and foliar spray against leaf spot of fenugreek.

Plant Growth Characters: All the treatments enhanced plant growth characters including yield in terms of seed weight per plant and reduced percent disease incidence.

Shoot Length: The maximum shoot length 53.6 cm was recorded with seed treatment and foliar spray of Annona squamosa (T_1) followed by 52.3 cm in T_4 (Ocimum extract), 52.1 cm in T_3 (Withania extract) and then 50.2 cm in T_2 (Aegle extract). In untreated healthy control the shoot length was recorded to be 40.00 cm. In treated plus inoculated fenugreek plants maximum shoot length was observed in T_1+ I followed by T_4 + I and T_3+ I and then T_2+I as 52.3 cm, 51.2 cm, 51.1 cm and 49.9 cm respectively as compared to only inoculated diseased plant (I) 32.8 cm.

Root Length: Among different treatments the maximum root length was observed in T_1 followed by T_4, then in T_3 and T_2 as 15.3 cm, 15.00 cm, 14.8 cm and 14.2 cm

as compared to control (12.5 cm). In treated plus inoculated plants maximum root length was 14.3 cm in T_1+I, then 14.1 cm in T_4+I, then 13.6 cm in T_3+I and 13.2 cm in T_2+I, However the minimum root length 8.9 cm was observed in I inoculated diseased plant.

Fresh Weight: Fenugreek plant treated with T_1 showed significant higher fresh weight 19.92 g followed by 19.09 g in T_4 and 18.98 g in T_3 and 18.01 g in T_2 treatment. In healthy control it was recorded as 16.2 g. The fresh weight of treated plus inoculated plants were observed as 19.90 g, 18.97 g, 18.32 g, 17.89 g in T_1+I, T_4+I, T_3+I and T_2+I respectively as compared to only inoculated (diseased) plant (I) as 12.9 g.

Dry Weight: Data on dry weight of plant observed was 5.27 gm as highest weight in T_1 followed by 4.81 g in T_4, 4.02 g in T_3 and 4.00 g in T_2 over healthy control (3.71 g). The dry weight of T_1+I, T_4+I, T_3+I and T_2+I was 5.01 g, 4.32 g, 4.00 g and 3.92 g as compared to only inoculated (diseased) plants 2.21 g.

Number of Leaves: The number of leaves per plant of fenugreek was almost same in all the treatments and observed was 40 except in the inoculated diseased plants (I) where it was recorded to be 28.

Leaf Area: Among the different treatment maximum leaf area was observed in T_1 followed by T_4, T_3 and T_2 as, 12.69 cm^2, 12.61 cm^2, 12.45 cm^2 and 12.41 cm^2 as compared to healthy control 12.35 cm^2. In treated plus inoculated plants maximum leaf area was observed in T_1+I, followed by T_4+I, T_3+I and T_2+I as 12.32 cm^2, 12.31 cm^2, 12.29 cm^2 and 12.26 cm^2 respectively as compared to the inoculated diseased plant, Where the leaf area was observed as 10.26 cm^2.

Seed Weight: The yield of plant in terms of seed weight was recorded. The maximum seed weight of 182.2 g was recorded with pre sowing seed treatment and foliar treatment of fenugreek plant with Annona extract (T_1) followed by 181.7 g at T_4 , 181.4 g at T_3 and 180.6 g at T_2 as compared to healthy control where seed weight was observed at 177.6 g. In treated plus inoculated plants

maximum seed weight was observed in T_1+I followed by T_4+I then T_3+I and T_2+I as 180.0 g, 179.2 g, 179 g, 178.3 g respectively. The maximum seed weight 152.2 g was observed in only inoculated (diseased) plant (I).

Percent disease Incidence (PDI) : Among the different treatments minimum PDI of 3.36% was observed in T_1+I, followed by 5.89% in T_4+I, 5.92% in T_3+I and 6.23% in T_2+I as compared to only inoculated (diseased) plants (I) where the highest PDI value that is 35.32% was observed.

Management through Biorationals :

Observations on plant growth character, including yield in terms of seed weight per plant and percent disease incidence were recorded.

Shoot Length : In healthy control the shoot length of 45.3 cm was recorded with minimum shoot length of 36.2 cm in inoculated showed higher value of 47.4 in T_5+I followed by 46.7 cm in T_6+I treatment with Trichoderma species.

Root Length: Among both the treatments on inoculated plants T_5 showed significant higher value of 15.13 cm followed by 15.01 cm in T_6. However treatment inoculated plants showed root length of 15.03 cm in T_5+I and 14.69 cm in T_6+I as compared to inoculated (diseased) plant (I) 8.69 cm.

Fresh Weight: Fenugreek plant treated with T_5 showed significant higher fresh weight 17.93 g followed by 17.41 gm in T_6 treatment. In healthy control it was recorded as 16.24 g. The fresh weight of treated plus inoculated diseased were observed as 17.64 g in T_5+I followed by 17.21g in T_6+I as compared to only inoculated (diseased) plant (I) as 14.31 g.

Dry Weight: Data on dry weight of plant observed was 4.25 g as highest weight in T_5, followed by 4.11 g in T_6 treatment over healthy control 3.73 g. The dry weight of T_5+I and T_6+I was 4.13 g and 3.92 g as compared to only diseased plant (I) 2.71 g.

Number of leaves: The number of leaves per plant of fenugreek plant was almost same in all the treatments and observed was 52 in control, whereas 41 in inoculated diseased (I) plant.

Leaf Area: Among the different treatments maximum leaf area was observed in T_5 followed by T_6 as 12.39 cm^2 and 12.28 cm^2 as compared to healthy control 12.22 cm^2. In treated plus inoculated plants maximum leaf area was observed in T_5+I, followed by T_6+I as 12.32 cm^2 and 12.26 cm^2 respectively as compared to the only diseased plant (I) where the leaf area was observed as 9.61 cm^2.

Seed weight: The yield of plant in terms of seed weight was recorded. The maximum seed weight was recorded 182.5 gm was recorded with pre sowing seed treatment of fenugreek plant with Trichoderma viride followed by 181.9 g in T_6 treatment as compared to control where the seed weight was observed as 177.6 g. In treated and inoculated plants higher seed weight was observed in T_5+I, followed by T_6+I as 180.6 g and 179.3 respectively. The minimum seed weight 153.3 g was observed in only inoculated plant I.

Percent Disease Incidence: According to the number of lesions on plant parts percent disease incidence was recorded with all treatments over inoculated and control. The Lower PDI 5.92 % was recorded with the pre sowing treatment of fenugreek plant with Trichoderma viride, followed by 6.23% at T_6+I, as compared to inoculated plant (I) where the highest PDI value that is 34.21% was observed.

Screening

In the present study, an attempt was made to locate the source of reisitance in fenugreek cultivars against leaf spot pathogen, Cercospora traversiana. Five variety namely RMT-1, RMT-305, RMT-303, RMT-143 and Hisar sonali were studied and results indicated that RMT-1 extremely cultivated variety was found to be susceptible to the leaf spot pathogen and other diseases also. RMT-303 cultivar was moderately susceptible followed with moderately resistant varities Hisar sonali, RMT- 143. The most resistant variety was recorded as RMT-305.

The results showed that all above treatments of different botanicals, bioagents and screening of accessions can be used as an alternative source in controlling diseases on host plant. The results of the present research work will be useful for detecting effective ecofriendly measures to manage leaf spot and other diseases in Fenugreek crop. The knowledge enlisted during the present investigation will well-head for further research work on biology, epidemiology and management of the disease and others.

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Table No. 2 Occurrence of Phytoparasitic fungi on fenugreek in different localities of Kota district, Rajasthan.

S.No.	Tehsil	Location	No. of Sampling Unit	No. of Plant Samples Studied	Leaf Spot	Powdery Mildew	Downey Mildew	Leaf Blight
1	Ladpura	Chandresal	10	50	-	-	+	+
2		Jhalkhera	10	50	+	+	-	+
3		Gangaycha	10	50	+	+	+	-
4		Borabas	10	50	-	-	-	-
5		Mandana	10	50	-	-	-	-
6		Morak	10	50	+	-	-	-
7		Nayagaon	10	50	+	+	-	+
8	Digod	Borkheda	10	50	+	+	+	-
9		Sogaria	10	50	+	-	+	-
10		Bhadana	10	50	+	-	-	+
11	Ramganjmandi	Chechat	10	50	+	+	-	+
12		Nemana	10	50	-	+	+	-
13		Panda	10	50	+	+	+	-
14	Pipalda	Arjunpura	10	50	+	-	+	+
15		Tirat	10	50	-	+	+	-
16	Sangod	Sankhera	10	50	+	-	-	+
17	Total		160	800	11	8	8	7

+ = Occurance of disease in the area, - = Absence of same disease in the area

Table No. 3 Incidence, Severity and Prevalence of Fungal Diseases of Fenugreek in different Tehsils of Kota district, Rajasthan.					
S.No.	**Disease**	**Location**	**Incidence**	**Severity**	**PDI in %**
1	Cercospora leaf spot	Ladpura	63.33	1-4	22.60
		Digod	33.33	1-5	16.66
		Ramganjmandi	26.66	1-4	12.33
		Pipalda	6.66	1	1.33
		Sangod	3.33	1	0.66
2	Powdery Mildew	Ladpura	23.33	1-2	5.33
		Digod	3.33	1-5	15.66
		Ramganjmandi	16.66	1-4	6.66
		Pipalda	3.33	1	11.33
		Sangod	0	0	0
3	Downey Mildew	Ladpura	13.33	1-2	11.33
		Digod	10.00	1-2	5.33
		Ramganjmandi	3.33	1	3.33
		Pipalda	23.00	1-5	23.33
		Sangod	6.66	1	4.00
4	Leaf Blight	Ladpura	33.33	1-5	10
		Digod	13.33	1-4	12.33
		Ramganjmandi	6.66	1-2	6.66
		Pipalda	0	0	0
		Sangod	1	1	3.33
5	Disease complex	Ladpura	16.66	1-4	13.33
		Digod	6.66	1	3.33
		Ramganjmandi	6.66	1	0.66
		Pipalda	0	0	0
		Sangod	0	0	0

Table - 4 Disease incidence and losses due to leaf spot (Cercospora traversiana) in Fenugreek crop at different localities of Kota district. (Results are mean of 3 replicates).

S.No.	Tehsil	Location	No. of Sampling Units	No. of Plant Samples Studied	No. of Infected Plants	PDI In %	Decrease in Yield plant (Seed weight)	
							(H)	(I)
1	Ladpura	Chandresal	10	50	17	16.00	75.6	57.1
2		Jhalkhera	10	50	25	35.00	80.5	67.7
3		Gangaycha	10	50	34	43.90	78.5	52.0
4		Borabas	10	50	13	18.82	75.2	59.5
5		Mandana	10	50	12	18.00	70.2	56.4
6		Morak	10	50	26	28.20	79.6	54.6
7		Nayagaon	10	50	12	18.00	74.2	56.5
8	Digod	Borkheda	10	50	43	50.67	78.2	42.6
9		Sogaria	10	50	34	43.90	78.5	52.0
10		Bhadana	10	50	27	36.00	76.0	50.0
11	Ramganjmandi	Chechat	10	50	16	15.92	76.9	67.2
12		Nemana	10	50	12	18.00	74.2	59.5
13		Panda	10	50	26	28.20	79.6	54.6
14	Pipalda	Arjunpura	10	50	25	35.00	80.5	67.7
15		Tirat	10	50	14	14.82	80.0	62.2
16	Sangod	Sankhera	10	50	21	22.67	78.5	65.0

Table No. 5 Physiochemical Analysis of soil samples collected from fenugreek fields of 16 different sites of Kota district, Rajasthan.

S.No.	Tehsil	Soil type	Location	pH	Electrical conductivity (EC) in µs/cm	Organic carbon (OC) in %	Total Nitrogen (N) %	Available Phosporous (P) in mg/ha	Available Pottasium (K) in mg/ha	PDI in %
1	Ladpura	Deep black clayey	Chandresal	7.8	380	0.79	0.072	0.53	492	40.33
2			Jhalkhera	7.7	220	0.63	0.071	0.45	389	50.67
3			Gangaycha	8.0	350	0.58	0.068	0.65	372	43.90
4			Borabas	8.2	400	0.85	0.082	0.40	383	28.22
5			Mandana	7.6	380	0.58	0.085	0.38	379	25.32
6			Morak	7.5	360	0.62	0.061	0.32	358	15.92
7			Nayagaon	7.9	350	0.46	0.063	0.57	385	17.76
8	Digod	Deep black clayey	Borkheda	8.2	380	0.57	0.035	0.44	389	49.10
9			Sogaria	7.9	400	0.64	0.073	0.40	350	36.01
10			Bhadana	7.8	430	0.63	0.087	0.34	376	13.67
11	Ramganjmandi	Deep brown clayey	Chechat	8.5	260	0.71	0.063	0.50	384	45.16
12			Nemana	8.7	240	0.82	0.067	0.45	396	29.21
13			Panda	8.2	280	0.61	0.066	0.28	372	18.83
14	Pipalda	Deep brown clayey	Arjunpura	8.2	340	0.58	0.073	0.34	358	15.82
15			Tirat	8.5	360	0.60	0.071	0.5	392	39.20
16	Sangod	Deep brown loamy	Sankhera	7.1	260	0.34	0.083	0.32	372	25.14

Table -6 List of weed species of 16 different study sites of Kota																	
S.No	**Botanical Name**	**Vernacular Name**	**Localities**														
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	**Spergula arvensis**	**Bhandhania**	-	+	+	-	+	-	+	-	+	+	-	-	-	+	-	-
2	**Melilotus indica (L.)**	**Senjimethi**	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-
3	**Anagalliis arvensis (L.)**	**Krishnaneel**	-	-	+	+	-	-	+	+	+	-	+	+	+	-	-	-
4	**Chenopodium album (L.)**	**Bathua**	+	+	+	-	-	-	+	+	+	+	-	-	-	-	+	+
5	**Chenopodium murale L.**	**Khartua**	-	-	+	-	-	-	+	+	+	+	-	-	-	-	+	-
6	**Convolvulus arvensis L.**	**Hiran Khuri**	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+	-
7	**Cynodon dactylon L.**	**Doob**	+	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+

+ Presence **- Absence**

Table - 7 Effect of C. traversiana on chlorophyll content of T. foenum graecum cultivar RMT-1 in mg/gm fresh weight								
S.No	**Time Interval**	**Plant Type**	**Chlorophyll a**	**% Decrease in over healthy plant**	**Chlorophyll b**	**% Decrease in over healthy plant**	**Total Chlorophyll**	**% Decrease in over healthy plant**
1	After 30 days	H	0.708	27.05	0.573	33.42	1.431	32.42
		I	0.513		0.348		1.007	
2	After 60 days	H	0.798	33.36	0.757	39.31	1.771	41.36
		I	0.526		0.420		1.230	
3	After 90 days	H	1.141	31.82	0.819	33.76	2.143	31.86
		I	0.732		0.475		1.281	
4	After 120 days	H	1.172	24.24	0.910	19.04	2.709	43.04
		I	0.893		0.784		1.466	
5	Mean			29.11		31.38		37.17

H = In Healthy Plant, I = Infected Plant

Table -8 Quantification of various metabolites in healthy and Cercospora traversiana infected leaves of T. foenum graecum cultivar RMT-1 plant at different stages of growth (results are mean of 3 replicates).

S.No.	Metabolite	Plant Type	Time Interval								Mean % interval or Decrease over healthy plant
			After 30 days	% Increase or decrease over healthy plant	After 60 days	% Increase or decrease over healthy plant	After 90 days	% Increase or decrease over healthy plant	After 120 days	% Increase or decrease over healthy plant	
1	Carbohydrate	H	282		369.1		481		591		
		I	212	-24.82	259	-29.82	383	-20.37	485	-17.93	-23.23
2	Protein	H	16.21		16.43		17.2		17.8		
		I	14.20	-12.39	14.48	-11.86	15.4	-10.46	15.9	-10.67	-11.34
3	Amino Acid	H	3.55		4.64		4.89		5.22		
		I	2.69	-24.22	3.25	-29.95	3.8	-22.29	4.18	-19.92	-24.09
4	Proline	H	0.51		0.59		0.62		0.64		
		I	0.68	+25	0.72	+18.05	0.87	+28.73	0.92	+30.43	+25.55
5	Phenol	H	2.32		3.19		3.78		3.98		
		I	3.48	+33.33	3.92	+18.62	4.52	+16.37	4.78	+16.73	+21.26

H = In Healthy Plant, I = Infected Plant, + = Increase, - = Decrease

Table – 9 Effect of different plant extract on % inhibition of mycelial growth of *Cercospora traversiana.*

S.No.	Plant Extracts	Percent inhibition of mycelia growth at concentration (%)		Mean
		10%	20%	
1.	Annona squamosa	25.4	64.4	44.9
2.	Aegle marmelosa	17.8	19.96	18.88
3.	Withania somnifera	18.4	20.33	19.36
4.	Ocimum Sanctum	22.93	44.96	33.94
SEm±		0.28	0.45	
CD 5%		0.88	1.38	
CV		4.04	3.59	

Table –10 Effect of Bioagents on % inhibition of mycelial growth of *C. traversiana.*

S.No.	Bioagents	Percent inhibition of mycelia growth.
1.	Trichoderma viridae	73.00
2.	Trichoderma harzianum	71.33
SEm±		0.39
CD 5%		1.23
CV		0.88

Table – 11 Effect of different plant extracts on various growth parameters of C. traversiana infected Fenugreek plants (results are mean of 3 replicates).

S.No.	Treatment	Length (cm)		Weight (g)		No. of leaves	Leaf area (cm^2)	Seed weight/plant (g)	Percent Disease Incidence (PDI %)
		Shoot	Root	Fresh	Dry				
1.	Control C)	40.0	12.5	16.20	3.71	40	12.35	177.6	0
2.	T_1	53.6	15.3	19.92	5.27	40	12.69	182.2	0
3.	T_2	50.2	14.2	18.01	4.00	40	14.41	180.6	0
4.	T_3	52.1	14.8	18.98	4.02	40	12.45	181.4	0
5.	T_4	52.3	15.0	19.09	4.81	40	12.61	181.7	0
6.	Infected	32.8	8.9	12.90	2.21	28	10.26	152.2	35.32
7.	T_1+ I	52.3	14.3	19.90	5.01	39	12.32	180.0	3.36
8.	T_2+ I	49.8	13.2	17.89	3.92	40	12.26	178.3	6.23
9.	T_3+ I	51.1	13.6	18.32	4.00	40	12.29	179.0	5.92
10.	T_4+ I	51.2	14.1	18.97	4.32	40	12.31	179.2	5.89
SEm±		0.58	0.17	0.23	0.05	0.52	0.16	2.38	0.13
CD 5%		1.72	0.50	0.69	0.16	1.54	0.46	7.03	0.40
CV		2.09	2.15	2.24	2.30	2.34	2.17	2.33	4.10

Table – 12 Effect of Bioagents on various growth parameters of C. traversiana infected Fenugreek plants (results are mean of 3 replicates).

S.No.	Treatment	Length (cm)		Weight (g)		No. of leaves	Leaf area (cm^2)	Seed weight (g/plant)	PDI (%)
		Shoot	Root	Fresh	Dry				
1.	Control (c)	45.3	12.20	16.24	3.73	52	12.22	177.6	0
2.	T_5	48.2	15.3	17.93	4.52	52	12.39	182.5	0
3.	T_6	47.9	15.01	17.41	4.11	52	12.28	181.9	0
4.	(I)	36.2	8.69	14.31	2.17	41	9.61	153.3	34.21
5.	T_5+ I	47.4	15.03	17.64	4.13	50	12.32	180.6	5.92
6.	T_6+ I	46.7	14.69	17.21	3.92	50	12.26	179.3	6.23
SEm±		0.49	0.19	0.20	0.05	0.69	0.16	2.46	0.09
CD 5%		1.50	0.57	0.61	0.15	2.12	0.49	7.58	0.28
CV		1.86	2.38	2.05	2.23	2.41	2.32	2.42	2.03

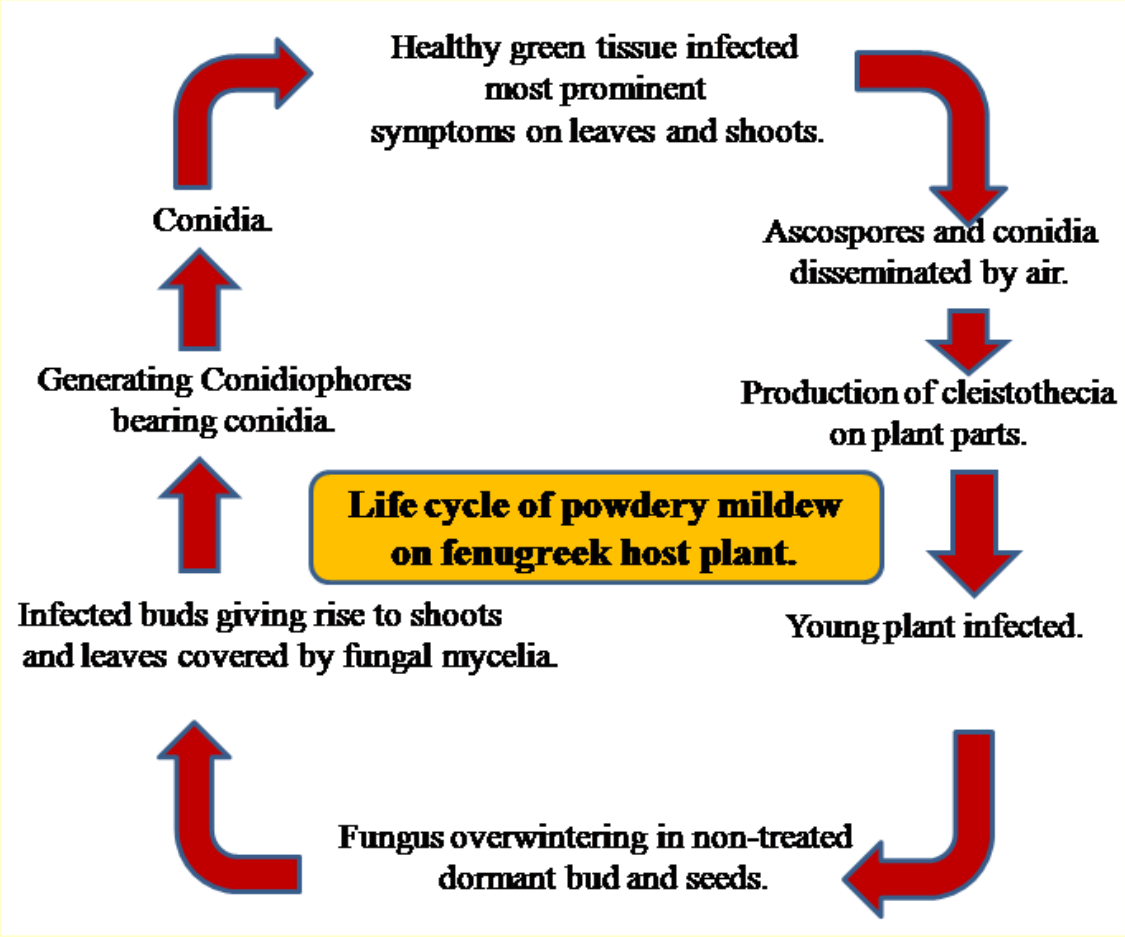

Healthy green tissue infected
most prominent
symptoms on leaves and shoots.
Ascospores and conidia
disseminated by air.
Production of cleistothecia
on plant parts.
Young plant infected.
Fungus overwintering in non-treated
dormant bud and seeds.
Infected buds giving rise to shoots
and leaves covered by fungal mycelia.
Generating Conidiophores
bearing conidia.
Conidia.
Life cycle of powdery mildew
on fenugreek host plant.

Figure -1

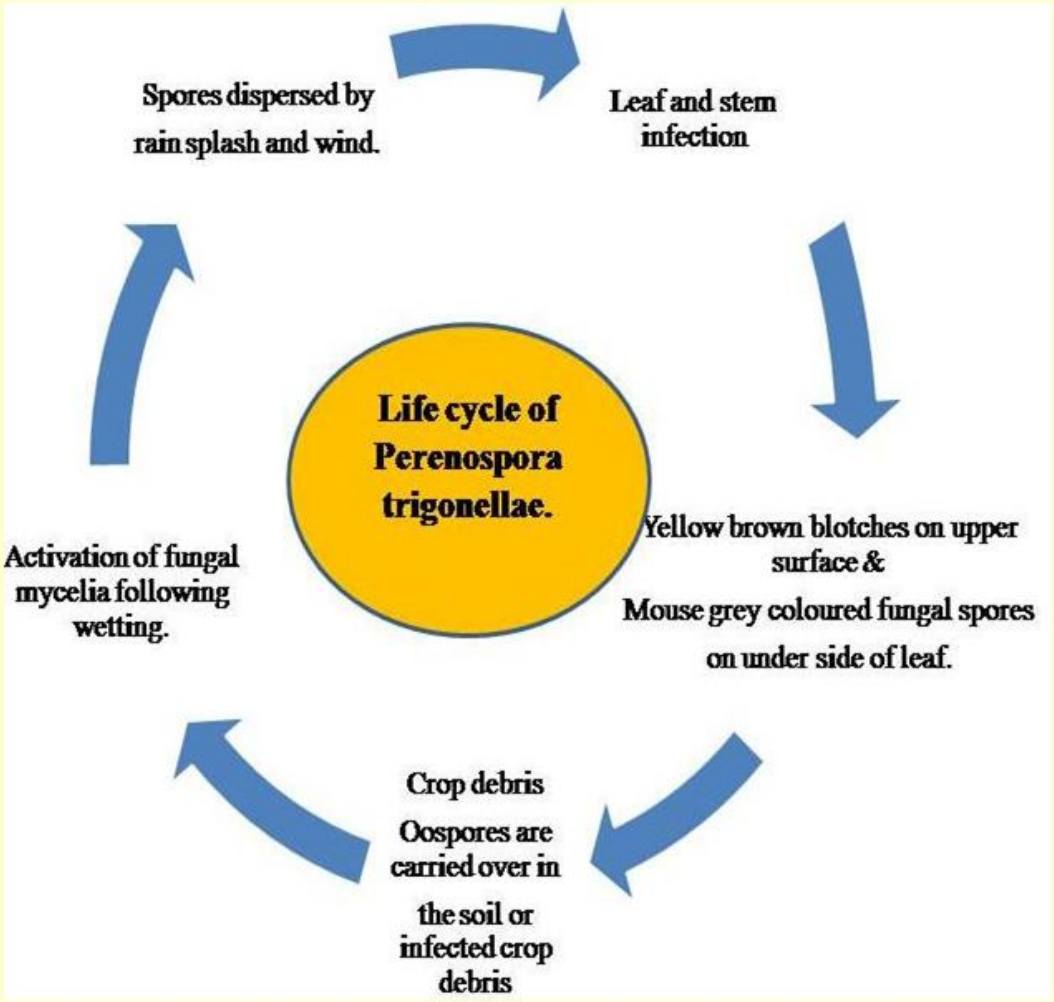

Spores dispersed by rain splash and wind.
Leaf and stem infection
Life cycle of Perenospora trigonellae.
Yellow brown blotches on upper surface &
Mouse grey coloured fungal spores on under side of leaf.
Activation of fungal mycelia following wetting.
Crop debris
Oospores are carried over in the soil or infected crop debris

Figure-2

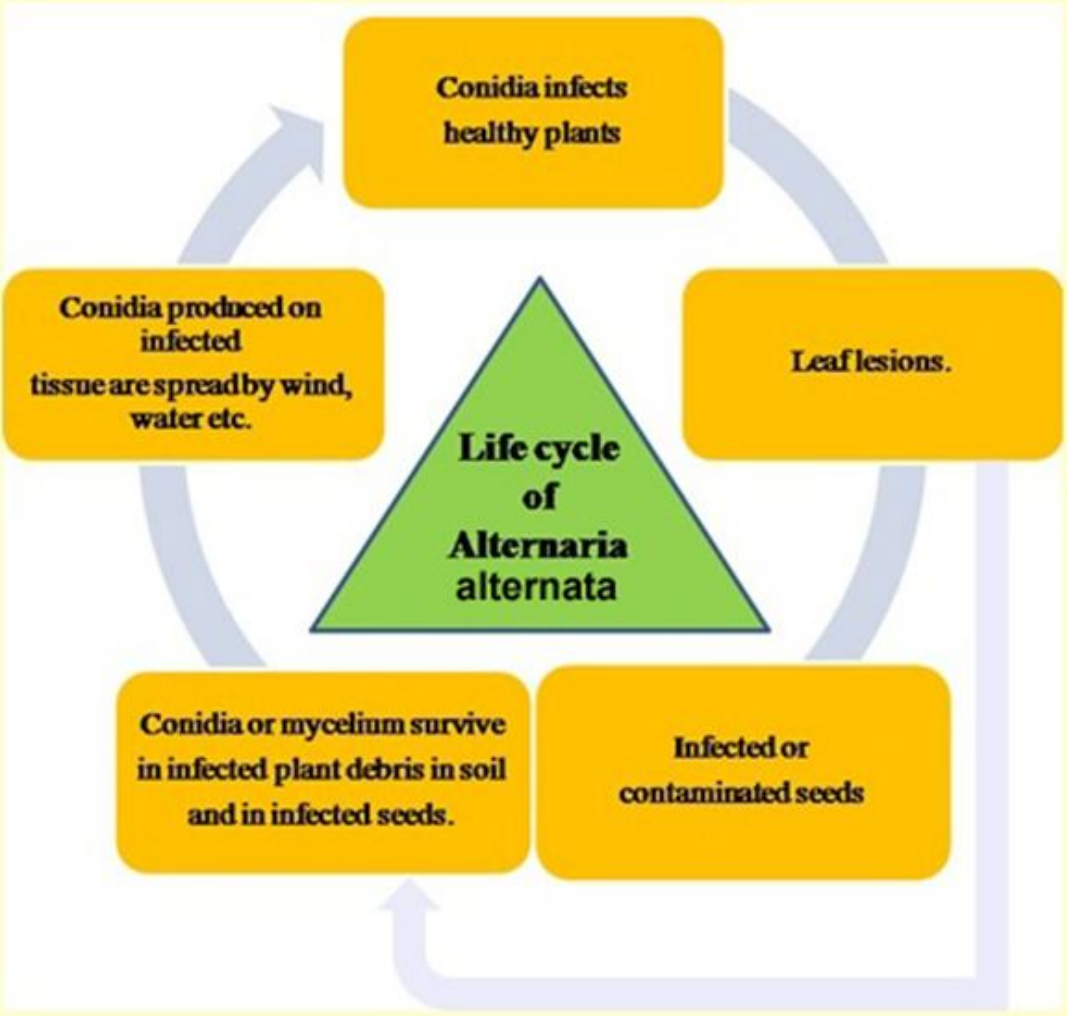

Conidia infects healthy plants
Leaf lesions.
Infected or contaminated seeds
Conidia or mycelium survive in infected plant debris in soil and in infected seeds.
Conidia produced on infected tissue are spread by wind, water etc.
Life cycle of Alternaria alternata

Figure - 3

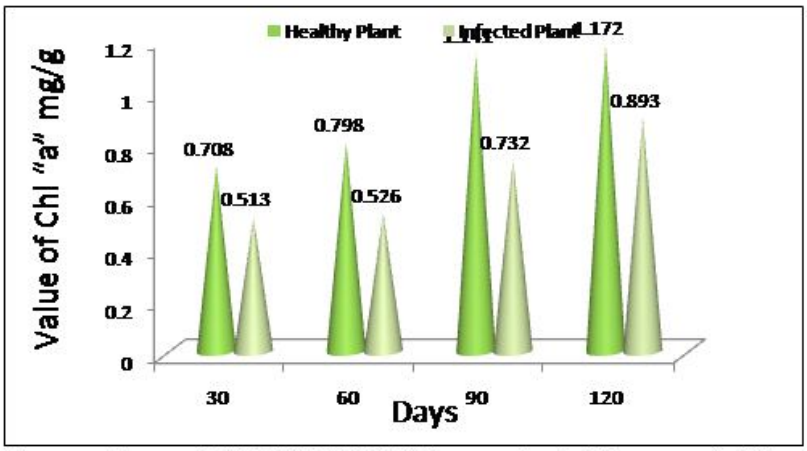


Figure: 4 Comparison of CHLORPHYLL a mg/g in Fenugreek Plant after C. traversiana Infection at different growth stages.

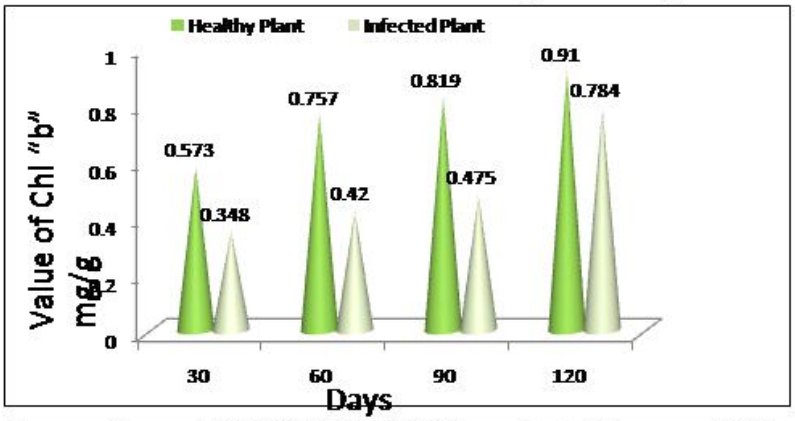


Figure: 5 Comparison of CHLORPHYLL b mg/g in Fenugreek Plant after C. traversiana Infection at different growth stages.

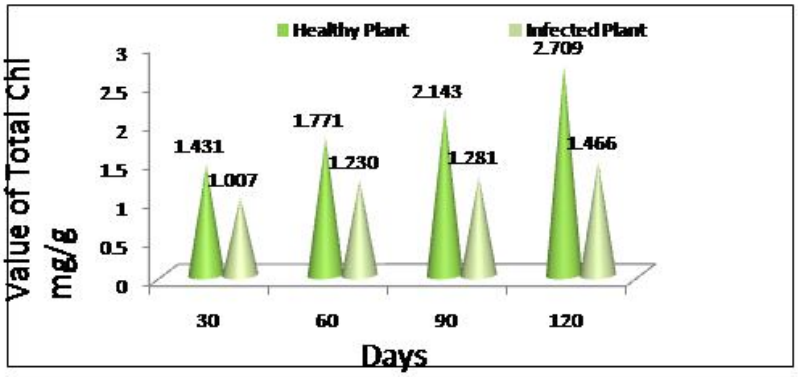


Figure: 6 Comparison of TOTAL CHLORPHYLL in Fenugreek Plant after C. traversiana Infection at different growth stages.

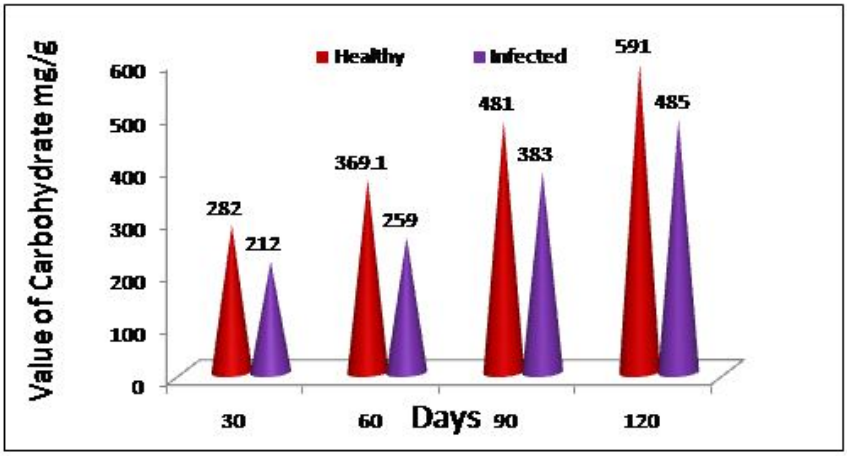


Figure: 7 Comparison of CARBOHYDRATE mg/g content in Fenugreek Plant after C. traversiana Infection at different growth stages.

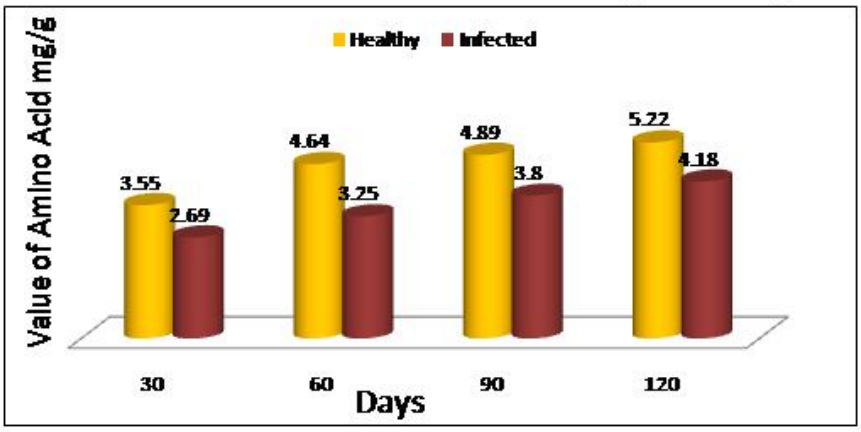


Figure: 8 Comparison of Amino Acid mg/g in content Fenugreek Plant after C. traversiana Infection at different growth stages.

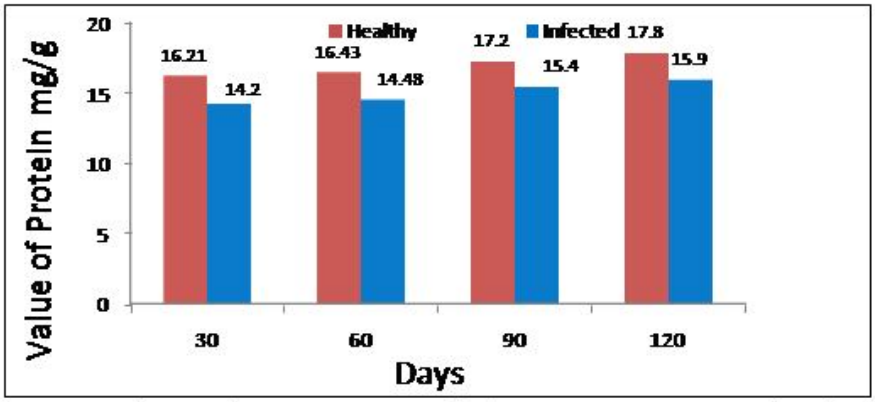


Figure: 9 Comparison of PROTEIN mg/g in content Fenugreek Plant after C. traversiana Infection at different growth stages.

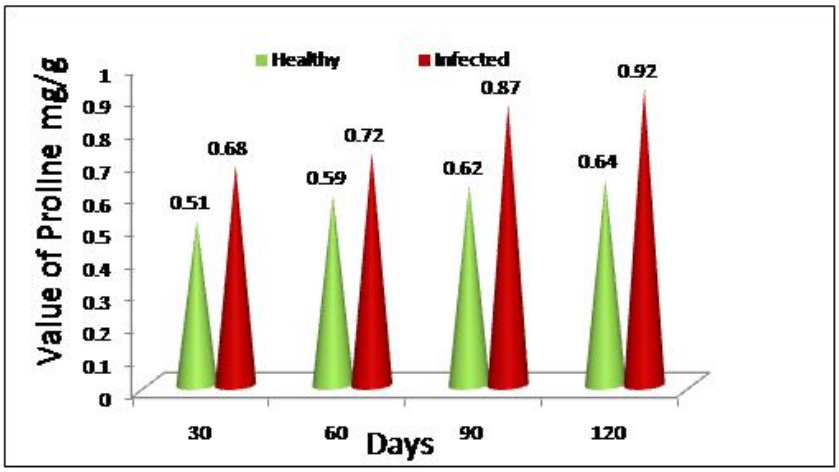


Figure: 10 Comparison of PROLINE mg/g content in Fenugreek plant after C. traversiana Infection at different growth stages.

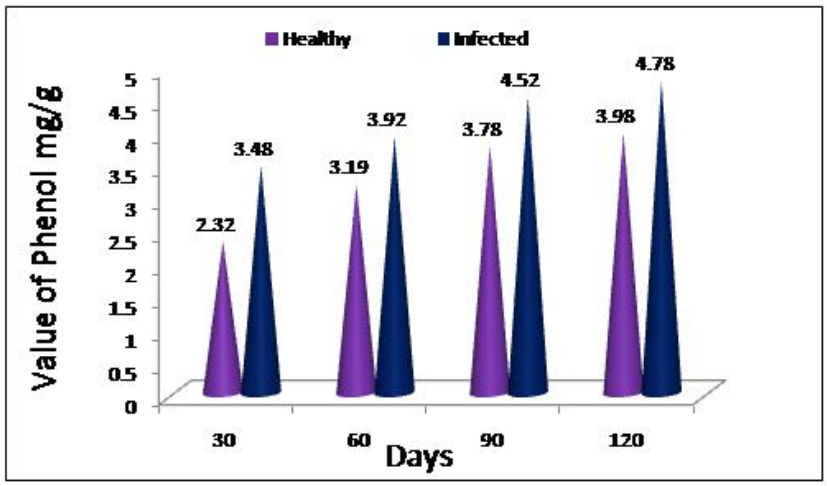


Figure: 11 Comparison of PHENOL mg/g content in Fenugreek Plant after C. traversiana Infection at different growth stages.

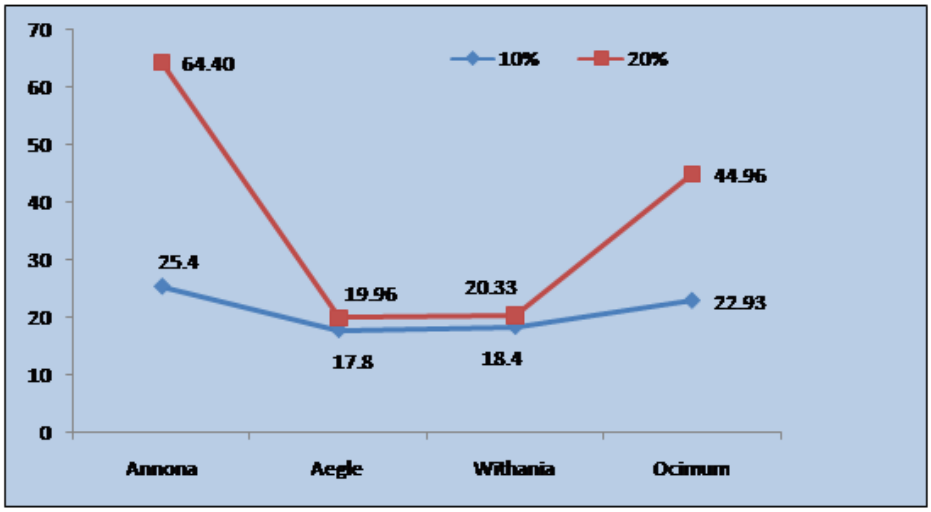


Figure: 12 Comparision of different botanicals on % inhibition mycelial growth of C.traversiana in culture experiment.

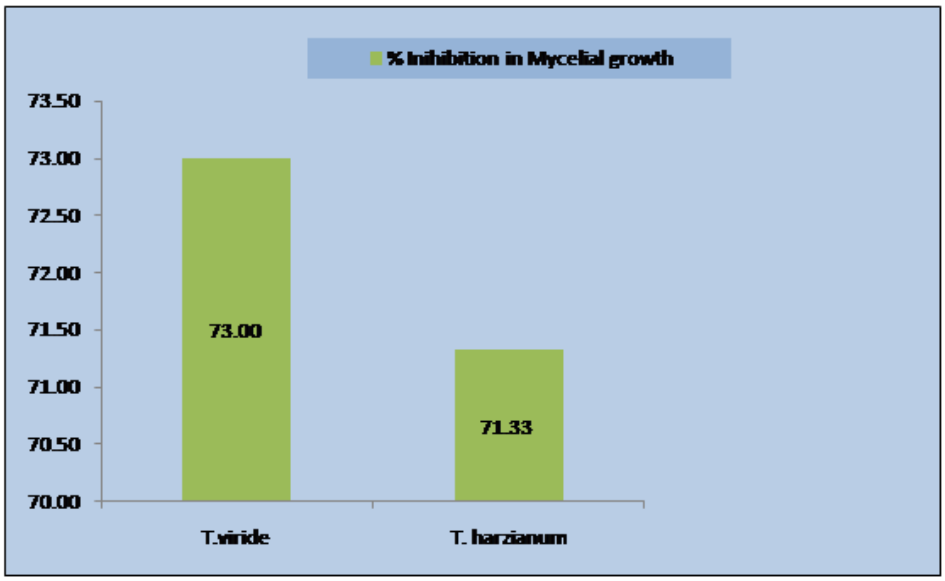


Figure: 13 Comparision of different bioagent on % inhibition mycelial growth of C.traversiana in culture experiment.

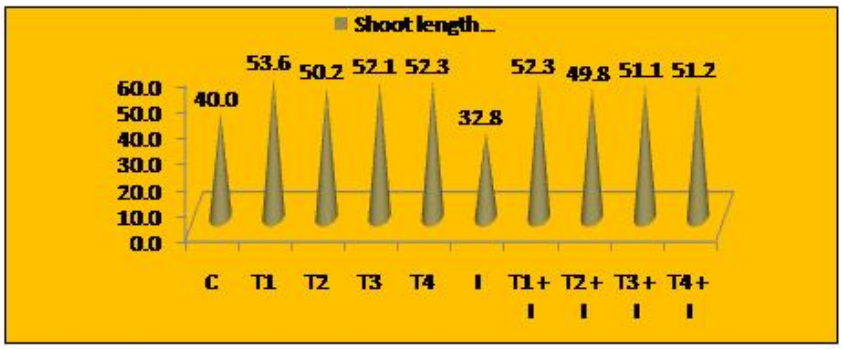


Figure – 14 Comparison of different plant extracts on shoot length in Fenugreek plant.

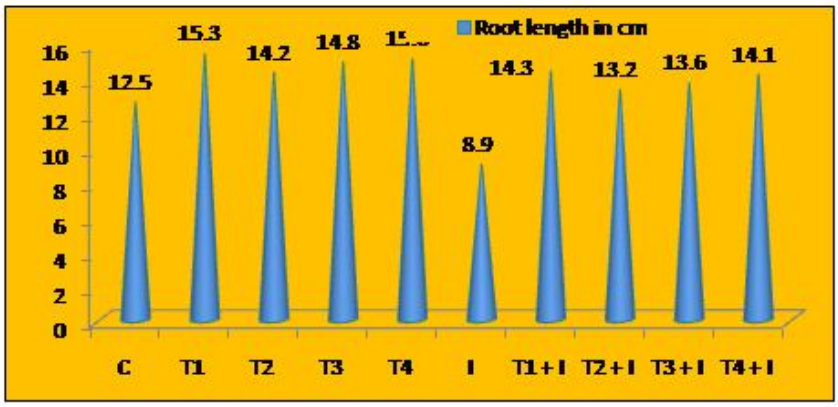


Figure –15 Comparison of different plant extracts on Root length in Fenugreek plant.

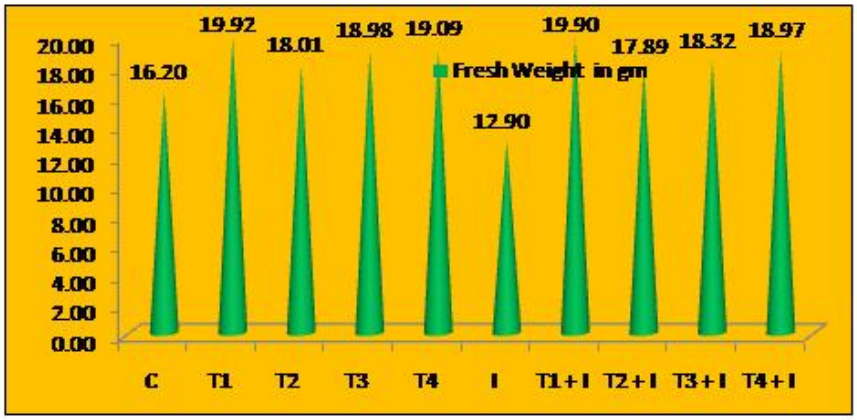


Figure –16 Comparison of different plant extracts on Fresh weight in Fenugreek plant.

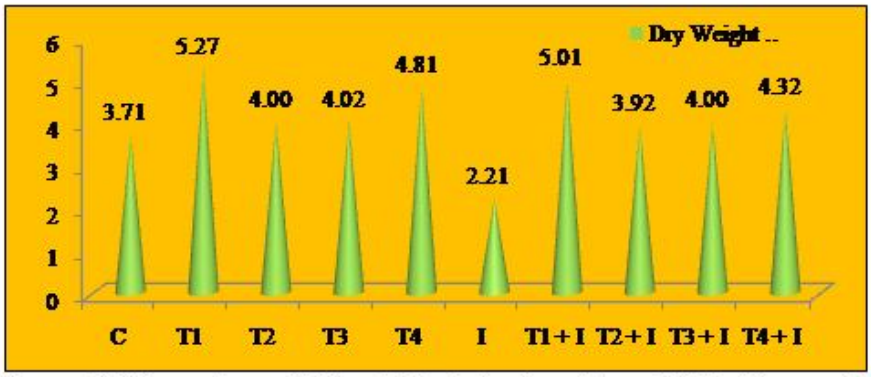


Figure – 17 Comparison of different plant extracts on Dry weight in Fenugreek plant.

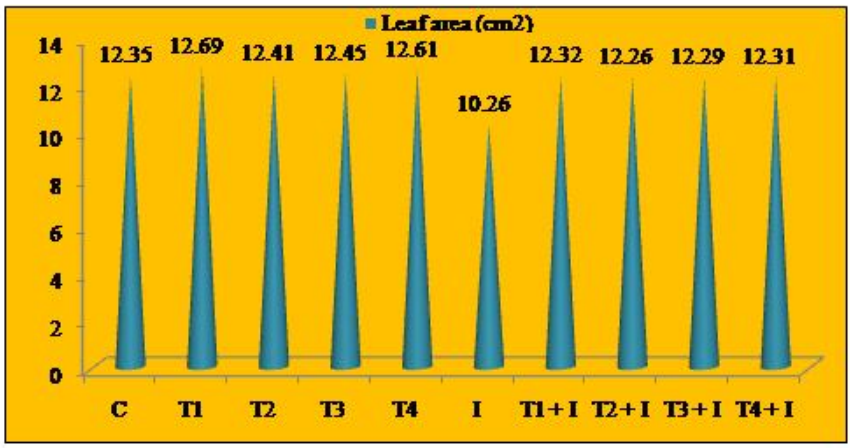


Figure –18 Comparison of different plant extracts on Leaf area in Fenugreek plant.

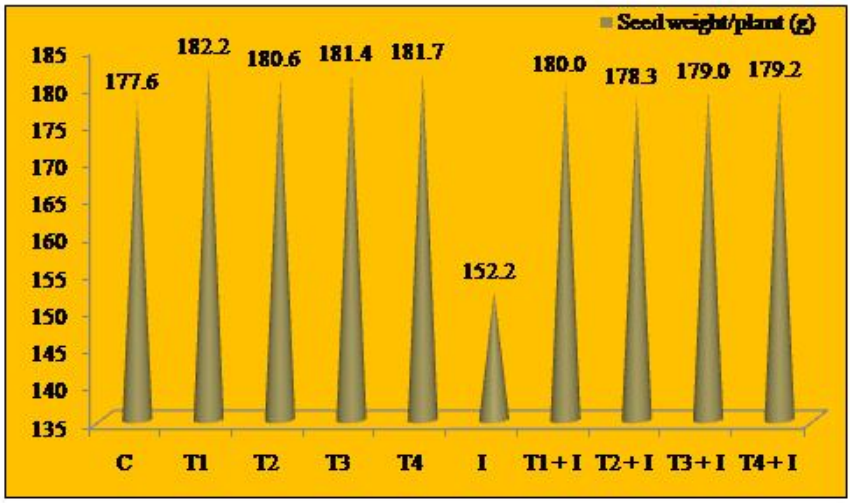


Figure –19 Comparison of different plant extracts on Seed weight in Fenugreek plant.

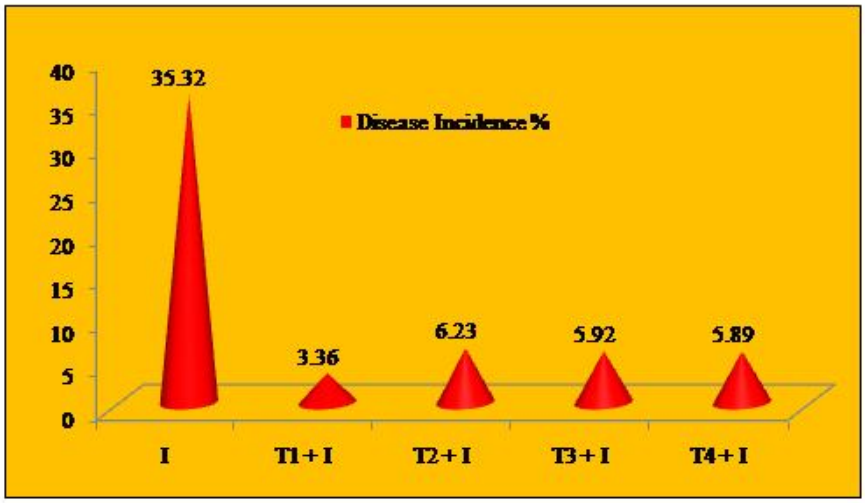


Figure –20 Comparison of different plant extracts on Disease incidence in Fenugreek plant.

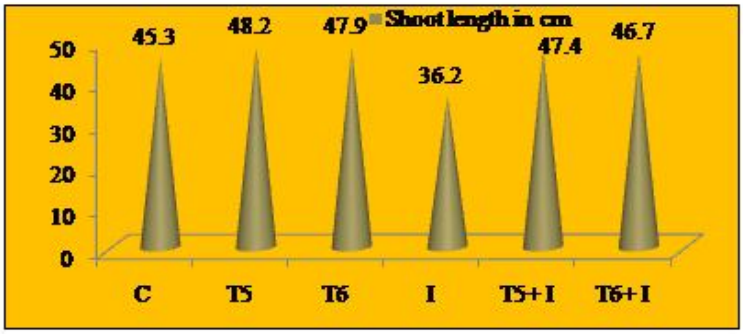


Figure –21 Comparison of Bioagents on Shoot length in Fenugreek plant.

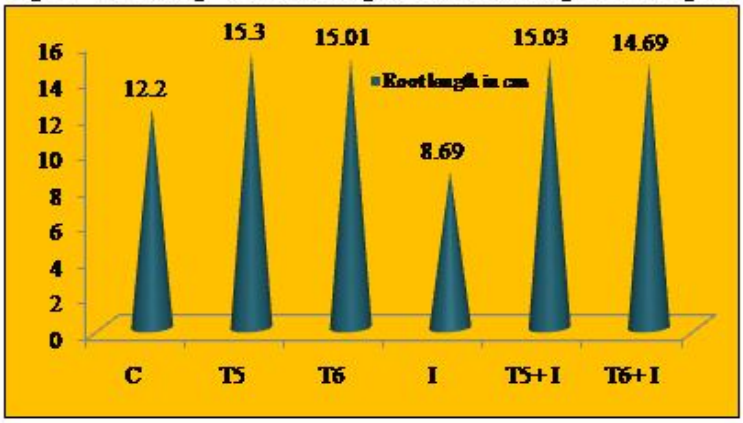


Figure –22 Comparison of Bioagents on Root length in Fenugreek plant.

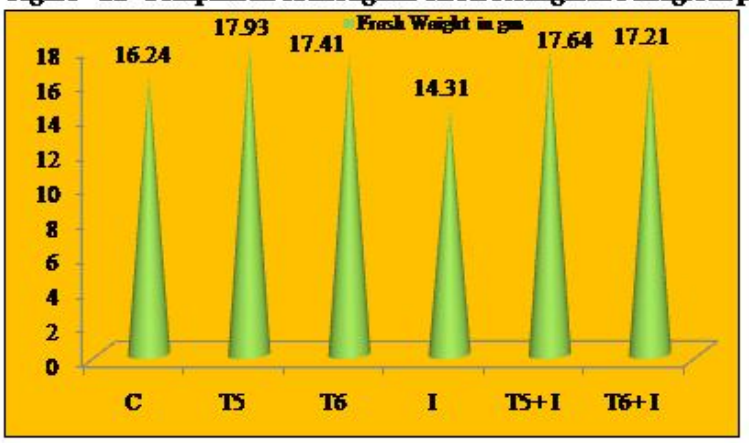


Figure –23 Comparison of Bioagents on Fresh weight in Fenugreek plant.

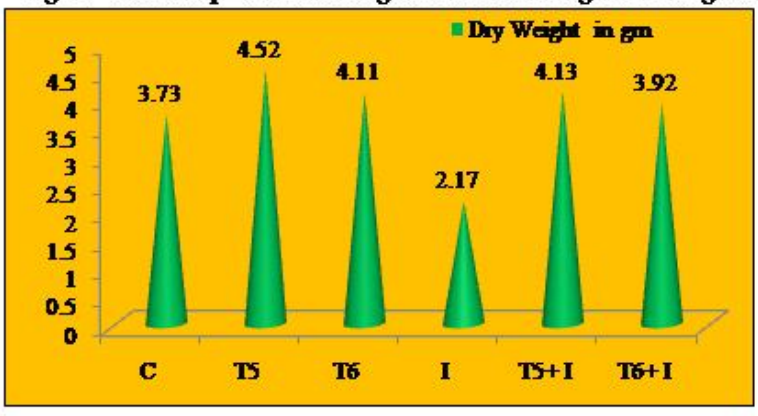


Figure – 24 Comparison of Bioagents on Dry weight in Fenugreek plant.

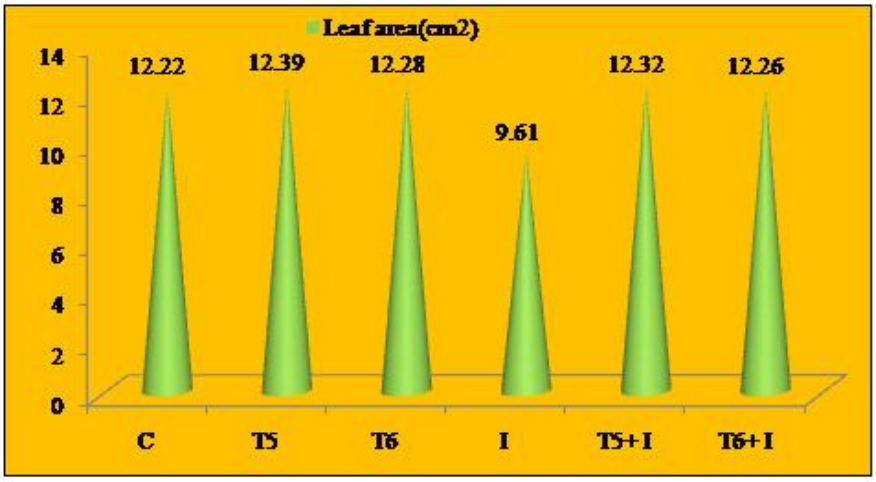


Figure –25 Comparison of Bioagents on Leaf area in Fenugreek plant

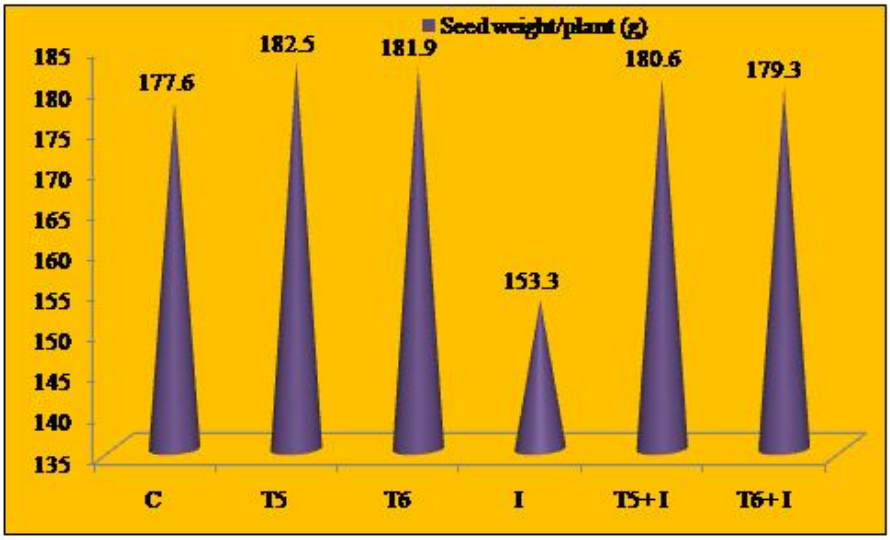


Figure – 26 Comparison of Bioagents on Seed weight in Fenugreek plant

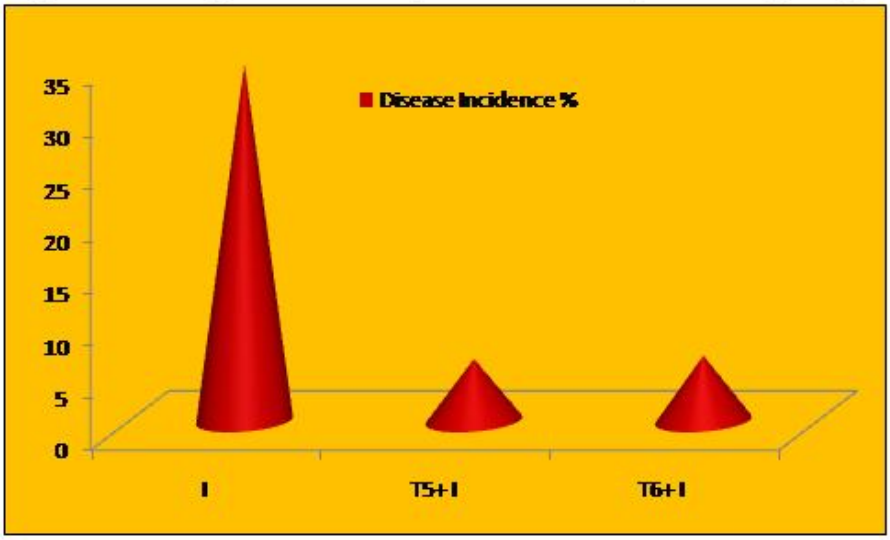
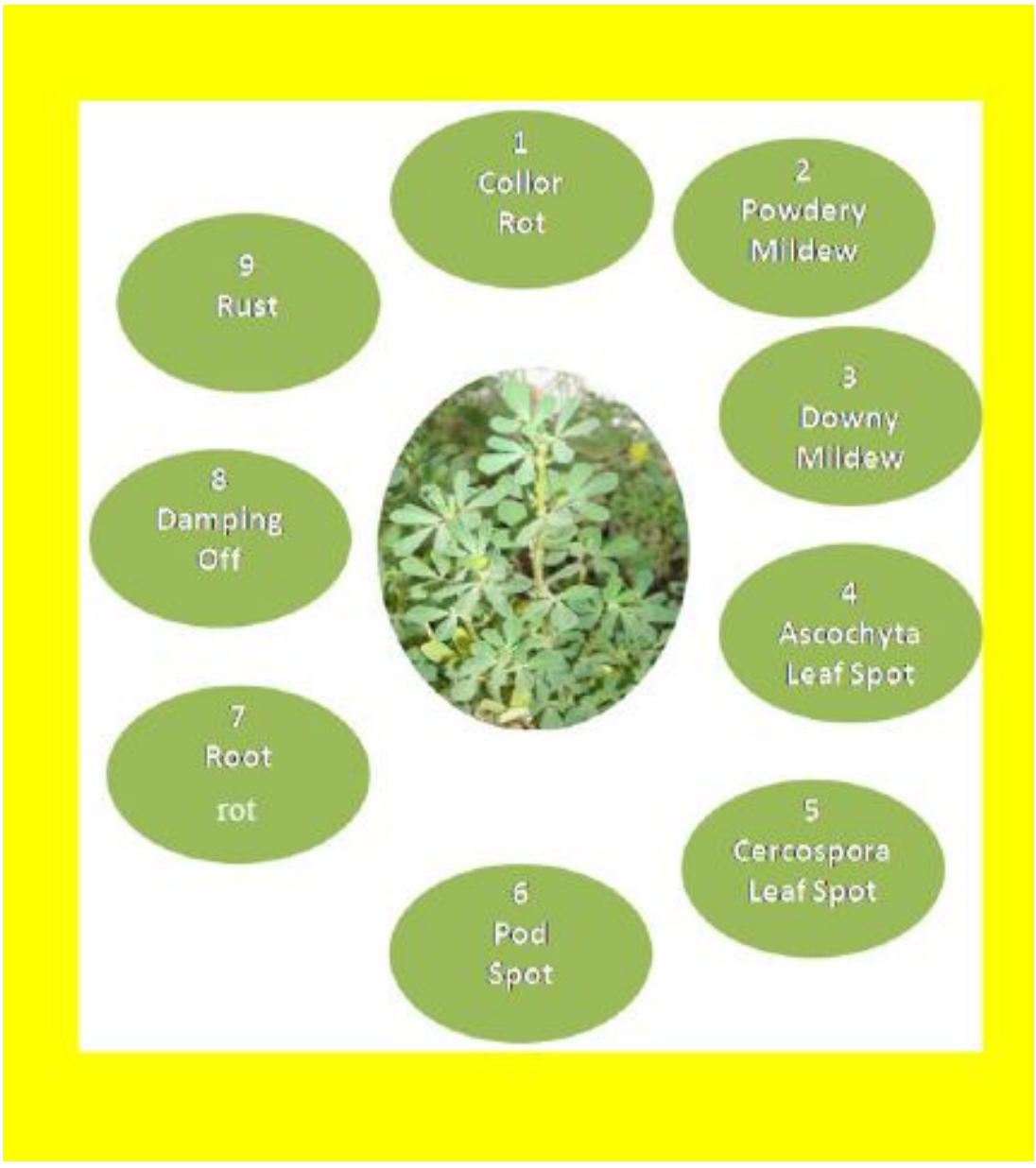


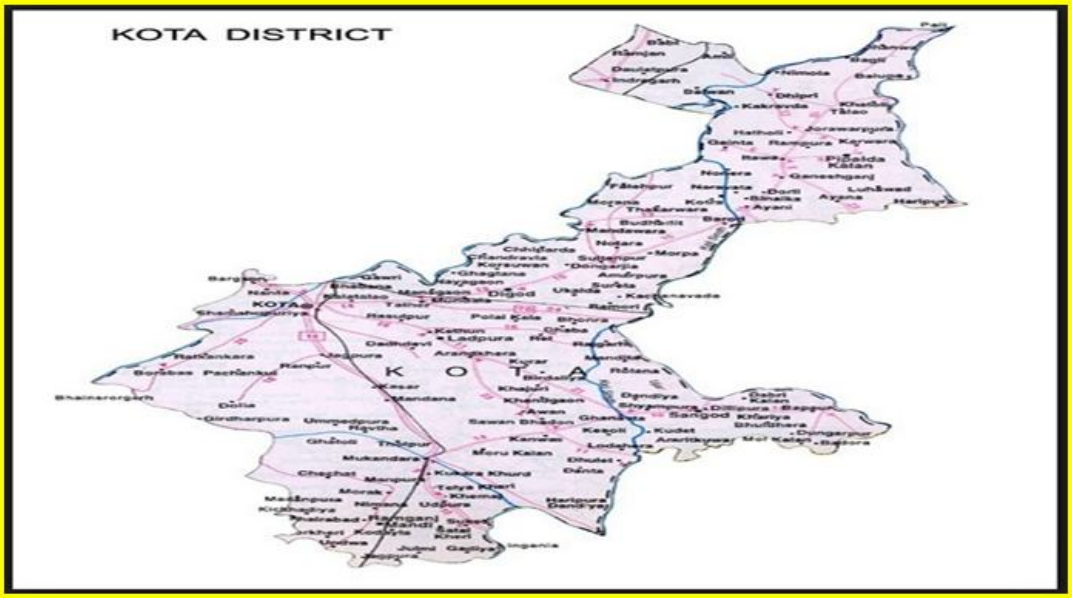
Figure –27 Comparison of Bioagents on Disease incidence in Fenugreek plant


1
Collor
Rot
2
Powdery
Mildew
3
Downy
Mildew
4
Ascochyta
Leaf Spot
5
Cercospora
Leaf Spot
6
Pod
Spot
7
Root
rot
8
Damping
Off
9
Rust

(A)

(B)

LOCATION MAP



Surveyed Localities of Kota District

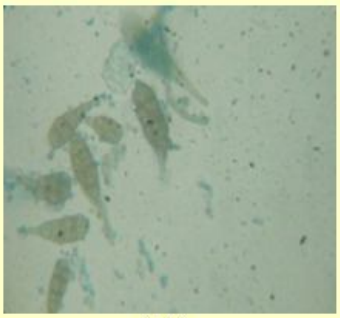
(A)

(B)

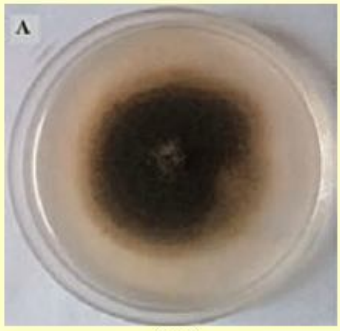
(C)

(D)

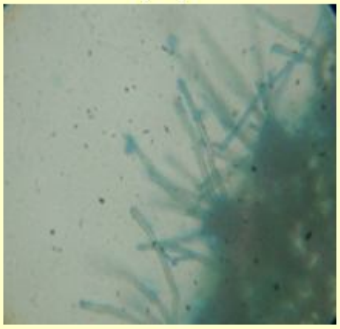
(E)



(A)



(a)

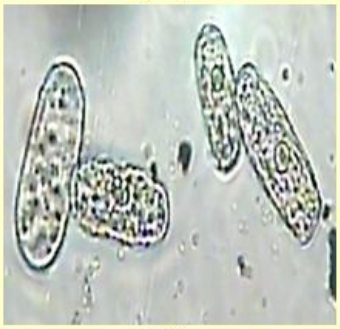


(B)

(b)

(C)

(c)



(D)

(d)

Intercropping of Fenugreek with Allium sativum

Soil drenching by Ash as a protective measure used by farmers

(A)

(B)

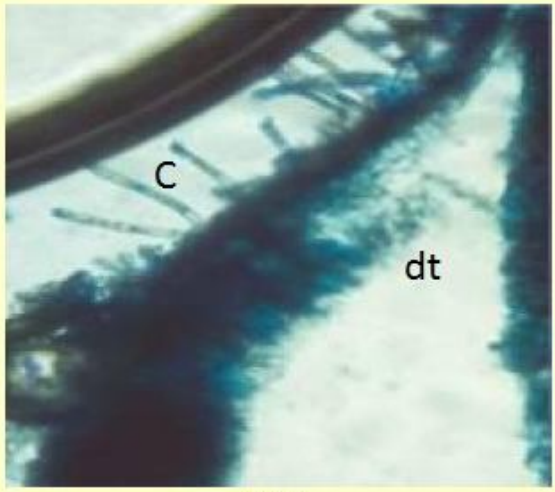
(C)

(D)

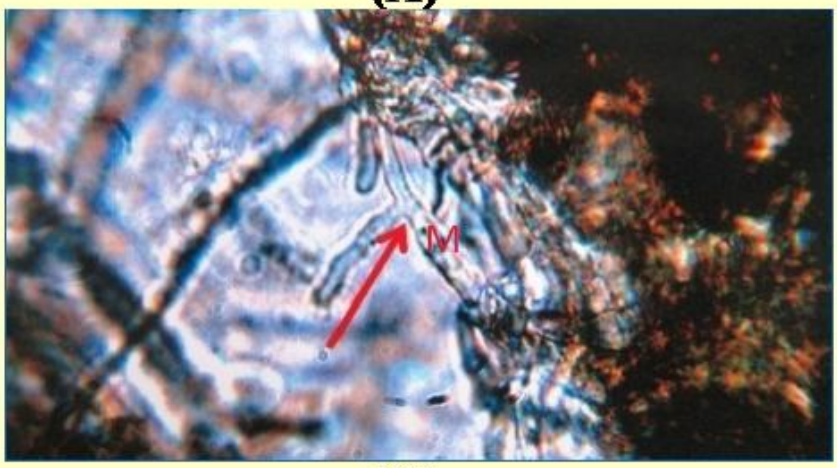
(E)

(F)

(G)



(A)



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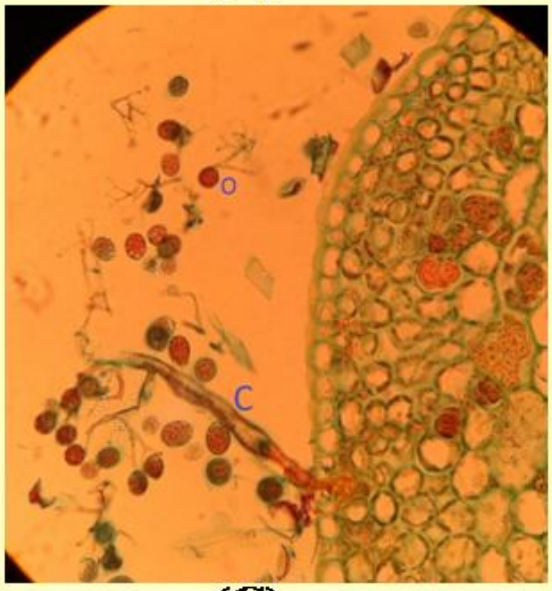
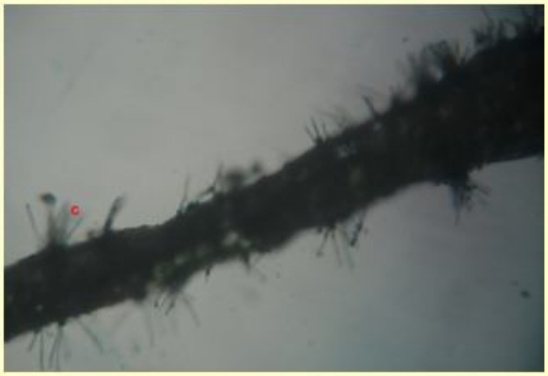


(C)

(A)



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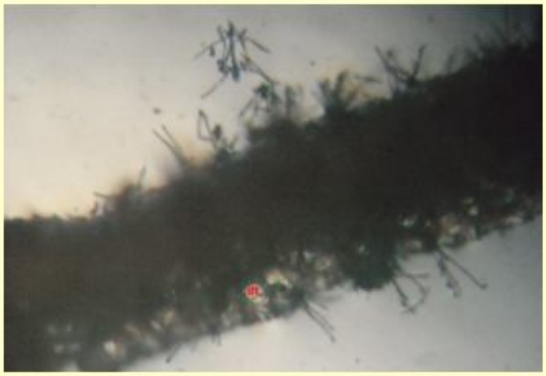
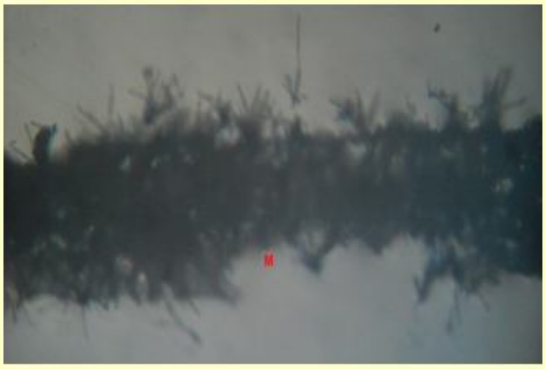


(C)



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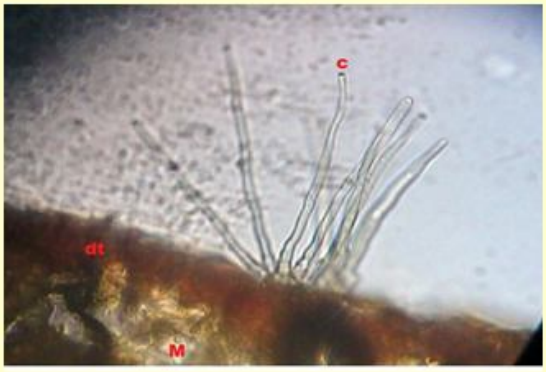
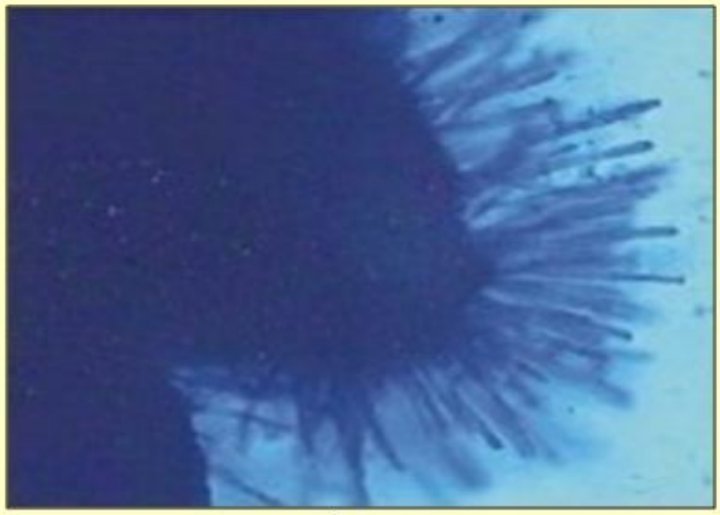


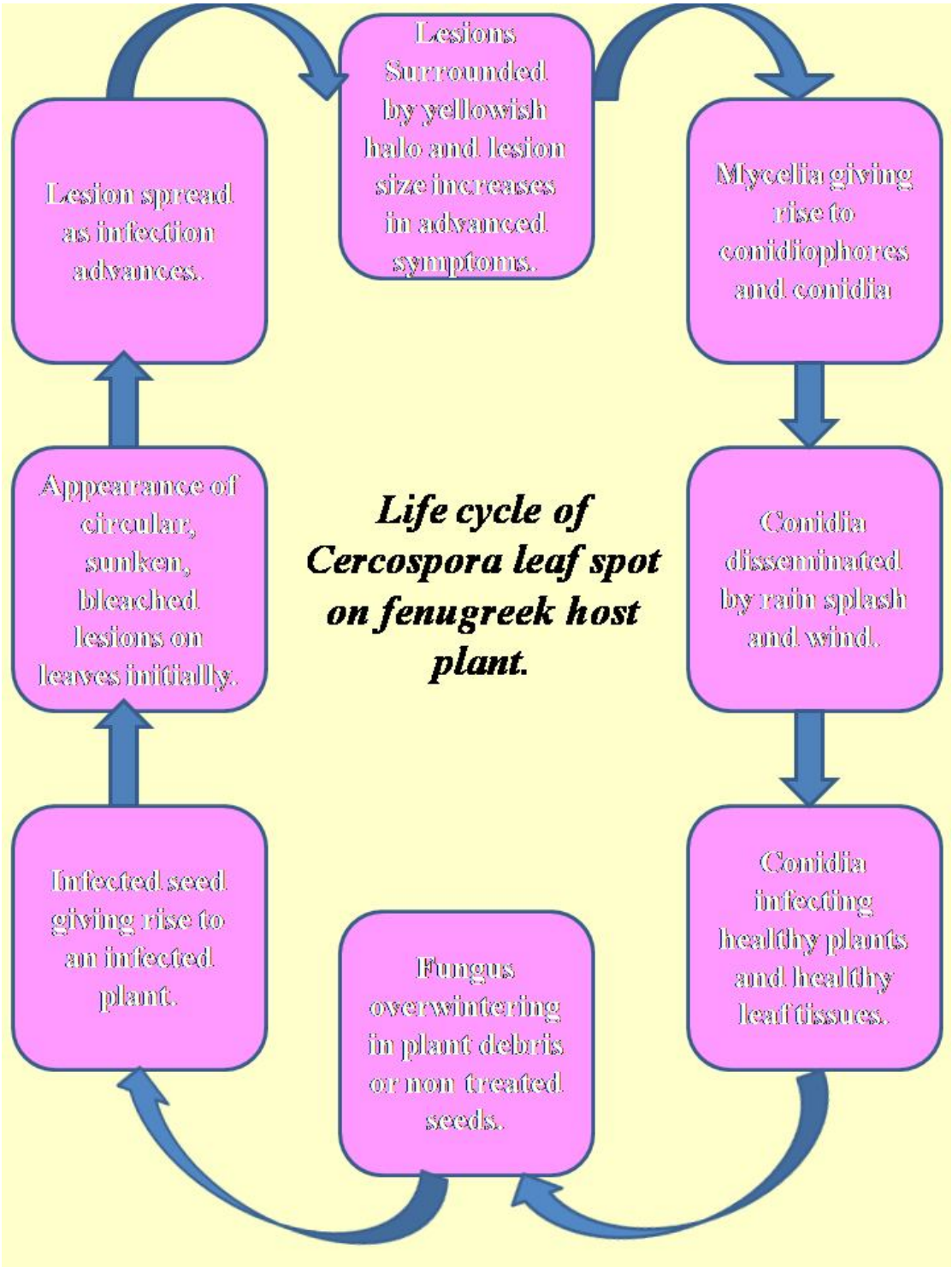
(E)

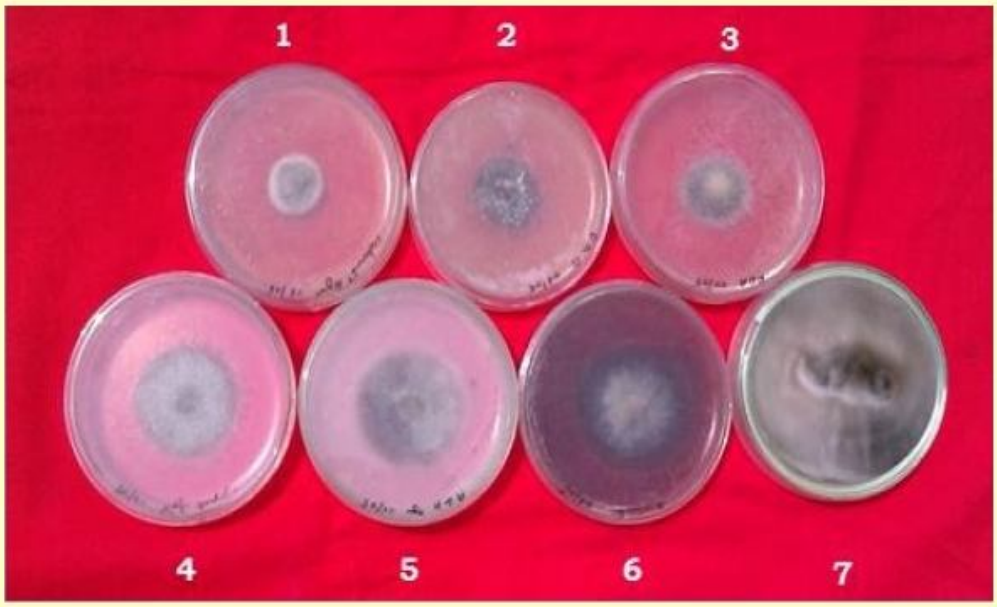


A

B

C


Lesions Surrounded by yellowish halo and lesion size increases in advanced symptoms.
Mycelia giving rise to conidiophores and conidia
Conidia dissemination by rain splash and wind.
Conidia infecting healthy plants and healthy leaf tissues.
Fungus overwintering in plant debris or non treated seeds.
Infected seed giving rise to an infected plant.
Appearance of circular, sunken, bleached lesions on leaves initially.
Lesion spread as infection advances.
Life cycle of Cercospora leaf spot on fenugreek host plant.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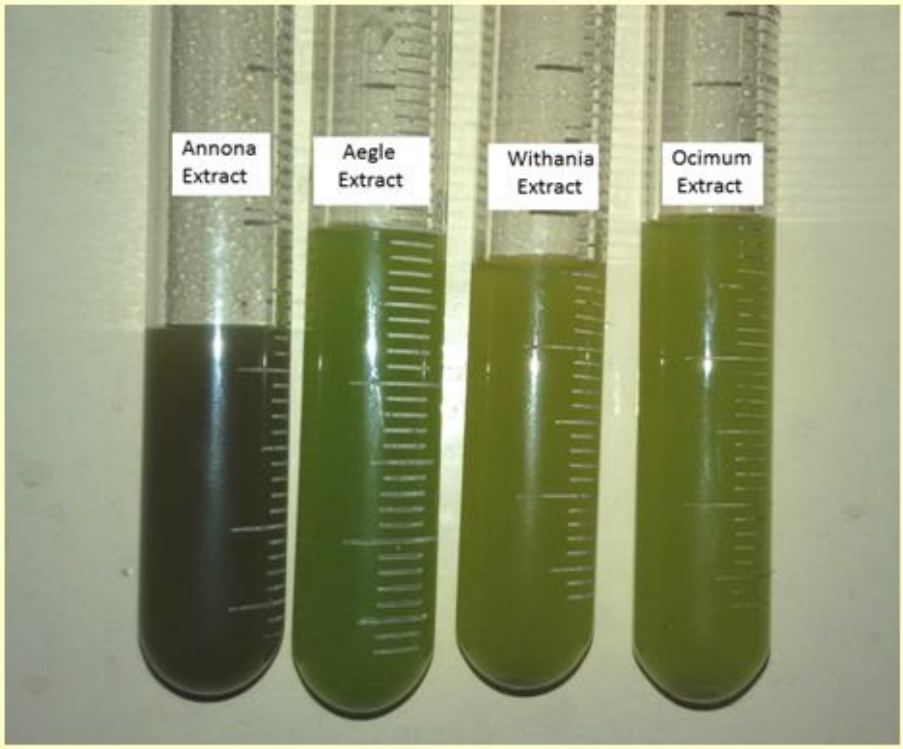


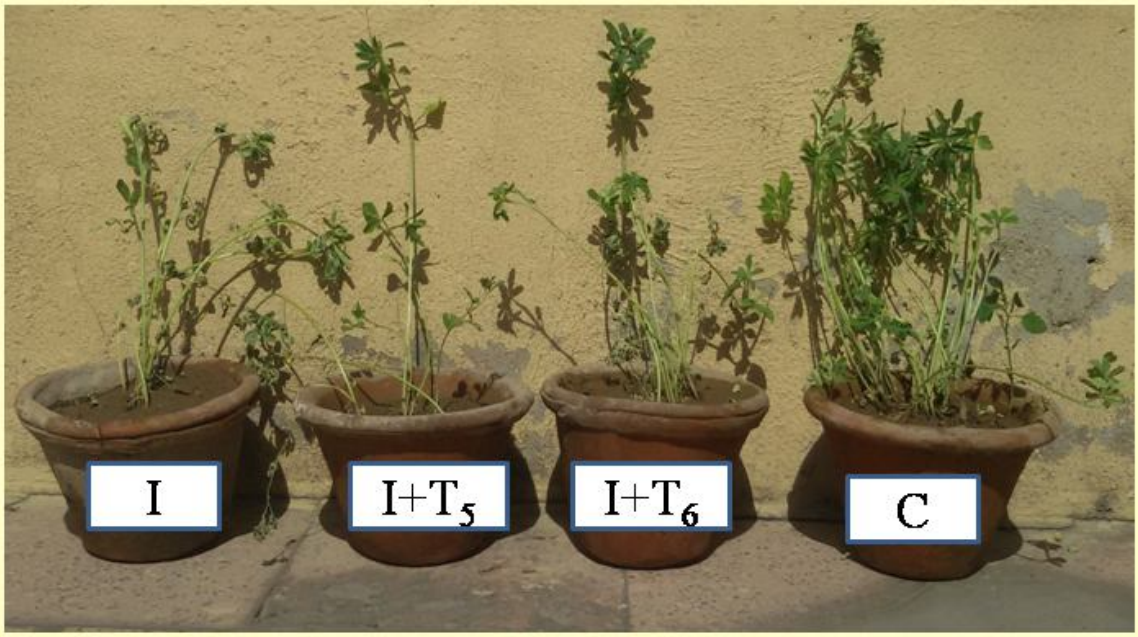
Botanicals as Control Agent

Seedlings in Pot Trials

In Vitro Evalution through different Botanicals against C. traversiana

In Vitro Evalution through Bioagent against C. traversiana

Treated With Botanicals



Treated with Bioagents

RMT- 1

RMT- 143

RMT- 303

HISAR SONALI

RMT- 305


RMT-1


RMT-143


RMT-303


HISARSONALI


RMT-305

Development of Leaf Spot on Fenugreek Leaves