Studies on Rhizosphere Mycoflora and AM Fungi associated with *Gossypium herbaceum* L.

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Abbreviations

mg	Milligram
mg/g	Milligram/ gram
cm	Centimeter
PDA	Potato Dextrose Agar Medium
pH	Potential of Hydrogen Ion
AM	Arbuscular Mycorrhizae
VAM	Vesicular Arbuscular Mycorrhiza
^{0}C	Degree in Celcius
mm	Millimeters
ml	Millilitre
PVLG	Polyvinyl Lactoglycerol
nm	Nanometre
ha	Hectare
mMT	Million Metric Tonnes

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Cotton (*Gossypium herbaceum* L.) belonging to the family Malvacae is one of the important fibre crop of global significance, which is cultivated in tropical and sub-tropical regions of more than seventy countries of the world. It is known as 'King of Fibres'. The major producers of cotton are China, India, USA, Pakistan, Uzbekistan, Argentina, Australia, Greece, Brazil, Mexico, and Turkey. These countries contribute about 85% to the global cotton production. India has the largest acreage (10.33 m. ha) under cotton at global level and has the productivity of 486 kg Lint /ha and ranks second in production 295 lakh bales (5.02 m MT) after China during 2009-10. Cotton plays a key role in the National economy in terms of generation of direct and indirect employment in the Agricultural and Industrial sectors (http:/www.cicr.org).

Cotton is a natural vegetable fibre of great economic importance as a raw material for cloth. Its widespread use is largely due to the ease with which its fibres are spun into yarns. Cotton's strength, absorbency, and capacity to be washed and dyed also make it adaptable to a considerable variety of textile products. Besides being a major natural fibre crop, cotton also provides edible oil and seed by-products for livestock food. Cottonseed oil is a vegetable oil ranking fifth in world use among edible oils (accounting for about 4% of world consumption of vegetable oil). The cotton seed meal is usually used as roughage in the diet of cattle for its high protein and energetic value.

About fifty species of cotton plants is known within the world of these only four are domestically cultivated for their fibres. The most commonly cultivated species of cotton in the world include *Gossypium hirsutum* and *G. barbadense* (also referred to as "New World" species), *G. herbaceum*, *G. hirsutum* originated in Mexico. It is the most important agricultural cotton, accounting for more than 90% of world fibre production. *Gossypium barbadense*, of Peruvian origin, accounts for about 5% of world fibre. It includes cotton fibres of the highest quality, such as the Jumel variety (from the Barbados), among the finest cotton in terms of quality and fibre length.

History

The genus *Gossypium* has a long history of taxonomic and evolutionary study. Our taxonomic understanding of the cotton tribe developed from more than a century of study involving traditional taxonomic methods as well as modern tools such as comparative analysis of DNA sequences. Speculation regarding the time and place of origin of *Gossypium* has a long history (Hutchinson *et al.*, 1947; Saunders, 1961; Fryxell, 1965; Edwards *et al.*, 1974; Valiček, 1978).

The place of origin of the genus *Gossypium* is not known, however the primary centers of diversity are west-central and southern Mexico (18 species), north-east Africa and Arabia (14 species) and Australia (17 species). DNA sequence data from the existing *Gossypium* species suggests that the genus arose about 10-20 million years ago (Wendel and Albert 1992; Seelanan *et al*., 1999). The antiquity of cotton in the Indian subcontinent has been traced to the 4^{th} millennium BC (Santhanam and Sundaran, 1997). The first reference to cotton is found in Rig Veda hymn (Khadi and Kulkarni, 2001). Most commercially cultivated cotton is derived from two species, *G. hirsutum* (Upland cotton, 90% of world plantings) and *G. barbadense* (Pima, or Long-staple cotton). Two other species, *G. arboreum* and *G. herbaceum*, are indigenous to Asia and Africa and are popularly referred as desi cottons in India.

India is the only country in the world where all the four cultivated species of cotton, viz. *G.hirsutum, G.arboreum, G.herbaceum* and *G.barbadense,* are cultivated on commercial scale, besides their hybrid combinations. The diversity of cotton cultivars and cotton agroclimatic zones in India is considerably larger as compared to other major cotton growing countries in the world.

Asiatic Cotton (*Gossypium arboreum, Gossypium herbaceum)*

India, China and the near east are the places which are the growers of this kind of cotton. It has coarse and harsh fibres and thus, is suitable for manufacturing products like blankets, filters, coarse clothes, padding materials and the like.

Under the rain fed growing conditions rainfall ranges from <400 to > 900 mm coupled with aberrant precipitation patterns over the years leading to large-scale fluctuations in production. In the irrigated tract canal and well irrigation is practiced including the use of micro-irrigation system.

The cultivated *G. herbaceum* was derived from the truly wild form of the diploid, *G. herbaceum* race *africanum* which has distribution in South Africa. It has been assumed that traders sailing between Mozambique and Western India introduced this wild form of *G. herbaceum* into Southern Arabia, where the first domestication in the Old World cotton took place. From here, the spread of the species led to the development of new races (Biology of cotton: www.dbtbiosafety.nic.in).

G. herbaceum is known primarily as a crop plant (grown from Ethiopia to Western India), with the exception of an endemic form from southern Africa, *G. herbaceum* sp. *africanum*. This morphologically distinct entity, which occurs in regions far removed from historical or present diploid cotton cultivation, has a unique ecological status in that it is fully established in natural vegetation in open forests and grasslands. Its small fruit, thick, impervious seed coats, sparse lint, and absence of sympatric cultivated *G. herbaceum* suggest that *G. herbaceum* sp. *africanum* is a wild plant. Consistent with the expectation that the site of original domestication lies within the range of the wild progenitors, this is generally accepted as the source of the original *G. herbaceum* cultigens (Hutchinson, 1954). The most agronomically primitive *G. herbaceum* cultivars, the perennial race *acerifolium* forms, are distributed along the coasts boarding the

Indian Ocean trade routes. This suggests that the primary dispersion involved the diffusion of *G. herbaceum* northward into northern Africa, Arabia and Persia. Hutchinson (1954) suggests that secondary agronomic development and diffusion led to expansion into western Africa and the development of annualized forms in more northerly temperate climates. The agronomic success of the annualized *G. herbaceum* races fostered a later dispersal into peninsular India that replaced perennial *G. arboreum* cultigens (Wendel *et al.*, 2010).

Cotton Cultivation in India

There has been a significant enhancement in production from 2004-05 onwards as compared to the earlier years (from 17.7 m bales in 2003-04 to nearly 29.5 m bales in 2009-10). Adoption of improved technologies IPM, IRM, new chemistry (including Bt cotton) coupled with favourable weather and low insect pest pressure in major cotton growing tracts has enabled this transformation in production and productivity. Punjab and Gujarat states recorded much higher productivity than national average and contributed to a large measure in enhancing productivity and production at the national level. The average national productivity showed a remarkable spurt from nearly 309 kg lint/ha (2001-02) to 560 kg lint per ha in 2007-08 and 486 kg lint/ha in 2009-10. A trend of continuous improvement is quite clear from 2002-03 onwards(Biology of cotton: www.dbtbiosafety.nic.in).

Bt cotton

Advancement of biotechnological tools and genetic engineering paved the way for development of transgenic cotton (Boll guard), which offers great promise in the control of bollworms. The commercial cultivation of such transgenic cotton conferring pest resistance began during 1996.

Bt cotton, which confers resistance to Lepidopteron pests of cotton, was first adopted in India as hybrid in 2002 after stringent assessment for bio-safety and profitability. In India, after extensive testing of Bt cotton hybrids (with Cry1 Ac gene) in All India Coordinated Cotton Improvement Project (AICCIP) and farmers field, Government of India has approved commercial cultivation of Bt cotton hybrid with effect from 2002 crop season. The transgenic hybrids released in the country can be categorized in different ways on the basis of transgene involved. They can be categorized into two groups *viz*., (i) Bollgard I (single gene) and (ii) Bollgard II (double gene).

Bt Cotton is a genetically engineered form of natural cotton. The main advantage of utilizing biotechnology in agriculture are the possibilities of increase in productivity through the use of newer varieties that possess properties such as resistance to pests, diseases, and other stressful conditions like drought, salinity, or water logging. Of these measures, imparting the property of insect (specific) resistance through the transfer of a gene from *Bacillus thuringiensis* Berliner (Bt) into target plants by modern biotech methods is presently considered to be one of the most advanced applications of biotechnology.

Bt or *Bacillus thuringiensis* is a gram positive, ubiquitous soil bacterium first discovered in 1901 by Ishiwata, a Japanese Microbiologist (Kumar *et al.*, 1996). Later it was found that some Bt strains (Cry+) were highly toxic to larvae of certain insect species which are also plant pests. Bt was first sold as a spray formulation in 1938 in France for the management of European corn borer. Subsequent research has revealed that Bt carries proteinaceous crystals that cause mortality in those insects which carry receptor proteins in gut membranes that bind to Bt proteins. Other organisms that do not contain receptors to Bt proteins are not affected by the toxin. The advent of genetic transformation technology made it possible to incorporate cry genes and thus the ability to produce Bt proteins in plant cells so that target insect larvae infesting the

crop plants are effectively killed. The first Bt crops *viz.*, Bt cotton, Bt corn and Bt potato were commercialized in USA in 1996. Bt crops are currently cultivated in 23 countries over an area of 46 mha (James, 2008).

Agro climatic conditions

Cotton requires a daily minimum temperature of 16°C for germination and 21°C to 27°C for proper crop growth. During the fruiting phase, the day temperature ranging from 27°C to 32°C and cool nights are needed. The sowing season of cotton varies considerably from tract to tract and is generally early (April-May) in northern India where it is mostly irrigated. It is delayed on proceeding to down south. It is cultivated largely under rainfed or dryland conditions. An annual rainfall of atleast 50 cm distributed through-out the growing season is required for good yield. It is mainly raised during tropical monsoon season, although in southern India it is cultivated during late-monsoon season in winter.

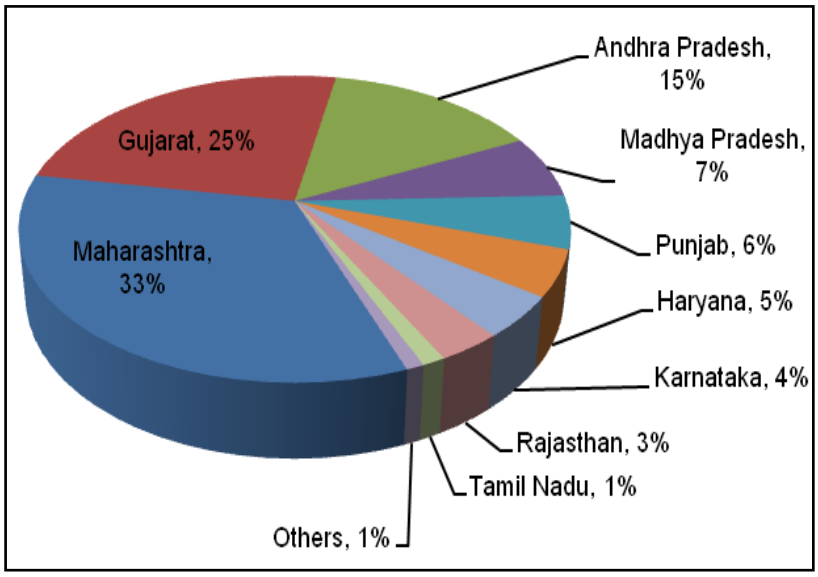
Cotton is successfully grown on all soils except sandy, saline or water logged types. It is grown in well drained deep alluvial soils in the north to black clayey soils of varying depth in central zone and in the black and mixed black and red soils in south zone. It is moderately tolerant to salinity and is sensitive to water logging as well as frost and chilling temperature in winter.

Zonal Distribution

Cotton is grown in India in three distinct agro-ecological zones *viz*., north zone (Punjab, Haryana, Rajasthan and Western Uttar Pradesh), central zone (Gujarat, Madhya Pradesh, Maharashtra and Orissa) and south zone (Karnataka, Andhra Pradesh and Tamil Nadu). The northern zone is totally irrigated, while the percentage of irrigated area in the central and southern zones is much lower, the lowest being in the central zone which has nearly 60% of cotton area. It is also grown in small area in the eastern region in Sundarbans of West Bengal and

in north-eastern states. Nearly 60% of cotton area is accounted by Central zone even though the irrigation source and potential are very much limited in central zone, ideal temperatures and ample sunshine during growth and maturity periods and the extended moderately cool, rain free dry weather prevailing during October to February are favourable for obtaining higher yields.

Fig 1. Pie chart showing state wise cotton acreage in India



Source: (www.dbtbiosafety.nic.in).

Table showing list of Bt cotton varieties grown in Gujarat

Sr. No.	Variety	Company Name
1	MECH 12 Bt*	M/s Mahyco
2	MECH 162 Bt*	M/s Mahyco
3	MECH 184 Bt*	M/s Mahyco
4	RCH 2 Bt	M/s Rasi Seeds Ltd
5	NCS –207 Mallika Bt	M/s Nuziveedu Seeds Ltd
6	NCS –145 Bunny Bt	M/s Nuziveedu Seeds Ltd
7	RCH –144 Bt	M/s Rasi Seeds Ltd
8	RCH –118 Bt	M/s Rasi Seeds Ltd
9	RCH -138 Bt	M/s Rasi Seeds Ltd
10	RCH –20 Bt	M/s Rasi Seeds Ltd
11	Ankur –651 Bt	M/s Ankur Seeds Ltd
12	Ankur – 09	M/s Ankur Seeds Ltd
13	RCH 377 Bt	M/s Rasi Seeds Ltd
14	GK 205 Bt	M/s Ganga Kaveri Seeds PvtLtd
15	GK 205 Bt	M/s Ganga Kaveri Seeds PvtLtd
16	KDCHH 9632 Bt	M/s Krishidhan Seeds Pvt Ltd
17	KDCHH 9821 Bt	M/s Krishidhan Seeds Pvt Ltd
18	ACH-33-1 Bt	M/s Ajeet Seeds Ltd
19	ACH-155-1	M/s Ajeet Seeds Ltd
20	Tulasi 4 Bt	M/s Tulasi Seeds Pvt Ltd
21	ACH-11-2 BG II	M/s Ajeet Seeds Ltd
22	JK Varun Bt	M/s JKAgri Genetics Seeds Ltd
23	Ankur 2226 BG	M/s Ankur Seeds Ltd
24	Sigma Bt	M/s Vibha Agrotech Ltd
25	VBCH-1010 Bt	M/s Vibha Seeds (P) Ltd
26	SP 504BI (Dhanno) Bt	M/s Proagro Seeds Co (P) Ltd
27	VICH-15 Bt cotton	M/s Vikram Seeds Ltd
28	322 Bt cotton	M/s Bioseeds Research India Pvt Ltd
29	NCHB-992	M/s Nuziveedu Seeds Pvt Ltd
30	Ajeet 155 BG II	M/s Ajeet Seeds Ltd
31	RCH-515 BG II	M/s Rasi Seeds (P) Ltd
32	JK Durga Bt	M/s J K Agri Genetics Ltd
33	Atal BG II	M/s Monsanto Genetics Pvt Ltd
34	Paras Lakshmi BG II	M/s Monsanto Genetics Pvt Ltd
35	Sarju BG	M/s Solar Agrotech Pvt Ltd

Table showing different parameters of cotton production in Gujarat (2008-2012)

Sr. No.	Parameters	2008-09	2009-10	2010-11	2011-12
1	Cotton area (lakh ha)	23.54	26.25	26.33	30.23
2	Cotton production (lakh bales)	90.00	98.00	103.00	114.00
3	Cotton productivity (kg/ha)	650	634	685	659

Source: www.cicr.org.in

Economic Importance of Cotton

The bulk of cotton production is consumed in the manufacture of woven goods, alone or in combination with other fibers. Cotton constitutes of one of the basic raw materials for cellulose industries including plastics, rayon and explosives. Sterilized absorbent cotton finds use in medical and surgical practice.

Cotton waste is a by-product of the spinning and weaving mills and consists principally of short fibres rejected by combing and carding machines. The amount of waste given by cotton is an important factor in its quality evaluation. Cotton waste of good grade is employed in making cotton blankets, sheets, towels and flannelettes. The stalks of the plant contain a fibre that can be used in paper making or for fuel, and the roots possess a crude drug. The seeds are of the greatest importance as oil is utilized. The hulls are used for stock feed; as fertilizer; for lining oil wells; as a source of xylose, a sugar that can be converted into alcohol and for many other purposes. The kernels yield one of the most important fatty oils, cottonseed oil and an oil cake and meal, which are use for fertilizer, stock feed, flour and as a dyestuff.

- **Cotton Pests and Diseases**

Cotton pests

Cotton insects are the principal cause of yield losses. Estimates indicate that the yield losses due to insect infections would amount to almost 15% of world annual production.

More than 1300 different species of insect pests attack the crop. Among the most common and endogenous species found in cotton fields are:

• **The pink bollworm** (*Pectinophora gossypiella* Saunders) was first described in 1843 by W.W. Saunders as *Depressaria gossypiella*, from specimens found to be damaging cotton in India in 1842. The pink worm withdraws nutrients from the inside of the cottonseed and may cause serious yield losses. Although the most sever infestations have occurred in Africa and India, the pink bollworm has been recorded in nearly all cotton-producing countries and is a key pest in many of these areas. Infestations may be reduced by the heating of cottonseeds at about 55°C, as well as by other management tactics, including plantation treatment and destruction of the infested crop.

• **The boll weevil** (*Anthonomus grandis* Boheman), also known as bollworm, is most common in American cotton plantations.

• The Egyptian (spiny) bollworm (*Earias insulana* Boisduval) and the red bollworm (*Diparopsis castanea* Hmp.) feed on the developing cotton bolls.

• **Cotton stainers** (*Dysdercus superstitious* Fabr.) attack maturing cotton bolls and seeds. They may cause the staining of the lint. In addition, feeding wounds may allow the entry to the boll of saprophytic fungi (organisms which draw nutrients from the host, but do not harm it, contrary to parasites).

• Other insect pests of cotton, such as the white flies (*Bemisia gossypiella* Saund.), may adversely affect lint quality and yield potential. They suck sap from leaves and pose the most serious threat in India and Africa.

• **The cotton aphid** (*Aphis gossypii* Glover), also known as the melon aphid, infests the cotton seedlings. Cotton aphids are among the most injuring insects found in cotton. They suck sap from leaves and secret honeydew on the undersides of leaves. Honeydew secretions may burn the leaves and interfere with photosynthesis. In addition, aphid is a vector of viruses and a carrier of other insects. In Africa, aphid infestations are among the most injuring insect pests in terms of economic yield lost.

• **Nematodes:** Nematodes or round worms are a diverse group of animanls belonging to the phylum Nematoda inhibiting a very broad range of environments. They are found in almost all habitats. There are approximately 128 species of nematodes associated with cotton. Five parasitic forms pose the most serious threat to the crop, including the *Meloidogyne incognita* Goldi (or root knot nematode) and the *Rotylenchulus reniformis* Lindford and Oliveria (or reniform nematode). These two species can become serious pests (in the United States, particularly in the State of Virginia, they accounted for 99% of the damage caused by cotton parasitic nematodes). These parasites live in the soil (the root knot nematode favours rough and arenaceous soil) and withdraw nutrients from the plant roots. Symptom patterns associated with nematodes include stunting, potassic deficiency or early maturity. Nematodes can reduce yields (in Alabama, United States, yield losses are estimated to average 10% or 20%, but can peak to 50% in arenaceous dry soil). In addition, depending upon the stage of development of the infested crop, they can hamper the quality of cotton. Root knot nematodes do produce plant damage symptoms that are rather easy to recognise, such as the yellowing or whitening of normally green plant tissue because of a decreased amount of chlorophyll. Damage symptoms caused by other kinds of nematodes (for

example, the reniform nematode) are more difficult to detect, since they are generally small and sparse. Besides the direct damage, nematodes are also an important factor in the incidence of *Fusarium* and other wilts of cotton. Nematodes may be controlled by cultural practices, such as crop rotations, soil tilling, and use of resistant varieties, or by chemical treatment through nematicides. The two types of nematodes seldom coexist in the same fields.

- **Bacterial and Fungal diseases in the cotton plant**

  - **Bacterial blight of cotton**

Also called angular leaf spot (*Xanthomonas malvacearum (*E.F. Smith) Dowson) is favoured by wet weather conditions (temperature above 25°C and relative humidity exceeding 85%). Disease incidence is higher in plants with injured tissues (due to insect pests or cold temperatures). The disease causes stunting and yellowing of the leaves (mainly lower leaves). As diseases progresses, it may result in defoliation. Affected bolls are smaller than normal and exhibit small black spots on their surface. Bolls may fail to open or produce bad quality lint.

Use of copper oxychloride and streptomycin sulphate has been suggested against the disease Singh *et al*., (2010) suggested use of fungicides against foliar pathogens.

  - **Boll rots** (*Diplodia gossypina* Berk and M.A. Curtis, *Colletotrichum* spp., *Fusarium* spp.)

Attacks lower bolls near maturity. Warm, humid conditions favour the disease. Affected bolls are dark brown, with a white to salmon-pink overgrowth. The fungus is capable of giving a brownish tint to the lint. This disease is a stress-related one, in the sense that it infects plants that have been previously damaged by insect pests.

- **Verticillium wilt of cotton**

The *Verticillium dahlia* Kleb, a common soil inhabitant, penetrates though roots and grows up along the stem tissue. Cooler temperatures, excessive soil moisture and excessive soil nitrogen levels favour the fungus. Symptoms first appear on the lower leaves, which turn yellow. Larger plants are stunted (as diseases progresses, defoliation may occur), whereas younger seedlings may die.

Management strategies include proper management of irrigation and the selection of resistant varieties. Under conditions favorable to the development of the disease, yield reductions of up to 30% are possible. Seedling diseases (fungi *Rhizoctonia solani, Pythium* spp.) may result in to seed and root rott. In the case of *Rhizoctonia solani*, girdling of the stem at ground level is observed. *Pythium spp.* is characterized by the similar symptom patterns, with a water soaked lesion at the soil line.

- **Fusarium wilts** (*Fusarium oxysporum f. sp. . vasinfectum*)

Wet weather conditions (temperature above 23°C and relative humidity exceeding 85%) are particularly conducive for disease development. Disease incidence can be higher in plants with injured tissues (for example, plants damaged by nematodes). The disease can affect plants at any stage during the season. The vascular tissue of infected plants exhibits a brown/chocolate discoloration through the main stem. Infected water-conducting stem tissues become inactive, causing wilted foliage. Plant death, wilting, yellowing and defoliation are typical of disease symptoms. Leaves turn yellow between veins and eventually shed to leave bare stems. Once the fungus has colonized the plant (diagnosis is confirmed by splitting the stem to reveal dark brown), it most likely causes the death of the host. There is no commercially viable way to eradicate the disease once established (apart

from soil fumigation, which is excessively expensive). The impact of the disease may nonetheless be reduced by the use of varieties with high levels of resistance *to Fusarium* wilt, or by avoiding crop stresses such as over-irrigation and over-application of nitrogen. *Fusarium* wilt is now an important constraint to sustainable cotton production, especially in Australia.

Fusarium wilt of cotton, caused by *F. oxysporum f sp.vasinfectum*, was first recognized in Australia in 1993. It is a soil-inhabiting fungus that invades cotton plants via the roots and causes a blockage of the water conducting tissues resulting in wilting and eventual death of affected plants. The pathogen can also be seed borne.

SYMPTOMS

External: Growth is stunted and leaves initially appear dull and wilted, before yellowing or browning progresses to eventual death from the top of the plant. Some affected plants may re-shoot from the base of the stem. External symptoms can appear in the crop at any stage but most commonly become apparent in the seedling phase when the plants begin to develop true leaves and after flowering when the bolls are filling.

Internal: Lengthwise cutting of the stem of an affected plant will reveal continuous brown discolouration of the stem running from the main root up into the stem. The internal discolouration is similar to that of Verticillium wilt but usually appears as continuous browning rather than flecking in the stem tissue. The severity of external symptoms does not always reflect the degree of internal discolouration that might be seen when the plant is cut open. Often the discolouration might only be visible up one side of the plant. Symptoms can appear as only a few individual plants or as a small patch, often but not always in the tail drain or low-lying (waterlogged) areas of a field.

Other Fungal Diseases

Of all diseases known to occur in cotton, cotton root rot (*Phymatotrichum omnivorum* (Duggar) Hennebert) is one of the most destructive and difficult to control. The fungus lives in alkaline soils low in organic matter. It occurs only at elevations below 1500m. The fungus has unique biological characteristics that contribute to management difficulties. Fungus *P. omnivorum* has a remarkably wide host range, although it attacks only mature plants and does not easily spread from field to field. Second, the fungus survives for long periods in the soil (much of the fungus is found as deep as 60cm to 2m in soils). This explains why fungicides are not effective treatment. The fungus is only active when air and soil temperatures are high (respectively above 40°C and 27°C). When environmental conditions are conducive to its development, the fungus invades the plants through the root system. Infected plants can die in two weeks. The first disease symptom is slight yellowing of the leaves, which then quickly turn to a bronze colour and begin to wilt.

List of Fungi already reported from *G. herbaceum* includes (Bilgrami *et al.*, 1981)

Chaetomium spiralotrichum Lodha.
Colletotrichum sp.
Helminthosporium gossypii Tucker
Myrothecium roridum Tode.
Sclerotium rolfsii Sacc.
Trichothecium roseum (Pers.)Link
Verticillium alboatrum var dahliae Reinke and Berthold.

Soil forms a rich and dynamic medium for all microorganisms. Soil being a complex ecosystem, is composed of multiple, minute habitat and harbours almost all major taxonomic groups of fungi. Considering all living forms, the diversity of soil microorganisms in general is

more extensive than any other environment in the world. It has been found that more number of genera and species of fungi exists in soil than any other environment, as soil is exposed to various conditions and basically receives all microorganisms present on this plane (Stotzky, 1997). Along with the bacteria, actinomycetes and algae , fungi are primary decomposers ; agents of biogeochemical transforms and recyclers of stored energy and nutrients of the organic matter already degraded by invertebrates and other microbes for plant growth. Fungi occur in soil either in mycelia state or reproductive stage (Nagamani *et al.,* 2006).

- **Rhizosphere Mycoflora**

The rhizosphere may be defined as that portion of the soil which is adjacent to the root system of a plant and is influenced by the root exudates. The area of this zone depends on the soil type and host plant under study and soil environment conditions. The roots exert influences on various type of microorganisms. The stimulatory effect on microorganisms is known as the "Rhizosphere effect" as indicated by the interaction of soil and rhizosphere microbes and their ratio. The chemical and physical nature of the root zone is quite different from the soil away from the root zone and the biology of this complex zone has been studied extensively. The term 'Rhizosphere' was proposed by Hiltner (1904). The phenomenon of accumulation of microorganisms around the root zone was reported by a number of earlier workers (Agnihothrudu 1955; Starkey 1958; Rouatt 1959; Katznelson 1946). Various compounds such as amino acids, vitamins, sugars, tannins etc. are exuded by the roots. Some root exudates are also known to affect certain microbial species adversely leading to their decrease in the root zone and, in return, microorganisms are known to exert profound influence on the plant itself by decomposition, affecting nutrient uptake, antagonistic effect on other microbes and by parasitism.

Interestingly different types of microbes like fungi, bacteria, nematodes and viruses may interact with the same plant simultaneously either independently, synergistically or antagonistically. Factors such as soil type, soil moisture, pH, temperature, plant age, relative humidity and several other factors are known to influence the rhizosphere effect.

According to Pinton *et al.* (2001), rhizosphere represents a poorly defined zone of soil with a microbiological gradient in which maximum changes in the population of microflora in soil is evident adjacent to root and decline with distance away from it (Newman 1978; Bowen and Rovira 1991). Root exudates stimulate microbial activity selectively in rhizosphere and rhizoplane regions (Bansal and Mukerji 1994). There is an intense competitive activity by the obligate saprobes, unspecialized root parasites and root inhabiting fungi depending on their behaviour towards exudates. In case of root diseases the pathogen has to react with the rhizosphere and rhizoplane fungi before entering the root tissues. These may show antagonism and check its advancement. Plant microbe interaction is a regular and continuous feature of the biological world. The beneficial fallouts of such interactions have been extensively exploited for economic gain in recent years.

The term ‘rhizoplane’ was proposed by Clark (1949) to refer to the immediate surface of plant roots together with any closely adhering particles of soil or debris. Using different isolation techniques microorganisms have been isolated and identified by a number of Mycologists.

Fungi are very large and diverse group of organisms which have a unique lifestyle. They have worldwide distribution and successfully exploit many different habitats. They are extremely variable in form and versatile in the ways they solve the problems posed by the environments they inhabit (Susan, 1992). Fungi are ubiquitous; some having beneficial effects on plants, while others may be detrimental (Anderson and Cairney, 2004; Ipsilantis and Sylvia, 2007).

Micro organisms are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soil.

Type of cultivation and crop management practices found to have greater influence on the activity of soil microflora (Mc Gill *et al.,* 1980).

The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance; particularly in the face of global climate change and human alteration of ecosystem processes. Fungi are the important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995, Saravanakumar and Kaviyarasan 2010). Soil is an important panorama of interactions between microbes and plants (Shekh *et al.,* 2012). It is one of the most important habitats for filamentous fungi are major contributors to soil biomass (Pandey *et al.,* 2013).

The term rhizosphere was first introduced by a German microbiologist, L. Hiltner (1904). It is describe the zone of metabolically active soil which contains higher microbial community that surrounds and is influenced by the roots of plants (Mishra, 1967; Chamle *et al.,* 2011). Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.,* 1997; Tokuda and Hayatsu, 2002).

Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems. It has been estimated that 1.5 million fungal species are present in natural ecosystems, but only 5 –10% have been described formally (Hawksworth 2001). Schmit and Mueller (2007) estimated that there is a minimum of 7, 12,000 fungal species worldwide. The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been described (Wang *et al.* 2008). Research on fungal diversity provides a basis for estimating the functional role of fungi in ecosystems.

One of the most important factors responsible for the growth of microorganisms is organic substances exuded by roots i.e. root exudates (Liljeroth and Baath, 1988). The exudates include simple sugars, amino acids, organic acids, vitamins and many other compounds

(Singleton and Sainsbury, 1991; Klein, 1992). The influence of exudates upon rhizosphere microorganisms varies with plant age as well as plant type (Abdel-Rahim *et al.,* 1983; Oyeyiola 2009). However soil factors, such as moisture influences the amount of exudation and hence colonization of the roots (Whipps and Lynch, 1986). The organisms inhabiting soil includes microalgae, fungi, bacteria, actinomycetes, protozoa etc (Garrett, 1981). They carry out numerous transformations as a part of their normal activities like addition of organic matter, nitrogen fixation, solubilization and immobilization of several nutrients (Katayama *et al.,* 1998; Lal, 1998; Muller *et al.,* 1998; Brady and Weil, 1999).

The fungi responsible primarily for the decomposition of organic compounds (Paul and Clark, 1989) actively participate in processes related to biodeterioration and biodegradation (Allsop and Seal, 1986; Eggins and Allsopp, 1975; Molin and Molin, 1997; Trevors, 1998; Wall and Virginia, 1999) and also influence above- ground ecosystem by contributing to soil fertility (Yao *et. al.,* 2000; O'Donnell, 2001; Van der Heijden, 1998; Cairney,2000; Klironomos *et.al.,* 2000; Ovreas, 2000).

Besides this soil type, macro and micronutrients may also adversely affect the mycoflora (Rama Rao, 1957). The plant type, age and soil type have a significant influence the nature and number of mycoflora. (Wahegaonkar 2009; Namdas and Bhosale, 2009; Abdul-Hafez, 1982). Most rhizosphere fungi are highly dependent on association with plants that are regulated by root exudates (Bais, 2004).

The rate of biodegradation depends on environmental factors, numbers and types of microorganisms present and the enzymatic processes leading to the disappearance of the parent molecular structure and the formation of smaller organic species (Sharma and Raju 2013).

Soil micro-organism has the capacity to detoxify and inactivate pesticide present in the soil (Hill *et al.,* 1995). The micro-organisms present in soil depend on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration pH and temperature etc. The main focus of the study is to isolate mycoflora from different agricultural fields and to observe the percentage contribution of different fungal species

Soil bore plant pathogenic fungi a major economic loss, which is a major problem among the agricultural community. Nowadays the diseases are managed with the application of chemical pesticides. Use of chemical pesticides causes environmental problem, as they don't undergo biodegradation. So minimizing the application of pesticides has become order of the day. To achieve this goal the biological control methods can be effectively used along with other methods of disease control. Antagonistic interactions and cell free culture filtrate have been used to demonstrate the role of antibiotics in biological control.

Knowledge on the modes of survival of pathogens and the ways by which they could be suppressed are important especially in the control of plant diseases. The pathogens, in the absence of their hosts, survive either as dormant propagules or actively as saprophytes on dead organic substrates of the host in the soil. The survival structures of the pathogens in the soil are suppressed either due to manipulation of the soil environment. The pathogen suppression in the soil is considered as important step in the control of diseases as it involves the direct disinfestations of the soil.

A decrease in crop yield as a result of a plant disease caused by a pathogen is a negative effect. Some fungi are the main pathogens responsible for plant diseases and they may cause high yield losses. There are many ways to reduce yield losses caused by fungal diseases, with the application of chemical fungicides, presently being the most common method. Chemical fungicides however, have a negative effect on human health and on the environment. The application of such fungicides over a long period may result in plant pathogenic fungi developing

resistance. When this happens the chemical fungicides become ineffective and other fungicides must be used for effective disease control. The use of microorganisms as biological control agents to control plant disease is a potentially powerful alternative method (Kulkarni *et al*., 2007). Over the past 30 years, microorganisms have been described, characterized, and tested for their use as biocontrol agents against diseases caused by soil borne plant pathogens. A wide range of biological control agents have been developed as commercial mycofungicide products in past few years (Benítez *et al*., 2004; Kim and Hwang, 2004; Fravel, 2005).

An alternative way to increase the crop yield besides using chemical fertilizers is biofertilizers. Biofertilizers promote increased absorption of nutrients in plants (Vessey, 2003; Hart and Trevors, 2005; Chen, 2006). Biofertilizers include materials derived from living organisms and microbial sources (Rola, 2000; Chen, 2006). Biofertilizers have various benefits, such as increased access to nutrients, providing growth-promoting factors for plants, and composting and effective recycling of solid wastes (Gaur and Adholeya, 2004; Das *et al*., 2007). Biofertilizers, commonly known as microbial inoculants are produced from cultures of certain soil organisms that can improve soil fertility and crop productivity such as mycorrhizae (Malik *et al*., 2005; Marin, 2006).

- **Use ofBiofertilizer and Mycorrhiza to increase the yield of cotton**

Complex interactions take place in the volume of soil around roots, which traditionally has been termed the ‘‘rhizosphere.’’ More appropriately, that soil volume constitutes a *mycorhizosphere* (Rambelli 1973) because of the dramatic influence an abundance of fungal external hyphae has on root and soil associated microorganisms, as well as the effects of those microorganisms, on the establishment and spread of mycorrhizae (Bagyaraj 1984). Mycorrhizal roots also have altered root exudation patterns (Marschner 1998). The *Rhizobium* - *Bradyrhizobium* association with legumes most notably is affected by mycorrhizal fungi, largely as a result of increased availability of phosphorus in host roots, which drives nitrogenase activity

in nodules (Aźcon 1994). Other indirect interactions affect both pathogens and beneficial organisms, either through effects of mycorrhizal formation on root exudates or through competition (Linderman 1988; Garbaye 1994).

The influence of fungal hyphae in the mycorrhizosphere is much greater than previously thought (Tisdall *et al*., 1997) with the discovery of 'glomalin,' a heat stable glycoprotein that coats hyphae and spore surfaces and accumulates in soil (Wright and Upadhyaya 1996). Evidence indicates a strong involvement of glomalin in soil aggregate stability; researches have revealed found a highly significant correlation between glomalin concentration and soil aggregate stability (Wright and Upadhyaya 1996).

Perhaps the largest obstacle to understanding the biology and ecology of Arbuscular Mycorrhiza (AM) fungi is our inability to culture them apart from their plant hosts. The plant provides carbon to the fungus largely via an arbuscule – plant cell plasmalemma interface. It also provides a protected site in root cells where the fungus can live. The external fungal hyphae improve phosphorus acquisition by the plant in soils with low levels of phosphorus (Safir 1987; Smith and Gianinazzi-Pearson 1988; Smith and Read 1997). In soils in which phosphorus levels exceed requirements of the host, however the AM symbiosis often is inhibited. Under those conditions for certain host-soil interactions, mycorrhizal development can reduce plant growth and thus become pathogenic (Modjo and Hendrix 1986).

In nature, fungal communities are taxonomically complex and rarely, if ever, consist of only one species (Morton 1988). When individual AM fungi are cultured on plants, host specificity appears to be minimal or absent (Smith and Gianinazzi-Pearson 1988; Brundrett 1991; Smith and Read 1997). Investigators at the International Culture collection of (vesicular) Arbuscular Mycorrhizal Fungi (IN VAM) in West Virginia showed that more than 1000 isolates of 98 species of fungi in all genera were able to grow and sporulate on one plant host, *Sorghum*

sudanese, or Sudan grass (Morton *et al.,* 1993). Roots of Sudan grass accommodate colonization by as many as 10 species of fungi at one time in pot culture using field soil as inoculum. And those fungi can be members of any genus (Morton 1988). Lack of host specialization may be the result of mutualistic co evolution of the plants and their fungal partners over the 400 million years since their origin (Simon *et al*., 1993; Taylor *et al.,* 1995).

A combination of host and environmental factors can differentially influence rates and degrees of colonization and/or sporulation by different AM fungi in a community (Johnson *et al*., 1992; Bever *et al*., 1996), which are manifested as changes in species richness and relative abundance in sporulation. In general, those responses represent compatibility adjustments rather than specificity, although Mc Gonigle and Fitter (1990) and Brundett (1991) explained the 'ecological specificity,' respectively. Divergence in physiological and life –history traits within a fungus species and between species is expected in these asexual organisms as a natural response to local and regional selection pressures (Morton *et al.,*1990).

Members of a small number of plants do not form AM associations (Tester *et al.,*1987). Many other orders, however, include both mycorrhizal and non-mycorrhizal families and genera. Non mycorrhizal taxa are assumed to have evolved away from the symbiosis, based on evidence from their distributions, relative to those of their mycorrhizal relatives (Trappe 1987), and from the presence of activated defense mechanisms in chemically induced mycorrhizal-resistant mutants (Peterson and Bradbury 1999). A few genera, however, support both arbuscular fungi and ecto mycorrhizaal fungi. One of the most widely studied plant being *Eucalyptus* (Lapeyrie and Chilvers 1985).

Mycorrhiza is a mutualistic beneficial association between fungi and plant roots. It is more or less a universal phenomenon throughout the plant kingdom (Mosse 1981). More than 80% of all land plant families are thought to have a symbiotic relationship with AM Fungi that

belong to Glomeromycota. This interaction of AM symbiosis is the evolutionary precursor of most of the mutualistic root-microbe associations (House and Fester, 2005).

Arbuscular Mycorrhozal association with plants is an ancient (>460 million years BC) and widespread terrestrial symbiotic association formed between fungi of the phylum Glomeromycota and the roots of vascular plants (Schussler *et al.,* 2001, Redecker *et al.,* 2000 a, b, Toljander, 2006) and they, therefore, represent an ancient phylogenetic clade within the fungi. It is estimated that about 250,000 species of plants, are capable of forming the symbiosis with AM fungi worldwide (Smith and Read 1997). The colonization of terrestrial ecosystems by the ancestors of modern vascular plants was facilitated by symbiotic fungi similar to modern endomycorrhizae. AM comprise of over 150 species that are not host specific and form symbiotic associations with a wide range of host species. AM bestow a selective advantage on their host over competing non-host species by making available nutrients, providing defence against several pathogenic organisms and by influencing the composition of the microflora of the rhizosphere (Kothamasi *et al.,* 2001).

The symbiotic relationship benefits both- the individual plant and the fungus (Francis and Read, 1995). Exchange of nutrients-mineral nutrients supplied by the fungal microsymbiont versus carbohydrates provided by the plant-is considered to be the main benefit for the symbiotic partners (Smith and Read 1997). According to the phylogenetic position of these partners and according to the symbiotic structures, several types of mycorrhiza have been defined such as arbuscular mycorrhiza (AM), ectomycorrhiza, ericoid mycorrhiza, and orchid mycorrhiza. The efficiency of each AM Fungi for increasing plant growth, nutrient contents, water stress tolerance (Vazquez *et al*., 2001) and providing defense against several pathogenic organisms (Kothamasi *et al*., 2001) is well documented. In endomycorrhiza, the fungus grows inter- and/or intra cellularly. Specific fungal structures of endomycorriza are produced within the cortical cells by non septate fungi which are commonly known as vesicles and arbuscules, hence it was called

earlier as vesicular- arbuscular mycorrhiza (VAM). As many of the endomycorrhiza fungi do not necessarily form internal vesicles, the abbreviated term 'VAM' was suggested to be replaced by 'AM' (Strack *et al.,* 2003).

Growth in plant communities is often governed by the availability of nutrients such as P and N. In contrast, C is growth-limiting element in fungal communities. It was but obvious for natural selection to have favoured the development of symbiotic associations between plants and fungi. Plants provide C to fungal symbionts and the fungi transfer nutrients from the soil to the host (Kumar *et al.* 1999; Pierzynski *et al.* 2000; Read 1990; Sen 2000). Mycorrhizae have been associated with vascular plants since the Palaeozoic era (Taylor 1990). The colonization of land by the ancestors of modern vascular plants seems to have been hastened by the origin of symbiotic associations between these plants and some phycomycetous fungi similar to those of modern endomycorrhizae (Malloch *et al.* 1980; Phipps and Taylor 1996; Simon *et al.* 1993). AM, the most prevalent plant-fungus association, comprise about 150 species, belonging to the order Glomales of Zygomycotina (Morton and Bentivenga 1994; Myrold 2000; Perry *et al.* 1989; Schenck 1981; Simon 1996). AM are one of the few plant-fungus associations with a fossil record (Taylor 1990) and are believed to have assisted vascular plants in their growth and survival (Simon *et al.* 1993). AM are present in most soils and are generally not considered to be host specific. However, population sizes and species composition are highly variable and influenced by plant characteristics and a number of environmental factors such as temperature, soil pH, soil moisture, P and N levels, heavy metal concentration (Boddington and Dodd 1999), the presence of other microorganisms, application of fertilizers and soil salinity (Barea and Azcon-Aguilar 1983; Bationo *et al.,* 2000). Species and strains of AM differ in their ability of tolerance to physical and chemical properties of soil (Abbot and Robson 1991), as a result they also differ in their effectiveness in improving plant growth.

AM forms the connecting link between the biotic and geochemical portions of the ecosystem (Miller and Jastrow 1994). Mycorrhizae aid the plant in better growth by assisting it in absorbing useful nutrients from the soil, in the competition between plants and in increasing the diversity of a given area. A number of reviews have appeared recently on AM, particularly dealing with the application of AM in agriculture. Information on the role of AM in plant adaptations has been scattered and the present review deals with the critical appraisal of the role of AM in plant community dynamics, nutrient mobilization and overcoming both abiotic and biotic stresses.

AM fungi are known to infect a wide range of host species. They have a large geographical distribution (Malloch *et al.,* 1980), being found even in the Arctic tundras and the Antarctic region (DeMars and Boerner 1995b; Gardes and Dahlberg 1996). Unlike most ectomycorrhizal species, AM are not host specific. This enables them to form associations with a large number of plant species. Mycorrhizae owing to their role in nutrient cycling, keep more nutrients in the biomass and in doing so increase the productivity of the ecosystem (Newman 1988). AM fungi regulate plant communities by affecting competition, composition and succession (Allen and Allen 1984; Kumar *et al,*. 1999).

- **Classification of Mycorrhizae**

Mycorrhizal fungal association widely varies in structure and function, but the AM fungi exhibit most common associations (Harrier, 2001). Six genera of AM fungi have been recognized based on the morphological characteristics of sexual spores and also based on various biochemical studies as well as molecular methods (Peterson *et al.,* 2004). Further, various criterion has been used for the identification of AMGF like hyphal charaters, auxillary cells subtending hyphae, spore or sporocarp ontogeny, morphology, germination, shield spore wall *etc.* (Mukherji *et al.,* 2002). AMF are zygomycetous belonging to the genera *Glomus, Gigaspora, Sclerocystis, Acaulospora, Enterophospora* and *Scutellospora* (Garbaye, 1994).

The classification of AMF is based on the structure of their soil borne resting spore, biochemical properties and molecular studies (Morton and Benny, 1990). The latest classification of AMF contains 4 orders and 9 families (Siverding and Ohel, 2006). Plant species belonging to the Cruciferae, Chenopodiaceae and Cactaceae are not known to form AMF symbiosis (Smith and Read, 1997). AMF reproduce asexually by spore production. There is no evidence that AMF reproduce sexually (Kuhn *et al.*, 2001).

Table showing Recent Classification of Arbuscular Mycorrhizal Fungi (Siverding and Ohel, 2006)

Phylum	Glomeromycota	
Class	Glomeromycetes	
Orders	**Families**	**Genera**
1 – Glomerales	Glomeraceae	*Glomus*
2 – Diversisiporales	Gigaspraceae Acaulospraceae Entrophosporaceae Pascisporaceae Diversisporaceae	*Gigaspora, Scutellospora* *Acaulospora, Kuklospora* *Entrophospora* *Pacispora* *Diversispora*
3 - Paralomerales	Paraglomeraceae Geosiphonaceae	*Paraglomus* *Geosiphon*
4 - Archaeosporales	Arthaeosporaceae	*Archaeospora, Inraospora*

- **Types of Mycorrhizae:**

Several types of mycorrhizal fungi have been recognized and the most important types are mentioned below:

- **Endomycorrhizae:**

Endomycorrhizae represents a group of fungi that are associated with most of the agricultural crops and provide biochemical protection against soil borne diseases (Smith and Read, 2008). They occur in most of the ecosystems of the world and are found in many important crop species

i.e. (cotton, wheat, maize, rice and soyabean) and horticultural species like grapes, roses and petunias etc. (Peterson *et al*., 2004). AMF are obligate biotrophs feeding on the products of their live host and those fungi are not specialized to their potential hosts. The host plants receive mineral nutrients from outside the root depletion zone via the extraradical mycelium, while the AMF obtains photo-synthetically produced carbon compounds from the host (Smith and Read, 1997).

Many endomycorrhizal fungi form terminal or intercalary vescicles in the root cortex. When the vesicles are expanded the thin walled structures, contain large quantity of lipids (Tahat *et al*., 2010). They may be oval, spherical or lobed in shapes and may become thick walled and resting spores (Pirozynski and Dalphe, 1989).

- **Ectomycorrhizae (ECM):**

ECM fungi form a thick mantle like structure and within the intracellular spaces of root cortex form network. These fungi do not penetrate living cells in the host roots, but can only surround them. They are most common in ornamental and forest tree species in the family Pinaceae, Myrtaceae, Salicaceae, Dipterocarpaceae, Fagaceae and *Gnetum* plants (Shalini *et al.,* 2000). Ectomycorrhizas are distinguished by the presence of mantle and harting net. Harting net develops in cortical cells or epidermal cells. Harting net consists of branch system which can provide a large surface contact between cells of the two symbionts (Peterson *et al*., 2004). Other type of mycorrhizal fungi includes Ecto-endo Mycorrhizae, Ericoid Mycorrhizae, Monotropoind, Arbutoid and Orchid mycorrhizae (Smith and Read, 2008).

- **Uptake of the nutrient by AM Fungi:**

When soil resources, such as P or N, limit photosynthesis, C is in excess, mycorrhizal fungal hyphae explore the soil volume for P and N, and transport the nutrient (over distances of cm to m) in exchange for excess plant C. Mycorrhizal hyphae are more efficient at exploring the soil volume then even fine roots. As long as P or N are limiting plants will support mycorrhizal

fungi. Even as the availability of the limiting resources shifts through time, mycorrhizal fungi similarly shift resource provisioning (Molina *et al*., 1992). Linking space and time is important because of exploring a neighboring patch (Aikkio and Ruotsalainen, 2002). Since a complex network of fungal mycelia and plant roots are distributed horizontally across a landscape and extend vertically into the soil and rock substrate (Egerton-Warburton *et al.*, 2003), resource extraction becomes dynamic.

Energy, in the form of C compounds is the currency for exchange of soil resources. This connection occur at the (fungus) membrane: interspace: (plant) membrane interface in the form of simple sugars or amino acids. Photosynthetic rates depend on the concentrations of N (as RuBP carboxylase and other enzymes), P (for ATP, ADP). Fe and Mg (for cholorophyll), internal CO_2 , and water (to keep stomata open to fix CO_2). These interactions create several important and well known linear and curvilinear relationships that form that basis of stoichiometeric ratios between elements. Mycorrhizae, by increasing P and N uptake, create a C sink and enhance the photosynthetic machinery. Mycorrhizae also increase water release through transpiration by, opening the stomata. Together these increase rates of total carbon gain by 10% to 40% (Allen *et al.*, 1981). In the field, this increased CO_2 fixation is associated with environmental change, such as drought, or as a function of particular fungal – plant species combination.

- **Effects and processes involved in the growth enhancement by Vesicular Arbuscular Mycorrhizas (Tinker *et al.*, 1994)**

1. Growth increase occurs by improved supply of elements of low mobility in growth medium, predominantly phosphate.
2. This arises by increased uptake rate per unit amount of root length (inflow).
3. This is caused by proliferation of a considerable length of external hyphae.

4. Hyphae absorb, translocate and transfer P to host, from soil outside the root depletion zone.
5. Uptake is normally from the isotopically labile pool of nutrient, from which the root also absorbs.
6. There is a feedback effect by absorbed Phosphorus on the percentage of infected root.
7. Infection of phosphate-deficient plants is accompanied by a rapid but temporary increase in internal P concentration.
8. Much of phosphorus in the fungal partners is in the form of phosphate.
9. The fungus is maintained by carbon supplies from its host, and infection results in a large proportion of total fixed carbon being allocated below ground.
10. The uptake efficiency should be large for this mechanism than for uptake by the uninfected root system.

- **Plants used for Biocontrol**

Plants are the richest source of renewable bioactive organic chemicals. The total number of plant based chemicals may exceed 4,00,000 of these 10,000 are secondary metabolites, whose major role in the plants is reportedly defensive (Swain, 1977).

The screening of plant extracts for antimicrobial activity has shown that a great number of these plants contain active compounds. The presence of antibacterial, antifungal, and other biological activities has been demonstrated in extracts of different plant species used in traditional medicine practices (Hashem, 2011).

Basic researches for over more than forty years in the fields of biological and biochemical have made it possible to envisaged not only how new pesticides may be synthesized but also a completely new approach for the protection of plants using secondary plant products, which may be toxic to a specific pest yet harmless to man. There has been a renewed interest in

botanical pesticides because of several distinct advantages (1) Pesticidal plants are generally much safer than conventionally used synthetic pesticides. These pesticides will not cause harm in nature. (2) Plant based pesticides will be renewal in nature and would be economical. (3) Some plants have more than one chemical as an active principle responsible for their biological properties. These may either be selected for one particular biological effect or may have diverse biological effects (Singh, 1993).

Efforts are being made these days to shift from the conventional use of chemicals to the use of eco-friendly botanicals for the management of plant parasitic nematodes. Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility (Trivedi, 2002).

Leaves of following plants were used to study the biocontrol activity:

1. ***Annona reticulata*** **L.**
   Family: Annonaceae

It is a small deciduous or semi-evergreen tree reaching 8 metres to 10 metres tall with an open, irregular crown. The slender leaves are not hairy, straight and pointed at the apex 10 cm to 20 cm long and 2 cm to 7 cm wide. The yellow-green flowers are generally produced in clusters of three or four 2 cm to 3 cm diameter, with three long outer petals and three very small inner ones. The fruits are variable in shape: heart-shaped, spherical, oblong or irregular.

2. ***Sapindus emarginatus*** **Vahl.**
   Family: Sapindaceae

Sapindus emarginatus is a deciduous tree. Commonly called as Soapnut tree and is the south Indian species of genus *Sapindus*. It is an economically significant tropical tree species meagerly distributed in diverse geographical provinces like Gangetic Plains, Western Ghats, and Deccan Plateau in India. The trunk of the tree is straight and cylindrical, approximately 4-5 m in height.

5-10 pairs of leaves, solitary alternate, 15–40 cm long, pinnate, with 14-30 leaflets, the terminal leaflet often absent. The flowers form in large panicles and each flower is small and creamy white in colour. It flowers during summer. The fruit is small leathery-skinned drupe which is 1–2 cm in diameter, which is yellow and turn blackish when ripen, containing one to three seeds. The members of genus *Sapindus* are well known for their medicinal values. Traditionally it is used as anti-inflammatory and antipyretic. Its fruits are natural substitute for chemical soaps and hair dyes.

3. *Cochlospermum religiosum* (L.) Alston
Family: Bixaceae

It is a flowering plant from the tropical region of Southeast Asia and the Indian Subcontinent. In India it is commonly found in Andhra Pradesh, Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar. It is a small tree growing to a height of 7.5 m usually found in dry deciduous forests. Also known as Silk-Cotton Tree because the capsules containing the seeds have a fluffy cotton-like substance similar to kapok. Plant can be identified by deeply furrowed bark, palmately 5-lobed leaves and bright golden yellow bisexual flowers. Quick growing tree yield a gum known as gum katira from a juice orange in colour exudes from the bark. The dried leafves and flowers are used as stimulants, antipyretic, laxative and sedative. Root powder mixed with water when applied to face reduce wrinkles.

4. *Gliricidia sepium* (Jacq.) Kunth ex Walp.
Family: Fabaceae

It is a medium-sized tree, semi deciduous tree that grows from 10 to 12 meters high. The bark is smooth and its color can range from a whitish gray to deep red-brown. It has compound leaves that can be 30 cm long. Flowers have a bright pink to lilac color that is tinged with white. Leaves are rich in protein and highly digestible for ruminants like goat and cattle, as they are low in fibre

and tannin. There is evidence of improved animal production (both milk and meat) in large and small ruminants when *Gliricidia* is used as a supplement to fodder. However, non-ruminants fed on *Gliricidia sepium* have shown clear signs of poisoning. The flowers attract honeybees (*Apis* spp.), hence it is an important species for honey production. Good for firewood and charcoal production. The wood burns slowly without sparking and with little smoke. Very durable and termite resistant; used for railway sleepers, farm implements, furniture, house construction and as mother posts in live-fence establishment. A traditional remedy for hair loss, boils, bruises, burns, cold, cough, debility, eruptions, erysipelas, fever, fractures, gangrene, headache, itch, prickly heat, rheumatism, skin tumours, ulcers and wounds

5. *Feronia accidissima* (L.) Swingle
Family: Rutaceae

The tree is native and common in India, Sri Lanka, China and Indonesia and widely distributed in most tropical and subtropical countries. Commonly known in India as wood-apple. It has economic as well as medicinal value. It contains important medicinal compounds like umbelliferol, dictamnine, xanthotoxol, scoparone etc. those could be used in the pharmaceuticals industries. The fruit is used in India as a liver and cardiac tonic. and when unripe, as an astringent means of halting diarrhoea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. Oil derived from the crushed leaves is applied on itch and the leaf decoction is given to children as an aid to digestion.

6. *Balanites roxburghii* Planch.
Family: Zygophyllaceae

It is a spiny, evergreen tree. It is common in open sandy plains. Commonly called as Hingoli/ Hingoru. Bark, Friuts, Seeds and Leaves are used. Fruits are used in treatment of whooping cough, skin diseases and in antifertility. Leaves are used for the treatment of jaundice. In case

of pain and swelling, the bark of plants is used as traditional healers. The paste of bark is prepared and applied externally on the affected part of the body to treat snake-bite and dog bite.

7. ***Tephrosia jamnagarensis* Sant.**
   Family: Fabaceae

   It ia an annual herb of 1mt with simple linear leaved covered by hairs. Flowers purplish blue in colour. Pods densely hairy and oblique at both the ends. Seeds reniform, brownish. Leaves contain glycosides and favanoides. The leaves could be used as insecticide, pesticide and is having hepatoprotective properties.

Objectives

1. Survey of different cotton fields in certain districts of Gujarat, to assess the varieties of cotton (Normal / Bt) under cultivation, yield obtained, and associated seed and soil borne diseases.
2. Isolation and identification of different fungi from the rhizospheric and non- rhizospheric soils.
3. Effect of certain fungi like *Trichoderma* sp., *Gliocladium* sp. *and Aspergillus niger* will be observed on percentage germination and growth performance of cotton.
4. Isolation and identification of different AM Fungi from soil associated with roots of Bt and Non Bt cotton plants.
5. Effect of AM fungi on increase in plant biomass/yield.
6. Role of pathogenic fungi both seed and soil borne in cotton and their control *in vitro*.
7. Effect of compost and vermicompost on increase in plant biomass.

Soil is a complex and dynamic environment in which the biological activities are mostly governed by microorganisms. The beneficial effects of soil microorganisms are manifold and range from nitrogen fixining and organic matter deposition to the breakdown of metabolic byproducts and enhancing the availability of sulphates, phosphates, nitrates and essential metals (Bridge and Spooner, 2001).

The root system is important for plant fitness because it provides anchorage, contributes to water use efficiency, and facilitates the acquisition of mineral nutrients from the soil (Lopez-Bucio *et al.,* 2005a)

Rhizosphere microbial communities can significantly influence phytopathogens development (Nehl *et. al.*, 1997; Glick, 1995), nutrient acquisition (Lynch, 1990), heavy metal resistance (Bradly *et.al.,* 1981) and ecological fitness of the plant. Qualitative as well as quantitative distribution of fungi in the rhizosphere and non-rhizosphere soil has been discussed in detail (Harley and Waid, 1955; Parkinson and Waid, 1960; Burges and Raw, 1976).

Fungi represent a very important component of the ecosystem, along with the other microbes of the biomass (Harrison *et al.,* 1994). Fungi are important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits (Diana, 1994). It is estimated that there are 1.5 million fungal species on earth of which only about 70,000 have been described up to now (Hawksworth and Rossman, 1997).

A mycorrhiza is a symbiotic association between fungus and the roots of a plant (Kirk *et al.,* 2001), where in this association the fungus may colonize the roots of host plant either intracellularly or extracellularly.

Azcon *et al.,* (1995) reported that inoculation of VAM fungi greatly increased shoot and root biomass and leaf area in micropropagated *Annona* plants. Bagyaraj (1984) generalized that modern high input agricultural practices are detrimental to AM fungi, while the low input sustainable agriculture methods enhance the population of AM fungi in the soil.

Hayman (1981) reported that the mycorrhizal association in groundnut improved growth and nutrition of plants. More than 100% increase in the dry matter yield and P uptake of groundnut plants inoculated with mycorrhizal fungi compared to non-inoculated control.

Arbuscular mycorrizal fungi are the most frequent in plants growing on mineral soils. The populations of AM fungi is greatest in plant communities with high diversity such as tropical rainforests and temperate grasslands, where they have many potential host plants and can take advantage of their ability to colonize a broad host range. There is a lower incidence of mycorrhizal colonization in very arid or nutrient rich soils. Mycorrhizas have not been observed in aquatic habitats; however, waterlogged soils have been shown to have colonization in some species (Smith and Read, 2002). In costal soils infection of AM has been influenced by soil temperature and moisture content of the soil (Mohankumar *et al.*, 1988a).

Literature survey showed that the work has also been carried out on colonization of AMF and its effects on growth performance of some tree species by various workers such as *Tectona grandis* L.f. by Rajan *et al.,* (2009) on *Eucalyptus* spp. and *Acacia* sp. by Malajczuk *et al.,* (1981) on *Acacia leucocephala* and *Moringa concanensis* by Pawar and Vyas (2002) ; on *Carica papaya* L. by Kennedy and Rangarajan (2001); on *Casuarina equisetifolia* J.R. and G. Forst, (Rajeshwari *et al.*, 2001).

Jain and Gupta (2002) reported effect of rhizosphere fungi on nodule number, shoot and root length of *Vigna mungo*. Inoculation of AM fungi and *Rhizobium* increases the percentage of

chlorophyll content in the leaves of *Arachis hypogea* L (Charitha and Reddy, 2004). AM fungi have the effect on control of disease infection in plants and it has been successfully shown in plants viz. tomato for nematode infection (Suresh *et al.,* 1985).

Nematode infection has also been reduced in arbuscular mycorrhiza inoculated plants of *Piper nigrum* L. (Sivaprasad *et al.,* 1990). Fusarium wilt disease severity in *Albizia procera* Benth. and *Dalbergia sissoo* Roxb. was significantly reduced when inoculated with mycorrhizal fungi (Chakravarty and Mishra, 1986). In case of papaya plants having arbuscular mycorrhizae showed drought resistance (Shivaputra *et al,* 2004).

In the experiments carried out by Edwards *et al.*, in 1998, plants grown in the presence of *Glomus mossae* had a significantly higher shoot dry weight than those grown in the absence of *G. mossae.* Colonization and the activity of *G. mossae* was unaltered in the presence of *Pseudomonas fluroscence* Migula isolated and presence of *G. mossae* increased the population of *P. fluroscence* in the rhizosphere.

Dodd *et al.,* (1996) in their investigations aimed at using morphological and molecular characters to study inter- and intraspecific variation within isolates of *G. mossae* and *Glomus coronatum* from different parts of the world. Morphological evaluations of various possible taxonomic characters including spore colour, size sporocarp architecture and hyphal attactment morphology, showed that only spore colour discriminate the two groups.

In 1982, Scheneck and Smith studied that there was considerable variation in the plant response to various combinations of temperature and fungus species, with both growth stimulatory and growth repressive and effects occurring. The use of AM fungi in ecological restoration projects have been shown by Jeffries *et al.,* (2003) to enable host plant establishment on degraded soil and improve soil quality and health (phytoremediation).

With the establishment of the facts that AM fungi has the role in phosphate solubilisation in the rhizosphere and thereby providing the host plant the solubilised form of the phosphorus has led to think regarding the means and ways to mass multiply the different strains of AM fungi. Mass multiplication methods at bioreactor (Jolicoeur *et al.,* 1999) and *invitro* (Gryndler *et al.,* 1998; Pawlowska et al., 1999; Gadkar and Adholeya, 2000) level apart from conventional way at pot level have been standardized (Gaur and Adholeya, 2000) along with the selection of suitable host (Singh and Pandya, 1995). The post harvest storage management of these AM fungi cultures has also been worked out (Mohankumar and Mahadevan, 1988b; Sreenivasa and Kulkarni, 1993).

Jakobsen *et al.,* (1992) indicated that the efficiency of phosphorus uptake by VAM fungus is strongly affected by its spatial distribution of hypahe in the soil and possibly also by differences in capacity for uptake by unit length of hyphae. Pearson and Jakobsen (1993) strongly indicated that the P uptake by root cells is influenced by the presence of mycorrhizal fungi and this effect varies from species to species.

According to Jensen (1982), AM fungi are universally associated with the crop plants. They helps the host in the uptake of Phosphorus and other minerals. The growth increase may be controlled more by available soil P than by endophytic species (Clarke and Mosse, 1981). Grant *et al.,* (2005) indicated that as the phosphorus availability levels in the soil increases, the amount of phosphorus also increases in the plant tissues and carbon drain on the plant by the AMF symbiosis become non-beneficial to the plants.

In 2002, Panwar and Vyas reported the use of AM fungi as a biological approach towards conservation of endangered plants in Thar desert of India. The efficiency of eight AM species, *Acaulospora mellea, Gigaspora margarita, Gigaspora gigantean, Glomus deserticola, Glomus fasciculatum, Sclerocystis rubiformis, Scutellospora calospora* collected from the

rhizosphereic soils of *Moringa concanensis*, was evaluated for nutrient uptake and enhancement of acid phosphatase, nitrate reductase, peroxidase and polyphenol oxidase activities in this endangered tree of the Thar desert.

George *et al.,* (1995) showed that AM symbiosis increases the phosphorus and micronutrient uptake and growth of their plant host. Uptake of micronutrients is enhanced by the same mechanisms that operate for P, Cu and Zn have consistently been shown to be increased by VAM fungal colonization was indicated by Menge *et al.,* (1978).

Wani and Lee (1995) reported for studying AM fungi colonization the best sampling time is flowering stage up to maturity and in field conditions inclusion of appropriate crop in crop rotations or intercropping can increase the native population of AM fungi in soil which is often objective of the artificial inoculation. Tian *et al.,* (2009) studied different AM species showing different distributions among the three plant communities and the results indicate that both biotic and abiotic factors were important in determining the AMF communities, with biotic factors possibly the more important.

Braunberger *et al.,* (1996) indicated that the development and function of mycorrhizas after late summer and early autumn rains may be limited by the occurrence and predominance of propagules of different AM fungi. In 2008, Mikkelsen *et al.,* showed ability of AM fungal mycelia to anastomose in soil for the formation of large plant-interlinking functional networks, long distance nutrient transport and retention of nutrients in readily plant –available pools.

In nature the Mycorrhizal condition is the rule and non- mycorrhizal condition is the exception according to Gerdemann (1969). The association of AM fungi is found with most of the vascular plant communities examined so far except members belonging to families Brassicaceae, Chenopodiaceae, Caryophyllaceae, Cyperaceae, Araceae, Asteraceae, Poaceae,

Onagraceae, Polygonaceae and Portulacaceae (Hirrel *et al.,* 1978; Berch, 1988). In recent times it has been reported that few members of Chenopodiaceae (Kruchkelmann, 1973; Ross and Harper, 1973; Williams *et.al.,* 1974), Cyperaceae (Mejstrik, 1972) and Cruciferae (Kruchkelmann, 1973; Ross and Harper, 1973) may have AM association.

Rhizosphere soil and roots were sampled for root colonization, AMF identification and spore counts Moreira *et al.,* (2006). They found that percent root colonization and spore numbers were inversely related to each other in all ecosystems. Guar and Adholeya (2000) reported that plants with sand particle sizes of 0.50-0.78mm has higher root fresh weights, spore production and percentage mycorrhizal colonization than with other particle sizes.

Dalal and Hippalgaonkar (1995) reported that the spore density was found more in rainy season as compared to winter and was high in soils collected under water stress in summer. Allelochemicals induced in mycorrhizal plants play an important role in disease resistance (Inderjit and Mukherji, 2006). Graham *et al.*,(1981) found that reduced root exudation limited the growth of pathogen in the rhizosphere and there by, its ability to cause disease.

Vyas (1990) on the basis of his observation reported that distribution of endomycorrhizal fungi in soybean and chick pea such as *Glomus monosporum, G. fasciculatum, G. epigaeum, G. constrictum* and *Acaulospora morrowae* were associated with soybean and chick pea. *Glomus monosporum, G. fasciculatum* occurred in 40% and 25% of samples and 50% and 25% of samples in soybean and chickpea respectively. *G. epigaeum* occurred in 25% of samples in soybean and 30% samples in chickpea. *Glomus mossae* and *G. constrictum* were found to be associated with all cultivars of soybean.

Singh and Mishra (1995) reported that in two paddy varieties plant biomass is increased when these are associated with AM fungi, the mean shoot and root dry weights of was

significantly higher than those of the non-mycorrhizal plants. They observed that the increasing soil phosphate levels reduced the percentage of root infection in both the varieties at all the host stages examined.

Ammani and Rao (1996) reported effect of eight varieties of upland rice in sterilized soil in pot culture with inocula of *Acaulospora spinosa* and *A. scrobiculata*. All the eight varieties showed a positive response to inoculation with the AM fungi in terms colonization of roots, formation of vesicles and spores, plant biomass and grain yield. Three of the eight varieties showed a greater response than the others. The traditional variety, *Mettasannalu* gave the least response. *A. spinosa* was found to be more effective than *A. scrobiculata* on all the varieties.

In 1988, Khan *et. al.*, studied mycorrhizal status of some Bangladesh soils and the effect of indigenous AM fungi on the growth of rice plants. Mycorrhizal infection greatly improved the growth and nutrient (nitrogen, phosphorus and zinc) content of rice.

Microorganisms growing on the plant roots can influence plant growth positively or negatively (Liljeroth and Baath, 1988). In this respect, the soil microflora can be manipulated and protected to improve the bio-physico-chemical characteristics and regulates decomposition process in the soil (Rezacova *et al.*, 2007). The rhizosphere mycoflora affect the health of the plants in many ways hence, it becomes necessary that there is adequate information on the rhizosphere mycoflora of crops (Odunfa, 1979).

Fungi are fundamental for soil ecosystem functioning (Warcup, 1950). Especially in forest and agricultural soils; they play a key role in many essential processes such as organic matter decomposition and elemental release by mineralization (Christensen *et al.*, 1989).

The enhancing effect of AM fungi on other crop plants has been studied by different workers from time to time. The effect of AM fungi in acid soils (Potty and Indira, 1990), soils with low available phosphate (Haymann and Mossae, 1972; Ross and William, 1973; Khan

1975; Mosse *et al.,* 1976; Swaminathan and Verma, 1977; Bagyaraj and Manujunath, 1980; Koide and Roger, 1985; Manjunath and Bagyaraj, 1980), rice crops (Khan *et al.,* 1988), microelements absorption (Arines *et al.,* 1989), enhancing biomass (Saif and Khan, 1977; Gaunt, 1978; Hall, 1978; Kough *et al.,* 1986; Sasa *et al.,* 1993; Wang *et al.,* 1989: Hooker *et al.,* 1922a; Sheoran *et al.,* 1992; Rocha *et.al*., 1993; Azcon *et al.,* 1995; Clark and Zeto, 1996; Sitaramaiah and Khanna, 1977), enhancing productivity (Khan, 1974; Mosse, 1976; Riazi *et al.,* 1977; Sukahda, 1978; Bagyaraj, 1989; Bryla *et al.,* 1990; Neeraj *et al.,* 1991; Singh and Tilak, 1990; Sulochana and Manoharachary, 1990; Allsopp and Stock, 1992; Edathil *et al.,* 1996; Selvaraj *et al.,* 1996; Clark *et al.,* 1999), minimal usage of fertilizers (Roncadori, 1971; Babu *et al*., 1988; Gisela and Honrubia, 1993; Bagyaraj, 1989; Chandraghatgi and Sreenivasa, 1955).

Saif and Khan (1977) observed effect of AM fungi on barley plant. They investigated that mycorrizal plants removed more phosphate from the soil and had greater dry matter. Yield was increased four fold by the fungus, but weight of individual grain was not affected. Number of tillers and ears per plant was more i.e. double in mycorrhizal plants as compared to non-mycorrhizal plants.

Swaminathan and Verma (1977) reported symbiotic effect of AM fungi on the phosphorus nutrition in potatoes. In an unfertile soil, mycorrhizal plants observed 8 times more phosphorus and showed greater growth than non-mycorrhizal ones. Gaunt (1978) compared growth of onion and tomato plants inoculated with the mycorrhizal fungus through seed and soil. Plants treated with the fungus grew better than the uninoculated ones. There were no significant differences between the two methods of mycorrhizal inoculation. Sukhada (1978) reported response of *Lycopersicon esculantum* Mill. to inoculation with the AM fungus *Glomus fasciculatum* and bacterium *Azotobacter vinelandii* singly and in combination in the field. *G. fasciculatum* as well as *A. vinelandii* significantly increased leaf area, shoot dry weight, nitrogen

content, phosphorus content and yield in respect to uninoculated control. While AM fungal treatment alone brought about substantial increase in growth, nitrogen content, phosphorus content and yield.

Bagyraj and Manjunath (1980) reported that the three crop plants i.e. cotton, cow pea and finger millet inoculated with AM fungus (*Glomus fasciculatum*) in an unsterile Indian soil low in available phosphorus significantly increased their biomass.

Arshi and Roy (2008) studied the effect of vermicompost and endomycorrhizae on growth performance of *Gliricidia sepium* and observed maximum growth in shoot height, number of branches and number of leaves.

Bhatia *et al.,* (1998) showed biomass production and changes in soil productivity during long term, cultivation of *Prosopis julifera* (Sw.) DC. inoculated with vesicular arbuscular mycorrhiza and *Rhizobium* species.

Manjunath and Bagyaraj (1986) observed response of black gram (*Vigna mungo*), chick pea (*Cicer arietinum*) and mung bean (*Vigna radiata*) to inoculation with mycorrhizal fungus *Glomus fasciculatum* with and without added phosphate (22kg per hectare) in a phosphate deficient unsterilized soil. Inoculation with a *G. fasciculatum* increased the dry weight and phosphorus content of the shoot and root significantly.

Sulochana and Manoharachary (1990) reported in sesame out of eight AM fungi used, *Gigaspora margarita* followed by *Glomus fasciculatum* were found to be the most beneficial in relation to plant height, number of leaves and dry matter production.

Neeraj *e .al.,* (1990) reported that *Glomus* sp. and *Gigaspora* sp., used as a source of inocula on *Cyamposis tetragonoloba* var. Pusa Navbahar, for shoot production the best response was obtained with *Glomus fasciculatum* followed by *G. mossae*. The total dry weight of leaves

increased with *G. fasciculatum* and thus they concluded that *G. fasciculatum* seems to be most suitable AM fungal species for *C. tetragonoloba.*

Champawat and Pathak (1993) reported shoot and root dry weight, phosphorus and nitrogen uptake was significantly greater in mycorrhizal plants than non-mycorrhizal control. The experiments performed in phosphorus deficient soil suggested that inoculation of pearl millet with AM fungi could be extremely useful in plants growth and nutrient uptake.

Druege and Schoenbeck (1993) studied the effect of arbuscular mycorrhizal fungi infection on transpiration, photosynthesis and growth of *Linum usitatissimum* L. (Flax) in relation to cytokinin levels. Their results lead to the conclusion that enhanced internal cyokinin levels were responsible for improved photosynthesis and growth of mycorrhizal roots in flax.

Gisela and Honrubia (1993) investigated that *Lygum spartum* L. inoculated with *G. fasciculatum* at several fertilization rates of phosphate as 0, 30, 60 and 90 mg of phosphate per kilogram of soil produced a significant growth improvement, especially at low fertility levels. The maximum yield was obtained in mycorrhizal plants growing in soil with 60 mg per kg of added phosphate.

Bethlenfalvay and Barea (1994) concluded that AM fungi effects on plants and soil determine if AM fungi mediate a relationship between changes in seed yield and soil aggregation. Their result suggested carbon allocation between the plant (measured as seed yield) and the soil (measured as the formation of water soluble aggregates) were influenced by AM fungus. The soil appeared to gain carbon at the expense of carbon lost by the plant. Mycorrhizal fungi thus seems to affect to biologically controlled aspects of sustainable agriculture, plant production and soil quality.

Champawat (1988) observed that plants treated with three mycorrhizal fungi viz. *Glomus fasciculatum, G. constrictum* and *Gigaspora calospora* in *Arachis hypogea* enhanced root and shoot dry weight and significantly, over non-mycorrhizal plants. *G. constrictum* was significantly superior over *G. fasciculatum* and *G. calospora* in enhancing growth, nutrient uptake and root colonization. Increased root and shoot growth was recorded in mycorrhizal plants and the same was absent in non-mycorrhizal controls.

Investigations by Van der Heijden *et al.,* (2006) showed that AMF play a key role in grassland by improving plant nutrition and soil structure and by regulating the make-up of the plant community.

Ojha *et al.,* (2008) showed that different growth parameters like height of the plant, fresh and dry weight of the roots and shoots were observed to be significantly high in *G. fasciculatum* treated plants compared to the respective controls. Kandasamy *et al.,* (1985) noticed 23,22 and 87% higher plant height, dry weight and shoot P content of the chilli seedlings respectively, inoculated with a mixture of *Glomus fasciculatum* and *Glomus mossae* over un inoculated chilli seedlings. Mohandas (1987) recorded significantly higher leaf area, shoot dry weight, nitrogen and phosphorus contents and yield of " Pusa Ruby" variety of tomato with the inoculation of *Glomus fasciculatum* than the control.

Rasal *et. al.,* (1988) observed significant increase in the shoot dry weight and P-uptake when the *Cicer arietinum* var, "Vishwas" plants were inoculated with VA mycorrhizae. AM inoculated plants recorded higher shoot dry weight which was comparable with the un-inoculated plants supplied with recommended level of phosphorus and had significantly higher P-uptake than the fertilized un-inoculated plants.

Baqual *et al.,* (2005) carried out the analysis of Chlorophyll a, b and total chlorophyll content of the leaves of mulberry. The results revealed a significant variation due to the different treatments. Both chlorophyll-a, chlorophyll-b and the total chlorophyll of the leaves were highly influenced due to co-inoculation of mulberry with different microorganisms.

Rajasekaran Nagarajan (2005) concluded that dual inoculation with mycorrhizal fungi and *Rhizobium* species is effective in increasing the chlorophyll content, leading to enhanced growth in legumes.

AMF are particularly important in tropical and sub tropical regions, where the soils are usually of low fertility and mycorrhizae is thought to play a crucial role for the growth, survival and development of plant species thus influencing plant secondary succession and community structure (Janos, 1996). Their benefits may involve better access to soil resources and enhancement of soil aggregation, stability (Rillig and Mummey, 2006) and protection against phytopathogens (Newsham *et al.,* 1995). In addition to their individual plant effects at plant community level, AMF can be mediators of competition influencing plant biodiversity (Van der Heijden *et al.,* 1998) and sustainability of terrestrial ecosystems. The ubiquitous presence of AMF and their taxonomic, genetic and functional diversity are directly related to plant and soil processes (Oehl *et al.,* 2003).Hence, there is an increasing interest in the assessment of the biodiversity and functions of AMF communities (Lovelock and Ewel, 2005).

The recent developments in the AM taxonomy with help of molecular techniques along with morpho-taxonomy, Arbuscular mycorrhizal fungi are placed in the Class Glomeromycetes of the phylum Glomeromycota consisting four orders namely Archaesporales, Diversisporales, Glomerales, and Paraglomerales with 14 families comprises of about 23 genera namely *Acaulospora, Entrophospora, Gigaspora, Scutellospora, Sclerocystis, Glomus, Paraglomus, Claroideoglomus, Ambispora, Archaeospora, Geosiphon, Pacispora, Kuklospora, Racocetra,*

Centraspora, Diversispora, Fuscutata, Dentiscutata, Quatunica, Redeckera, Octospora, Rhizophagus, and *Funneliformis*.

Root characters, either morphological or physiological, affect plant uptake of nutrients from soil (Muthukumar *et al.,* 1999). The influence of root morphology on development of "Magnolioid root hypothesis" which predicts that plants with coarse root and with no or few short root hairs develop intense mycorrhizal colonization in natural soil compared to those with fine roots and abundant long root hairs. Peat and Fitter (1993) have indicated significant differences in root characters between mycorrhizal and non-mycorrhizal plant species. Mycorrhizal associations in cotton (*Gossypium hirsutum*) have been studied by various researchers and several studies have shown that AMF can promote cotton nutrient uptake and growth (Rich and Bird 1984; Smith and Roncadori 1986). *Gossypium herbaceum* showed heavy mycorrhizal colonization with almost every cortical cell of the white roots included an arbuscule (Jeffries *et al.,* 1988).

In agriculture seeds of many crops are known to carry various types of pathogenic and non-pathogenic fungi which are commonly known as seed mycoflora or seed-borne fungi. It is observed from the literature on seed pathology and seed biodeteioration that due to association of seed borne fungi several abnormalities occurred on the seeds are toxic and poor in quality for consumption as well as for seed industry (Rathod 2012). The most vital input in a crop production programme is seed, it should be of high quality and pathogen free. Pathogen free sound seeds are preferred for sowing to have desired germination, emergence, health seedlings and plant population (Hanuman *et al.*, 2005; Morel *et. al.,* 2005; Basm *et.al.,* 2000). Fungi form the largest group among such microorganisms causing seed damage, seed rot diseases at later stages of crop growth till maturity. Seed borne fungi may be present in the form of hyphae, conidia, oospores, chlamydospores, sclerotia, microspores, hyalospores and phaeospores (Behura *et al.,* 2000; Bhamaeapravati *et. al.,* 2006). Seed-borne diseases have been found to affect the

growth and productivity of crop plants (Kubiak and Korbas, 1999; Weber *et al*., 2001; Dawson and Bateman, 2001).

A seedborne pathogen present externally or internally or associated with the seed as contaminant, may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Khanzada *et al.,* 2002; Bateman and Kwasna, 1999). Seeds provide natural substrate for the growth of associated fungi, they get associated with seed externally on the seed surface, seed coat and internally with the endosperm, cotyledons, plumule, radical, embryo. Some are on the seed surface as contaminant this influences the seed to plant transmission of the pathogen. Seed borne pathogens result in heavy losses in crop yield and seed quality (Sangvikar 2012).

Fungi form a major group of pathogens that can be seed-borne or transmitted through seeds. Fungi are multicellular plants without roots, leaves or chlorophyll. Therefore they must live off other materials including grains. The vegetative parts of fungi produce enzymes that interact with seeds to extract nutrients need for the growth. Fungi reproduce primarily by means of small, light airborne spores that are easily distributes by the wind (Ramesh and Marihal, 2012).

A large number of fungal pathogens are transmitted through seeds and vegetative propagating parts. Some of the fungal pathogens transmitted through seeds and vegetative propagating parts. Metabolic products of seed-borne microorganisms may affect the seed itself or sometimes may have other serious consequences such as toxicity to animals and human beings. Poor storage facilities add substantially to this loss in different parts of the country.

The antagonistic activities of *Trichoderma harzianum* against several pathogenic fungi have been reported by many workers [Henis and Chet, (1975); Backman and Rodrigues-Kabana, (1974); Hadar *et al.,* (1979) and Elad *et al.,* (1981)]. Kakde and Chavan (2011) studied the

antagonistic activity of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi and found that growth of *Curvularia lunata, Rhizopus stolonifer, Fusarium oxysporum Macrophomina phaseolina* and *Penicillium chrysogenum* was retarded due to *Trichoderma* species.

The studies on antagonism between *F. oxysporum* f. sp. *ciceri* and the fungal antagonists viz., *T. viride, T. harzinaum, T. hamatum* were carried out by applying 'Direct Bit Placement Method' (Brodbent *et al*. 1971).

The increased growth parameter in crop plants by the application of *Trichoderma* might be due to biological control of minor plant pathogen or by the production of growth regulatory metabolites by *Trichoderma* (Widham and Baker, 1986). Sinaga (1986) reported that *Gliocladium* sp. constitute another group of soil fungi investigated for their potential as biocontrol agents against *Rhizoctonia solani, Sclerotium rolfsi* and *F. oxysporum.* Antifungal response of *G. virens* isolate most likely is due to the production of gliotoxin and / or an antibiotic and also the hyper parasitic and antibiotic activity of *G. virens* with eight phytopathogenic fungi including *F. solani* and *R. solani* were confirmed. The action of bioagent on soil borne pathogen has been discussed by Benhamou *et al*. (2000) who stated that in addition to mycoparasitism, antagonistic process might rely on the dual action of the antibiotics and hydrolytic enzymes.

2.1 SURVEY

A survey was carried out in certain districts of Central Gujarat and Saurshtra namely 1) Vadodara 2) Bharuch and 3) Jamnagar to find out the different varieties of cotton (Normal / Bt) under cultivation, yield obtained, and associated seed and soil borne diseases.

2.2 ISOLATION OF FUNGI

3 samples from each field were collected from 30 cm depth in clean polythene bags from different locations and at a distance of 50 m and brought to the laboratory for further studies. Both rhizospheric and non rhizospheric soil of the cotton was used for the isolation.

Following method was incorporated for the isolation of different soil fungi:

- **Serial Dilution Technique (Waksman, 1961)**

To study soil mycoflora, serial dilution method is commonly used. The method is based upon the principle that when material containing microorganisms are cultured each viable propagule viz. spore, hyphae, sclerotia etc. will develop into a colony. Hence, the number of colonies appearing on the plates represents the number of living colony forming units (CFU) present in the sample.

10 g of soil sample was dissolved in 100 ml of sterile distilled water. After settling of the soil, 1ml of soil suspension was transferred into tubes containing 9ml of sterile distil water. Similar procedure was repeated 4 times to get the desired dilution.

0.5 ml of soil suspension from each tubes having 10^{-3} and 10^{-4} dilution was spread into the plates containing PDA medium. These plates were incubated at 25±2°C for 7days. Once fungal colonies were formed in PDA plates, each colony was transferred to a new agar slant to obtain a pure culture.

The following culture medium was employed:

- **Potato Dextrose medium: (pH- 6.0-7.0)**

200g of potato were peeled and sliced into small pieces. It was boiled in an autoclave for 40 min in 500 ml of distilled water and then filtered through a cloth. Twenty grams of dextrose was added and total volume was raised to 1000ml.

Borosil glassware and pure reagents supplied by Qualigens or SRL were used throughout the present investigation.

For cultural studies Petri dishes of (100 mm diameter, containing 20 ml agar) were inoculated with a piece of mycelium at the edge kept in diffused daylight at room temperature (20-25^{o}C) and examined at 7 days intervals. Separate slides were prepared and mounted in lactophenol.

LACTOPHENOL – COTTON BLUE STAIN

Phenol : 20 g

Lactic acid : 20 ml

Glycerol : 40 ml

Distilled water: 20 ml

The above mentioned chemicals were mixed and heated at 70 ^{0}C and then 5 ml of 1 % aqueous cotton blue was added.

2.2.1. Identification of fungi based on morphological characters

During field survey the Materials were collected in clean polythene bags from different locations and brought to the laboratory. The identification of fungal cultures was done based on morphological characters of conidia/ spore and final confirmation was done from IARI, New Delhi and Agharkar Research Institute, Pune.

2.3 Isolation of Seed Mycoflora

Before the isolation of the seed mycoflora, cotton seeds were tested for their viability. For seed viability test, four replications of 100 seeds were fully immersed in distilled water for 18 hours to start activity of dehydrogenase enzyme and for the facilitation of the penetration of tetrazolium solution (1% of TTC was used) for three hours at room temperature i.e. 25 ± 2 ºC in dark. After three hours the solution was decanted and excess reagent was removed, the seeds were then rinsed with water and evaluated. Individually the seeds were assessed for their viability. On the basis of staining of embryo, staining of cotyledon assessment on basis of necrosis, assessment on the basis of necrosis, and on the basis of color intensity was done, (Khare and Bhale 2000).

Isolation of seed mycoflora was done using-

(i) Blotter Method and (ii) Agar Plate Method

Blotter Method

In this method the Petri dish was lined with 3 layers of filter paper and then moistened with distilled water, the seeds were then placed and the dishes are kept at 25 ± 2 ^{o}C temperature and the germination of seeds was recorded on 4th and 7th day. Altogether 300 seeds were used (3 replicates of 100 seeds each) for this experiment. However, according to ISTA a sample size of 400 seeds is recommended. If any fungal mycelium was seen associated with seeds it was isolated under sterile conditions in to PDA slants.

Agar Plate Method

In this method, hundred seeds in four replictes were placed on potato dextrose agar. Before placing in Petri plate, seeds were surface sterilized with 0.1% mercuric chloride solution for one or two minutes and washed thrice with sterile distilled water to avoid surface contaminants and for the isolation of internal mycoflora, which were placed at equidistance in Petri plates. These

plates were then incubated for seven days. Different fungal colonies were examined under microscope for further identification.

2.4 Isolation of Arbuscular Mycorrhizal Fungi

Soil samples were collected from different locations of cotton growing area (Plate I) to study the natural distribution of AM Fungi. Soil was dug out with a trowel to a depth of 30 cm. Three representative samples were taken in clean polythene bags, labelled and stored until they were processed further. The number of AM spores found in rhizospheric and non rhizospheric soil are depicted in Tables 6-9. Isolation of AM Fungal spores from the rhizospheric and non rhizospheric soil of cotton was done.

- **Wet Sieving and Decanting Method (Gerdemann and Nicolson, 1963)**

The method includes mixing of 100g soil with 1litre of tap water and then decanting it in a stack of sieves. In the present case 5 sieves ranging from 63 to 500 µm were used. The AM spores were separated on different sieves, based on their size. They were then picked up by hypodermic syringe using a stereo microscope. Permanent slides were prepared in polyvinyl alcohol glycerol. Similar types of 5 spores were placed in a vial containing 0.05% streptomycin sulphate for further use.

- **Mounting of AM spores**

AM spores were mounted with polyvinyl-lacto-glycerol (PVLG) and were examined for their various characteristics and fungi were identified using the standard keys (Schenck and Perez, 1990; Mehrotra and Baijal, 1994)

Ingredients	Quantity
Lactic acid	100 ml
Glycerol	10 ml
Polyvinly alcohol (PVA)	16.6 g
Distilled Water	100 ml

PVLG is used to mount the spores semi -permanently on glass slides. For the best results, mounted specimens should not be studied for 2-3 days after they were mounted to give time for spore contents to clear. Whole spores will change color, generally darkening to varying degrees, and shrink or collapse with plasmolysis of spore contents. Broken spores are also needed to mounted as discrete layers of the spore wall or flexible inner walls of broken spores will swell to varying degrees and appear fused after long storage in some instances.

It is most important to mix all ingredients in a dark bottle before adding the polyvinyl alcohol. The PVA should have the following properties: 99-100% hydrolyzed, and a viscosity of 24 - 32 centipoise in a 4% aqueous solution at 20^{o}C. The PVA is added as a powder to the other liquid ingredients. The PVA dissolves slowly, and then only when placed in a hot water bath (70 - 80^{o}C). The solution will be clear in 4-6 hours. The solution was prepared by the mixture in the evening and letting it incubated in the water bath overnight. PVLG stores well in dark bottles for approximately one year. According to Koske in 2013 (http://invam.wvu.edu/methods/recipes), PVA powder can be added to the water, followed by autoclaving for 15 minutes. The lactic acid and glycerin are added, and the solution then is stored at room temperature for at least 24 h before using.

- **Storage of AM spores**

Isolated spores were collected in 0.1% Streptomycin sulphate solution and were preserved in small injection vials at low temperature, in a refrigerator (10-15^{0}C).

2.4.1. Identification of AM spores

Spores are the main structures used for the Identification of AM fungi. AM fungi were identified by using following morphological structures i.e. color, size, shape, wall structure, presence or absence of hyphae, surface ornamentations, nature and size of subtending hyphae and bulbous

suspensor attachment. For identification Manual of Scheneck and Perez (1990) and Mehrotra and Baijal (1994) and identification key of http://invam.caf.wvu.edu was used. Spores were photographed using Leica DME Research Microscope. The details are presented in Table 10.

2.5. Percentage Root Colonization by Root Clearing and Staining Technique (Phillips and Hayman, 1970)

The roots were washed and rinse in several changes of tap water. Then add 10% KOH and autoclave at 120^{o} C for 15 min. Decant KOH and rinse with water to remove KOH. Acidify roots by adding 1% HCl for 5 min. Decant HCl, do not rinse with water because the specimens must be acidified for proper staining. Add 0.05% trypan blue in lacto glycerol and simmer for 10 min. Decant stain and add lacto glycerol. Examine under microscope for mycorrhizal colonization.

2.6 Mass Multipication of AM fungi

50: 50 sand + soil mixture was sterilized for 1 hr. After cooling down fill the pots (30 cm dia) upto 3/4th with this mixture. AM spores were sterilized by keeping in 200 ppm Streptomycin sulphate solution for 5 min. After disinfection, the spores were thoroughly washed in sterilized distilled water and were used as inoculant for initiating mycorrhization in maize plant. Add AM spores (20 spores in each pot) in center and spread one layer of soil (3cm) over to it. Sow the five maize seeds and again spread one layer of soil (2cm) to it and add water to it. The pots were kept in green house under constant observation. After 90 days, soil containing AM spores was used to inoculate nursery seedlings. Further, after 90 days, the maize roots were cut into pieces of 1cm and were mixed with the soil for preparing the AM inoculum. This incoulum was also used in plots containing garden soil without any fertilizer for mass multiplication of AM spores.

2.7 Mass culture of Fungi

For mass culture, the fungi were inoculated in to maize meal sand medium (Singh 1977). In 250ml conical flask, 150 g of sand and 4.5 g of crushed maize grains. 20ml of distilled water was added. The 150 g sand maize meal mixture gives 65% saturation. Then the flasks were autoclaved for 30 min at 15 *p.s.i* and were then inoculated with agar inoculum discs (Garrett, 1936) with 10 day old cultures flasks were incubated for 21 days at room temperature and in between they were shaken so that the fungus gets distributed equally inside the flask. When the fungal mycelium was well grown then it was ready to be incorporated in the soil. 5 g of upper 10cm inoculum was used per pot and mixed with the pot soil. Then seeds of Cotton varieties were sown. The pots were then watered once in a day to maintain appropriate moisture and then the effect of fungus on plant biomass was observed.

2.8.Biomass Study:

Plant Analysis:

Morphological and Biochemical Analysis of the Plants.

The plants were harvested on 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} day and morphological and biochemical analyses of *G. herbaceum* L. were considered in three replicates. The parameters that were taken into considerations during the plant analysis were as follows:

(A) Morphological Parameters:

I. **Shoot Length (cm):** At every harvest shoot length of all three varieties of G. *herbaceum* were noted.

II. **Root Length (cm):** At every harvest the root length of the plants was noted.

III. **Number of leaves per plant**: At every harvest number of leaves per plant was counted.

(B) Biochemical Parameters:

I.Fresh weight /plant (g):

The whole plant after harvesting was washed with water properly and air dried till the water evaporates from the plant surface. This plant was then cut to separate root and shoot and was weighed on the accurate digital balance. This procedure was repeated with 3 plants and the average of all the plants was calculated.

II.Dry weight /plant (g):

After noting the fresh weight of these plants , they were kept in oven for drying at 60^0 C for 48 hr separately and then the plant was weighed again and the average dry weight was calculated.

III. Estimation of Total Chlorophylls (Sadasivam and Manickam, 1996)

Weigh 1g of fresh leaf tissue and grind in the mortar. Add a pinch of $CaCO_3$ and the tissue was grinded to make fine pulp with the addition of 20 ml of 80% Acetone. The acetone extract was centrifuged at (5000 rpm for 5 min) and transfer the supernatant to a 100ml volumetric flask. The same procedure was repeated till the residue became colourless. The volume of volumetric flask was make up to 100ml with 80% acetone. The absorbance of the solution was read at 645 and 663 nm against the solvent (80% acetone) as blank in the spectrophotometer.

Calculations:

The amount of chlorophyll present in the extract mg chlorophyll per gram tissue was calculated using the following equations:-

Chlorophyll-a (mg/g tissue) : $[\ 12.7(A_{663}) - 2.69(A_{645})] \times \frac{V}{1000 \times W}$

Chlorophyll-b (mg/g tissue) : $[\ 22.9(A_{645}) - 4.68(A_{663})] \times \frac{V}{1000 \times W}$

Total Chlorophyll (mg/g tissue) : $[\ 20.2(A_{645}) + 8.02\ (A_{663})] \times \frac{V}{1000 \times W}$

Where,

A= Absorbance at specific wavelength

V= Final volume of chlorophyll extract in acetone

W= Fresh weight of tissue extracted

2.9 Effect of Fungal Cultural Filtrates on cotton seed germination

Agar blocks of equal size (5 mm) was cut from the actively growing margin of the individual species of soil fungi namely *Aspergillus niger, Fusarium oxysporum, Gliocladium virens, T. viride* and *T. harzianum*, and inoculated separately into the 250 ml conical flasks containing 100 ml sterile potato dextrose broth. The flasks were incubated at 25 ± 2^0C for 15 days. After incubation, the fungal cultures were filtered through Whatman filter paper No.1. The filtrates were transferred to conical flasks and stored at 4°C for further use.

2.10 Antagonistic Effect

To study the antagonistic effect, an experiment was laid out in petriplates poured with sterilized PDA. Solidified medium in the petri plates was inoculated by placing the discs (5 mm diameter) of bioagents culture. It was exactly opposite to this disc of test fungus (7 days old culture) were placed in such a manner that both organisms would get equal opportunity for their growth. The experiment was conducted with four replications and one control plate containing only test fungus. The growth rate of both test fungus and antagonistic fungi were recorded at 24 h intervals. Assessment was made when the fungi had achieved an equilibrium after which there was no further alternation in the growth. These plates were then incubated at 25 ± 2^0C. Observations were recorded after seven days of inoculation on area covered by the antagonist fungi and pathogen and percent growth inhibition was calculated as per formula (Skidmore and Dickinson,1976).

The percentage inhibition of growth was calculated as follows.

$$\text{Percent inhibition} = \frac{r - r_1}{r} \times 100$$

where, r = colony diameter of pathogenic fungi in control set

r_1 = colony diameter of pathogenic fungi in treated set

2.11. Bio – control of Pathogenic fungi (Poisoned Food Technique)

Effect of aqueous leaf and Methanolic extracts were obtained by Soxhlet Extraction method of 7 plants was observed on test organisms. It was tested on 3 different pathogenic fungi. The healthy leaves of *Annona reticulata, Balanites roxburghii, Cochlospermum religiosum, Gliricidia sepium, Ferronia limonia, Sapindus emarginatus* and *Tephrosia jamagerensis* were collected and washed well and dried in oven at 60 ^{0}C for 48h. The dried leaves were powdered and stored in plastic bags. Twenty grams of leaf powder was extracted in a Soxhlet extractor with 200 ml methanol for 8 hours. The extract was concentrated then the residue was treated with 20 % of methanol. It was added to dry residue and water soluble compounds were filtered out. The leaf extracts were mixed with appropriate volume of medium (PDA) to obtain concentrations ranging from 5 to 25 % in the final volume of 100 ml of medium. This 100 ml medium was dispensed into 100 mm Petri plates with triplicates **(**Nene and Thapliyal, 1979).

Fungal isolates of was placed in the centre of each plate. Control sets were also prepared without plant extract. The plates were incubated at 25 °±2°C and growth of colony was measured after 7 days of inoculation. The radial growth of mycelium was measured at two points along the diameter of the plate and the mean of these two readings was taken as the diameter of the colony. The growth of the colony in control sets was compared with that of various treatments and the difference was converted into percent inhibition by following formula

$$\text{Percent inhibition} = \frac{\text{Diameter of control set - diameter of treated set}}{\text{Diameter of control set}} \times 100$$

4.1. Survey

Gujarat is a prosperous state in western India. It was part of Bombay before 1st May 1960, when the two states Gujarat and Maharashtra were formed. It has an area of 196,077 km² with a coastline of 1,600 km, most of which lays on the Kathiawar peninsula, and a population in excess of 50 million (2010 census). The state is bordered by Rajasthan to the north, Maharashtra to the south, Madhya Pradesh to the east and the Arabian Sea as well as the Pakistani province of Sindh on the west. Gujarat played an important role in the economic history of India throughout the history of India. Gujarat has some of the largest businesses in India. Gujarat is the main producer of tobacco, cotton and groundnuts in India. Groundnut research station is situated in Junagadh. Other major food crops produced are rice, wheat, jowar, bajra, maize, pigeonpea, and gram. Gujarat has an agricultural economy; the total crop area amounts to more than one-half of the total land area. In India 31% of cotton production is contributed by Gujarat.

To find out the different varieties of cotton (Non Bt/ Bt) under cultivation, yield obtained and associated seed and soil borne diseases, a survey was conducted in different fields of 1) Vadodara 2) Bharuch and 3) Jamnagar districts of Gujarat during 2011 and 2012. The location of these districts is depicted in Fig. 2. All the fields surveyed in different districts of Gujarat 95% fields were cultivated with the Bt cotton and results are recorded in Tables 1, 2 and 3 respectively.

About 3-4 fields cultivated with the non Bt cotton showed the presence of the wilted plants in Jambusar area of Bharuch district. Wilting was also observed in the fields of Jamnagar where Bt cotton was cultivated.

1. Vadodara district:

Vadodara District is a district in the eastern part of the state of Gujarat in western India. Vadodara District covers an area of 7,794 km².The district is bounded by Panchmahal and Dahod districts to the north, Anand and Kheda districts to the west, Bharuch and Narmada districts to the south, and the state of Madhya Pradesh to the east. The tallest point in the region is the hill of Pavagadh. The Mahi River passes through the district. Extensive surveys were conducted in the following areas.

Kayavarohan village

Kayavarohan is a village in the Vadodara district of the state of Gujarat, India. Kayavarohan is popularly known as Karvan and is situated on the National Highway 8 at a distance of 30 km from Vadodara. It is famous for its Shiva temple.

Por

Por is a Village in Vadodara Taluka in Vadodara District of Gujarat State, India. It is located at $22^0$08'25.32" N, $73^0$11'08.66" E . Por is surrounded by Padra Taluka towards west , Vadodara Taluka towards North , Dabhoi Taluka towards East , Waghodia Taluka towards East . Gujarati is the Local Language here. Crops grown in this area includes mainly of Jowar, Bajra and cator.

Padra

Padra is a city in Vadodara district in the Indian state of Gujarat. Padra is located about 16 km from Vadodara city. Padra is located at 22.23°N 73.08°E. It has an average elevation of 79 metres (259 feet). Padra is also known for producing cotton and tobacco like cash crops.

Dabhasa village

Dabhasa is one of the Village in Padra Taluka, Vadodara District, Gujarat State . Dabhasa is 17 km distance from its district headquarter Vadodara. It is situated on baroda-jambusar highway located at 22°14'42"N 73°2'13"E.

Dabka Village

It is a village in Padra taluka of Vadodara district. Dabka is 60.45 km from the maincity vadodara.

Dabhoi

Dabhoi also called as Darbhavati is a city in Vadodara district in the state of Gujarat, India. It was originally known as Darbhavati. It is located at 22.18°N 73.43°E. It has an average elevation of 99 metres (324 feet). It is famous for its Jain temple of Shri Lodhan Parshvanath. Major crops cultivated in this include Cotton, Castor, Maize, Bajra and Jowar.

Kunvarpura Village

It is a village in Dabhoi Taluka in Vadodara district of Gujarat State, India. It is located at $22^0$05'45.44" N $73^0$15'21.08" E and is 38 km towards East from district head quarters Vadodara.

2. Bharuch district

Bharuch (formerly commonly known as Broach) in India, is a district in the southern part of the Gujarat peninsula on the west coast of state of Gujarat with a size and population comparable to that of Greater Boston. The Narmada River outlets into the Gulf of Khambat through its lands and that shipping artery gave inland access to the kingdoms and empires located in the central and northern parts of the sub-continent of India. Administratively, it contains the talukas (administrative subdistricts similar to a borough or township) of Ankleshwar, Hansot, Jambusar, Jhagadia, Amod, Valia and Vagra. Bharuch district is the fourth major cotton producing district in Gujarat.

Jambusar

Jambusar is a city and a municipality in Bharuch district in the Indian state of Gujarat. It is located at 22.05°N 72.8°E. It has an average elevation of 4 metres (13 feet). The villages in

Jambusar Taluka are Degam , Hamadpor Kanthariya , Islampore , Jafarpara , Jantran , Kahanava , Kaliari , Kansagar , Kapuria , Kareli , Karmad, Kavi.

Kavi village

Kavi is one of the costal village in Jambusar Taluka of Bharuch District. Kavi is situated at a distance of 63.30 km from the main city Bharuch. Place kavi is situated on the banks of Arabian Sea. It is famous for the Stambheshwar Mahadev Temple.

3. Jamnagar district

Jamnagar district is located in state of Gujarat, India on the southern coast of the Gulf of Kutch. This district has 10 sub districts and 600 villages.

Table 1: Survey of different cotton fields in Vadodara district of Gujarat.

Village	Name of Farmer interviewed	Land owned (Acres)	Variety Sown	Yield (quintal)
Por	Sunil Patel	1	Rashi	5
	Surendra Bhai Patel	0.5	Mallika	3
Kayavarohan	Shanker Bhai	2	Ajeet -11	11
	Praful Bhai	3	RCH	17
	Ashok Bhai	3.2	Ajeet-155	14
Padra	Jayesh Bhai	20	Bollgard	40-50
	Ghanshyambhai Patel	3.5	Ajeet-11	43
Dabhasha	JayantiBhai Patel	2.5	Bt- Sigma	18
	Kalubhai	5	RCH	19
Dabhoi	Lalji Bhai Patel	3.5	Bt- Gabbar	12
	Mohan bhai	5	Ajeet-11	37
Kunvarpura	Ramji bhai Patel	2	Bt- Mallika	10
	Labhu Bhai	4	Vikram-5	28
Muval	Bachu Bhai Patel	1.5	Bt- Drona	9

Table 2: Survey of different cotton fields in Bharuch district of Gujarat.

Village	Name of Farmer interviewed	Land owned (Acres)	Variety Sown	Yield (quintal)
Simor	Shivabhai Vasava	2	Bt- Sigma	6.4
Amdada	Chiman Vasava	2	Rasi-2	7
Matar	Mohan Bhatt	10	Rasi	100
Jambusar	Badri bhai Joshi	4	Desi kapas	12
	Bakabhai Patel	16	Desi	48
	Dasrath Sinh	2.27	Desi kapas	27
Kavi	Damji Bhai	3	Vikram-5	30
	Chandu bhai Solanki	2.5	Ajeet-155	19
Umber	Fatehsinh Solanki	30	Ajeet-11	184
	Hasmukh Patel	4.21	Ajeet-11	54.2
Dabhali	Khushal bhai	3.42	Bt- Pratik	20
	Chandrashanker Rawal	3.42	Ajeet-11	30
Asanvad	Ramanbhai Makwana	0.38	Ajeet-155	13
Anastu	Amit Bhai Bhatt	2.8	Ajeet-155	23
Karjan	Hamjibhai Raval	4.21	Vikram-5	54. 2
	Ajinbhai	1.23	Obama	18
Chhapra	Bhagwan Bhai Pawar	3.38	Vikram-5	400
Haripura	Magan Bhai Patel	2.37	Obama	36
Intola	ParagbhaiVankar	2.3	Desi	12

Table 3: Survey of different cotton fields in areas of Jamnagar district of Gujarat.

Village	Name of Farmer interviewed	Land owned (Acres)	Variety Sown	Yield (quintal)
Chella	Ishwarbhai Pawa	3.30	Ajeet-11	47
	Yashwantbhai Gohil	3	Rashi	35
Taraghadi	Mahesh Harishanker Bhatt	5	Ajeet-11	62
	Dilipsinh Jadeja	2.32	Ajeet-11	45
	Khushab Bhai	1.11	Ajett-155	15.7
Falla	Magan Bhai Rohit	2.37	Obama	36
	Raman bhai	1.27	Mallika	30
Dhunvav	Ambalal Gohil	4.05	Bt- Shanker	250
Gaduka	Shanker bhai Manek	3.22	Obama	43
	Narayan Bhai	2.3	Bt- Shanker	23
Hadmatiya	Ebrahimbhai	3.02	Obama	47
	Valjibhai Pawa	2.11	Bt- Tulsi	14
Lonthiya	Narpatbhai	3.23	Bt- Vikram	62
	Virsinh Gohil	1.10	Shanker-6	12
Sapara	Jeevan bhai Pawa	3.30	Ajjet-155	42
	Lachmi bhai Khamar	1.09	Bt- Rasi	15
Umrali	Ramesh Bhai Gosai	5.5	Rasi-2	35
	Meghjibhai Menpara	17	Bt- Pratik	150

4.2. Studies on Soil borne fungi associated with cotton:

Soil is a complex system. It is the most precious natural resource and contains the most diverse assemblages of living organisms. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Singh *et al.,* 1999). It is an important panorama of interactions between microbes and plants (Shekh *et al.,* 2012). Many biological processes take place in soil and determine functions that provide various services within ecosystems: turn-over of organic matter, symbiotic and non-symbiotic atmospheric nitrogen fixation, denitrification, aggregation, etc (Chenu and Stotzky, 2002). It regulates global biogeochemical cycles, filters and remediates anthropogenic pollutants, and enables food production (Kennedy and Smith, 1995; Richards, 1987). One significant component of soil is occurrence of microorganisms. Soil is a medium with solids, liquids and gases in which the mineral and organic particles form differently-sized aggregates that delimit pores (Tisdall and Oades, 1982; Feller and Beare, 1997). This organization creates micro-environments that are suited to microbial activity to varying extents (Chotte *et al.,* 1997).

Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils (O'Donnell *et al*. 1994) because of their involvement in such key processes as soil structure formation, organic matter decomposition, nutrient cycling and toxic removal (Van Elsas, 1997; Doran and Zeiss 2000).

Rhizosphere and non Rhizosphere soil samples were collected from cotton fields of study area. The soil samples were collected with the help of a soil augur upto a depth of 30 cm. To compare the fungal population present in the rhizosphere with that of the non rhizosphere regions, soil around the plant roots and for non rhizosphere fungal studies one meter away from the root zone is selected. Three soil samples for each (around the root and away from the root zone) were

collected, placed separately in fresh polythene bags, labeled and brought to the laboratory. In the present investigation the survey was conducted to find out the fungal diversity in different cotton fields of Vadodara, Bharuch and Jamnagar districts.

Table 4: List of Fungi Isolated from the rhizospheric and non rhizospheric soil of different Cotton fields of 3 districts in Gujarat

Sr. No	Fungi Isolated	Rhizospheric soil			Non Rhizospheric soil		
		Vadodara	Bharuch	Saurashtra	Vadodara	Bharuch	Saurashtra
Zygomycetes							
1.	*Rhizopus stolonifer* (Ehrneb) Vuill.	++	++	+	++	++	++
Ascomycetes							
2.	*Aspergillus terreus* Thom.	++	++	-	+	+	-
3.	*A. niger* Van. Tiegh	+	+	+	+	+	+
4.	*A. flavus* Link.	+	+	-	+	-	-
5.	*A. fumigates* Fresen.	+	-	++	-	+	-
6.	*Alternaria alternata* (Fr.)Keissl.	+	+	+	+	-	+
7.	*Curvularia lunata* Bat.	++	+	-	-	+	-
8.	*Chaetomium globosum* Kunze	+	+	-	-	-	-
9.	*Cladosporium cladosporoides* (Pers).Link	++	++	-	+	-	-
10.	*Fusarium oxysporum* Schlecht.	+++	+++	++	-	-	+
11.	*F .roseum* (Schwein) Petch	-	++	+	+	+	-
12.	*Fusarium* sp.	++	-	-	-	-	-
13.	*Myrothecium verrucaria* (Alb. And Schwein) Ditmar	++	-	++	-	+	+
14.	*Penicillium* sp.	+	+	-	+	+	-
15.	*Penicillum citrinum* Thom	-	+	+	+	-	-
16.	*Penicillium* sp.	+++	-	-	+	-	-
17.	*Penicillium* sp.	-	+++	-	+	-	-
18.	*Trichothecium roseum*	++	-	-	-	-	-
19.	*Trichoderma viride* Pers.	+	+	-	+	-	+
20.	*T. harzianum* Rafai	+	+	+	-	+	-
Deuteromycetes							
21	Black sterile mycelium	+	++	+	+	-	-

*Readings based on 3 replicates
+++: Dominant ++: Abundant +: Present -: Absent

Serial dilution method (Waksman, 1916) for rhizospheric and non rhizospheric soil revealed the presence of total 21 species belonging to 10 genera. The results depicted that maximum number of fungal strains were isolated from the rhizospheric soils of Vadodara district (18) followed by Bharuch district (16) and areas of Saurashtra viz Rajkot and Jamnagar (10). Various Ascomycetes fungal members like *Aspergillus niger, A. terreus, A. flavus, A. fumigatus, Alternaria alternata, Curvularia lunata, Chaetomium globosum, Cladosporium cladosporaoides, Fusarium oxysporum, F. roseum, Myrothecium verrucaria, P. citrinum, Tricothecium roseum, T. harzianium, T. viride and Rhizopus stolonifer*- Zygomycetes member were isolated which are depicted in Table 4. The occurrence of these fungi was found to more in the rhizospheric soil as compared to non rhizospheric soil.

The most dominant species *F. oxysporum* known to cause wilting was found in cotton fields of all three districts. Other than *F. oxysporum , Penicillium* sp . was found dominating in Vadodara district. *A. terreus, Curvularia lunata, Cladosporium herabceum, Fusarium* sp., *M. verrucaria, R. stolonifer, Trichothecium roseum* were found abundantly, whereas, fungi like *A. niger, A. flavus, A. fumigatus, Alternaria alternata, C. globosum, Penicillum* sp., *Trichoderma viride, T. harzianum* and Black sterile mycelium was found but in lesser amount, whereas, *Fusarium roseum* and Penicillium sp. were not found from the rhizospheric soils of Vadodara district.

The non rhizospheric soil of Vadodara district showed the presence of 13 fungal genera which included four species of *Penicillium* followed by three species of *Aspergillus* viz. *A. terreus, A. niger, A. flavus* and other single species of *Alternaria alternata, Cladosporium herbaceum, Fusarium roseum, Rhizopus stolonifer, Trichoderma viride* and black sterile

mycelium. Among the non rhizospheric soils of Vadodara, *Rhizopus stolonifer* was recorded abundantly.

Aspergillus fumigates, Curvularia lunata, Chaetomium globosum, Fusarium oxysporum, Fusarium roseum, Fusarium sp., *Myrothecium verrucaria, T. roseum* and *T. viride* and sterile mycelium was not found in the non rhizospheric soil as compared to the rhizospheric soils.

In Bharuch district, rhizospheric soil showed presence of 16 fungal strains of which *Fusarium oxysporum* was found dominant. As depicted in Table 4, *Rhizopus stolonifer* was found abundantly, whereas *A. terreus, A.niger, A. fumigatus, C. lunata, F. roseum, M. verrucaria, Penicillium* sp. and *Trichoderma harzianum* was found from the non rhizospheric soils. Fungi like *A. flavus, A. alternata, C. globosum, C. herbaceum, F. oxysporum, Fusarium* sp., *P. citrinum, Trichothecium roseum, T. viride* and sterile mycelium were not found in non rhizosphereic soil of Bharuch.

Rhizospheric soil of Saurashtra region viz . Jamnagar showed presence of 10 different fungi of which 3 fungal genera namely *A. fumigatus, F. oxysporum* and *Myrothecium verrucaria* were dominantly found. Whereas other fungi like *A. niger, A. alternaria, P.citrinum, R. stolonifer, T. harizanum* and Sterile mycelium were also recorded in less amount.

Similar results reported by Shekh *et. al.,* (2012) from the rhizospheric and non rhizospheric soil samples of cotton fields in Nanded district and also found that total number of fungal species were higher in rhizosphere soil as compared to non rhizosphere soil. The results obtained in the present investigation are similar to the investigations made by Oyeyiola (2009) in case of *Hibiscus esculentus* The results obtained in the study for *Sorghum bicolor* (Odunfa, 1979), cowpea (Odunfa and Oso, 1979), Sugarcane (Abdel- Rahim et al., 1983) and *Amaranthus hybridus* (Oyeyiola, 2002) were similar to present results obtained for cotton.

Kalich (1988) investigated soil fungi of some low-altitude desert cotton fields and found forty two taxa from which genera like *Aspergillus*, *Penicillium* and *Fusarium* were found predominantly. These three genera were present in all the soil samples of three districts in Gujarat.

4.2.1. Studies on Fungi Associated with seeds of cotton varieties

The significance of sustainable agricultural production is hidden in the use of quality of seed. It is the most crucial and vital input for enhancing crop productivity. Since, seed is the custodian of the genetic potential of the cultivar thus, the quality of the seed determines the limits of productivity realized in a given system. The shortage of quality of seed, absence of techno-infrastructure facilities needed for processing, storage and distribution of seed are amongst the major considerable attributes for such outbreaks.

Seeds are the basic input for crop production and are of great economic interest and also constitutes a major part of diet, they play a vital role in associating microorganisms which prove hazardous for seed or the new plant created from it. So any infectious agent (bacteria, fungi, nematode, etc), which is associated with seeds having potential agent of causing a disease of a seedling or plant, is termed as seed borne pathogen (Agarwal and Sinclair, 1987). The associating microorganisms may be pathogenic, weak parasites or saprophytes.

Seeds are regarded as highly effective means for transporting plant pathogens over long distances. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of the importation of seeds that were infected or contaminated with pathogens (Agarwal and Sinclair, 1996).

Neergaard (1973) reported several types of abnormalities occurred to the seeds, which mainly include seed discoloration, necrosis, seed abortion, seed toxification, seed rotting, etc. He

further reported that these types of abnormalities occur due to dominate fungi like *Aspergillus, Curvularia, Drechslera, Fusarium, Penicillium, Rhizoctonia, Verticilastum and Alternaria*.

Seed-borne mycoflora of wheat reported recently included *Alternaria alternata, Drechslera sorokiniana, Fusarium moniliforme, F. avenaceum, F. graminearum, F. nivale, F. culmorum, F. equiseti, F. sporotirchioides, Cladosporium herbarum, Stemphylium botryosum* (Nirenberg et al., 1994; Glazek, 1997; Mirza and Qureshi,1978). Seed-borne mycoflora of sorghum reported from different parts of the world include *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium* sp., *Fusarium moniliforme, F.oxysporum, F. pallidoroseum, Drechslera tetramera, Nigrospora* sp., *Phoma* sp., and *Rhizopus* sp., (Abdullah and Kadhum, 1987; Ahmed *et al.,* 1992).

Cotton seeds are also used as oil seeds. The economic value of cotton seed is greatly influenced by the presence of fungi in the seed. Fungi or associated metabolites may reduce the vigor of planting seed (Davis, 1982; Halloin and Bourland, 1981) increase the amount of free fatty acid in the seed thereby reducing the quality of the oil (Ashworth *et al.,* 1971; Roncadori *et al*., 1971), or produce mycotoxins that render the seed unsuitable for consumption (Diener *et al.,* 1976). An understanding of the distribution and frequency of the mycoflora could lead to practical measures for control of the fungi which devalue cotton seed. Concomitant with the need to assess the mycoflora of cotton seed, an understanding of the conditions conducive to their presence is important for both predictive purposes and to develop methods of reducing seed infection (Klich, 1986).

Cotton seedling diseases are a worldwide problem; they are caused by a complex of microorganisms. Fungi are the widest pathogens that affect cotton crop especially at the seedling disease stage causing pre or post emergence damping off (Aly *et al.,* 2008). Cotton seedling

diseases may lead to stand losses when the disease is not managed or environmental conditions are highly conducive for disease occurrence and development (Blasingame and Mukund, 2001; Rothrock *et al*., 2007).

Cotton seedlings are vulnerable to disease injuries upto one month after sowing. Of these, fungal diseases are the most widespread and devastating diseases that affect crop yield quantitatively and qualitatively (Aly *et al.,* 2000; Nehl *et al.,* 2004).

Cotton seedlings damping off, the serious problem in most of the cotton producing regions often attributes to *Rhizoctonia, Pythium, Fusarium* (Disfani and Zangi, 2006; Omar et al., 2007). *Alternaria, Fusarium, Macrophomina, Rhizoctonia* and several other fungi were frequently isolated from cotton seeds and seedlings (Colyer and Vernon, 2005; Asran-Amal, 2007; Mikhail *et al.,* 2009; Fard and Mojeni, 2011). Palmateer *et al*., (2004) isolated fifty eight species of fungi belonging to 37 genera, including 9 species of *Fusarium. Fusarium oxysporum, F. solani* and *F. equiseti* were the most common members of this genus occurring at seedling stage.

Table 5(A): Showing Percentage Occurrence of seed mycoflora of three varieties of *G. herbaceum* by Blotter Method.

Sr. No.	Fungi Isolated	Non- Bt		Ajeet-11		Vikram-5	
		Control	Treated	Control	Treated	Control	Treated
1.	*Aspergillus niger*	28	16	19	9	11	5
2.	*A. flavus*	32	16	23	12	24	11
3.	*Alternaria alternata*	10	8	6	4	-	-
4.	*Chaetomium globosum*	-	-	3	-	1	1
5.	*Curvularia lunata*	-	-	2	-	1	1
6.	*Fusarium oxysporum*	19	11	13	9	10	7
7.	*F. roseum*	9	2	11	7	14	10
8.	*Mucor hemalis*	12	-	12	-	9	-
9.	*Rhizospus stolonoifer*	10	8	17	7	13	6
10.	*Trichoderma harzianum*	3	1	1	1	-	-

**Readings based on 3 replicates of 100 seeds each -: Absence of colony*

Table 5 (B): Showing Percentage Occurrence of seed mycoflora of three varieties of *G. herbaceum* by Agar Plate Method

Sr. No.	Fungi Isolated	Non- Bt		Ajeet-11		Vikram-5	
		Control	Treated	Control	Treated	Control	Treated
1.	*Aspergillus niger*	30	18	26	10	22	9
2.	*A. flavus*	35	20	24	18	20	12
3.	*Alternaria alternata*	22	8	11	5	14	9
4.	*Chaetomium globosum*	-	-	-	-	3	1
5.	*Curvularia lunata*	13	7	14	9	10	2
6.	*Fusarium oxysporum*	33	20	29	16	30	18
7.	*F. solani*	4	12	5	10	7	8
8.	*Mucor hemalis*	18	-	12	-	15	-
9.	*Rhizospus stolonoifer*	17	8	19	9	15	10
10.	*Trichoderma harzianum*	18	5	16	3	13	5
11.	*Penicillium citrinum*	-	-	-	-	4	2
12.	Sterile Mycelium	10	-	6	-	4	-

*Readings based on 3 replicates of 100 seeds each -: Absence of colony

Fungi were isolated from seeds using the standard blotter method as recommended by ISTA (2003). Three replications of 100 seeds each from each variety were placed on three layers of well moistened filter paper r and agar plate methodusing Potato Dextrose Agar (PDA) from unsterilized and sterilized seeds.

It is evident from the Tables 5A and 5B that total 12 different fungal organisms were obtained from the untreated and treated three varieties of cotton of which 2 fungi belonged to Zycomycetes, 8 fungal organisms belonged to Ascomycetes and Black non spourlating

unidentified fungi was also recorded. . In Blotter method less number of fungal organisms were isolated and observed in comparison to agar plate method.

In blotter method as depicted in Table 5A, in-total 10 fungal isolates were observed. *Aspergillus flavus, A. niger, Fusarium oxysporum* and *Rhizopus stolonifer* were found dominant in all the three varieties of cotton. *Alternaria alternata* was from the seeds of two varieties namely Non-Bt and Ajeet-11, it was not recorded from Vikram-5 variety. *Chaetomium globosum* was not recorded from Non Bt variety. It was isolated from both Bt varieties Vikram-5 variety and treated seeds of Ajeet-11.

Curvularia lunata was isolated from the Vikram-5 variety and untreated seeds of Ajeet-11, whereas it was totally absent in the Non Bt seeds. *Fusarium roseum* was observed from all three varieties of cotton.

Presence of *Mucor hemalis* was recorded in all the control sets. Seeds of all three varieties after treated with 1% NaOCl did not show the presence of *Mucor.* Contrary to it *Rhizopus stonlonifer* was recorded in both treated and untreated seeds and was found to be one of the dominant fungal species. *Trichoderma harzianum* was found to present in Non Bt and Ajeet-11 variety.

From Table 5(B) it is depicted that 13 different fungal isolates were recorded with maximum colonies of *Aspergillus flavus* and *A. niger* followed by *F. oxysporum* which was recorded in both treated and untreated seeds of all three varieties of cotton.

Alternaria alternata was isolated from Non Bt and Ajjet-11 variety of cotton where as it was not at all recorded from the Bt variety Vikram-5. *Chaetomium globosum* was recorded only in Vikram-5 variety in comparison to other two cotton varieties.*Curvularia lunata* was isolated from all hybrid and Bt variety.

F. solani was recorded only in Agar plate method where it was isolated maximum from the treated seeds of all three varieties. It was found more in Non Bt variety followed by Bt varieties Ajeet-11 and Vikram-5.

Mucor hemalis was found present in untreated seeds of all three varieties as compared to the seeds treated with 1% NaOCl. *R. stolonifer* and *T. harzianum* was recorded in all three varieties of seeds, both treated and untreated seeds of Ajeet -11 showed maximum presence followed by Non Bt and Vikaram-5 respectively.

Penicillium citrinum was recorded only in the agar plate method and it was found only in the seeds of variety Vikram-5. In both Non Bt and Ajeet-11 varieties it was not found. Black non sporulating mycelium was also recorded from the untreated seeds of all three varieties.

The results obtained in the present investigation are similar to the results of Tomar *et al.,* (2012), where they have investigated the presence of eleven fungal flora viz., *Aspergillus niger, A. flavus, Penicillium* spp, *Altemaria alternata, Chaetomium* spp, *Rhizopus niger, Fusarium solani, Macrophomina phaseolina, Myrothecium roridum, Trichothecium roseum* and *Curvularia lunata* from JK 4 cotton cultivar.

Crawford (1923) isolated eight taxa, predominantly *Colletotrichum* sp. and *Fusarium* spp. from the interior of cotton seed in Arkansas. *Colletotrichum gossypii* , a seed-borne pathogen causing leaf anthracnose have been reported to produce myco toxins (Diener *et al.,* 1976; Suzuki *et al.,* 1980).

Templeton *et al., (*1967) reported *Alternaria alternata* from seed coat of cotton. In 1979 Padaganur found *Alternaria macrospora* on cotton seeds. Similarly (Gawade *et al,*. 2006) reported *Alternaria macrospora* from cotton seeds.

Davis (1977) reported *Alternaria* as a dominant member of the mycoflora of cotton seed in Mississippi. However, *Alternaria* was listed as an infrequent fungus by Roncadori et al. (1971) and was present in more than 10% of the seeds from only one location and *Aspergillus niger* was a dominant fungus at several locations infecting up to 23% of the seed in the study by Simpson *et al.*, (1973).

Fulton and Bollenbacher (1959) isolated *Rhizoctonia solani, Pythium ultimum, Fusarium oxysporum, F. moniliforme, F. semitectum* and several other fungi from cotton seeds and seedlings and found that the most isolated fungi were pathogenic to cotton seedlings. Also, Alfred (1963) indicated that fungi belonging to Alternaria, *Aspergillus*, *Diploidia*, *Fusarium* and *Rhizoctonia* were associated with the seed hairs and the actual seed during boll development. Kuch (1986) isolated *Fusarium equiseti* and *Fusarium semitectum* for more than 10% of the seed at any sampling of delinted surface sterilized cotton seeds in the southern USA.

Mansoori and Hamdolahzadeh (1995) isolated *Alternaria alternata, Aspergillus niger, Fusarium acuminatum, Fusarium solani, Pythium ultimum, Rhizopus arrhizus* and *Rhizoctonia solani* from cotton seeds.

4.2.2. Identification of fungus based on morphological characters

i. *Alternaria alternata* (Fr.) Keissl. (Plate-III, Fig.- A)

Colonies growing rapidly reaching 6cm in diameter in one week olive black without aerial mycelium, conidiophores short, simple, straight, 3 –septate, branched or unbranced, upto 50µm long, 3-6 µm wide; conidia forming often in branched chains of 2-10 or more, muriform with 3-8 transverse septa, walls rough in lower part with longitudinal or oblique septa, obclavate, ovoid, ellipsoidal, often with a short or cylindrical beak, smooth or verruculose, medium golden brown, pale, beak pale and upto 1/3 the length of conidium, 2-5 µm wide.

ii. *Apergillus flavus* Link. (Plate- IV , Fig.-B)

Synonym*: Aspergillus fasciculatus* Bat.and Maia

Colonies growing rapidly, 6-7 cm in 10 days, conidial heads yellow when young, becomes dark yellow in age, in older culture deep green, conidial heads radiate, splitting into poorly defined column, conidiophores arising separately from the substratum, 0.5-1.5 mm long, metuale

present predominantly, only phialides arising on metuale, conidia globose to subglobose, echinulate, yellowish green 4.5-14x 3-5.5µm.

iii. *Aspergillus fumigatus* Fresen.

Colonies white at first becoming dull blue green, velvety to floccose, conidial heads columnar, compact, often densely crowded upto 400x 50 µm, conidiophores short, smooth, light green upto 300 µm in length, septate, gradually enlarging into flask shaped vesicle, phialides closely packed, 6-8x2-3 µm, conidia globose to subglobose, green inmass, echinulate, 2.5-3 µm, cleistothecia are absent.

iv. *Aspergillus niger* Van. Tiegh. (Plate- IV, Fig.- E)

Synonym: *Sterigmatocystis nigra* Tiegh.

Colonies growing moderately 3.5-4.5 cm in 10 days on PDA, with abundant submerged mycelium, conidial heads carbon black; conidial heads large and black, at first globose then radiate or splitting in well defined columns; conidiophores arising directly from the substratum, smooth, non septate, thick walled; vesicles globose, walls thick, occasionally longer, bearing two series of fully packed phialides; conidia globose, spinulose with colouring substance, black; globose to subglobose sclerotia are produced .

v. *Aspergillus terreus* Thom. (Plate- III , Fig.- D)

Synonym: *Aspergillus boedijnii* Blochwitz.

Colonies attaining 3.5-5cm in 10 days, plain or with radial furrows, velvety, floccose in some strains, cinnamon-buff to wood brown; conidial heads long columnar, cinnamon brown;conidiophores smooth, colourless; vesicles hemispherical, dome like, phialides biseriate; metuale crowded, parallel; phialides closely packed; conidia globose to subglobose, smooth, relatively heavy walled hyaline cells globose to ovate or even truncate, produced singly or in small clusters on submerged vegetative mycelium, sclerotia like mass absent.

vi. ***Chaetomium globosum*** **Kunze** **(Plate- III, Fig.-E)**

Synonym: Chaetomium coarctatum **Sergeeva,**
Chaetomium olivaceum **Cooke and Ellis**

Colonies well growing, aerial mycelium pale brown, producing perithecia after 5 days, perithecia scattered or gregarious, globose to subglobose to broadly oval, olive green to grayish green, 160-390x 230-300µm, thickly clothed with hairs, terminal hairs abundant, light coloured, finely roughened, obscurely septate, lateral hairs light coloured, straight to slightly flexed or undulate, asci oblong, clavate, ascospores dark, lemon shaped, broadly ovoid, apiculate at both ends.

vii. ***Cladosporium cladosporoides*** **(Pers.) Link** **(Plate- III , Fig.-F)**

Colonies velvety, oliviaceous green to oliviaceous brown, with a greenish black reverse, *ca.* 2.5 cm diam when 5-day old and 5.5 cm diam when 10-day old and grown on potato dextrose agar (PDA) at a room temperature.

Conidiophores macronematous, semimacronematous, straight or flexuous, simple or branched, intercalary or terminal, smooth, sometimes verruculose, septate, up to 360 µm long and 3.3-4.1 µm wide, not geniculate nor nodose, apically truncate with 1-3 denticles, or sympodially denticulate, with scars at conidiogenous.

Conidia in heads of densely crowded, profusely branched chains, oblong, limoniform, ellipsoid or fusiform with truncate ends, light olive, 0-1 septate, smooth, 5.0-6.8 x 2.3-3.5 µm in the broadest part with prominent, protuberant, dark scar at each end.

viii. ***Curvularia lunata*** **Bat., J.A. Lima and and C.T. Vasconc.**

Synonym: ***Curvularia aeria*** **(Bat., J.A. Lima and and C.T. Vasconc.) Tsuda.**

Colonies on PDA dark gray, usually zonate; stroma regularly and abundantly formed in culture; mycelium branched, septate, conidiophores long, conidia elliptic, curved, septa 2-3, middle cells broad and darker than other cells, middle septum not median, smooth.

ix. ***Fusarium oxysporum*** **Schlecht.** **(Plate- III , Fig.-B)**

Colonies reaching 4.5 cm diameter in 4 days at 25^0C, aerial mycelium sparse to floccose, white or peach, but usually purple or violet tinge, conidiophores unbranched or sparsely branched, monophialidic, stroma white, smooth, effuse, microconidia usually abundant, mostly 0-septate, oval, ellipsoidal, kidney shaped or straight, 5-12 x2.5-3.5 µm; macroconidia 2-5 septate, spindle to fusiform, curved or almost straight, pointed at both ends, definitely or weakly pedicellate, 27-60x3-5 µm; chlamydospores mostly terminal, globose, smooth or roughened.

x. ***Fusarium roseum***

Synonym***: Gibberella zeae*** **(Schwein.) Petch**

Colonies growing very fast, reaching 9 cm diameter in four days, grayish rose to livid red crimson, often becoming vinaceous with a brown tinge, aerial mycelium floccose, somewhat lighter cfoloured and becoming brown, sporulation often scarce, conidiphores densely branched along with solitary phialides, conidia slender, falcate, moderately curved, with pointed curved apical cell, basal cell pedicillated, mostly septate, chlamydospores scarce, often completely absent, perithecia dark blue, tuberculate, ascospores 3-septate.

xi. ***Fusarium pallidoroseum***

Colonies growing very fast, aerial mycelium sparse to floccose, white, conidiophores unbranched or sparsely branched, monophialidic, stroma white, smooth, effuse, microconidia usually abundant.

xii. ***Myrotehcium verrucaria*** **(Alb. and Schwein.) Ditmar**

Synonym*: Gliocladium fimbriatum* Gilman and Abbott

Colonies reaching 4-5 cm diam on PDA at 25 ^{0}C after 14 days, conidiomata sporodochial or rarely synnematous, spore mass wet, black, convex, surrounded by white, floccose margin; mycelium absent ort floccose, white to rosy buff; sporulation diffuse or coalesced into pale olivaceous to black sporodochia with a white margin, hyphae hyaline , smooth, thin walled, rarely branched, septate, conidiophores branched repeatedly, usually forming 2-4 branches at each level, phialides 3-6 in a whorl, closely packed in a dense parallel layer, conidia broadly fusiform, one end pointed, other protruding and truncate.

xiii. ***Penicillium citrinum*** **Thom.** **(Plate- IV, Fig.- A)**

Synonym: ***Penicillium baradicum*** **Biourge.**

Colonies growing 25-30 mm in 7 days at 25^{0}C, sometimes smaller, radially sulcate, marginal areas ventunous, centrally floccose, mycelium white in peripheral areas, at the centres white to grayish orange or apricot, conidiation moderate, turquoise grey to greyish turquoise or glaucous sky blue, exudates clear, pale yellow to reddish brown, conidiophores borne from surface to subsurface hyphae, phialides in compact verticils, conidia spheroidal to subspheroidal, smooth or finely roughened, typically borne in long well defined columns, one per metula, arranged in a characteristic whorl on each conidia.

xiv. ***Rhizopus stolonifer*** **(Ehrenb.) Vuill.** **(Plate- IV, Fig.-C)**

Synonym: ***Mucor mucedo*** **L.**

Colonies growing profusely, white at first, turning brownish black, stolons spreading, internodes brown, with well branched rhizoides at each node; spornagiophores in clusters of 3-10 unbranced, white, becoming pale to dark at maturity; sporangia globose, hemispherical, granular, olivaceous, black; columella hemispherical, very often becoming pilate; spornagiophores irregular, round to oval, angular, straight.

xv. ***Trichoderma harzianum*** **Rafai.**
Synonym: ***Sporotrichum narcissi*** **Tochinai andShimada**

Colonies growing rapidly upto 9cm in 4 days, smooth surfaced, watery white, with sparse mycelia mat but soon developed aerial hypahe on their surface; conidiation predominantly effuse, appearing granular or powdery with formation of conidia, rapidly turning to yellowish green to dark green, producing tufts fringed by sterile white mycelium, hypahe septate, branched, smooth walled, hyaline, chlamydospores fairly abundant, intercalary or terminal on short branches, conidiophores hyaline, smooth walled, straight or flexuous, much branched, phialides in false verticils, conidia produce singly and successively, accumulate at the tip of each phialide and form into globose conidial head, subglobose or short obovoid, often with truncate base, pale green singly, darker in mass, 2.8-3.2x2.5-2.8 µm.

xvi. ***Trichoderma viride*** **Pers.** **(Plate- IV, Fig.- F)**
Synonym: ***Trichoderma lignorum*** **(Tode) Harz,**

Colonies growing rapidly upto 9 cm, watery white becoming hairy from the formation of loose scanty aerial mycelium, floccose, somewhat whitish; conidiation effuse or in compact tuffs, galucous to dark bluish green; reverse uncoloured, conidiophores much branched, arise in compact or loose tufts, main conidiophores 4-5 µm wide, producing smaller branches , ultimately a conifer- like branching system is formed, all the branches stand at wide angles to

the bearer, tip terminated by phialides, conidia globose or short obovoid, or broadl;y ellipsoidal, blueish green to dark green.

xvii. *Trichothecium roseum* (Pers.) Link (Plate- III, Fig.- C)

Colonies growing fast, reaching 9 cm diameter in ten days, pinkish, powdery due to conidial formation; reverse colourless to light pink; exudate lacking; conidiophores erect, produced singly or in groups, mostly simple, hyaline, septate, smooth, upto 2 mm long (base difficult to trace), 4-5 µm wide often with three septa in the lower part; conidia ellipsoidal to pyreform, pear shaped, with an obliquely prominent truncate basal scar, 2 celled, upper cell slightly larger, smooth, thick walled, hyaline.

4.3. Studies on Arbuscular Mycorrhizal fungi associated with cotton

For millennia, diverse microorganisms have yielded important biological materials useful to human beings such as antibiotics, drugs, enzymes, herbicides and growth promoters. Microbial diversity is the key to human survival and economic well being and provides a huge reservoir to resources which can utilize for our benefit. Diverse microorganisms are essential to a sustainable biosphere. Microbes are able to recycle nutrients, produce and consume gases that affect global climate, destroy pollutants, treat our wastes and they can be used for biological control of plant and animal pests (P. Jones Nirmalnath, 2010).

Soil microbiota plays a fundamental role for the productivity and stability of horticulture and agroecosystems (Castillo *et. al*., 2006 b, 2009). Within this microbiota AM fungi stand out because they are important for the phosphate (P) nutrition of the plants (Borie *et al.,* 2010; Castillo *et al.,* 2010). Among the soil beneficial microorganism, Arbuscular mycorrhizal fungi (AMF) are one of the most important components of the soil biota in natural and agricultural systems. They are obligate symbionts currently placed within the division Glomeromycota (Schüßler *et al*., 2001), that establish endomycorrhizal associations with up to 90% of plant families (Smith and Read, 1997). AMF are recognized as an important, widespread component of terrestrial ecosystems, benefiting plant establishment by enhancing plant nutrient acquisition, improving soil quality and increasing resistance to environmental stresses (Smith and Read, 1997), and also playing an important role in plant biodiversity, ecosystem variability and productivity (Wang and Zhao, 2008) .

The diversity of AMF species is measured mainly by identifying the characters of asexual spores, which are the fungal propagules that possess morphological characters that help to define species in this group of organisms (Morton *et al.,* 1995). Molecular techniques serve as an useful

tool for characterization and identification of AMF (Kowalchuk *et al.,* 2002). To the date, although only fewer than 200 species of AM fungi have been described (Redecker and Philipp, 2006), numerous studies on AM fungal diversity in different ecosystems worldwide have shown that AM fungi distribute globally (Treseder and Cross, 2006).

In order to study AM fungal diversity, representative soil samples were collected from various cotton fields of Vadodara, Bharuch and Jamnagar districts of Gujarat (Fig. 4).

The present study represents 4 genera and 28 species were observed in the soils associated with cotton in the area studied (Table 9).

In the present study maximum number of AM fungal spores was isolated from the rhizospheric soil of Kayavarohan area of Vadodara district and minimum number of spores was found in the soil of Padra agricultural fields (Table- 6). Three AM fungal genera namely *Acaulospora, Glomus* and Gigaspora were isolated from the soils of Vadodara district of which *Glomus fasciculatum* was found in maximum numbers, followed by G. *aggregatum* and *G. mossae. Acaulospora laevis* was recorded only from the soils of Kayavarohan and Muval village of Padra in the Vadodara District (Table-6, Fig. 5).

As depicted in table 7 and Fig. 6, from the field soils of Bharuch district, three AM fungal genera were isolated of which species of Glomus were found maximally. Fields of Karjan village showed highest number of AM fungal spores which belonged to the *Glomus* genera. *G. fasciculatum, G. fugienaum* and *G. melanosporum* were found in this area. Minimum AM fungal spores were observed in the Kavi area of Bharuch district. In the entire district survey, *Glomus mossae* was found frequently in the fields. In total 15 species of Glomus, 4 species of *Gigaspora* and 1 species of *Acalulospora* was found in Bharuch district. Acaluospora laevis was found only

in the soils of Asanvad village in the sites surveyed of this district. As depicted in table 8, from the field soils of Jamnagar district, species of only *Glomus* genera were found.

4.3.1. Morphological Characters of AM FSpores

Isolation studies showed presence of 3 genera in rhizospheric and 3 genera in non rhizosperic soil of different places. The details of various species are described below.

Acaulospora

Acaulospora Gerd. and Trappe *emend*. Berch, Mycotaxon 23: 409, 1985.
Kuklospora Oehl and Sieverd., J. Applied Bot. Food Quality 80: 74, 2006.

Azygospores produced singly in soil, large generally globose, or sub globose, with oily contents, borne laterally on the stalk of a large, terminal thin walled vesicle. Vesicles about the same size as the spores, with vesicle contents transferred to spore at maturity. Spore walls continuous except for a small occluded pore. Germ tube produced directly through walls near spore base. Forming endo mycorrhizae with lobed vesicles and arbuscules.

1. ***Acaulospora laevis*** **Gerd. and Trappe.** **(Plate- VIII , Fig.- B)**

Hyphae hyaline, thin walled, 6-8 µm broad, fertile hyphae terminate with the vesicles. Vesicles globose, up to 320 µm diam., 30-40 µm broad at the base, contents flown into the spore and collapsed as the spore matures. Spores formed singly and laterally on the hyphae just below the base of the vesicles, smooth, globose to subglobose, ellipsoid, occasionally reniform to irregular, initially dull yellow, turn deep yellowish-brown, red-brown to dark olive green at maturity, 119-300 x 119-520µm; spore wall three layered, continuous to the hyphae except for the occluded opening, outer wall 3-4 µm thick, rigid, yellowish-brown to reddish brown, inner layers hyaline, the inner most layer sometimes minutely roughened. In older spores, wall minutely perforated and the outer surface sloughing away. Spore contents globular to polygonal in appearance.

The family Gigasporaceae

Gigasporaceae J.B. Morton and Benny *emend.* Sieverd, Souza and Oehl, Mycotaxon 106: 328, 2008.

Gigasporaceae J.B. Morton and Benny, Mycotaxon 37: 471, 1990.

Gigaspora Gerd. and Trappe *emend*. C. Walker and F.E. Sanders Mycotaxon 27:179, 1986.

Sporocarps unknown. Spores formed singly in soil or rarely in roots. Spores formed on bulbous suspensor cell arising from subtending hyphae (sporophore). Spores with one wall consist of three layers: a unit, semi-persistent to persistent outer layer, a laminate middle layer and a thin inner germinal layer. The germinal layer has multiple, irregularly arranged germ pores. Most of the germ pores produce germ tubes, which penetrate the spore wall and branch profusely in the soil. Axillary cells round, spiny, with nodulous elevations. Form arbuscular mycorrhiza, vesicles unknown.

1. *Gigaspora ramisporophora* Spain, Sieverd. and N.C. Schenck. (Plate- VIII , Fig.-D)

Hyphae sub hyaline to pale yellow, up to 10µm broad, subtending hyphae septate, simple, branched, with 1-3 suspensor cells, light brown, 9-14 µm broad, wall up to 3 µm thick. Spores produced singly on the apex of bulbous suspensor cells, predominantly globose, often subglobose, smooth, golden yellow to yellowish brown, 150-400 x 200-450 µm. Spore wall three layered, 9-20 µm thick. Outer layer hyaline to subhyaline, brittle, up to 4 µm thick, continuous with outer layer of suspensor cell and usually adherent to middle layer. Middle layer yellow to yellowish brown, 4-25 µm thick, adherent to inner layer; inner layer yellow to yellowish brown, up to 3 µm thick. Suspensor cells globose to ovate, 60-80 x 40-60 µm in diam., usually with three walls, 6-10 µm thick; outer and middle walls hyaline; innermost wall brown. Auxiliary cells round to clavate.

2. *Gigaspora albida* Scenck and Smith (Plate- VIII , Fig.- C)

Spores globose to subglobose, 200 – 280 µm in size, Cream with pale green tint in colour, spore wall consists of three layers, the first two layers adherent and of equal thickness, hyaline to pale yellow in colour.

3. *Gigaspora candida* Bhattacharjee, Mukherjee (Plate- VIII , Fig.-E)

Azygospores found singly in soil, white, globose, 200-300 µm diameter, spore wall smooth, 2-layered, the two layers distinctly visisble in fractured azygospores, suspensor like cell attached to the azygospore, white globose to subglobose, usually detached during wet sieving. Soil borne vesicles not isolated.

The genus *Glomus*

Glomus Tulasne and Tulasne *emend*. C. Walker and Schüßler

Spores glomoid, produced terminally on undifferentiated, non-gamitangial hyphae, solitary, in clusters or produced in Sporocarps. Peridium complete or imcomplete. Spore contents at maturity separated from attached hyphae by a septum or occluded by spore wall thickening.

1. *Glomus aggregatum* N.C. Schenck and Smith *emend*. Koske. (Plate- V , Fig.-A)

Hyphae hyaline to subhyaline, up to 8 µm broad, 8-12 µm wide at the point of attachment of the spore. Spores formed in loose clusters or in Sporocarps without peridium; Sporocarps hyaline to light yellow with a greenish tint, becoming yellow with age. 660-1500 x 330-1000 µm. Spores globose, subglobose, obovate, cylindrical to irregular, hyaline to yellow, 73-105 x 60-85 µm in diam; wall yellow to yellowish brown, 1-3 µm thick, outer walls slightly thicker and lighter than the inner wall; walls separable with slight pressure and most apparent in stained preparations.

2. *Glomus formosanum* Wu and Chen. (Plate- VIII , Fig.-F)

Hyphae pale yellow to honey yellow, up to 9 µm broad; subtending hyphae 1-4 in numbers, 7-18 µm broad, with opening at the attachment, occluded by spore wall thickening, two nearby attached hyphae fused together or closely separated at the attachment. Spores produced in Sporocarps or in aggregates without peridium. Sporocarps yellowish brown to reddish brown, globose, subglobose to irregular, 360-500 x 450-500 µm, peridium composed of septate, thin walled, loosely interwoven hyphae, up to 10 µm broad. Spores globose to sub globose, 82-125 x 95-135 µm diam., yellowish brown to reddish brown; Spore wall single, yellowish brown to reddish brown, 5- 12 µm thick, thickest at attachment, up to 20 µm broad, surface smooth.

3. *Glomus glomerulatum* Sieverd., (Plate- V, Fig.- D)

Hyphae up to 6 µm broad. Sporocarps dark brown, without peridium, become compact with age, globose, subglobose, rectangular, flattened or some times irregular in shape, surface knobby, 330 x 460 µm diam., formed by the interwoven hyaline hyphae, 2-6 µm in diam., walls up to 0.5 µm thick. Spores in the sporocarps are clustered in the mycelium and embedded in an unordered gleba, globose to subglobose, 40-70 µm diam., yellow to brown; wall consists of two walls in one group; yellow to brown, laminated and 4-9 µm thick, the spore surface smooth; second wall hyaline, membranous, up to 0.5 µm thick and adherent to first wall. Chlamydospores formed in sporocarps, have two hyphal attachment at irregular distance along the hypha; hyphal attachments yellow to brown, 5-7 µm broad, straight to recurved; hyphal attachment pore 1-2 µm in diam. and are closed by the second wall or by a septum.

4. *Glomus macrocarpum* Tulasne and Tulasne. (Plate- V, Fig.- F)

Hyphae yellow to light brown, up to 12 μm broad, subtending hyphae up to 15 μm broad. Sporocarps globose, subglobose, elongate to irregular, 10x10 mm in diam. Spores sub globose, globose to irregular, 70-150 x 75-120 μm in diam.; spore wall composed of two distinct layers: outer layer thin, hyaline, 1-2 μm thick in water or glycerol mount, swell enormously in lactic acid mount. Inner wall layer yellow, 6-12 μm thick, laminated, rarely seen as two layers, swell slightly in lactic acid. Spores taper towards the attachment, attachment hyphae single and persistent, wall thickening continues into the subtending hyphae, subtending hyphae up to 90 μm long from the spore proper. The pore closed by a thinner septum.

5. *Glomus rubiformis* Gerd. and Trappe. (**Plate- VI, Fig.-F)**

Hyphae hyaline to light yellow, up to 9 μm broad. Sporocarps dark brown, subglobose to ellipsoid, 150-175 x 190-410 μm, consisting of a single layer of chlamydospores surrounding a central plexus of hyphae. Peridium absent, individual spores at times partially enclosed in a thin network of tightly appressed hyphae. Chlamydospores dark brown, obovoid, ellipsoid to subglobose, 30-40 x 80-100 μm, with a small pore opening into the thick walled subtending hyphae. Spore wall laminate, 3-8 μm thick, up to 12 μm thick at spore base, often perforated projections on the inner surface. A variable stalk-like projection produced near the base of some spores. Hyphal attachment simple, thickening of wall extended along subtending hyphae. Pore occluded at maturity.

6. *Glomus fasciculatum* (Thaxt.) Gerd and Trappe (**Plate- V , Fig.- C)**

Zygospores found in loosely coherent spongy mass, pale yellow to pale yellow-brown in colour, globose to subglobose. 60-110 μm in diameter. Spore wall consisting of three layers, subtending hypae is cylindrical to slightly flared.

7. *Glomus intraradices* N.C.Schenek andG.S. Smith (Plate- VIII , Fig.- A)

Spores pale cream to yellowish brown sometimes with green tint, globose to subglobose with some elliptical spores. Spore size ranges from 40-400 µm. Spore wall consists of 3 layers, in juvenile spores, initial sublayer is thin and become thick due to formation of additional sublayers. Thickness of the wall layers varies from 3.2-12 µm in mature spores, subtneding hyphae is cylindrical, occasionally slightly constricted.

8. *Glomus mosseae* (T.H. Nicolson and Gerd.) Gerd. and Trappe (Plate- V, Fig.-B)
Syn: *Funneliformis mosseae*

Spores are surrounded by tight peridium to form sporocarps, spores found singly in soil, spores straw to dark orange borwn in colour, majority are yellow- brown, globose to subglobose in shape, 100-260 µm in diameter, spore wall three layered, subtending hyphae funnel shaped with 14-32 µm thickness.

9. *Glomus hoi* Berch and Trappe

Spores borne singly in soil, globose, subglobose, ellipsoidal or irregular, (50)-80-120 (-155) µm, yellow- brown. Wall of spore composed of two distinct, seperable layers,subtending hypahe cylindrical or slightly flared toward the point of attachment to the spore where it is (5-)8-11(-13) µm wide.

10. *Glomus geosporum* (Nicol.and Gerd.) Walker

Spores borne singly in soil, yellow- orange, globose to subglobose (120-)175(-260) µm diameter, sometimes ovoid, with single subtending hypahe.

11. *Glomus etunicatum* Becker and Gerd.

Spores borne singly, pale yellow to yellow, globose to subglobose, (75-)95(-135) µm diam; occasionally ovoid, with one subtending hypha.

12. *Glomus caledonium* (Nicol. and Gerd.) Trappe and Gerdemann

Spores single in the soil; pale yellow to golden yellow; globose to subglobose; (90-)224(-370) µm diam; with a single subtending hypha. Spore wall consist of three layers.

Table 6: Isolation of AM Spores from the Rhizospheric and Non Rhizospheric soil of different cotton fields of Vadodara district.

Location	Sample	No. of AM Spores/ 100g soil		Major AM fungi isolated
		Rhizospheric soil	Non Rhizospheric Soil	
Por	1	135	53	*G. aggregatum, G. mossae, G. fasciculatum*
	2	147	68	
	3	180	60	
Kayavarohan	1	243	98	*G. aggregatum, G. intraradices, Acoulospora laevis, G. fasciculatum*
	2	197	78	
	3	210	89	
Kunvarpura	1	159	71	*G.maculosum,G.fugienaum, Gigaspora* albida
	2	130	65	
	3	165	83	
Dabhoi	1	180	88	*G.melanosporum,G.fugienaum, G.fasciculatum*
	2	172	79	
	3	183	85	
Varnama	1	192	102	*G.aggregatum, G.mosseae, G.geosporum*
	2	189	99	
	3	183	89	
Sundarpura	1	218	119	*G. geosporum, G.convolutum, G. fasciculatum, G. aggreagatum, G.etunicatum*
	2	201	109	
	3	189	99	
Padra	1	102	67	*G.glomerulatum, G.clarum, G.fuegianum,, G.hoi*
	2	130	78	
	3	172	85	
Dabhasa	1	210	98	*G. fecundisporum, G. mossae, G. melanosporum, G. etunnicatum, G. intraradices, G. microcarpa*
	2	198	93	
	3	190	81	
Muval	1	155	79	*G. fasciculatum, G. tenerum, Acaluospora laevis*
	2	168	86	
	3	184	92	
Dabka	1	166	72	*G.fasciculatum, G.mosseae*
	2	172	79	
	3	181	82	

** Data based on average of three samples*

Table 7: Isolation of AM Spores from Rhizospheric and Non Rhizospheric soil of different cotton fields of Bharuch district.

Locations	Sample	No. of AM Spores/ 100g soil		Major AM Fungi isolated
		Rhizospheric soil	Non Rhizospheric Soil	
Jambusar	1	102	86	*G. clarum, Gigaspora ramisporophora, G.etunicatum*
	2	91	74	
	3	88	69	
Kavi	1	82	49	*G. mossae, Gigspora sp., G. microcarpa*
	2	96	45	
	3	76	52	
Umber	1	102	67	*G. aggregatum, G. rubiformis*
	2	93	71	
	3	110	82	
Simor	1	97	58	*G. fasciculatum, G. tenerum, G. segmentum*
	2	106	62	
	3	103	77	
Andada	1	84	59	*G. mossae, Gigaspora candida*
	2	93	57	
	3	101	69	
Matar	1	91	53	*G. fasciculatum, G. glomerulatum*
	2	104	61	
	3	94	50	
Karjan	1	180	88	*G.melanosporum,G.fugienaum, G.fasciculatum*
	2	172	79	
	3	183	85	
Anastu	1	113	90	*G. aggregatum, Gigaspora albida, G. tenerum*
	2	129	72	
	3	120	69	
Asanvad	1	145	60	*G. melanosporum, G. etunnicatum, G. intraradices, Acaulospora laevis*
	2	122	53	
	3	133	57	
Chhapra	1	107	59	*G. aggregatum*
	2	104	85	
	3	103	77	
Haripura	1	89	31	*G. mossae*
	2	97	64	
	3	98	58	
Intola	1	92	40	*Gigaspora albida, Glomus*

	2	102	89	*clarum*
	3	76	56	

** Data based on average of three samples*

Table 8: Isolation of AM Spores from the Rhizospheric and Non Rhizospheric soil of different cotton fields of Jamnagar district

Location	Sample	No. of AM Spores/ 100g soil		Major AM Fungi isolated
		Rhizospheric soil	Non Rhizospheric Soil	
Chella	1	71	47	*G. mossae, G. intraradies, G. aggregatum*
	2	62	39	
	3	58	51	
Taraghadi	1	140	96	*G. fasciculatum, G. caledonium G. aggregatum,*
	2	43	23	
	3	58	27	
Falla	1	102	89	*G. intraradices, Acaulospora laevis, G. fasciculatum, G. glomerulatum*
	2	118	92	
	3	103	86	
Dhunvav	1	97	64	*G. melanosporum G. etunnicatum*
	2	71	45	
	3	83	56	
Gaduka	1	112	87	*G. fecundisporum, G. mossae, G. melanosporum, G. etunnicatum, G. intraradices,*
	2	105	79	
	3	127	100	
Hadmatiya	1	111	90	*G. fasciculatum, G. aggregatum ,G. microcarpa*
	2	97	60	
	3	91	54	
Lonthiya	1	100	77	*G. etunnicatum G. fasciculatum, G. aggregatum*
	2	108	86	
	3	127	103	
Sapara	1	113	98	*G. microcarpa, G. mossae, G. melanosporum*
	2	121	102	
	3	98	79	
Umrali	1	98	58	*G. aggregatum, G. rubiformis G. fasciculatum, G. aggregatum*
	2	111	89	
	3	108	95	

** Data based on average of three samples*

Table 9: Characteristics of AM spores based on different morphological features

Sr. No.	Name of AM Fungi	Colour of the Spore	Size (µm)	Wall layers	Thickness of wall /Hyphae (µm)
1	*Glomus agrregatum* (Schenck Smith) emend. Koske	Yellow-Brown	131.2	2	12.8 - W --- - H
2	*Glomus mosseae* Nicol.& Gerd.	Yellow- Brown	127.3	2	9.6 – W 15.2 – H
3	*G. fasciculatum* (Thaxter) Gerde.& Trappe emend. Walker & Koske	Yellow-Brown	92.8	2	9.6 – W 9.99 - H
4	*Glomus glomerulatum* Sieverding	Yellow-Brown	64	2	6.4 – W --- - H
5	*G. hoi* Berch. & Trappe	Yellow- Brown	92.8	3	12.8 –W --- - H
6	*G. macrocarpum* (Tul. & Tul.) Berch & Fortin	Hyaline- Brown	121.6	2	9.6 –W --- - H
7	*G. geosporum* (Nicol. & Gerd.) walker	Yellow-Brown	123.2	2	9.6 – W --- - H
8	*G. etunicatum* Becker & Gerd.	Yellow	83.2	2	6.4 –W --- - H
9	*G. claroides* Schenck& Smith	Yellow-Brown	76.8	2	12.8 – W --- - H
10	*G. fuegianum* (Spegazzii)	Yellow- Brown	66.6	3	9.99 – W --- - H
11	*Glomus microcarpa* Iqbal & Bushra	Yellow - Brown	112	1	12.8 –W --- - H
12	*Glomus rubiformis* Gerdemann & Trappe	Brown	169.6	1	6 – W --- - H
13	*Glomus convolutum* Gerde.& Trappe.	Yellow to brown	83.2	2	9.6 – W --- - H
14	*Glomus monosporum* Gerdemann & Trappe	Yellow to brown	169.7	2	12.1 – W --- - H
15	*G. caledonium* (Nicol. & Gerd) Trappe & Gerdemann	Brown	139.3	2	9.0 – W --- - H
16	*G. segmentum* Trappe, Spooner and Ivory	Yellow	80	2	6.4 – W --- H
17	*G.tenerum* (Tandy) Mcgee	Orange	109.1	2	9.0 – W

					--- - H
18	*G. formosanum* Wu & Chen	Yellow to brown	73.6	2	6.4 – W --- - H
19	*G. intraradies* Schenek & Smith	Yellow to Brown	109.1	2	9.0 –W --- - H
20	*Acaulospora laevis* Gerde & Trappe	Hyaline to yellow	102.4	2	9.6 – W --- - H
21	*Gigaspora albida* Scenck & Smith	Yellow to brown	92.8	2	6.6 – W --- - H
22	*Gigaspora albida* Scenck & Smith	Brown	262.4	2	16 – W 12.8 - H
23	*Gigaspora ramisporophora* Spain, Siverding & Schenck	Brown	284.8	2	12.8 – W 12.8 - H
24	*Gigaspora candida* Bhattacharjee,Mukherji, Tiwari & Skoropad	Brown	204.8	2	16 – W 16 - H
25	*Glomus fecundisporum* Schenck & Smith	Hyaline-Yellow	92.8	2	9.6 – W --- -H
26	*G. claroides* Schenck& Smith	Yellow-Brown	70.4	2	6.4 – W --- -H
27	*G. melanosporum* Gerde. & Nicolson	Yellow-Brown	166.5	2	13.32 –W --- -H
28.	*Glomus fecundisporum* Schenck & Smith	Yellow-Brown	83.2	2	6.4 –W --- - H

W: Width , H- Hyphae , --- : Absent

4.4. Effect of Arbscular Mycorrhizal Fungi on Growth of Cotton Varieties

The importance of mycorrhizal fungi in sustainable agriculture is based on their role as a link between plant and soil (Bethlenfalvay 1992). The symbiotic fungi that predominate in the roots of agricultural, native and weed plants are of the Vesicular-arbuscular mycorrhizal type, recently.

AM fungi have gained much importance in the field of agriculture as this play an important role in the capture of nutrients from the soil of all ecosystems. AM has beneficial role in providing soil nutrients for the host-plantpartner, mycorrhizal fungi are an important, and much overlooked, contributor to this process. AMF are natural plant growth regulators and stimulants (Wood and Cummings 1992). The main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and the supply of these nutrients to the host roots (Javot *et al.,* 2007; Sundar *et al.,* 2010). The AM symbiosis confers resistance to the plant against abiotic stresses such as drought, salinity, metal toxicity and environmental stresses.

AM fungal colonization of plant roots has also been suggested to increase plants tolerance to pathogens thereby acting as a biocontrol agent (Azcon-Aguilar and Barea, 1996; Chhabra *et al.,* 1992).

AMF mycelium in soil results in greater efficiency of nutrient absorption particularly for slowly diffusing mineral ions, especially phosphorous as observed by Smith *et al.,* (2003). In addition to phosphorous, AMF mycelium also enhances the uptake of nitrogen in the form of NO_3 (Frey and Schuepp, 1993; Morte *et al.,* 2001) and also increase the potassium content in plants (Azcon and Barea, 1992; Maksoud *et al.,* 1994). AM fungi helps in water regulation of plants by extending their hyphae towards the available moisture zone for continuous water

absorption and translocating them to plants. AM association can affect the host plants in terms of stomatal movement and photosynthesis of leaves and has been shown to increase the rate of transpiration, photosynthesis and chlorophyll content (Panwar, 1991; Bethlenfalvay *et al*., 1992).

Cotton is a monotrophic plant in which growth and nutrient uptake is usually increased by mycorrhizal colonization (Belgard and Williams, 2002; Nehl *et al.,* 2004). Generally, AMF show little specificity and the factors that determine mycorrhization appear to depend on the genotype of the host plant (Damodaran *et al.,* 2012; Koide and Schreiner 1992; Klironomos 2002). Host preference may be under the genetic control of the host, the fungus or a complex of interactive effect of both symbiotic partners with edaphic factors (Sylvia and Chellemi 2001). Host dependant sporulation among common lawn plants is also well demonstrated (Bever *et al.,* 1996). Evidence for this is provided by the existence of non-host plant species (Giovannetti and Sbrana 1998) and Myc- mutant *Pisum sativum* plants unable to form AM symbiosis (Gollotte *et al.,* 1993). Mycorrhizal colonization patterns in inbred lines of Z*ea mays* selected for resistance to fungal pathogens indicate that they had significantly low levels of mycorrhizal colonization and larger root systems (Toth *et al.,* 1990).

Earlier studies on vesicular-arbuscular mycorrhiza (VAM) reported the beneficial effect of inoculation on plant growth in sterilized soil with low available phosphorus (Gerdemann, 1964; Mosse and Haymann, 1976). Since most of the natural soils usually harbour AM it was felt that plants might not respond to mycorrhizal inoculation in unsterile soils. But later investigations indicated that even in unsterile soils plants do respond to inoculation with efficient strains of AM (Mosse and Hayman, 1976; Khan, 1974). Rich and Bird (1974) reported that early-season root and shoot growth of cotton was increased in the presence of mycorrhizal fungi and that these plants flowered and matured bolls earlier. Zak *et al.,* (1998) suggested that the

fungus forms a hyphal network in the soil that can serve as an extension of the plant root system. Thus a seedling that is colonized early can explore a much greater soil volume than is possible with an uncolonized newly developing root system. Inorganic ions such as Phosphorus (P) and Zinc (Zn) are absorbed by the fungus and transferred to the plant (Kumar *et al.,* 2001; Qureshi *et al.,* 2012). This improvement of P nutrition is a critical factor in soils with low P content. In turn, this can lead to reduced fertilizer requirements and more efficient use of soil nutrients (Marschner and Dell, 1994). Such seedlings are likely to be more persistent in adverse conditions than non mycorrhizal associated seedlings counterparts.

The growth of cotton plant will be the better, when the seeds will be grown in the presence of AM along with bioinoculants (Vazquez *et al.,* 2000). This is because seeds with AM and bioinoculant have better adaptability to critical sites since they have better tolerance to harsh conditions. In mycorrhizal associations, the hyphae of the fungal species invade plant roots and form arbuscles, which facilitate ready exchange of nutrients between the host and the fungus, resulting in the association known as AM (Arbuscular Mycorrhizae) (Linderman, 1998). This association may be parasitic, benign or beneficial (Siqueira, 1986), but it is commonly mutualistic with the fungus receiving energy from the plant. The plant in turn, may receive several benefits from the association (Sutton, 1973).

VAM fungi significantly increase the net photosynthesis by increasing total chlorophyll and carotenoid contents ultimately increasing carbohydrate accumulation. The VAM fungi have also increased stomatal resistance, thereby reducing the rate of transpiration (Mathur and Vyas, 1995). Shrestha *et al.,* (1995) have shown that photosynthesis and transpiration rates of mycorrhizal *Satsuma mandarian* trees are higher than non-mycorrhizal trees. Mycorrhizal turf, creeping bent grass has maintained significantly higher chlorophyll concentration than non-

mycorrhizal turf during the drought period. Wright *et al.* (1998) also showed that mycorrhizal *Trifolium repens* L. exhibited a higher specific leaf area and increased rate of photosynthesis compared with nonmycorrhizal plants.

Mycorrhizal plants very often show higher rate of photosynthesis than non mycorrhizal plants Huixing Song (2005). Arbuscular mycorrhizal symbiosis increased the rate of photosynthesis, and so as to increase the rates of photosynthetic storage and export at the same time . It has been proved that the amount of chlorophyll in mycorrhizal plants was higher than non mycorrhizal plants Gemma *et al.*, (1997); Davies *et al.*, (1993); Mathur and Vyas (1995) and higher concentration of chlorophyll is associated with higher photosynthesis rate Davies *et al.*, (1993).

AM pure cultures are single spore cultures isolated from rhizosphere soil of cotton surveyed cotton fields surrounding Vadodara district. These pure *Glomus* cultures are always maintained in active stage by sowing Maize seeds at regular time intervals. A thin layer of the mycorrhizal consortium of infected maize roots and rhizosphere soil of pot was mixed in top soil upto 2 cm.

The present study reflects on effect of AM fungi on the growth of cotton plant seedlings The experiment was conducted in Arboretum of Department of Botany in campus premises of The Maharaja Sayajirao University of Baroda. Two different hybrid varieties (Ajeet-11, Vikram-5) of cotton seeds were procured and sown in plots of 2x2 m size containing nursery soil under natural condition without providing any chemical or biological fertilizers. These plants were routinely watered and all the routine nursery precautions were taken.

Till 90 days the seedlings were carefully extricated and different growth parameters were recorded. Philips and Hayman (1970) procedure was employed for clearing and staining the

roots. Percentage of infection was calculated by the formula of Gioventti and Mosse (1980). Among all the three variety of seedlings treatment of AM with Vikram-5 showed maximum shoot length and fresh weight and its 90% roots had AM colonization. The results are presented in table-10.

Measurement of growth parameters:

Growth measurement of seedlings of cotton varieties were recorded after every 15 days. The length of the shoot was adjusted by taking the physical count of the length of the shoot from colour region to apical bud. The length of the root was adjusted by taking the physical count of the length of root from collar region to the tip of the tap root. The fresh weight of shoot increased to almost double in treatment with AM fungi. Root colonization was 68% in non Bt cotton after 90 days while in inoculated saplings it became 87%. This increase was more in Vikaram-5 (90%) and Ajeet-11 (88%). Thus we can conclude that inoculation of AM fungi will increase the shoot biomass and thus yield of cotton.

It is evident from table -11 that bacterial count was 131 x 10^4 after 90 days and fungal cfu was 14 x 10^4 which increased to 160 x 10^4 and 20 x 10^4 after 90 days in non Bt+ AM treatment. Maximum fungal colonies were recorded in rhizosphereic soil of Vikram-5 + AM fungi.

Table10: Growth responses on 3 varieties of *G. herbaceum* by AM fungi in Plot of 2x2 m

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves	Total Chlrophyl Content (mg/g)	Root colonization (%)
		Shoot	Root	Shoot	Root					
Non Bt (Control)	15	0.71	0.038	0.10	0.018	12.1	3.23	4	0.30	0
	30	1.195	0.114	0.162	0.020	16.2	6.1	6	0.61	0
	45	6.732	0.874	1.703	0.201	19.33	7.0	9	0.91	20
	60	8.964	1.165	2.271	0.269	25.77	8.6	11	1.12	38
	75	11.205	1.456	2.839	0.336	32.21	11.0	14	1.53	56
	90	13.447	1.748	3.407	0.404	38.66	13.0	17	1.83	68
Non Bt+ AM	15	0.72	0.122	0.018	0.019	13.2	4.19	4	0.40	0
	30	1.2	0.24	0.025	0.025	19.6	7.0	6	0.62	0
	45	18.90	1.95	10.5	0.820	27.40	13.0	8	1.0	36
	60	26	2.59	14	1.109	36.54	18.32	11	1.39	58
	75	31.80	3.19	17.21	1.39	45.80	22.11	15	1.75	79
	90	37.6	3.72	20.82	1.66	54.62	28.0	19	2.10	87
Ajeet- 11	15	0.77	0.082	0.11	0.027	12.0	3.0	4	0.31	0
	30	1.29	0.145	0.133	0.022	18.3	6.4	6	0.72	0
	45	9.985	1.254	2.135	0.366	21.0	8.5	9	0.93	26
	60	13.314	1.672	2.874	0.488	28.0	11.33	12	1.24	44
	75	16.642	2.090	3.559	0.610	35.00	14.166	15	1.55	64
	90	19.971	2.509	4.271	0.733	42.0	17.0	17	1.87	76
Ajeet-11 + AM	15	1.271	0.123	0.17	0.029	14.0	4.16	5	0.36	0
	30	1.49	0.23	0.183	0.032	19.2	7.0	7	0.62	0
	45	18.810	1.821	4.525	0.553	25.16	10.0	10	1.08	30
	60	25.080	2.482	6.03	0.738	33.55	13.33	14	1.44	50
	75	31.350	3.035	7.541	0.922	41.941	16.66	17	1.81	72
	90	37.621	3.642	9.05	1.107	50.33	20.0	21	2.17	88
Vikram-5	15	0.610	0.06	0.072	0.014	10.3	3.0	3	0.35	0
	30	1.31	0.081	0.165	0.015	19.3	5.93	6	0.70	0
	45	17.585	1.683	5.626	0.511	22.16	9.0	10	1.05	28
	60	23.447	2.244	7.501	0.681	29.55	12.0	13	1.40	40
	75	29.309	2.805	9.376	0.851	36.94	15.0	16	1.75	54
	90	35.171	3.366	11.252	1.022	44.33	18.0	19	2.10	70
Vikram-5 + AM	15	1.065	0.153	0.095	0.018	13.4	4.15	4	0.47	0
	30	1.35	0.160	0.100	0.025	19.9	7.1	6	0.95	0
	45	34.671	2.489	10.41	0.832	27.33	15.16	11	1.43	34
	60	46.22	3.318	13.88	1.109	36.44	20.22	14	1.91	56
	75	57.785	4.418	17.350	1.368	46.0	25.27	17	2.38	76
	90	69.342	4.978	20.82	1.664	54.66	30.33	20	2.87	90

Table 11: Effect of AM fungi on the rhizospheric microorganisms on three varieties of *G. herbaceum*

Variety	No. of Days	Bacteria CFU/g soil	Fungi CFU/g soil
Non Bt (Control)	15	24.5×10^4	2×10^4
	30	53.5×10^4	3×10^4
	45	89.5×10^4	5×10^4
	60	115×10^4	8×10^4
	75	125×10^4	10×10^4
	90	131×10^4	14×10^4
Non BT + AM	15	44.5×10^4	6×10^4
	30	114.5×10^4	10×10^4
	45	132.5×10^4	13×10^4
	60	149.5×10^4	16×10^4
	75	155×10^4	18×10^4
	90	160×10^4	20×10^4
Ajeet-11 (Control)	15	20×10^4	1×10^4
	30	28×10^4	3×10^4
	45	34×10^4	4×10^4
	60	36.5×10^4	6×10^4
	75	38×10^4	8×10^4
	90	40×10^4	11×10^4
Ajeet-11 + AM	15	39×10^4	3×10^4
	30	49×10^4	10×10^4
	45	110×10^4	14×10^4
	60	132×10^4	17×10^4
	75	206×10^4	20×10^4
	90	249×10^4	22×10^4
Vikram-5 (Control)	15	25×10^4	2×10^4
	30	30×10^4	4×10^4
	45	33×10^4	5×10^4
	60	38×10^4	7×10^4
	75	41×10^4	11×10^4
	90	47×10^4	13×10^4
Vikram-5 + AM	15	44×10^4	6×10^4
	30	53×10^4	11×10^4
	45	129×10^4	19×10^4
	60	186×10^4	22×10^4
	75	234×10^4	25×10^4

	90	261 x10^4	27 x10^4

Mycorrhizal colonization, height of the plant, fresh and dry weight of the plant was studied and data are presented inTable-10.

Vikram-5 seedling with *Glomus* sp. colonization is 90%, showed maximum shoot length 54.66 cm, highest root length 30.33 cm, highest dry weight of shoot i.e. 20.82 g and highest dry weight of root i.e. 1.66 g is recorded. The effect of inoculations of AM pure culture on plant height, root length and chlorophyll content of cotton was studied and data are presented in Table-10.

In single combination of AM+ hybrid seedling the data clearly indicated that Vikram -5 seedling which was provided pure culture of *Glomus* sp. showed maximum chlorophyll content of 2.87 mg/g compare to 2.10 mg/g for Vikram -5 without AM.

In non Bt seedling after 90 days of growth the colonization in combination of Non Bt + AM is 79% compare to only 56 % colonization in control set of plants.

There is a vast difference recorded in fresh and dry weight of shoot and root of non Bt seedlings. Shoot length after 90 days in AM inoculated plants is 45.80 cm compare to 38.66 cm in control sets.

Similarly length of root is 28.0 cm higher as compare to 13 cm in control (non Bt) seedlings. Even in Ajeet -11 + AM combination colonization is 88% with plant shoot length 50.3 cm, root length is 20 cm, shoot fresh weight is 37.62 g, root fresh weight is 3.6, shoot dry weight is 9.05 g, root dry weight is 1.1 g, chlorophyll content is 2.10 (mg/g) is recorded. The readings are higher compare to Ajeet -11 seedling growth without any Glomus association.

Non mycorrhizal plants showed significantly less biomass than the mycorrhizal plants. Roots inoculated with AM fungi showed maximum colonization. Differences in host genome can

control the degree of mycorrhizal colonization as per (Toth *et al.,* 1990) and bean (Sutton 1973) on maize.

The growth of cotton plant will be the better, when the seeds will be grown in the presence of AM along with bioinoculants (Vazquez *et al.,* 2000). This is because seeds with AM and bioinoculant have better adaptability to critical sites since they have better tolerance to harsh conditions. The plants inoculated with AM fungi showed increase in the chlorophyll content in comparison to the control variety. Plants inoculated with AM fungi brought about significant changes in chlorophyll a, b and total chlorophyll content. As reported by Gemma *et al.,* (1997) mycorrhizal *Agrostis plastids* had higher chlorophyll concentration compared with non mycorrhizal.

The increase in root length and root biomass directly indicates improvement of the health of the plant. AM inoculated plants were having better root and shoot growth. Plant dry weight is influenced by AMF as a result of enhanced efficiency of resource acquisition by mycorrhizal plants. The data in Table.10 clearly indicate that among all cotton seed varieties with AM inoculated hybrid grown best in all parameters. Similar results were reported by Damodaran *et al.,* 2012) for certain Indian cotton cultivars.

4.5. Studies on *in vivo* effect of three fungi on growth performance of cotton

Cotton is the third largest important economic crop in India produced for cloth and other kind of things of human need serving many other important uses (Hutchinson *et al.,* 1947). Cotton is the fast turning out to be a cash-crop in this region. So it is thought to find out growth promoter from rhizosphere. Studies on the same line have been carried out by Hande (2000) on *Cajanus cajan* and Subhedar *et. al.,* (2006) successfully. Recently, efficient and exploitive agriculture throughout the world is practiced at great cost to the environment. After decades of warning, the inappropriate usage of pesticides has led to development of more than 500 resistant pathogens (Georghiou, 1990).

Seed germination and seedling establishment are determined by several factors including quality of seeds and environmental factors. Within the environment of the seed and seedling are physical, chemical and biological factors that influence growth (Okoth *et. al.,* 2011). The rhizosphere, is relatively rich in nutrients, because as much as 40% of plant photosynthetic products are exudates from roots (Bais *et al*., 2006). Consequently the rhizosphere supports large microbial populations capable of exerting beneficial, neutral, or detrimental effects on plant growth.

Some bacteria and fungi prevent diseases and enhance plant growth. Beneficial microbes associate with plants in several ways. Some may inhabit the rhizosphere, taking advantage of root exudates; others may live on root or leaf surfaces and some may colonize intracellular spaces and vascular tissues inside the plant (Preston, 2004). Plant-associated microbial diversity encompasses symbionts, protecting their host against various aggressions and to determine the ecological success of plants. They drastically modify plant communities and to improve the

inventory of diversity and functions of *in situ* plant-associated microorganisms (Selosse *et al.*, 2004).

Plant-associated microorganisms fulfil important functions for plant growth and health (Gabriele, 2009) such as enhancement of plant growth and protection of plants from various plant pathogens in several crops such as cucumber, radish, tomato, sugar cane, and rice as reported by Viswanathan and Samiyappan (1999), Ongena *et al.* (2000) and Ramamoorthy *et al.* (2001).

Many resistance-inducing fungi and bacteria increase both shoot and root growth, some non-pathogenic root-colonizing fungi also have similar effect (Harman *et al.*, 2004**).** The increased growth response induced by *Trichoderma* sp. has been reported for many crops such as beans (*Phaseolus vulgaris*) cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), carnation (*Dianthus carophyllus*), maize (*Zea mays*), and wheat (*Tritichum aestivum*) (Lo and Lin, 2002).

The effectiveness of the use of microorganisms as biofertilizers and biocontrols however, is determined by a myriad of factors including virulence of the isolate, environmental factors, time of application, ability to survive in the environments other than their origin and colonize plants roots during certain period of time to control plant pathogens (Kredrics *et al*., 2003; Nemec *et al*., 1996; Stephan *et al*., 2005; Vinale *et al*., 2008) suggesting that augmenting of a local virulent strain would be more successful.

Fungi are ubiquitous; some having beneficial effects on plants, while others may be detrimental (Anderson and Cairney, 2004; Ipsilantis and Sylvia, 2007). Chemical fungicides however, have a negative effect on human health and on the environment (Voorrips *et al*., 2004; Soytong *et al*., 2005; Gavrilescua and Chisti, 2005; Calhelha *et al*., 2006; Haggag and Mohamed, 2007). The application of chemical fungicides over a long period may result in plant pathogenic fungi developing resistance (Benítez *et al*., 2004, Agrios, 2005; Kim and Hwang, 2007). An

alternative way to increase the crop yield besides using chemical fertilizers is biofertilizers. Biofertilizers promote increased absorption of nutrients in plants (Vessey, 2003; Hart and Trevors, 2005; Chen, 2006). Biofertilizers include materials derived from living organisms and microbial sources (Rola, 2000; Chen, 2006). Biofertilizers have various benefits, such as increased access to nutrients, providing growth-promoting factors for plants, and composting and effective recycling of solid wastes (Gaur and Adholeya, 2004; Das *et al*., 2007). Biofertilizers, commonly known as microbial inoculants are produced from cultures of certain soil organisms that can improve soil fertility and crop productivity (Malik *et al*., 2005; Marin, 2006).

Trichoderma species are common in soil and root ecosystems and are ubiquitous saprobes (Harman *et al*., 2004; Thormann and Rice, 2007; Vinale *et al*., 2008; Kodsueb *et al*., 2008) and they are easily isolated from soil, decaying wood, and other organic material (Howell, 2003; Zeilinger and Omann, 2007).

Trichoderma species can not only reduce the occurrence of disease and inhibit pathogen growth when used as mycofungicides, but they also increase the growth and yield of plants (Elad *et al*., 1981; Harman *et al*., 2004; Vinale *et al*., 2008). They are also increase the survival of seedlings, plant height, leaf area and dry weight (Kleifeld and Chet, 1992). *Trichoderma* species improve mineral uptake, release minerals from soil and organic matter, enhance plant hormone production, induce systematic resistance mechanisms, and induced root systems in hydroponics (Yedidia *et al*., 1999). For these reasons *Trichoderma* species are known as plant growth promoting fungi (Hyakumachi and Kubota, 2004; Herrera- Estrella and Chet, 2004) or are increasing plant growth (biofertilization) (Benítez *et al*., 2004).

Trichoderma also has various applications and important sources of antibiotics, enzymes, decomposers and plant growth promoters (Daniel and Filho, 2007).

Trichoderma spp. produce a growth regulating factor that increases the rate of seed germination and dry weight of shoot and stems (Windham *et al.,* 1986).

Trichoderma has been widely studied for their capacity to enhance plant growth, produce antibiotics, parasitize other fungi and compete with deleterious plant microorganisms (Adams *et al.,* 2007; Chang *et al.,* 1986; Harman *et al.,* 2004 a; Yedidia *et al.,* 2001).

Table 12: Effect of *Trichoderma viride* on 3 varieties of *G. herbaceum* (Pot Study)

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves	Total Chlorophyll Content (mg/g)
		Shoot	Root	Shoot	Root				
Non Bt (Control)	15	0.71	0.038	0.10	0.018	12.1	3.23	4	0.20
	30	1.195	0.114	0.162	0.020	16.2	6.1	6	0.41
	45	6.732	0.874	1.703	0.201	19.33	7.0	9	0.71
	60	8.964	1.165	2.271	0.269	25.77	8.6	11	1.02
	75	11.205	1.456	2.839	0.336	32.21	11.0	14	1.23
	90	13.447	1.748	3.407	0.404	38.66	13.0	17	1.53
Non Bt+ T.viride	15	0.72	0.122	0.018	0.019	13.2	4.19	4	0.30
	30	1.2	0.24	0.025	0.025	19.6	7.0	6	0.52
	45	18.90	1.95	10.5	0.820	27.40	13.0	8	1.0
	60	26	2.59	14	1.109	36.54	18.32	11	1.29
	75	31.80	3.19	17.21	1.39	45.80	22.11	15	1.45
	90	37.6	3.72	20.82	1.66	54.62	28.0	19	1.90
Ajeet- 11	15	0.77	0.082	0.11	0.027	12.0	3.0	4	0.31
	30	1.29	0.145	0.133	0.022	18.3	6.4	6	0.52
	45	9.985	1.254	2.135	0.366	21.0	8.5	9	0.83
	60	13.314	1.672	2.874	0.488	28.0	11.33	12	1.14
	75	16.642	2.090	3.559	0.610	35.00	14.166	15	1.35
	90	19.971	2.509	4.271	0.733	42.0	17.0	17	1.67
Ajeet-11 + T. viride	15	1.271	0.123	0.17	0.029	14.0	4.16	5	0.36
	30	1.49	0.23	0.183	0.032	19.2	7.0	7	0.62
	45	18.810	1.821	4.525	0.553	25.16	10.0	10	1.18
	60	25.080	2.482	6.03	0.738	33.55	13.33	14	1.44
	75	31.350	3.035	7.541	0.922	41.941	16.66	17	1.71
	90	37.621	3.642	9.05	1.107	50.33	20.0	21	2.01
Vikram-5	15	0.610	0.06	0.072	0.014	10.3	3.0	3	0.35
	30	1.31	0.081	0.165	0.015	19.3	5.93	6	0.70
	45	17.585	1.683	5.626	0.511	22.16	9.0	10	1.05
	60	23.447	2.244	7.501	0.681	29.55	12.0	13	1.40
	75	29.309	2.805	9.376	0.851	36.94	15.0	16	1.75
	90	35.171	3.366	11.252	1.022	44.33	18.0	19	2.10
Vikram-5 + T.viride	15	1.065	0.153	0.095	0.018	13.4	4.15	4	0.47
	30	1.35	0.160	0.100	0.025	19.9	7.1	6	0.95
	45	34.671	2.489	10.41	0.832	27.33	15.16	11	1.43
	60	46.22	3.318	13.88	1.109	36.44	20.22	14	1.71
	75	57.785	4.418	17.350	1.368	46.0	25.27	17	2.28
	90	69.342	4.978	20.82	1.664	54.66	30.33	20	2.67

As depicted in table 12 and Fig.10,11 growth parameters of cotton varieties i.e. Conventional and Hybrid both showed the maximum growth reponse to *Trichoderma viride.* All the growth parameters were recorded till 90 days of the plant growth.

Seedlings of Vikram-5 cotton variety showed maximum shoot length (30.33 cm), root length (20 cm), highest dry weight of shoot (20.82g) and dry weight of root (1.66 g). Chlorophyll content of the plants treated with *T. viride* was found maximum (2.67 mg/g) in comparison to Ajeet-11 and Non Bt variety. Trichoderma exerted beneficial effects on the plant growth and development. Increased root size resulted into increased shoot size which translates into shoot biomass production indicating beneficial effect of inoculation on plant growth and developemt. The positive influence of *Trichoderma* on root system architecture would therefore relate to increased yield of plants.

Shanmugaiah *et al.,* (2009) showed similar results where single application of *T. viride* showed the growth promotion of cotton plants.

Okoth *et al.,* 2011 reported similar results for maize and beans where Trichodrma increased the rate of germination and seedling growth of maize and beans. The increased root and shoot length illustrated the direct effect of the fungi on the plant.

The increased growth response induced by *Trichoderma* sp. has been reported for many crops such as beans (*Phaseolus vulgaris*) cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), carnation (*Dianthus carophyllus*), maize (*Zea mays*), and wheat (*Tritichum aestivum*) (Lo and Lin, 2002).A number of mechanisms for plant growth promotion by *Trichodema* have been proposed by Harman *et al.* (2004) and Jaleed *et al.,* (1988).

We conclude that the *Trichoderma* spp. isolate tested against cotton varieties increased the rate of seed germination and shoot and root growth.

Table 13: Effect of *Gliocladium virens* on 3 varieties of *G. herbaceum* (Pot Study)

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves	Total Chlorophyll Content (mg/g)
		Shoot	Root	Shoot	Root				
Non Bt (Control)	15	0.51	0.028	0.10	0.018	12.5	3.23	4	0.20
	30	1.175	0.104	0.152	0.020	16.7	6.1	5	0.31
	45	6.832	0.864	1.603	0.201	19.81	7.0	7	0.61
	60	8.978	1.155	2.171	0.269	25.57	8.6	9	1.12
	75	11.211	1.446	2.739	0.336	32.21	11.0	13	1.33
	90	13.450	1.738	3.307	0.404	38.66	13.0	16	1.73
Non Bt+ G.virens	15	0.72	0.112	0.28	0.019	13.8	4.19	5	0.30
	30	1.26	0.28	0.163	0.025	19.6	7.0	6	0.42
	45	18.95	2.03	2.456	0.820	27.40	13.0	9	1.0
	60	26.52	2.63	8.667	1.109	36.54	18.32	10	1.29
	75	32.80	3.25	11.21	1.39	45.80	22.11	14	1.65
	90	38.6	3.81	13.82	1.66	54.62	28.0	18	2.12
Ajeet- 11	15	0.67	0.062	0.11	0.027	12.0	3.0	4	0.21
	30	1.39	0.135	0.133	0.022	18.3	6.4	6	0.42
	45	9.98	1.264	2.135	0.366	21.0	8.5	8	0.73
	60	13.35	1.662	2.874	0.488	28.0	11.33	11	1.14
	75	16.68	2.19	3.559	0.610	35.00	14.166	14	1.35
	90	19.98	2.53	4.271	0.733	42.0	17.0	17	1.77
Ajeet-11 + G.virens	15	1.274	0.129	0.17	0.029	14.0	4.16	5	0.36
	30	1.53	0.27	0.183	0.032	19.2	7.0	7	0.62
	45	18.86	1.831	4.525	0.553	25.16	10.0	9	1.05
	60	25.15	2.49	6.03	0.738	33.55	13.33	11	1.24
	75	31.38	3.025	7.541	0.922	41.941	16.66	15	1.61
	90	37.67	3.62	9.05	1.107	50.33	20.0	19	1.97
Vikram-5	15	0.62	0.09	0.062	0.014	10.3	3.0	4	0.35
	30	1.37	0.081	0.155	0.015	19.3	5.93	6	0.60
	45	17.59	1.683	3.626	0.511	22.16	9.0	7	1.09
	60	23.46	2.244	7.521	0.681	29.55	12.0	10	1.46
	75	29.32	2.805	9.366	0.851	36.94	15.0	13	1.78
	90	35.19	3.366	11.262	1.022	44.33	18.0	16	2.17
Vikram-5 + G.virens	15	1.073	0.153	0.095	0.018	13.4	4.15	5	0.47
	30	1.42	0.160	0.178	0.025	19.9	7.1	7	0.85
	45	34.682	2.489	3.898	0.832	27.33	15.16	9	1.33
	60	46.38	3.318	8.013	1.109	36.44	20.22	11	1.71
	75	57.79	4.418	12.343	1.368	46.0	25.27	14	2.18
	90	69.41	5.978	14.421	1.664	54.66	30.33	19	2.27

As depicted in table 13, growth parameters of cotton varieties i.e. Conventional and Hybrid both showed the good growth reponse to *Gliocladium virens.* All the growth parameters were recorded till 90 days of the plant growth.

Seedlings of Vikram-5 cotton variety showed maximum shoot length (54.66 cm), root length (30.33 cm), highest dry weight of shoot (20.82 g) and dry weight of root (1.66 g). Chlorophyll content of the plants treated with *G.virens* was found maximum (2.27 mg/g) in comparison to Ajeet-11 and Non Bt variety.

Table 14: Effect of *Aspergillus niger* on 3 varieties of *G. herbaceum* (Pot Study)

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves	Total Chlorophyll Content (mg/g)
		Shoot	Root	Shoot	Root				
Non Bt (Control)	15	0.71	0.038	0.10	0.018	12.1	3.23	4	0.10
	30	1.195	0.114	0.162	0.020	16.2	6.1	6	0.31
	45	6.732	0.874	1.703	0.201	19.33	7.0	9	0.51
	60	8.964	1.165	2.271	0.269	25.77	8.6	11	0.73
	75	11.205	1.456	2.839	0.336	32.21	11.0	14	0.96
	90	13.447	1.748	3.407	0.404	38.66	13.0	17	1.06
Non Bt+ A.niger	15	0.72	0.122	0.018	0.019	13.2	4.19	4	0.30
	30	1.2	0.24	0.025	0.025	19.6	7.0	6	0.52
	45	18.90	1.95	10.5	0.820	27.40	13.0	8	0.78
	60	26	2.59	14	1.109	36.54	18.32	11	1.03
	75	31.80	3.19	17.21	1.39	45.80	22.11	15	1.19
	90	37.6	3.72	19.82	1.66	54.62	28.0	19	1.31
Ajeet- 11	15	0.77	0.082	0.11	0.027	12.0	3.0	4	0.21
	30	1.29	0.145	0.133	0.022	18.3	6.4	6	0.62
	45	9.985	1.254	2.135	0.366	21.0	8.5	9	0.93
	60	13.314	1.672	2.874	0.488	28.0	11.33	12	1.14
	75	16.642	2.090	3.559	0.610	35.00	14.166	15	1.45
	90	19.971	2.509	4.271	0.733	42.0	17.0	17	1.77
Ajeet-11 + A.niger	15	1.271	0.123	0.17	0.029	14.0	4.16	5	0.36
	30	1.49	0.23	0.183	0.032	19.2	7.0	7	0.72
	45	18.810	1.821	4.525	0.553	25.16	10.0	10	1.24
	60	25.080	2.482	6.03	0.738	33.55	13.33	14	1.56
	75	31.350	3.035	7.541	0.922	41.941	16.66	17	1.84
	90	37.621	3.642	9.05	1.107	50.33	20.0	21	2.17
Vikram-5	15	0.610	0.06	0.072	0.014	10.3	3.0	3	0.35
	30	1.31	0.081	0.165	0.015	19.3	5.93	6	0.70
	45	17.585	1.683	5.626	0.511	22.16	9.0	10	1.15
	60	23.447	2.244	7.501	0.681	29.55	12.0	13	1.49
	75	29.309	2.805	9.376	0.851	36.94	15.0	16	1.76
	90	35.171	3.366	11.25	1.022	44.33	18.0	19	2.13
Vikram-5 + A.niger	15	1.065	0.153	0.095	0.018	13.4	4.15	4	0.47
	30	1.35	0.160	0.100	0.025	19.9	7.1	6	0.98
	45	34.671	2.489	10.41	0.832	27.33	15.16	11	1.49
	60	46.22	3.318	13.88	1.109	36.44	20.22	14	1.83
	75	57.785	4.418	17.35	1.368	46.0	25.27	17	2.09
	90	69.342	4.978	21.82	1.664	54.66	30.33	20	2.25

A.niger was found to be most common in the soils of cotton fields. The mass cultres of A. niger was grown on Maize Sand Meal medium for the further experiment. It was observed that cotton seeds gown in the pots treated with *A. niger* germinated faster in comparision to control pots. As depicted in table 14, growth parameters of cotton varieties *i.e.* Conventional and Hybrid both showed the maximum growth reponse to *Aspergillus niger*. All the growth parameters were recorded till 90 days of the plant growth.

Seedlings of Ajeet-11 cotton variety showed maximum shoot length (50.33 cm), root length (22 cm), highest dry weight of shoot (20.09 g) and dry weight of root (1.117 g). Chlorophyll content of the plants treated with *A.niger* was found maximum (2.27 mg/g) in comparison to Vikram-5 and Non Bt variety.

Hande D.V. (2010) showed similar results in cotton palnts where *A. niger* was found to enhance the growth of cotton.

4.5.1. Studies on *in vitro* effect of fungal metabolites on cotton seeds.

A large number of microorganisms are known to produce toxic metabolites when cultivated on synthetic media. Fungal metabolites are substances discharged by fungi in their metabolic processes. The metabolites are products of some amino acids, cyclic peptides, aromatic, phenols, terpenoids and plant growth regulators (Graffin, 1981; Madhosing, 1995; Nema, 1992). These metabolites are many and diverse and they are known to cause diseases in plants, animals and humans who eat infected food (Jalander and Gachande, 2012).

Fungi of the genera *Aspergillus, Fusarium, Penicillium* and *Rhizoctonia* are commonly known to produce toxic substances, such as aflatoxin B1 and B2, Aspergellic acid, cyclopiczonic acid, kojic acid, naphthoquinones, fumonizin and fusaric acid (Singh *et al.,* 1991) that threaten the health of our plants and animals. The role of toxic metabolites of pathogenic fungi in plant disease development has been reported by several workers. Anaso *et al.,* (1981) found out that toxic metabolites of *Drechslera rostrata* and *Fusarium equiseti* retarded root growth of wheat. Reduction in percentage seed germination of soybean seeds was observed in seeds soaked in filtrates of *Phomopsis phaseoli* (Hilty *et al.,* 1988). Soybean seeds soaked in cultures filtrates of *Fusarium solani, F. oxysporum, Aspergillus flavus, A. niger, Alternaria tenuis* and *A. alternata* for 24 hours showed reduction in percentage seed germination (Ibraheem *et. al.,* 1987). Filtrate from mycelial cultures of *Verticillum alboatrum* was found to inhibit cell growth and reduced the viability of alfalfa (*Medicago sativo*) seeds (Frame *et al.,* 1991) reported that the culture filtrate of seed-borne strains of *Alternaria alternata* (Fr.) Keissler inhibited the germination and vigour of sunflower seeds and maize kernels. Culture filtrates of *Fusaruim moniliforme, F. semitectum* and *F. oxysporum* gave very high percentage reduction in seed germination and also inhibited root and shoot growth of *Sorghum.*

The fungi have been found to cause quantitative and qualitative changes in chemical composition of the seeds. This is called Biodeterioration of seeds. These fungi degrade the chemicals present in the seeds, which are rich in protein, carbohydrate and fats by producing enzymes and toxins (Patil *et al.,* 2012).

Table 15: Effect of different fungal culture filtrate on percentage germination of 3 varieties of Cotton seeds.

Name of Fungus	Percentage germination					
	Non Bt		Ajeet-11		Vikram-5	
	30 min	1 hr	30 min	1 hr	30 min	1 hr
Fusarium oxysporum	70	20	90	30	90	23
Alternaria alternata	60	30	70	40	67	42
Chaetomium globosum	50	25	55	23	52	21
Trichoderma viride	90	85	100	93	97	91
T. harzianum	70	66	90	85	80	77
Gliocladium virens	60	60	74	70	80	70
Aspergillus niger	63	50	93	73	80	75
Control	100	100	100	100	100	100

In the present study, the seed samples of three cotton varieties were treated with culture filtrates of *Aspergillus niger, Alternaria alternata, Chaetomium globosum, Fusarium oxysporum, Gliocaldium virens, Trichoderma harzianum* and *T. viride* and their effect on percentage of seed germination and seedling growth was studied.

The effect of presoaking seeds in undiluted filtrates of seed-borne fungi for time period of 30 min and 1 hour on seed germination. The percentage of germination varied with presoaking period as depicted in Table 15.

It was found that *F. oxysporum* was the most pathogenic fungi which inhibited seed germination in all three cotton varieties. 30% germination was observed in both Non Bt variety and Bt variety Ajeet-11 after 1hour of seed soaking whereas in Vikram -5 the germination percentage was recorded 40%. *Alternaria alternata and Chaetomium globosum* was found to inhibit the seed germination in all three varieties of cotton seeds after 1hour of presoaking of the seeds. Rate of seed germination observed was 30 % in Non Bt , 40% and 42% in Ajeet-11 and Vikram-5 respectively when the seeds were presoaked in the filterate of *A. alternata*.whereas,in case of *C.globosum* it was recorded 25%, 23% and 21% in Non Bt, Ajeet-11 and Vikram-5 respectively.

Fungal filtrates of *T. harzianum, T.viride* and *Aspergillus niger* were not found to inhibit much of the seed germination in all three varities of cotton seeds.

4.5.2. Effect of different organic composts on the growth of cotton

In developing countries like India, the situation is comparatively grimmer as it has limited resources to feed the burgeoning population (Saini *et al.,* 2004). The maximum yield of crops can be achieved by introducing high yielding varieties with the application of the suitable fertilizers and growth promoters. Use of chemical fertilizers has been the kingpin of modern agriculture over the past 100 years (Mathivanan *et al.,* 2012). In today's era, heavy doses of chemical fertilizers and pesticides are being used by the farmers to get a better yield of various field crops (Joshi and Vig, 2010). Enviornmental degradation – a major threat conforting the world and the rampant use of chemical fertilizers contribute largely to the deterioration of the environment through depletion of fossils fuels, generation of carbon dioxide and contanmination of water. These chemical fertilizers and pesticides decrease the soil fertility that has adversely impacted agricultural productivity and caused soil degradation.

The long term use of inorganic fertilizers without organic supplements damages the soil physical, chemical and biological properties and causes the environmental pollution (Albiach *et al.,* 2000). Due to the adverse effects of chemical fertilizers, interest has been stimulated for the use of organic manures (Follet *et al.,* 1981). Organic manures are one more important factor which influences soil microflora (Chamle *et al.,* 2011). They not only act as source of nutrients and organic matter but also increase the size, biodiversity and activity of the microbial population in the soil, influence structure, nutrients get turnover and many other change related to physical, chemical and biological parameters of the soil (Albiach *et al.,* 2000). Organic fertilizers like plant residues, manures and composts play a vital role in changing the soil ecosystem, physico-chemical properties and soil mycoflora are of great importance in soil microbiology (Anastasi *et al.,* 2005). Microbial community composition can be more sensitive to

soil amendment with plant residues than microbial biomass. The different kinds of soil amendments such as compost, vermicompost, farm yard manure (FYM) etc., stimulate soil microbial growth and activity with successive mineralization of soil nutrients (Randhawa *et al.*, 2005). In the previous studies application of FYM (Toyota *et al.*,1999) and spent mushroom compost (Piqueres *et al.*, 2006) significantly affected soil microflora.

Vermicompost is the microbial composting of organic wastes formed through the earthworm activity to organic fertilizer which contains higher level of organic matter, organic carbon, total and available N,P,K and micronutrients, microbial and enzyme activities (Edwards and Bohlen, 1996; Ranganathan ,2006; Parthasarthi *et al.*, 2007; Orozco *et al.*, 1996; Parthasarthi ,2004).

Decomposition of leaf litter is an integral and significant part of biochemical (i.e., intrasystem) nutrient cycling and food webs of floodplain forests. Decomposition refers to both the physical and chemical breakdown of litter and the mineralization of nutrients (Boulton and Boon, 1991). Through decomposition the nutrients within leaf litter are converted into a form available for uptake by vegetation, thereby exercising a critical control on vegetation productivity (Mitsch and Gossselink, 1993 and Groffman *et al.*, 1996). Litter plays a fundamental role in the nutrient turnover and in the transfer of energy between plants and soil, the source of the nutrient being accumulated in the upper most layers of the soil (Singh, 1971).

In the present study two organic manures namely vermicompost and dried leaf litter had been selected for conducting the experiments and observing their effects on conventional and hybrid variety of cotton. A plot study was conducted in the Arboretum, of The M.S. University of Baroda. The 1x1 m plots were used for the experiments and to study the effect plot soil was

mixed with 2.5 kg vermicompost and leaf litter both against the control plot, where no compost was mixed.

Different plant growth parameters such as height of the plant, fresh and dry weight of the plant and chlorophyll estimation was studied and data are presented inTable-16. Convention as well as hybrid variety showed maximum response in the soil mixed with vermicompost.

In hybrid variety maximum shoot length of 45.3 cm, highest root length 20 cm, highest dry weight of shoot (3.38 g) and dry weight of root (0.48 g) is recorded.

As depicted in Table 16 chlorophyll content of both conventional and hybrid cotton variety was recorded highest in the plants treated with vermicompost as compared to dried leaf litter and control plants. Chlorophyll content of 5.146 mg/g was recorded in the hybrid variety with vermicompost where as the conventional variety (Non Bt) 2.680 mg/g cholorphyll content.

Table 16: Growth parameters of Bt and Non Bt variety of *G. herbaceum* to different treatments of fertilizers in Plot of 2x2m

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves	Total Chlorophyll Content (mg/g)
		Shoot	Root	Shoot	Root				
Non Bt (Control)	15	0.801	0.056	0.107	0.011	13.73	4.1	4	1.828
	30	1.134	0.091	0.113	0.021	15.98	5.36	5	1.942
	45	1.471	0.123	0.127	0.034	18.33	6.66	7	2.096
	60	2.265	0.124	0.495	0.041	27.0	9.0	9	2.889
	75	2.344	0.504	0.798	0.111	31.0	10.5	13	3.109
	90	2.378	0.561	0.877	0.149	34.0	12.5	16	3.670
Bt (Control)	15	0.952	0.074	0.14	0.011	16.0	4.766	5	1.896
	30	2.096	0.158	0.433	0.042	22.04	6.49	6	2.311
	45	3.239	0.239	0.722	0.074	26.66	8.0	9	2.707
	60	4.879	0.688	1.727	0.226	29.33	9.5	10	3.056
	75	5.549	0.746	1.814	0.27	34.5	17	14	3.829
	90	5.827	1.385	1.822	0.415	38.0	19.0	18	5.143
Non Bt + Vermicompost	15	0.836	0.076	0.125	0.017	13.46	4.5	4	1.917
	30	2.010	0.150	0.413	0.043	18.01	5.0	6	2.172
	45	3.166	0.222	0.707	0.068	23	6.5	8	2.237
	60	4.237	0.455	1.124	0.119	29	9.5	11	2.498
	75	4.275	0.600	1.282	0.247	33.5	13.5	14	2.541
	90	4.554	0.661	1.447	0.277	39.5	17	17	2.662
Bt+ Vermicompost	15	0.693	0.056	0.186	0.016	13.26	7.1	5	2.486
	30	1.621	0.121	0.302	0.045	20.17	7.6	7	2.504
	45	2.569	0.190	0.614	0.055	27.3	8	9	2.543
	60	3.546	0.303	0.762	0.094	29	9.16	11	3.563
	75	7.635	0.911	2.279	0.372	37.5	17.3	15	3.580
	90	12.543	1.255	3.381	0.481	45.3	20	19	5.146
Non Bt + Leaf litter	15	0.457	0.134	0.095	0.008	10.5	3.93	4	1.455
	30	1.635	0.145	0.445	0.031	17.01	5.85	6	1.643
	45	2.808	0.157	0.654	0.053	22.66	7.66	7	1.825
	60	2.359	0.304	0.919	0.097	30	10	10	2.120
	75	3.77	0.613	0.863	0.291	30.5	12.5	13	2.315
	90	4.246	0.725	2.061	0.332	35	16	16	2.680
Bt + Leaf litter	15	0.908	0.07	0.136	0.029	14.13	6.5	5	2.779
	30	1.865	0.164	0.435	0.057	19.17	8.10	7	2.962
	45	2.818	0.246	0.631	0.066	24	9.66	9	3.157
	60	4.538	0.353	1.114	0.114	32	11.33	11	3.264
	75	4.686	0.725	1.561	0.285	34	17.5	14	3.474
	90	14.14	1.16	4.303	0.396	40.66	21.33	19	3.892

4.7. *Studies* on *in vitro* antagonistic activity of pathogenic fungi

In recent years, large number of synthetic fungicides have been banned in the world because of their harmful toxicity (Jat and Agalave, 2013). Many pathogenic microorganisms have developed resistance against chemical fungicides (Gaigole *et al.*, 2011). This seriously hinders the management of diseases of plants and agricultural crops. Worldwide traditional agricultural practices are increasingly being affected by various problems such as diseases, pests, droughts, decreased soil fertility due to use of hazardous pesticides, pollution and global warming. There is a need for some eco-friendly biocontrol agent that may help to resolve some of these problems, Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection (Harman *et al.*, 2004; Saba *et al.*, 2012).

The word antagonism means hostility that results in active resistance, opposition, or contentiousness. In terms of phytopathology it is the action of any microbe that suppresses the activity of a plant pathogen due to an opposition in physiological action. Antagonistic microorganisms play an active role in micro environment. If a plant surface harbours few microbes these can compete with the pathogens for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyperparasitism. This kind of interaction between microorganisms leads to effective control of several diseases. The intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment, and also in the build-up of resistance of the pathogens. In view of this, investigation and the application of biological control agents (BCAs) seems to be one of the promising approaches (Cook, 1985). Biocontrol involves the use of

naturally occurring nonpathogenic microorganisms that are able to reduce the activity of plant pathogens and thereby suppress plant diseases. In this direction fungal endophytes may prove one of the most appropriate biological control agents (BCA).

Antagonists have been frequently used as biocontrol agents. Important constraints being adverse environmental conditions such as extreme dryness, heat and cold limited shelf life and inability to control latent infections. For suitability of an antagonists to be used as a commercial product, Hofstein *et al.,* (1994) has outlined following criteria-

1. It should be genetically stable.

2. Effective at low concentration.

3. Effective against wild rang of pathogens.

4. Does not produce metabolites that are deleterious or harmful to human health.

5. Compatible with commercial processing procedures.

In the present study the antagonistic activity of three pathogenic fungi viz. *F. oxysporum, C. globosum* and *A. alternata* isolated from the field soil and seeds of cotton as examined using the dual culture method.

Table 17 : Antagonistic effect of four different fungi against three pathogens.

Sr. No.	**Fungi**	**Percentage Inhibition**		
		Fusarium oxysporum	***Chaetomium globosum***	***Alternaria alternata***
1.	*Trichoderma viride*	50.98	64.66	70.33
2.	*T. harzianum*	47.66	41.66	61.33
3.	*Gliocadium virens*	23.07	35.33	57.66
4.	*Aspergillus niger*	51.33	59.33	63.66

The observation based on the comparative analysis revealed that *T. viride* hampered the growth of pathogenic fungi and showed the maximum percentage of inhibition against all three

pathogens. It reduced the growth of *F. oxysporum* by 50.98%, in case of *C. globosum* 64.66% and in case of *A. alternata* 70%. Two other fungi *T. harzianum* and *A. niger* also inhibited the growth of three pathogenic fungi and least inhibition was recorded by antagonist *Gliocladium virens* as depicted in the above table-17.

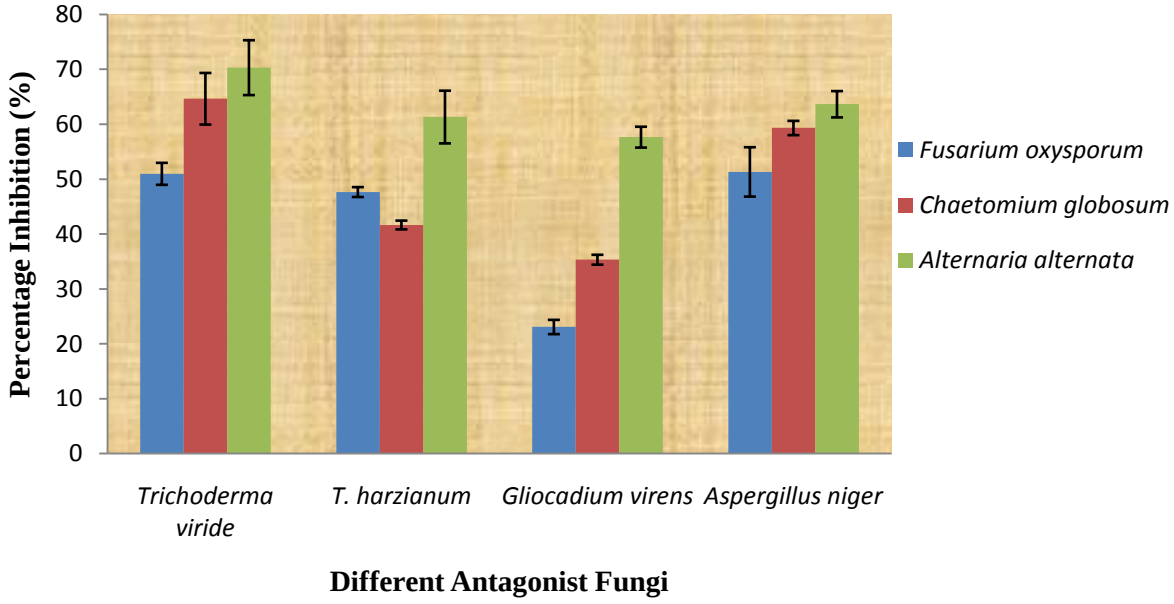
Similar results were obtained by Tapwel *et al.,* (2011) for *T. viride* against five phytopathogens isolated from the nursery seedlings; Dhar *et al.,* (2006) reported similar results for *Trichoderma* and *Gliocladium* against *Fusarium udum*.

Three *Trichoderma* spp. *viz., T. viride, T. harzianum, T. hamatum* were tested against the *F. oxysporium* f. sp. *ciceri* in laboratory applying direct bit placement method. The results indicated that all *Trichoderma* spp. significantly inhibited the growth of *F. oxysporum* f. sp. *ciceri* as against 90 mm radial growth in control treatment. The combined effect of three *Trichoderma* spp. (*T. viride + T. harzianum + T. hamatum*) was found to be most effective in checking the growth (11 mm) of *F. oxysporum* f. sp. *ciceri* over control (90 mm). It was also revealed all *Trichoderma* spp. when used individually as biocontrol agents also exhibited antagonistic effect against *F. oxysporum* f. sp. *ciceri* leading to reduced radial growth of the fungus.

The conidia of *Fusarium oxysporum* were found to be inhibited by all the three antagonistic microorganisms. Among them, highest percent inhibition of conidial germination was brought out by *Trichoderma viride* [89.4%] *followed by Trichoderma harzianum* [85.7%]. This inhibition is due to the volatile and non volatile metabolites and cell wall degrading enzymes produced by *Trichoderma* spp. (Rajeshwari *et al.,* 2011). Inhibition of colony growth of *F. oxysporium* was earlier reported (Brasier 1975). Fakhrunnisa *et al.* (2006) confirmed that *T. harzianum* inhibited radial growth of *F. oxysporum* to the extent of 79.97%. Pawar (2011)

investigated antifungal property of leaf extracts from 18 plants against 5 seed borne pathogenic fungi *viz. Alternaria alternata, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Trichoderma viride.* Deepa *et al.*, (2012) investigate antifungal activity of methanolic and aqueous extracts of *Sapindus emarginatus* leaves against *Aspergillus niger*, they reported that the methanolic extract showed inhibitory effect on *A. niger* in comparision to aqueous extract.

Fig. 17: Histogram showing *in vitro* antagonistic activity



4.7.1. Biocontrol Studies of Seed borne and soil borne fungi

India is the largest consumer of pesticides in the world. Pesticides which include insecticides, fungicides, herbicides, rodenticides and fumigants, are undoubtedly the largest group of toxic chemicals that are introduced profusely into the environment. They are defined as any substance or mixture of substances used for preventing, destroying, repelling or mitigating the pest. Most of the chemicals products fall within four main categories *viz.* organochloride insecticides, organophosphate insecticides, carbamate insecticides and pyrethroid. Pesticides have an inmate capacity to cause damage to the biological system, which may involve human health or environment. The most dramatic of such effects on human are accidental acute poisoning (Sinha and Choudhary, 2008).

Synthetic fungicides are currently used as the primary means for the control of plant diseases. However, the alternative control methods are needed because of the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens, and high development cost of new chemicals.

Some fungicides are not readily biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi. Because of these problems associated with the use of chemicals, researches are now trying to use environmentally safe alternative methods of fungal control.

The commonly used synthetic fungicides have been found to display side effects in form of carcinogenicity, teratogenecity and pollutive effects. Uses of less harmful and true eco – friendly products of plant origin are replacing the routine fungicides (Fawcett and Spencer 1970, Khanna and Chandra 1972, Dixit *et al.*, 1983, Arya and Mathew 1990, Arya *et al.*, 1995). Efforts

are on to find out substitutes for chlorine containing, pentachlorophenol, ethylene dioxide, Gammexane and Dieldrin like pesticides. Use of synthetic pesticide is increasing day by day to meet the challenges of agriculture sector. Modern scientific developments are in no way less than concern with the health of common man.

Plants produce diverse range of pre-infectional metabolites including alkaloids, chalcones, flavanones, organic acids, saponins, sesquiterpene lactones, steroids, sulphur containg amides, and terpenoids, many of which display a broad spectrum antifungal activity (Ebel, 1986). These secondary metabolites with no direct effect on the growth and development of plants in which they have produced, have a potential bioactivity (Nychas, 1995) and are attempted as natural fungicides (Benner, 1993). Wilkins and Board (1989) reported approximately 1400 plants as potential sources of microbial agents with different classes of compounds and several other metabolites from new plant species being identifies every year (Aqil and Ahmad, 2003; Eksteen *et al.,* 2001; Qasem and Abu-Blan, 1996; Ushiki *et al.,* 1996).

A detailed description of the plant derived antifungal metabolites representing different classes of compounds has been described by numerous workers earlier (Grayer and Harbone, 1994; Kishore and Pande, 2004). Majority of the identified natural fungicides are terpenes, phenolic compounds or nitrogen containing secondary products such as alkaloids. Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Bobbarala *et al.,* 2009; Mann *et al.,* 2008).

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. The secondary metabolites of

the plants are a vast repository of biologically active compounds. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al.,* 1998). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy *et al.,* 2001; Ateb and ErdoUrul, 2003). Much work has been done on ethno medicinal plants in India (Maheshwari *et al.,* 1986; Negi *et al.,* 1993). Interest in a large number of traditional natural products has increased (Taylor *et al.,* 1996). Plants are the sources of natural pesticides that make excellent leads for new pesticide development (Arokiyaraj *et al.,* 2008; Gangadevi *et al.,* 2008; Satish *et al.,* 2008; Brinda *et al.,* 2009; Jagadish *et al.,* 2009; Pande *et al.,* 2009; Shanmugavalli *et al.,* 2009; Swarna Latha and Neelakanta Reddy, 2009; Rajan *et al.,* 2009).

The Botanical pesticides like pyrethrum, rotenone, ryania and nicotine, but thereafter, these botanicals were relegated to insignificant position in pest control. Pyrehthrum is extracted from flowers of *Chrysenthemum cinerieolium* and rotenone is derived from rhizomes of *Derris* and *Lonchorpus*. It has been promising source of biopesticide. Neem owes its toxic attributes azadirachtin, nimbin, salannin, meliantriol etc. Neem seed kernels are richest source of meliacins and contain 0.2 – 0.3 % azadirachtin and 30 – 40% oil. Though neem leaves and seeds contain azadirachtin, bark also contains this yet in smaller quantities. George (1999) reported Swallow root *(Decalepis hamiltonii*) of family Asclepediaceae causing protection of food grains against insect infestation. Rice borer (*Sitophilius oryzae*) and Red rust of beetle (*Tribolium*) were controlled by the application of Swallowrot. Inhibition of growth was observed on garlic extract (Tansy and Appleton 1975). Electron microscopic studies revealed thickening in cell wall in *Rhizoctonia solani,* wheras, *Colletotrichum lindemuthianum* revealed a singular accumulation of

osmiophill bodies immediately under the cell membrane when subjected to suspension of micronized garlic powder in distilled water (Bianchi *et al.,* 1997).

Extracts of *Azadiracthta indica, Lantana camara, Lawsonia inermis, Datura* spp., *Acacia* spp. *Trachyspermum ammi* etc are commonly available in India and other tropical countries, are widely used as natural fungicides. There are several increasing reports on the potent antagonistic activity of extracts from many other several plant spp. (Afolayan *et al.,* 2002; Dhaliwal *et al.,* 2002; Letessier *et al.,* 2001; Pinto *et al.,* 1998; Singh and Tripathi, 1999).

Antifungal spectrum and stability of natural fungicides is dependent on the chemical nature of their constituents. Antifungal activity of aqueous extracts *of Padus aviam*, *Populus tremata* and *Chelidonium majus* against *Puccinia tritica* can be correlated with the high phenolic content and peroxidise activity. Fungicide potential of extracts from different parts of *Heraceum sibiricum* was in correlation with the phenolic compounds (Karavaev *et al.,* 2002). Leaf extract was evaluated, owing to its high content of phenols and flavonoids (Parimelazhagan, 2001).

Essential oils, the complex mixture of volatile compounds, mainly monoterpenes (C_{10}) and sesquiterpenes (C_{15}), and their oxygenated derivatives such as alcohols, aldehydes, ketones, acids and esters (Wijesekara *et al.,* 1997), are a major group of natural fungicides. Multiple components rather than a single component, were responsible for fungicidal activity of essential oils. Majority of the essential oils were broad spectrum antifungal. However, the composition of these active components is affected by the genotype, geographical location, environment and agronomic conditions and even with diurnal rythm.

To control fungal pathogens of fruit crops Arya (2010) suggested use of natural fungicides like plant extracts, essential oils, gel and latex etc. Arya *et al.,* (2005) found fruit

peelings (at 25 % conc. for *Myrothecium roridum* and *Chaetomium ganglegarum*) and seeds (at 25% against *Phoma multirostrata* and *Eurotium chevalieri*) of bitter gourd (*Momordica charantia* L. and Cucurbitaceae) effective against 4 fungi. The effect may be due to presence of alkaloid momordicine (0.038%) and some saponines in the fruit (Sabnis and Daniel, 1990) and Elaterin a (Cucurbitacin) present in seeds and fruit wall.

Mode of action of Natural fungicides

Though the chemical nature of several natural fungicides is available, very few attempts have been made to determine the mechanisms operating to control the fungal pathogens. Based on the available findings we can conclude that any one or more than one of the following mechanisms are responsible to restrict (fungistatic) or kill fungicidal) the phytopathogeneic fungal agents.

A) Inhibition of fungal Metabolic pathways

Chemical fistulosin (Octadecyl 3 – hydroxyvindole) isolated from the roots of *Allium fistulosum*, inhibits the protein synthesis of *Fusarium oxysporum*. Eugenol (4 – allyl – 2 methoxy phenol), a major component of several medicinal and aromatic plants, inhibits the involved in free radical scavenging, lipid peroxidation and maintenance of redox potential, which together reduce the aflatoxigenecity of the fungus (Jayshree and Subrmanyam, 1999).

B) Alteration in cell wall composition and structure

The cell wall protects the fungi against external agents including antifungal metabolites. Many antifungal concentration target at cell wall composition and affects the integrity of cells resulting in fungal death.

C) Changes in Membrane Permeability

Membranes act as barrier between the cell and its external environment and also separate various organelles of the cell. Natural fungicides, particularly essential oils and their monoterpenoid components affect the structure and function (Knobloch *et al.,* 1989). This happens due to inhibition of membrane enzymatic reactions such as respiratory electron transport, proton transport and coupled phosphorylation steps (Knobloch *et al*., 1986). Essential oils can degenerate hyphal tips and promote cytoplasmic retraction (de Bilerbeck *et al.,* 2001)

D) Alterations in the Hyphal structure

Treatments with natural fungicides result in microscopically detectable and often macroscopically visible changes in the hyphal structure. The hyphal deformations are mainly due to altered or lysed cell wall, and vacuolization or evacuation of the cytoplasm. Trypsin and chmotrypsin inhibitors from cabbage foliage cause leakage of intracellular contents of *Botrytis cinera and Fusarium solani*. Kaemoferol – 3 – O-b-D- apiofuranosyl -12) –b-D-glucopyranoside, a flavonol diglycoside from the leaves of *Phytolacca americana*, lyse the cell walls diverse pathogenic fungi such as *B. cinera, Magnaporthe grisea, Penicillium italicum, Diaporthe actinidiae, Botryosphaeria dothidea* and *Colletotrichum gloeosporiodes* (Bae *et al.,* 1997).

E) Inhibition of Fungal Cell Wall degrading enzymes

Pathogenic fungi produce cell wall degrading fungi produce cell wall degrading enzymes that degrade the plant cell wall polymers and facilitate the pathogen penetration and further colonization. Production of (CWDE) cell wall degrading enzymes is of significance in the pathogenisis of necrotrophic fungal pathogens, and is of minor significance in case of biotrophic pathogens. Important CWDE involved in the pathogenesis of necrotrohic fungi is

polygalacturonases, pectinlyase, pectimethylesterase,β – 1,4 – glucanase and celulase. The virulence of several necrotrohioc is often related to the differences in their production of CWDE (Carder *et al.*, 1987).

Extracts of *Allium cepa* and *A. porrum* inhibits the production of polygalacturonase by *Sclerotinia scleroternum, B. cinerea, Fusarium moniliforme, Phoma terrastris, P. lycopersici, D. Bryoniae, Sclerotium cepivorum* and *Rhizoctonia bataticola* mediated by the heat labile and protease inhibitor in sensitive factors (Flavaron *et al.,* 1993). Aqueous extracts of *Ocimum sanctum* inhibits the production of pectinolytic and cellulolytic enzymes of *Rhizopus arrhizus* and *Botryodiplodia theobromae* (Patil *et al.*, 1992). Putrescine reverses the inhibitory effect of *O. sanctum* extract suggesting its effect on fungal fungal ornithin decarboxylases pathway. Fruit and flower extracts of *Datura innoxia* inhibits the *in vitro* production of endo and exo pectinolytic and cellulotytic enzyme of *Colletotrichum capsici*. (Chitra *et al.,* 2001). Purified chestnut cystatin strongly affects the protease activity of *B. cinerea.* However unlike biocontrol agents (Elad and Kapt, 1999, Kapat *et al.,* 1998) the inhibitory action of natural fungicides on fungal CWDE in the infection courts has not studied and needs further investigation.

In the present study 7 types of leaf extracts were used against three pathogenic fungi *viz. Alternaria alternata, Chaetomium globosum and Fusarium oxysporum.* Table 18 shows the list of plants used for the antifungal study.

Table 18: List of Plants used as Biocontrol against Pathogenic Fungi

Sr. No.	Plants Used	Family	Chemical Constituents
1	*Annona reticultata* L.	Annonaceae	Alkaloids, Flavanoids, Glycosides, Triterpenoides
2	*Balanites roxburghii* Planchon.	Zygophyllaceae	Alkaloids, Glycosides, Saponins, Flavones and Phenolic compounds.
3	*Cochlospermum religiosa* (L.) Alst.	Cochlospemaceae	Flavanoids, Phytosterols, Saponins and Tanins
4	*Gliricida sepium* (Jacq.) Kunth ex Walp.	Papilionaceae	Tanins, Isoflavanoids like Aformosin, Formentin, Glyricidin A and B and Medicarpin.
5	*Limonia acidissima* L.	Rutaceae	Essential oil containing estragol, Flavone, 3'OMe-quercitin nad Phenolic acids like p-hydroxy benzoic, vanillic, syringic, p-coumaric and ferulic acids.
6	*Sapindus emarginatus* Vahl.	Sapindaceae	Alkaloids, Phenols, Flavonoids, Saponins.
7	*Tephrosia jamnagarensis* Sant.	Fabaceae	Alkaloids, Phenolic acids, Flavonoids and mucilage.

Methanolic fractions exhibited more promising results than aqueous fractions in suppressing the fungal growth. The periodic data regarding fungal growth, exposed to various concentrations of plant extracts of *Annona reticultata, Balanites roxburghii Cochlospermum religiosa, Gliricida sepium, Limonia acidissima, Sapindus emarginatus and Tephrosia jamnagarensis* are present in below table.

Table 19: Percentage inhibition of *Fusarium oxysporum in vitro* at different concentration of seven leaf extracts

Sr. No.	Plants Selected	Methanolic Extract			Aqueous Extract		
		5%	10%	25%	5%	10%	25%
1	*Annona reticulata*	12.7	23.6	72.3	9.4	13.2	15.6
2	*Balanites roxburghii*	31.2	79.2	100	12.0	19.0	35.3
3	*Cochlospermum religiosa*	43.3	68.0	100	19.3	36.6	49.8
4	*Gliricidia sepium*	28.01	61.05	87.9	1.3	5.8	7.6
5	*Limonia accidissima*	32.19	61.91	100	3.2	9.0	16.3
6	*Sapindus emarginatus*	24.9	42.8	66.7	12.0	27.0	39.0
7.	*Tephrosia jamnnagerensis*	27.67	51.36	90.4	16.06	38.97	69.96

*Each mentioned values are based on results of three replicates
Results were significant at $P \leq 0.05$ level by one way ANOVA

F. oxysporum showed 100% inhibition to three plant extracts *B. roxburghii, C. religiosa* and *L. accidissima* followed by *T. jamnnagerensis* which showed 90.4 % inhibition at 25% extract, minimum inhibition of 66.7 % was recorded in plant extract of *S. emarginatus.*

Table 20: Percentage inhibition of *Chaetomium globosum in vitro* at different concentration of seven leaf extracts

Sr. No.	Plant Selected	Methanolic Extract			Aqueous Extract		
		5%	**10%**	**25%**	**5%**	**10%**	**25%**
1	*Annona reticulata*	27.2	23.6	72.3	6.17	16.7	62.0
2	*Balanites roxburghii*	100	100	100	18.0	30.16	62.7
3	*Cochlospermum religiosa*	100	100	100	56.7	78.6	100
4	*Gliricidia sepium*	39.18	63.48	100	28.2	41.5	50.3
5.	*Limonia accidissima*	82.27	100	100	9.1	52.0	65.9
6	*Sapindus emarginatus*	17.5	37.4	100	18.0	30.16	62.7
7.	*Tephrosia jamnagarensis*	65.2	86.5	100	37.4	46.5	70.9

*Each mentioned values are based on results of three replicates
Results were significant at $P \leq 0.05$ level by one way ANOVA

C. globosum showed 100% inhibition to two plant extracts *B. roxburghii, C. religiosa* in all concentrations viz 5%, 10% and 25% , *Gliricidia sepium L. accidissima, S. emarginatus and T. jamnnagerensis* showed 100 % inhibition at 25% extract, maximum inhibition (72.3%) was recorded in plant extract of *A. reticulata* in 25% concentration of methanolic extract.

Table 21: Percentage inhibition of *Alternaria alternata in vitro* at different concentration of seven leaf extracts

Sr. No.	Plant Selected	Methanolic Extract			Aqueous Extract		
		5%	10%	25%	5%	10%	25%
1	*Annona reticulata*	20.4	48.5	75.2	5.9	14.5	32.0
2	*Balanites roxburghii*	8.3	38.8	100	27.3	26.0	32.8
3	*Cochlospermum religiosa*	1.3	25	100	18.7	33.2	60.2
4	*Gliricidia sepium*	29.77	40.81	100	5.2	9.7	13.5
5.	*Ferronia accidissima*	2.0	37.5	100	8	12	43
6	*Sapindus emarginatus*	11.3	33.1	65.0	7.8	12.2	15.3
7.	*Tephrosia jamnnagerensis*	5.0	21.0	100	5.0	19.6	52.5

**Each compound values are based on results of three replicates*
Results were significant at $P \leq 0.05$ level by one way ANOVA

A. alternata depicted 100% inhibition to five plant extracts *B. roxburghii, C. religiosa, G. sepium, L. accidissima and T. jamnnagerensis*, whereas *S. emarginatus* showed minimum inhibition of 65 % . It is evident from Table 17 that leaf extracts of 7 different plants were tested against three pathogenic fungi *in vitro*. In most of the cases 25% methanolic extract was more effective than 5% and 10%. From the above results it is depicted that the extracts of *B. roxburghii, C. religiosa* and *L. accidissima* were most effective in the against all three pathogenic fungi as compared to the other plants.

Earlier Jamuna Bai *et.al*, (2011) showed the antimicrobial activity of *Cochlospermum religiosum.* Sivia *et al.,* (2002) showed the antifungal activity of *A. reticulata* leaf and stem extract against *Colletotrichum gleospoeoides*.

The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (Didry *et al*., 1998). Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

The variation in antifungal activity of the extracts in different solvents may be attributed to the different chemical nature of the solvents. It is likely that different types of chemical were dissolved in different solvent that resulted in variable activity of the extracts of same part of the plant in different solvents.

Survey was conducted in three districts *viz.* Vadodara, Bharuch and Jamnagar of Gujarat to find out different cotton varieties (Bt/non Bt/ hybrid) under cultivation. Data of their yield obtained and associated seed and soil borne fungi. The results depicted that most of the farmers preferred to grow Bt cotton variety in all the present three districts and maximum yield was obtained from the seeds belonging to Ajeet-11 variety.

For the study Bt cotton varieties Ajeet-11 and Vikram -5 were used. Comparison was made with non Bt cotton.

Soil and Seed Borne fungi associated with cotton

Isolation was done from the rhizospheric and non rhizospheric soils to find out the associated fungi. Twenty one different types of fungi were isolated belonging to twelve genera. Various Ascomycetes fungal members like *Aspergillus niger, A. terreus, A. flavus, A. fumigatus Chaetomium globosum and P. citrinum* were present in the rhizospheric soil. Members belonging to Deuteromycetes were *Alternaria alternata, Curvularia lunata, Cladosporium cladosporaoides, Fusarium oxysporum, F. roseum, Myrothecium verrucaria, Tricothecium roseum, T. harzianium* and *T. viride.* Presence of Mucorale member *Rhizopus stolonifer* of Zygomycetes group was interesting feature.

The seed borne fungi were isolated by using Blotter method and Agar Plate method as recommended by ISTA. In blotter method ten types of fungi and in case of Agar plate method thirteen types of fungi were isolated.

Occurrence of Arbuscular Mycorrhizal fungi in rhizosphere soil of cotton

To find out the occurence of mycorrhizal fungi with rhizospheric and non rhizospheric soils of different cotton fields. Method suggested by Gerdemann and Nicolson was followed. Arbuscular Mycorrhizal fungi belonging to 4 genera and 28 species were observed in the soils of different cotton fields of Gujarat.

Isolated different arbuscular mycorrhizal fungi were identified on the basis of morphological characteristics of the fungal spores and their hyphal attachment.

Effect of Arbscular Mycorrhizal Fungi on Growth of Cotton Varieties

The effect of inoculations of AM fungi on plant height, root length, mycorrhizal colonization and chlorophyll content of cotton was studied. Mycorrhizal colonization was found to be superior in hybrid seedling inoculated with consortium of *Glomus* sp. in comparison to hybrid cotton plants without AM inoculation. The data clearly show that the plants which were inoculated with *Glomus* consortium, showed maximum mycorrhizal colonization, better shoot and root length and more dry weight. The chlorophyll content in leaves also increased as compared to non mycorrhizal plants.

Studies on *in vivo* effect of three fungi on growth performance of cotton

Effect of three selected fungi viz. *A. niger, T. viride* and *G. virens* was observed on growth performance of cotton plants. All the three varieties of cotton viz. Non Bt and Hybrid varieties Ajeet-11 and Vikram-5 showed maximum shoot length, root length of both fresh and dry weight and highest chlorophyll content in plants treated with *T. viride* followed by *G. virens* and *A. niger* as compared control plants. Thus we can conclude that the *Trichoderma viride* increased the rate of seed germination and shoot and root growth.

Effect of fungal metabolites on germination and growth of cotton

Effect of fungal metabolites on seed samples of three cotton varieties and their effect on percentage of seed germination and seedling growth was observed. *F. oxysporum* was found to be the most pathogenic and inhibited the seed germination in all three cotton varieties. *Alternaria alternata and Chaetomium globosum* were also found to inhibit the seed germination in all three varieties of cotton seeds after 1hour of presoaking of the seeds. Fungal filtrates of *T. harzianum, T. viride* and *Aspergillus niger* were not found to inhibit the seed germination of all three varieties of cotton.

Effect of two different organic composts on the growth of cotton

Effect of organic composts like vermicompost and dried leaves was observed on plant height, root length and chlorophyll content of cotton plants. The data evidently indicated that the plant treated with vermicompost showed maximum, shoot length, root length of both fresh and dry weight. The chlorophyll content was more in leaves of the plants grown with vermicompost as compared to plants supplemented with dried leaf powder and control plants.

Studies on *in vitro* antagonistic activity of pathogenic fungi

Study on *in vitro* antagonistic activity of three pathogenic fungi viz. *F. oxysporum, C. globosum* and *A. alternata* isolated from soil and seeds of cotton were examined using the dual culture method. The observation based on the comparative analysis of the radial growth three pathogens revealed that *T. viride* hampered the growth of pathogenic fungi and showed the maximum percentage of inhibition against all three pathogens. It reduced the growth of *F. oxysporum* by 50.98%, in case of *C. globosum* 64.66% and in case of *A. alternata* 70% of growth inhibition. Fungi *T. harzianum* and *A. niger* also inhibited the growth of three pathogens and least inhibition of pathogens was recorded by *Gliocladium virens.*

Biocontrol Studies of Seed borne and Soil borne fungi

Botanical pesticides obtained from leaf extracts aqueous and methanolic using Soxhlet method were used. The activity was tested by Poisoned Food technique. Best results were obtained in the methanolic extract of *Balanites roxburghii*, *Cochlospermum religiosa* and *Limmonia accidissima.* These extracts were highly effective against all three test fungi used.

Bio-fertilizers are being viewed as the future of fertilizers, as they have the ability to solve the problems of salinity of the soil, chemical-run offs from the fields. They ensure the well being of the nutrients present in the soil, therefore making the soil more fertile with time. These are more in demand for organic farming. During the past two decades advances in biological sciences have generated a new set of tools that allows us to use our biological resources. One of the biological tools that is now being integrated into biotechnology is the development of commercial Mycorrhizal inoculants for the use in agricultural and frest plants. The impetus for commercialization comes largely from the scientific literature and reports showing that AM associations benefit the plant growth, development and vigor. Benefits derived by AM fungi inoculums include the increase in root surface area for water and nutrient uptake, increased growth rate and yield of the plants etc. AM fungi also contribute to soil stabilization, mechanical aggregation and soil fertility. AM fungi are inoculated into wastelands, degraded sites, polluted soils and mine soils so as to convert them into green pastures. Therefore, mycorrhizae can be used as biofertilizers in the fields so as to decrease the use of chemical fertilizers, pesticides and fungicides. AM fungi can be multiplied in the form of soil pot culture and root organ culture.The soil and the root pieces containing the mycorrhizal infection can be supplied to the farmers for implications in their fields. Another method for supplying the mycorrhiza is by mixing the soil inoculum with several carriers like vermiculite, sawdust and clay etc. These carriers can be directly applied to the field before sowing the seeds.

Other than AM fungi use of *Trichoderma* sp., which is an eco-friendly fertilizer and also acts as a biocontrol agent and is hyper parasitic against different pathogens in the field should be applied to the fields for the better growth and yield of the plants. It can be supplied to the farmers in form of powder so that at the time of sowing seeds it can be directly apply in the soils.

Aspergillus and *Gliocladium* can also be used as mineral solubilizing agents. Present study is indicative of use of othr fungi than *Trichoderma*. Also they can be mixed with the seeds that farmers store so that seeds can be protected from the storage fungi and thus, they can be sotred for a long period of time.

Natural products or eco-friendly pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. The concept of **"Green Pesticides**" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. Pesticides based on essential oils obtained from plants or their constituents have demonstrated efficacy against some plant pathogenic fungi responsible for pre and post-harvest diseases. They may be applied as fumigants, granular formulations or as direct sprays.

In the present study extracts from the plants like *Balanites roxburghii, Cochlospermum religiosum* and *Limmonia accidissima* were found to be effective against the pathogenic fungi. Farmers may be encouraged to use these plants with the proper information. Cultivation of these plants should be ensured and the extracts should be used as foliar spray to achieve integrated control against the pathogenic fungi.

Developing protocol for mass production of PSB, mycorrhizal consortium for effective field application should be tried. New research advances may be linked to call centre services for farmers by KVK (Krishi Vigyan Kendra) a unit of ICAR in Central Gujarat region.

The use of biopesticide will reduce the diseases in cotton and prevent the farmers from harmful effect of pesticides

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Illustration of Plates

Plate I

A – Cotton Plant
B – Field sown with cotton plants during the initial stage of its growth
C and D – Cotton Fields of Vadodara district
E – Wilted cotton plant found in Jamnagar district
F – Leaf Spot disease in Cotton Plant

Plate II

A – Isolation of Soil Mycoflora from the rhizospheric soil of Jamnagar cotton Fields
B – Soil Mycoflora isolated from the cotton field soils of Bharuch
C – Cotton Fields of Area Padra in Vadodara district

Plate III

A – Conidia of *Alternaria alternata* (400x)
B – Macroconidia and Microconidia of *Fusarium oxysporum* (1000x)
C – Spores of *Tricothecium roseum*
D – Conidiophores of *Aspergillus terreus* (400x)
E – Perithecia of *Cheatomium globosum* (400x)
F – Fungal spores of *Cladosporium cladosporoides* (1000x)

Plate IV

A – Conidia of *Penicillium* sp.(400x)
B – Conidial head of *Aspergillus flavus* (400x)
C – *Rhizopus stolonifer*
D – Conidia of *Penicillium citrinum* (400x)
E – Conidiophore and Conidial head of *Aspergillus niger* (400x)
F – Fungal spores of *Trichoderma viride* (1000x)

Plate V

A – *Glomus aggregatum*
B – *Glomus mosseae*
C – *Glomus fasciculatum*
D – *Glomus glomerulatum*
E – *Glomus hoi*
F – *Glomus macrocarpum*

Plate VI

A – *Glomus geosporum*
B – *Glomus etunicatum*
C – *Glomus claroides*
D – *Glomus fuegianum*
E – *Glomus microcarpa*
F – *Glomus rubiformis*

Plate VII

A – *Glomus convolutum*
B – *Glomus monosporum*
C – *Glomus caledonium*
D – *Glomus segmentum*
E – *Glomus tenerum*
F – *Glomus formosanum*

PlateVIII

A – *Glomus intraradices*
B – *Acaulospora laevis*
C – *Gigaspora albida*
D – *Gigaspora ramisporophora*
E – *Gigaspora candida*
F – *Glomus fecundisporum*

Plate IX

Plate showing biomass study of cotton plants of the variety Ajeet-11 and Vikram-5 treated with AM fungi along with control.

Plate X

Plate showing biomass study of cotton plants treated with two different organic manures
A and B – Growth of Cotton plants at 15^{th} day treated with vermicompost and dried leaf litter respectivey
C and D –Cotton plants after 30 days of the sowing with vermicompost and dried leaf litter respectivey
E and F – Cotton plants at 90 days with vermicompost and dried leaf litter Respectivey

Plate XI

A to C- Biomass study of cotton variety treated with different fungi viz. A. *Aspergillus niger*, B. *Gliocladium virens* and C. *Trichoderma viride*
D and E- Root colonization in roots of cotton plants treated with AM fungi

Plate XII
A – Fungal Filterate of different fungi
B and C – Effect of *Fusarium oxysporum* on seeds of Non Bt and Bt variety
D and E – Effect of *Trichoderma viride* on seeds of Non Bt and Bt variety
F and G – Effect of *Gliocladium virens* on seeds of Non Bt and Bt variety

Plate XIII
List of Plants used for the experiment of Biological Control against three pathogenic fungi
A – *Annona reticulata* L.
B – *Balanites roxburghii* Planchon
C – *Cochlospermum religiosa* (L.) Alst
D – *Limonia acidissima* L.
E – *Sapindus emarginatus* Vahl.
F – *Tephrosia jamnagarensis* Sant.

Plate XIV
A – Growth of *F.oxysporum* in methanolic extract of *Annona reticulata*
B – Growth of *F.oxysporum* in methanolic extract of *Balanites roxburghii*
C – Growth of *F.oxysporum* in methanolic extract of *Cochlospermum religiosa*
D – Growth of *F.oxysporum* in methanolic extract of *Feronia acidissima*

Plate XV
A – Growth of *A.alternaria* in methanolic extract of *Tephrosia jamnagarensis*
B – Growth of *F.oxysporum* in methanolic extract of *Cochlospermum religiosa*
C – Growth of *F.oxysporum* in methanolic extract of *Balanites roxburghii*
D – Growth of *F.oxysporum* in methanolic extract of *Feronia acidissima*

Illustration of Plates

Plate I

A – Cotton Plant
B – Field sown with cotton plants during the initial stage of its growth
C and D – Cotton Fields of Vadodara district
E – Wilted cotton plant found in Jamnagar district
F – Leaf Spot disease in Cotton Plant

Plate II

A – Isolation of Soil Mycoflora from the rhizospheric soil of Jamnagar cotton Fields
B – Soil Mycoflora isolated from the cotton field soils of Bharuch
C – Cotton Fields of Area Padra in Vadodara district

Plate III

A – Conidia of *Alternaria alternata* (400x)
B – Macroconidia and Microconidia of *Fusarium oxysporum* (1000x)
C – Spores of *Tricothecium roseum*
D – Conidiophores of *Aspergillus terreus* (400x)
E – Perithecia of *Cheatomium globosum* (400x)
F – Fungal spores of *Cladosporium cladosporoides* (1000x)

Plate IV

A – Conidia of *Penicillium* sp.(400x)
B – Conidial head of *Aspergillus flavus* (400x)
C – *Rhizopus stolonifer*
D – Conidia of *Penicillium citrinum* (400x)
E – Conidiophore and Conidial head of *Aspergillus niger* (400x)
F – Fungal spores of *Trichoderma viride* (1000x)

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