

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°08'25.32" N, 73°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°05'45.44" N 73°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	Paragbhai Vankar	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
Umrli	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.	<i>Rhizopus stolonifer</i> (Ehrneb) Vuill.	++	++	+	++	++	++
Ascomycetes							
2.	<i>Aspergillus terreus</i> Thom.	++	++	-	+	+	-
3.	<i>A. niger</i> Van. Tiegh	+	+	+	+	+	+
4.	<i>A. flavus</i> Link.	+	+	-	+	-	-
5.	<i>A. fumigates</i> Fresen.	+	-	++	-	+	-
6.	<i>Alternaria alternata</i> (Fr.)Keissl.	+	+	+	+	-	+
7.	<i>Curvularia lunata</i> Bat.	++	+	-	-	+	-
8.	<i>Chaetomium globosum</i> Kunze	+	+	-	-	-	-
9.	<i>Cladosporium cladosporoides</i> (Pers).Link	++	++	-	+	-	-
10.	<i>Fusarium oxysporum</i> Schlecht.	+++	+++	++	-	-	+
11.	<i>F. roseum</i> (Schwein) Petch	-	++	+	+	+	-
12.	<i>Fusarium</i> sp.	++	-	-	-	-	-
13.	<i>Myrothecium verrucaria</i> (Alb. And Schwein) Ditmar	++	-	++	-	+	+
14.	<i>Penicillium</i> sp.	+	+	-	+	+	-
15.	<i>Penicillium citrinum</i> Thom	-	+	+	+	-	-
16.	<i>Penicillium</i> sp.	+++	-	-	+	-	-
17.	<i>Penicillium</i> sp.	-	+++	-	+	-	-
18.	<i>Trichothecium roseum</i>	++	-	-	-	-	-
19.	<i>Trichoderma viride</i> Pers.	+	+	-	+	-	+
20.	<i>T. harzianum</i> 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. harzianum*, *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*, *Tricothecium roseum* were found abundantly, whereas, fungi like *A. niger*, *A. flavus*, *A. fumigatus*, *Alternaria alternata*, *C. globosum*, *Penicillium* 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. harizantum* 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. sporotrichioides*, *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 Mojani, 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.	<i>Aspergillus niger</i>	28	16	19	9	11	5
2.	<i>A. flavus</i>	32	16	23	12	24	11
3.	<i>Alternaria alternata</i>	10	8	6	4	-	-
4.	<i>Chaetomium globosum</i>	-	-	3	-	1	1
5.	<i>Curvularia lunata</i>	-	-	2	-	1	1
6.	<i>Fusarium oxysporum</i>	19	11	13	9	10	7
7.	<i>F. roseum</i>	9	2	11	7	14	10
8.	<i>Mucor hemalis</i>	12	-	12	-	9	-
9.	<i>Rhizopus stolonifer</i>	10	8	17	7	13	6
10.	<i>Trichoderma harzianum</i>	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.	<i>Aspergillus niger</i>	30	18	26	10	22	9
2.	<i>A. flavus</i>	35	20	24	18	20	12
3.	<i>Alternaria alternata</i>	22	8	11	5	14	9
4.	<i>Chaetomium globosum</i>	-	-	-	-	3	1
5.	<i>Curvularia lunata</i>	13	7	14	9	10	2
6.	<i>Fusarium oxysporum</i>	33	20	29	16	30	18
7.	<i>F. solani</i>	4	12	5	10	7	8
8.	<i>Mucor hemalis</i>	18	-	12	-	15	-
9.	<i>Rhizopus stolonifer</i>	17	8	19	9	15	10
10.	<i>Trichoderma harzianum</i>	18	5	16	3	13	5
11.	<i>Penicillium citrinum</i>	-	-	-	-	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 and agar plate method using 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 stolonifer* 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 Ajeet-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, *Alternaria 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*,

Diplodia, *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 unbranched, 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. *Aspergillus 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; metulae 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, olivaceous green to olivaceous 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 aerea* (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 25⁰C, 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 x 2.5-3.5 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 zae* (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 coloured and becoming brown, sporulation often scarce, conidiophores 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. *Myrothecium verrucaria* (Alb. and Schwein.) Ditmar

Synonym: *Gliocladium fimbriatum* Gilman and Abbott

Colonies reaching 4-5 cm diam on PDA at 25 °C after 14 days, conidiomata sporodochial or rarely synnematosus, spore mass wet, black, convex, surrounded by white, floccose margin; mycelium absent or 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°C, sometimes smaller, radially sulcate, marginal areas venturous, 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; sporangiophores in clusters of 3-10 unbranched, white, becoming pale to dark at maturity; sporangia globose, hemispherical, granular, olivaceous, black; columella hemispherical, very often becoming pilate; sporangiophores irregular, round to oval, angular, straight.

- xv. ***Trichoderma harzianum* Rafai.**
Synonym: *Sporotrichum narcissi* Tochinai and Shimada

Colonies growing rapidly upto 9cm in 4 days, smooth surfaced, watery white, with sparse mycelia mat but soon developed aerial hyphae 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, hyphae 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, glaucous 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 broadly 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 pyriform, 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. fugiense* 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 *Acaulospora* was found in Bharuch district. *Acaulospora 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 rhizospheric 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 119-520 μm ; 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 visible 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 incomplete. 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)

Zygosporangia 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 hyphae is cylindrical to slightly flared.

7. *Glomus intraradices* N.C.Schenek and G.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, subtending 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 brown 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 hyphae 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 hyphae.

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	<i>G. aggregatum, G. mossae, G. fasciculatum</i>
	2	147	68	
	3	180	60	
Kayavarohan	1	243	98	<i>G. aggregatum, G. intraradices, Acaulospora laevis, G. fasciculatum</i>
	2	197	78	
	3	210	89	
Kunvarpura	1	159	71	<i>G. maculosum, G. fugienaum, Gigaspora albida</i>
	2	130	65	
	3	165	83	
Dabhoi	1	180	88	<i>G. melanosporum, G. fugienaum, G. fasciculatum</i>
	2	172	79	
	3	183	85	
Varnama	1	192	102	<i>G. aggregatum, G. mosseae, G. geosporum</i>
	2	189	99	
	3	183	89	
Sundarpura	1	218	119	<i>G. geosporum, G. convolutum, G. fasciculatum, G. aggregatum, G. etunicatum</i>
	2	201	109	
	3	189	99	
Padra	1	102	67	<i>G. glomerulatum, G. clarum, G. fuegianum, G. hoi</i>
	2	130	78	
	3	172	85	
Dabhasa	1	210	98	<i>G. fecundisporum, G. mossae, G. melanosporum, G. etunicatum, G. intraradices, G. microcarpa</i>
	2	198	93	
	3	190	81	
Muval	1	155	79	<i>G. fasciculatum, G. tenerum, Acaulospora laevis</i>
	2	168	86	
	3	184	92	
Dabka	1	166	72	<i>G. fasciculatum, G. mosseae</i>
	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	<i>G. clarum, Gigaspora ramisporophora, G.etunicatum</i>
	2	91	74	
	3	88	69	
Kavi	1	82	49	<i>G. mossae, Gigaspora sp., G. microcarpa</i>
	2	96	45	
	3	76	52	
Umber	1	102	67	<i>G. aggregatum, G. rubiformis</i>
	2	93	71	
	3	110	82	
Simor	1	97	58	<i>G. fasciculatum, G. tenerum, G. segmentum</i>
	2	106	62	
	3	103	77	
Andada	1	84	59	<i>G. mossae, Gigaspora candida</i>
	2	93	57	
	3	101	69	
Matar	1	91	53	<i>G. fasciculatum, G. glomerulatum</i>
	2	104	61	
	3	94	50	
Karjan	1	180	88	<i>G.melanosporum, G.fugienuum, G.fasciculatum</i>
	2	172	79	
	3	183	85	
Anastu	1	113	90	<i>G. aggregatum, Gigaspora albida, G. tenerum</i>
	2	129	72	
	3	120	69	
Asanvad	1	145	60	<i>G. melanosporum, G. etunicatum, G. intraradices, Acaulospora laevis</i>
	2	122	53	
	3	133	57	
Chhapra	1	107	59	<i>G. aggregatum</i>
	2	104	85	
	3	103	77	
Haripura	1	89	31	<i>G. mossae</i>
	2	97	64	
	3	98	58	
Intola	1	92	40	<i>Gigaspora albida, Glomus clarum</i>
	2	102	89	
	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	<i>G. mossae</i> , <i>G. intraradies</i> , <i>G. aggregatum</i>
	2	62	39	
	3	58	51	
Taraghadi	1	140	96	<i>G. fasciculatum</i> , <i>G. caledonium</i> <i>G. aggregatum</i> ,
	2	43	23	
	3	58	27	
Falla	1	102	89	<i>G. intraradices</i> , <i>Acaulospora laevis</i> , <i>G. fasciculatum</i> , <i>G. glomerulatum</i>
	2	118	92	
	3	103	86	
Dhunvav	1	97	64	<i>G. melanosporum</i> <i>G. etunicatum</i>
	2	71	45	
	3	83	56	
Gaduka	1	112	87	<i>G. fecundisporum</i> , <i>G. mossae</i> , <i>G. melanosporum</i> , <i>G. etunicatum</i> , <i>G. intraradices</i> ,
	2	105	79	
	3	127	100	
Hadmatiya	1	111	90	<i>G. fasciculatum</i> , <i>G. aggregatum</i> , <i>G. microcarpa</i>
	2	97	60	
	3	91	54	
Lonthiya	1	100	77	<i>G. etunicatum</i> <i>G. fasciculatum</i> , <i>G. aggregatum</i>
	2	108	86	
	3	127	103	
Sapara	1	113	98	<i>G. microcarpa</i> , <i>G. mossae</i> , <i>G. melanosporum</i>
	2	121	102	
	3	98	79	
Umralli	1	98	58	<i>G. aggregatum</i> , <i>G. rubiformis</i> <i>G. fasciculatum</i> , <i>G. aggregatum</i>
	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	<i>Glomus agrregatum</i> (Schenck Smith) emend. Koske	Yellow-Brown	131.2	2	12.8 - W --- - H
2	<i>Glomus mosseae</i> Nicol.& Gerd.	Yellow- Brown	127.3	2	9.6 – W 15.2 – H
3	<i>G. fasciculatum</i> (Thaxter) Gerde.& Trappe emend. Walker & Koske	Yellow-Brown	92.8	2	9.6 – W 9.99 - H
4	<i>Glomus glomerulatum</i> Sieverding	Yellow-Brown	64	2	6.4 – W --- - H
5	<i>G. hoi</i> Berch. & Trappe	Yellow- Brown	92.8	3	12.8 –W --- - H
6	<i>G. macrocarpum</i> (Tul. & Tul.) Berch & Fortin	Hyaline- Brown	121.6	2	9.6 –W --- - H
7	<i>G. geosporum</i> (Nicol. & Gerd.) walker	Yellow-Brown	123.2	2	9.6 – W --- - H
8	<i>G. etunicatum</i> Becker & Gerd.	Yellow	83.2	2	6.4 –W --- - H
9	<i>G. claroides</i> Schenck& Smith	Yellow-Brown	76.8	2	12.8 – W --- - H
10	<i>G. fuegianum</i> (Spegazzii)	Yellow- Brown	66.6	3	9.99 – W --- - H
11	<i>Glomus microcarpa</i> Iqbal & Bushra	Yellow - Brown	112	1	12.8 –W --- - H
12	<i>Glomus rubiformis</i> Gerdemann & Trappe	Brown	169.6	1	6 – W --- - H
13	<i>Glomus convolutum</i> Gerde.& Trappe.	Yellow to brown	83.2	2	9.6 – W --- - H
14	<i>Glomus monosporum</i> Gerdemann & Trappe	Yellow to brown	169.7	2	12.1 – W --- - H
15	<i>G. caledonium</i> (Nicol. & Gerd) Trappe & Gerdemann	Brown	139.3	2	9.0 – W --- - H
16	<i>G. segmentum</i> Trappe, Spooner and Ivory	Yellow	80	2	6.4 – W --- H
17	<i>G.tenerum</i> (Tandy) Mcgee	Orange	109.1	2	9.0 – W --- - H
18	<i>G. formosanum</i> Wu & Chen	Yellow to brown	73.6	2	6.4 – W --- - H

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

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

4.4. Effect of Arbuscular 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 (Bethlenfalvai 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 they play an important role in the capture of nutrients from the soil of all ecosystems. AM has a beneficial role in providing soil nutrients for the host-plant partner, 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 phosphorus as observed by Smith *et al.*, (2003). In addition to phosphorus, AMF mycelium also enhances the uptake of nitrogen in the form of NO₃ (Frey and Schuepp, 1993; Morte *et al.*, 2001) and also increases the potassium content in plants (Azcon and Barea, 1992; Maksoud *et al.*, 1994). AM fungi help 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 *Zea 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 131×10^4 after 90 days and fungal cfu was 14×10^4 which increased to 160×10^4 and 20×10^4 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 Chlrophyll 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 x10 ⁴	2 x10 ⁴
	30	53.5 x10 ⁴	3 x10 ⁴
	45	89.5 x10 ⁴	5 x10 ⁴
	60	115 x10 ⁴	8 x10 ⁴
	75	125 x10 ⁴	10 x10 ⁴
	90	131 x10 ⁴	14 x10 ⁴
Non BT + AM	15	44.5 x10 ⁴	6 x10 ⁴
	30	114.5 x10 ⁴	10 x10 ⁴
	45	132.5 x10 ⁴	13 x10 ⁴
	60	149.5 x10 ⁴	16 x10 ⁴
	75	155 x10 ⁴	18 x10 ⁴
	90	160 x10 ⁴	20 x10 ⁴
Ajeet-11 (Control)	15	20 x10 ⁴	1 x10 ⁴
	30	28 x10 ⁴	3 x10 ⁴
	45	34 x10 ⁴	4 x10 ⁴
	60	36.5 x10 ⁴	6 x10 ⁴
	75	38 x10 ⁴	8 x10 ⁴
	90	40 x10 ⁴	11 x10 ⁴
Ajeet-11 + AM	15	39 x10 ⁴	3 x10 ⁴
	30	49 x10 ⁴	10 x10 ⁴
	45	110 x10 ⁴	14 x10 ⁴
	60	132 x10 ⁴	17 x10 ⁴
	75	206 x10 ⁴	20 x10 ⁴
	90	249 x10 ⁴	22 x10 ⁴
Vikram-5 (Control)	15	25 x10 ⁴	2 x10 ⁴
	30	30 x10 ⁴	4 x10 ⁴
	45	33 x10 ⁴	5 x10 ⁴
	60	38 x10 ⁴	7 x10 ⁴
	75	41 x10 ⁴	11 x10 ⁴
	90	47 x10 ⁴	13 x10 ⁴
Vikram-5 + AM	15	44 x10 ⁴	6 x10 ⁴
	30	53 x10 ⁴	11 x10 ⁴
	45	129 x10 ⁴	19 x10 ⁴
	60	186 x10 ⁴	22 x10 ⁴
	75	234 x10 ⁴	25 x10 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 in Table-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 (*Triticum 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; Nemeč *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 Sylvania, 2007). Chemical fungicides however, have a negative effect on human health and on the environment (Voorrips *et al.*, 2004; Soyong *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 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+ <i>T. viride</i>	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 + <i>T. viride</i>	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 + <i>T. viride</i>	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 response 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 development. 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 Trichoderma 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 (*Triticum aestivum*) (Lo and Lin, 2002). A number of mechanisms for plant growth promotion by *Trichoderma* 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+ <i>G.virens</i>	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 + <i>G.virens</i>	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 + <i>G.virens</i>	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 response 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+ <i>A.niger</i>	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 + <i>A.niger</i>	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 + <i>A.niger</i>	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 cultures of *A. niger* was grown on Maize Sand Meal medium for the further experiment. It was observed that cotton seeds grown in the pots treated with *A. niger* germinated faster in comparison to control pots. As depicted in table 14, growth parameters of cotton varieties *i.e.* Conventional and Hybrid both showed the maximum growth response 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 plants 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, Aspergelic 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 *Fusarium 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
<i>Fusarium oxysporum</i>	70	20	90	30	90	23
<i>Alternaria alternata</i>	60	30	70	40	67	42
<i>Chaetomium globosum</i>	50	25	55	23	52	21
<i>Trichoderma viride</i>	90	85	100	93	97	91
<i>T. harzianum</i>	70	66	90	85	80	77
<i>Gliocladium virens</i>	60	60	74	70	80	70
<i>Aspergillus niger</i>	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*, *Gliocladium 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 filtrate 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 varieties 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). Environmental degradation – a major threat confronting the world and the rampant use of chemical fertilizers contribute largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide and contamination 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; Parthasarathi *et al.*, 2007; Orozco *et al.*, 1996; Parthasarathi, 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 Gosselink, 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 in Table-16. Conventional 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 chlorophyll 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 wide range 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		
		<i>Fusarium oxysporum</i>	<i>Chaetomium globosum</i>	<i>Alternaria alternata</i>
1.	<i>Trichoderma viride</i>	50.98	64.66	70.33
2.	<i>T. harzianum</i>	47.66	41.66	61.33
3.	<i>Gliocadium virens</i>	23.07	35.33	57.66
4.	<i>Aspergillus niger</i>	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

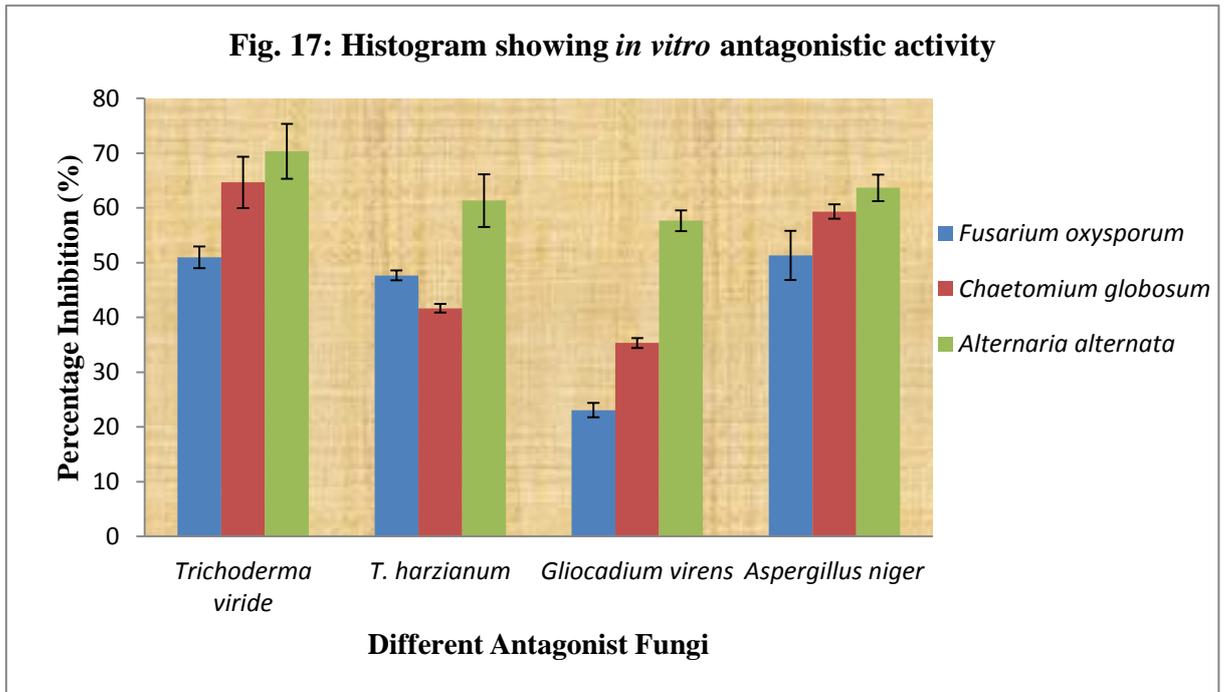
inhibited the growth of three pathogenic fungi and least inhibition was recorded by antagonist *Gliocladium virens* as depicted in the above table-17.

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. oxysporium* 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. oxysporium* 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. oxysporium* f. sp. *ciceri* leading to reduced radial growth of the fungus.

The conidia of *Fusarium oxysporium* 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. oxysporium* 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 comparison to aqueous extract.



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 innate 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, teratogenicity 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-infectious metabolites including alkaloids, chalcones, flavanones, organic acids, saponins, sesquiterpene lactones, steroids, sulphur containing 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 identified 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. Pyrethrum is extracted from flowers of *Chrysanthemum cinerifolium* and rotenone is derived from rhizomes of *Derris* and *Lonchocarpus*. 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 Asclepiadaceae causing protection of food grains against insect infestation. Rice borer (*Sitophilus oryzae*) and Red rust of beetle (*Tribolium*) were controlled by the application of Swallowroot. Inhibition of growth was observed on garlic extract (Tansy and Appleton 1975). Electron microscopic studies revealed thickening in cell wall in *Rhizoctonia solani*, whereas, *Colletotrichum lindemuthianum* revealed a singular accumulation of osmiophil bodies immediately under the cell membrane when subjected to suspension of micronized garlic powder in distilled water (Bianchi *et al.*, 1997).

Extracts of *Azadirachta 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 peroxidase 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 (C₁₀) and sesquiterpenes (C₁₅), 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 rhythm.

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 phytopathogenic 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 aflatoxigenicity 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 chymotrypsin inhibitors from cabbage foliage cause leakage of intracellular contents of *Botrytis cinera* and *Fusarium solani*. Kaempferol – 3 – O- β -D- apiofuranosyl -12) – β -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 gloeosporioides* (Bae *et al.*, 1997).

E) Inhibition of Fungal Cell Wall degrading enzymes

Pathogenic 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 pathogenesis of necrotrophic fungal pathogens, and is of minor significance in case of biotrophic pathogens. Important CWDE involved in the pathogenesis of necrotrophic fungi is polygalacturonases, pectinlyase, pectinmethylesterase, β – 1,4 – glucanase and cellulase. The virulence of several necrotrophic 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 ornithin decarboxylases pathway. Fruit and flower extracts of *Datura innoxia* inhibits the *in vitro* production of endo and exo pectinolytic and cellulolytic 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	<i>Annona reticulata</i> L.	Annonaceae	Alkaloids, Flavanoids, Glycosides, Triterpenoides
2	<i>Balanites roxburghii</i> Planchon.	Zygophyllaceae	Alkaloids, Glycosides, Saponins, Flavones and Phenolic compounds.
3	<i>Cochlospermum religiosa</i> (L.) Alst.	Cochlospermaceae	Flavanoids, Phytosterols, Saponins and Tanins
4	<i>Gliricida sepium</i> (Jacq.) Kunth ex Walp.	Papilionaceae	Tanins, Isoflavanoids like Aformosin, Formentin, Glyricidin A and B and Medicarpin.
5	<i>Limonia acidissima</i> L.	Rutaceae	Essential oil containing estragol, Flavone, 3'OMe-quercetin nad Phenolic acids like p-hydroxy benzoic, vanillic, syringic, p-coumaric and ferulic acids.
6	<i>Sapindus emarginatus</i> Vahl.	Sapindaceae	Alkaloids, Phenols, Flavonoids, Saponins.
7	<i>Tephrosia jamnagarensis</i> 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 reticulata*, *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	<i>Annona reticulata</i>	12.7	23.6	72.3	9.4	13.2	15.6
2	<i>Balanites roxburghii</i>	31.2	79.2	100	12.0	19.0	35.3
3	<i>Cochlospermum religiosa</i>	43.3	68.0	100	19.3	36.6	49.8
4	<i>Gliricidia sepium</i>	28.01	61.05	87.9	1.3	5.8	7.6
5	<i>Limonia accidissima</i>	32.19	61.91	100	3.2	9.0	16.3
6	<i>Sapindus emarginatus</i>	24.9	42.8	66.7	12.0	27.0	39.0
7.	<i>Tephrosia jamnagerensis</i>	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. jamnagerensis* 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	<i>Annona reticulata</i>	27.2	23.6	72.3	6.17	16.7	62.0
2	<i>Balanites roxburghii</i>	100	100	100	18.0	30.16	62.7
3	<i>Cochlospermum religiosa</i>	100	100	100	56.7	78.6	100
4	<i>Gliricidia sepium</i>	39.18	63.48	100	28.2	41.5	50.3
5.	<i>Limonia accidissima</i>	82.27	100	100	9.1	52.0	65.9
6	<i>Sapindus emarginatus</i>	17.5	37.4	100	18.0	30.16	62.7
7.	<i>Tephrosia jamnagarensis</i>	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. jamnagerensis* 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	<i>Annona reticulata</i>	20.4	48.5	75.2	5.9	14.5	32.0
2	<i>Balanites roxburghii</i>	8.3	38.8	100	27.3	26.0	32.8
3	<i>Cochlospermum religiosa</i>	1.3	25	100	18.7	33.2	60.2
4	<i>Gliricidia sepium</i>	29.77	40.81	100	5.2	9.7	13.5
5.	<i>Ferronia accidissima</i>	2.0	37.5	100	8	12	43
6	<i>Sapindus emarginatus</i>	11.3	33.1	65.0	7.8	12.2	15.3
7.	<i>Tephrosia jamnagerensis</i>	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. jamnagerensis*, 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 gleosporoides*.

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.