Studies on Seed Borne and Wilt Diseases of Castor

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Certificate

The thesis entitled "**Studies on Seed Borne and Wilt Diseases of Castor**" *submitted by* **Mrs. Renu Misra,** *contains his original research work carried out in the Phytopathology Laboratory. It has been prepared in accordance with the University norms under my direct supervision. It is further certified that her work has not been submitted to any other University/ Institute for any degree.*

(Prof. Arun Arya)

Head, Department of Botany

Dedicated to

the almighty God and my parents

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Contents

Chapter I Introduction

Global challenges faced by mankind today include food security, bio-fuels, climate change and plant health research.

Globalisation has led to a dramatic increase in trade and travel and this demands more alternative sources for transport fuel. Biodiesel obtained from *Jatropha, Ricinus* and *Pongamia* holds a great potential to be exploited in future**.**

Castor oil's application range is very wide. The uses range from cosmetics, paints, synthetic resins and varnishes, to the areas of national security involving engineering plastics, jet engine lubricants and polymers for electronics and telecommunications. Castor is a versatile, renewable resource having vast and varied applications such as lubricating grease, surfactants, surface coatings, telecom, engineering plastics, pharma, rubber chemicals, nylons, etc. Castor oil and its derivatives find major application in soaps, lubricants, grease, hydraulic brake fluids and polymers and perfumery products. The primary use of castor oil is as a basic ingredient in the production of nylon 11, jet engine lubricants, nylon 6-10, heavy duty automotive greases, coatings and inks, surfactants, polyurethanes, soaps, polishes, flypapers, lubricants, and many other chemical derivatives and medicinal, pharmaceutical and cosmetic derivatives. Castor oil is obtained by pressing the seeds, followed by solvent extraction of the pressed cake. Castor oil is one of the world's most useful and economically important natural plant oils**.**

Castor is a plant that is commercially very important to the world. Castor seed oil cake is a very good fertilizer alternative containing optimum levels of Nitrogen, Phosphorous and Potassium which is suitable for cultivation of paddy, wheat, maize and sugarcane

It is said that this plant originated in the tropical belt of India and Africa. The Sanskrit language gave the ancient name of castor *i.e.* 'Eranda'. It has been referred by other

names in different languages. Herodotus, the father of History, stated that the Egyptians used a type of oil to burn their lamps and for other purposes, which was made from the seeds of *Ricinus* in the fourth century BC. He named it 'Kiki' and till now castor is cultivated and is known in Greece by the same name. Theophrastus and Dioscorides, who stated that the oil of the plant is not worth eating but is very good if applied externally as a medicine, gave the detailed description of the plant at around 1st century A.D. They also stated the process of extracting oil from the seeds of the plant. This plant was cultivated till fifteenth century but due to the negative impacts that it has, it slowly started becoming unpopular and by the eighteenth century, its production practically ceased in Europe and the countries started fulfilling their requirements by importing the oil from Jamaica. This is the period in which its name changed to castor. The name *Ricinus* is a Latin word for tick; the seed is so named because it has markings and a bump at the end which resembles certain ticks. The common name "castor oil" probably comes from its use as a replacement for castoreum, a perfume base made from the dried perineal glands of the beaver (castor in Latin). It has another common name, 'Palm of Christ', or 'Palma Christi', that derives from castor oil's ability to heal wounds and cure ailments.

Castor is an important non-edible oilseed crop and is grown especially in arid and semi arid region. It is cultivated in different countries on commercial scale, of which India, China and Brazil are major castor growing countries accounting for 90 per cent of the world's production. Historically, Brazil, China and India have been the key producing countries meeting the global requirements. However, in early 90's, Brazilian farmers moved away to more lucrative cash crops, and surge in domestic demand in China made them net importers, leaving India to meet the global demand. The production of this crop is concentrated in the hands of a fewer countries and that is why the world production of

castor and its derivatives is highly fluctuating. Any change in the trend of the production of any of those countries leads to change in the level of world production. The world production of castor seed hovers around at an average of 12.5 lakh tons and of castor oil is 5.5 lakh tons. The major producer countries of castor are India, China and Brazil. The top most country in the list is India with around 65% of the share in production followed by China with 23% and Brazil with 7% of share. Since last few years, the countries China and Brazil are having an increase in their domestic consumption demand and hence are consuming a greater share of their production and exporting lesser leaving India to be the dominant player in the international market.

It is believed that agriculture had not diffused from one centre to the rest of the world but there were 7 centres of evolution of different crops. About 12000 to 9500 years ago castor is supposed to have evolved in Ethiopia. According to Smartt and Simmonds (1995) and Mehra (2000) castor was grown in Gangetic plains of India during 2200-800 B.C.

Cultivation aspect of castor

In India, it is grown as a khariff crop and is planted generally during the months of July and August. The crop has a duration period of 4 to 5 months and is generally harvested in the months of December and January. However, the sowing and harvesting periods within the country differ according to the different regions where the crop is grown. Also, the region only decides that whether the crop is to be grown as a sole crop or a mixed crop. It starts to reach the market in the month of October and is sold till April.

Castor crop needs a tropical type of climate to develop. That's why the castor is largely found in the countries lying in the tropical belt of the world. In India, the seed pods are dried, de-podded and brought to the market yards during December or January for trading. Traditionally, sowing of castor with onset of monsoon is found most

beneficial in rain fed condition. However, sowing can be done up to first fortnight of August without reduction in yield under irrigated condition. Castor crop loves heat and humidity and does best in regions where both are ample. India is gifted with an ideal climatic condition for castor seed. Castor seed production during the past decade remained around 7 to 8 lakh tonnes. More than 9 lakh tonnes castor was produced in 2005-06, which was the highest during the decade, this resulted into fall of Prices in 2005. Thereafter, the total production declined by about 19% during 2006-07 from about 9.9 lakh tonnes in the previous year. One may note that the area under production also declined by 15%. The average annual castor seed and castor oil prices declined during 2005. As per estimates, India produced 9.2 lakh tonnes in 2007-08 the area under castor production declined, leading to drop in production in the following year. Gujarat is the leading state in castor seeds production in India followed by Rajasthan and Andhra Pradesh. Gujarat contributed 71% of the total production of castor seed in India in 2007-08, followed by Rajasthan at 16%, Andhra Pradesh at 9% and other states sharing 4%.. In Gujarat castor cultivation comes from 6 districts of North Gujarat, viz., Mehsana, Banaskantha, Sabarkantha, Gandhinagar, Patan and Kutch. Mehsana and Banaskantha are the largest castor producing districts in Gujarat

To derive the oil, the castor fruits (regma) are separated from the plant and allowed to dry for about 10-15 days. The dried fruit is split open to collect castor seeds. These seeds are processed prior to extraction of oil. The seeds generally contain up to 48% oil and the rest becomes the part of oil cake. Though India meets most of the world's requirement of castor oil we are not capitalizing it. We still continue to export the oil rather than add value to it. We buy back our own castor oil as formulated products and derivatives at many times the price of the oil.

This oil is unique among vegetable oils and its uniqueness is derived from the presence of a hydroxyl fatty acid known as ricinoleic acid (12- hydroxyl-cis-9-octadecenoic acid) which constitute around 90% of the total fatty acids of the oil. Castor oil is also distinguished from other vegetable oils by its high specific gravity, thickness and hydroxyl value. The Indian variety of castor seed has an oil content of 48% and 42% can be extracted, while the cake retains the rest. Castor oil is used either in its crude form, or in the refined hydrogenated form. Typically, 65% of it is processed. About 28% is refined, 12% is hydrogenated, 20% is dehydrated, and the balance 5% is processed to manufacture other derivatives. The major derivatives of Castor oil used in the industry hydrogenated castor oil (HCO), dehydrated castor oil (DCO), sebacic acid etc. The demand for castor oil derivatives is perennial in nature since they are indispensable to the manufacture of various products. Besides, as castor does not have a synthetic or any other alternative, it has an assured market. Domestic demand of castor oil has unfortunately not kept pace with growth in castor seeds output. Developed economies are major uses of castor oil.

Castor crop plays an important role in the agricultural economy of the earning substantial foreign exchange through export of castor beans and oils. India's exports of castor oil and derivatives are estimated at over Rs.800 crores p.a. The global castor derivatives market is highly dependent on India. India's export of castor oil and derivatives is 2.6 lakh tons in 2007-08. India is the first country in the world to exploit hybrid vigor on commercial scale in this crop. Major markets include European Union, USA, Japan and now China and Thailand. Though, India is a dominant player in the world market, it is just a price taker and not a price setter due to its poor infrastructure but it has the capability to improve on the exports of the derivatives of castor and overcome this limitation.

The protein content of castor seed meal varies from 21-48% depending upon the extent of decortications. It has an ideal amino acid profile with moderately high Cystine, Methionine, and Isoleucine. But its anti nutritional substances, ricin, ricinin and an allergen restrict its use in poultry feed, even at a very low level of inclusion. Castor cake is an excellent fertilizer because of high content of N (6.4%), Phosphoric acid (2.55%) and Potash (1%) and moisture retention. The solvent extracted cake, although rich in protein cannot be used as cattle fodder because of its toxicity. However, it can be used as a fertilizer.

The seeds contain 2.8–3% toxic substances, 2.5–20 seed killing a man, 4 a rabbit, 5 a sheep, 6 an ox, and a horse, 7 a pig, 11 a dog, but 80 for cocks and ducks. The principal toxin is the albumin, ricin. However, it produces antigenic or immunizing activity, producing in small doses an antitoxin analogous to that produced against bacteria.

Castor bean is both self- and cross-pollinated by wind, varying from 5–36% depending on the weather conditions. Pollen sheds readily between 26–29°C, with a relative humidity of 60%. For single cross hybrid seed production, strains giving a 1:1 ratio or pistillate and heterozygous monoecious plants are used, the latter being rouged 1–5 days before flowering begins. Three-way cross hybrids can also be used. For open pollinated types, rouging of all off-types is done after the last cultivation, and for pure seed production isolation necessity depends on the wind velocity. For hybrid and open pollinated types in the United States, stands are isolated 300–720 m, but in areas of less wind velocity, less distance may be sufficient.

Fungi known to attack Castor bean plants include*: Alternaria compacta, A. ricini, A. tenuis, A. tenuissima, Aspergillus itaconicus, A. niger, A. quercinus, ricinicola, Botrysphaeria ribis, Botrytis cinerea (Gray mold), Cephalosporium curtipes, Cercospora*

canescens, C. coffeae, C. ricinella, Cercosporella ricinella (Leaf spot), Cladosporium herbarum, Clitocybe tabescens, Colletotrichum bakeri, C. erumpens, C. ricini, Corticium solani, Didymella ricini, Diplodia natalensis, D. organicola, D. ricinella, D. ricini, Discosporella phaeochlorina, Epicoccum nigrum, Erysiphe cichoracearum, Fusarium moniliforme, F. orthoceras, F. oxysporum, F. sambucinum, F. semitectum, Gibberella pulicarus, Glomerella cingulata, G. ricini, Haplosporella manilensis, Lecanidion atratum, Leveillula lanata, L. taurica, Macrophomina phaseoli, Macrophoma phaseoli, Phoma ricini, Macrosporium cavarae, M. ricini, Melampsora euphorbiae, M. ricini, Melampsorella ricini, Mecrostroma minimum, Mucor fragilis, Mycosporella ricinicola, M. tulasnei, Myrothecium roridum, Oidiopsis taurica, Peniophora cinerea, Phoma macropyrena, Phomopsis ricini, Phyllosticta bosensis, P.ricini, Phymatotrichum omnivorum (Root rot), Physalospora abdita, P. propinqua, P. rhodina, P. ricini, P. obtusa, Phytophthora cactorum, P. cinnamomi, P. palmivora, P. parasitica, Pleospora herbarum, Pythium aphanidermatum, P. debaryanum, P. gracile, P. intermedium, P. proliferum, P. ultimum, P. vexans, Rhabdospora ricini, Rhizoctonia solani, Schiffnerula ricini, Schizophyllum commune, Sclerotinia fuckeliana, S. minor, S. ricini, S. sclerotiorum, Scierotium rolfsii, sphaceloma ricini.

Disease caused by: *Agrobacterium tumefaciens, Bacterium lathyri, B. ricini, Pseudomonas solanacearum, Xanthomonas ricini, X. ricinicola. Striga lutea* parasitizes the plants.

Damage caused by insect pests

Several insects are pests. In India the Capsule borer (*Dichocrocis punctiferalis*) bores into young and ripening capsules; and the Castor semilooper (*Achoea janata*) are the worst pests. In Tanganyika damage by capsid and myrid bugs are a limiting factor causing immature fruit to drop. Green stinkbugs, leaf-hoppers, leaf-miners and

grasshoppers are pests that feed on the leaves. Most insects may be controlled by insecticides. Because some of the varieties are quite tall, wind storms are a potential hazard to a crop.

Damage caused by Nematodes

Nematodes isolated from Castorbean include*: Aphelenchoides asterocaudatus, A. bicaudatus, A. subtenuis, Helicotylenchus cavenssi, H. pseudorobustus, H. schachtii, Meloidogyne arenaria and var. thamesi, M. hapla, M. incognita, M. incognita acrita, M. javanica, M. thamesi, Merlinius brevidens, Pratylenchus brachyurus, P. neglectus, P. pratensis, P. scribner, P. vulnus, P. zeae, Radopholus similes, Scutellonema clathricaudatum, Tricephalobus longicaudatus,* and *Tylenchorhychus mashhoodi* (Duke 1983).

Synergistic effect on wilt intensity due to *Verticillium dahliae* Kleb and *Fusarium oxysporum* f. sp. *vasinfectum* with reniform nematode *Rotylenchulus reniformis* Linford and Oliveira has been observed in cotton (El-Gindi *et al.,* 1974; Krishnaprasad and Padaganur, 1980; Tachatchoua ans Sikora,1978). Reniform nematode was also found to be involved in the wilt complex of castor. Castor plants in carbofuran treated soil at 3 Kg.a.i. per ha remained free from wilt, while those without carbofuran were severely stunted and wilted. Thus castor plants attacked by *R. reniformis* are predisposed for *F. oxysporum* f. sp. *ricini* infection (Chattopadhyay and Reddy, 1995; Chattopadhyay *et al*.,1996)

Fusarium oxysporum, also referred to as 'Panama disease' or 'Agent Green', is a fungus that causes *Fusarium* wilt disease in more than a hundred species of plants. It does so by colonizing the water-conducting vessels (xylem) of the plant. As a result of this blockage and breakdown of xylem, symptoms appear in plants such as leaf wilting, yellowing and eventually plant death. Interest in *Fusarium oxysporum* as a herbicide was

first raised after the discovery in the 1960s that it was the causative agent in the destruction of the Hawaiian cocoa population.

The United States government was involved in a controversial program to use *Fusarium oxysporum* for the eradication of Cocoa in Colombia and other Andean countries, but these plans were cancelled by President Bill Clinton who was concerned that the unilateral use of a biological agent would be perceived by the rest of the world as biological warfare. The Andean nations have since banned its use throughout the region. Use of biological agents to kill crops is potentially illegal under the biological weapons convention.

Gros michel or 'Big Mike' was an early export cultivar of banana. This cultivar was wiped out by Panama disease in the 1950s. The disease first appeared in Surinam, then made its way to the Caribbean, and by the 1920s, to Honduras, the world's largest producer of bananas at the time. Nations a search for a substitute located the Vietnamese Cavendish cultivar which is resistant to the disease. However, more care is required for shipping the Cavendish banana and its quality compared to Gros Michel is debated. Recently, a new strain called 'Tropical race four Panama disease' has begun to attack Cavendish banana plants in South Asia. Given the high volume of modern International trade, banana producers expect this strain to spread through Africa and into South America and the Caribbean. The disease in Australia is restricted to a very small area, geographically near Darwin in the Northern Territory.

Taxonomic Classification

Kingdom: Fungi

Phylum: Ascomycota

Order: Hypocreales

Family: Hypocreaceae

Genus: *Fusarium*

Fusarium is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops (Pitt *et al*. 1994). While most species are more common at tropical and subtropical areas, some inhabit in soil in cold climates. Some *Fusarium* species have a teleomorphic state (Larone 1995, Sutton *et al*. 1998). As well as being a common contaminant and a well-known plant pathogen, *Fusarium* spp. may cause various infections in humans. *Fusarium* is one of the emerging causes of opportunistic mycoses. The genus *Fusarium* currently contains over 20 species. The most common of these are *solani*, and *Chlamydosporum* (de Hoog *et al* 2000).

Being a common plant pathogen, *Fusarium* spp. are causative agents of superficial and systemic infections in humans. Infections due to *Fusarium* spp. are collectively referred to as fusariosis. The most virulent *Fusarium* spp. is *Fusarium solani* (Mayayo, *et al*. 1999). Trauma is the major predisposing factor for development of cutaneous infections due to *Fusarium* strains. Disseminated opportunistic infections, on the other hand, develop in immuno suppressed hosts, particularly in neutropenic and transplant patients (Austen *et al.* 2001, Boutati 1997, Girmenia *et al.* 1999, Vartivarian *et al.*1993, Venditti *et al*.1988). *Fusarium* infections following solid organ transplantation tend to remain local and have a better outcome compared to those that develop in patients with hematological malignancies and bone marrow transplantation patients (Sampathkumar and Paya. 2001). Outbreaks of nosocomial fusariosis have also been reported. Existence of *Fusarium* in hospital water distribution systems may result in disseminated fusariosis in immunosuppressed patients (Squier *et al*. 2000). *Fusarium* may also exist in soil of potted plants in hospitals. These plants constitute a hazardous mycotic reservoir for

nosocomial fusariosis (Summerbell *et al*.,1989).

Fusarium spp. produce mycotoxins. Ingestion of grains contaminated with these toxins may give rise to allergic symptoms or be carcinogenic in long-term consumption. Fumonisins are the mycotoxins produced by *F. moniliforme* and *F. proliferatum* in maize. They may cause oesophageal cancer (Pitt, 2000). Another group of mycotoxins, zearalenones, may also be produced by some *Fusarium* spp. growing in grains (Schaafsma *et al*.1998). Studies on reduction or elimination of *Fusarium* mycotoxins from contaminated agricultural and food commodities are in progress (Visconti *et al*. 2000).

Fusarium spp. grow rapidly on Sabouraud dextrose agar at 25°C and produce woolly to cottony, flat, spreading colonies. The only slow-growing species is *F. dimerum*. From the front, the color of the colony may be white, cream, tan, salmon, cinnamon, yellow, red, violet, pink, or purple. From the reverse, it may be colorless, tan, red, dark purple, or brown. A sclerotium, which is the organized mass of hyphae that remains dormant during unfavorable conditions, may be observed macroscopically and is usually dark blue in color. On the other hand, sporodochium, the cushion-like mat of hyphae bearing conidiophores over its surface, is usually absent in culture. When present, it may be observed in cream to tan or orange color, except for *F. solani*, which gives rise to blue-green or blue sporodochia (de Hoog *et al*. 2000, Sutton *et al.* 1998). Phialides are cylindrical, with a small collarette, solitary or produced as a component of a complex branching system. Monophialides and polyphialides (in heads or in chains) may be observed. Macroconidia (3-8 x 11-70 µm) are produced from phialides on unbranched or branched conidiophores. They are 2- or more celled, thick-walled, smooth, and cylindrical or sickle- (canoe-) shaped. Macroconidia have a distinct basal foot cell and pointed distal ends. They tend to accumulate in balls or rafts. Microconidia

(2-4 x4-8 µm), on the other hand, are formed on long or short simple conidiophores. They are 1-celled, smooth, hyaline, ovoid to cylindrical, and arranged in balls (occasionally occurring in chains). Chlamydospores, when present, are sparse, in pairs, clumps or chains. They are thick-walled, hyaline, intercalary or terminal. Macroscopic and microscopic features, such as, color of the colony, length and shape of the macroconidia, the number, shape and arrangement of microconidia, and presence or absence of chlamydospores are key features for the differentiation of *Fusarium* spp. (de Hoog *et al.,* 2000). Molecular methods, such as 28S *r RNA* gene sequencing, may be used for rapid identification of *Fusarium* strains to species level (Hennequin. *et al* 1999).

Fusarium is one of the most drug-resistant fungi. Among the *Fusarium* spp., *Fusarium solani* in general tends to be most resistant of all. *Fusarium s*trains yield quite high MICs or flucytosine, Ketoconazole, miconazole, fluconazole, itraconazole and posaconazole (Arikan *et al.,* 1998, Pujol *et al.,* 1997, Van Cutsem, 1992). *Fusarium* infections are difficult to treat and the invasive forms are often fatal. Amphotericin B alone or in combination with flucytosine or rifampin is the most commonly used antifungal drug for treatment of systemic fusariosis (Sampathkumar and Paya. 2001). Despite its limited *in vitro* activity, posaconazole appears effective in murine fusariosis (Lozano *et al.* 1999). Human data are awaited for verification of this finding. Topical natamycin is used for treatment of keratitis due to *Fusarium* (Rahman *et al.,* 1998). In addition to antifungal therapy, keratoplasty is required for several patients (Singh and Malik, 1972). Patients with mycetoma due to *Fusarium* may respond to Itraconazole (Restrepo, 1994). Onychomycosis due to *Fusarium*, on the other hand, may be treated with itraconazole and ciclopirox nail lacquer.

Fungal Diseases of Castor

A number of fungal diseases in castor are reported they are-

1. Seedling blight. Castor seeds may be infected by various fungi. These fungi may cause seedling blight. To control seedling blight avoid low lying or water logged areas. Treating seeds with Thiram or Captan @ 3 g per kg of seed is suggested.

2. *Alternaria* leaf blight: Treatment of the seeds with Thiram @ 3 g/ kg of seed. Spray Mancozeb (0.2%) as and when the disease is noticed it is suggested that additional one or two sprays are done at intervals of 15 days if required.

3. Wilt and Root rot Avoid water logged conditions. Follow recommended soil and water conservation practices. Do not adopt short term crop rotations; follow 4 to 5 year crop rotations. Avoid planting castor immediately after sorghum if the latter was infected with charcoal rot. Uproot and destroy affected plants as and when they are detected.

Castor wilt was first time recorded in Morocco (Reiuf, 1953). In India castor wilt was first reported by Nanda and Prasad in 1974. The causal organism was established as *Fusarium oxysporum* f. sp. *ricini* by Nanda and Prasad. Occurrence of castor wilt from USSR was reported by Andreeva (1979) and the causal organism for the disease was identified as *F. oxysporum* f.sp. *ricini* on the basis of morphological characters and host specialization. Wilt of castor was later recorded from Gujarat state during 1980-81. Gujarat is the leading castor growing state of the country and shares about 54 percent area and 82 percent of castor seed production of the country. The productivity (1972 kg/ha) is the highest in Gujarat state, because more than 90 percent cultivated area is covered by castor hybrids, under irrigated conditions. This state is pioneer in the development and release of hybrids where in the first ever castor hybrid, GCH 3 was released for general cultivation in 1968-69. There after 4 more hybrids, viz; GAUCH 1, GCH 2, GCH 4, GCH 5 and GCH-6 have been developed and released for general

cultivation in the state. Recently, a nematode-wilt complex resistant higher yielding hybrid, GCH 7, was released (Dange 2003).

Wilt is most important disease of castor at present in India. Monocropping followed due to its high economic return resulted to the endemic development of wilt and this became the limiting factor for castor cultivation in the state. As high as 85 percent wilt incidence has been recorded in castor growing area of North Gujarat. In our country 23 different crops including cotton, rapeseed, mustard, groundnut, linseed, sunflower, gram, mung, moth, lentil, pea, guar, potato, chilly, tomato, cucurbits (vine crops *e.g.* melons, cucumber etc.), citrus, fruits, banana and guava are attacked by wilt. Symptoms similar to wilt disease also occur in Wheat, Maize, Sugarcane, Sesame, Castor, Soybean, Pigeon pea, Mango and Papaya.

Wilt is a common feature in those fields which are infested by this disease once and where crops are sown without rotation. The disease causes immense loss to crops. In. most cases the farmers are not aware of the problem.

The symptoms, preventive and curative measures are given here for the guidance of farmers as well as a reminder to research fellows and extension workers, so that they may try to find an easy and economical way to fight the disease. It is also expected to guide the breeders to develop and select wilt resistant varieties.

Wilt may be caused by fungi, viruses, nematodes, parasitic flowering plants and insects. These usually confused with root and crown rots, stem cankers, insect injuries, drought or excess water, soil compaction, and other non-infectious problems.

The *Fusarium* spp., predominantly common soil fungi, is found all over the world as a not detrimental colonizer of root surfaces or a weak invader of the root cortex of many plants. There are over 80 known strains only of *F. oxysporum*, which show specific pathogenicity to a particular crop, causing the vascular wilt diseases. Mirza and Qureshi

(1978) found that most of root infecting fungi including Fusarium spp. are known to attack many cultivated plants and parasitized 36 hosts in Pakistan. The cotton husks, used as cattle feed are identified as a potential source for spread of wilt causing fungus (*F. oxysporum* f.sp. *vasinfectum*).

Most *Fusarium* species are soil fungi and have a world-wide distribution. Some are plant pathogens causing root and stem rot, vascular wilt or fruit rot. Other species cause storage rot and are important mycotoxin producers.

Fusarium wilt is a solid dark brown ring around the outer portion of the stem, in cross section in the area of the vascular bundles, and that the infected areas are observed as irregular in shape and size. Wilt disease mostly spreads in patches, but entire field may also be affected in severe conditions. Some *formae speciales* are not primarily vascular pathogens but *Fusarium* spp. May cause foot and root rot or ball rot in cotton.

Complex interactions take place in the volume of soil around roots, which traditionally has been termed the ‘‘rhizosphere.’’ More appropriately, that soil volume constitutes a *mycorhizosphere* (Rambelli 1973) because of the dramatic influence an abundance of fungal external hyphae has on root and soil associated microorganisms, as well as the effects of those microorganisms, on the establishment and spread of mycorrhizae (Bagyaraj 1984). Mycorrhizal roots also have altered root exudation patterns (Marschner 1998). The *Rhizobium -Bradyrhizobium* association with legumes most notably is affected by mycorrhizal fungi, largely as a result of increased availability of phosphorus in host roots, which drives nitrogenase activity in nodules Aźcon 1993). Other indirect interactions affect both pathogens and beneficial organisms, either through effects of mycorrhizal formation on root exudates or through competition (Linderman 1988; Garbaye 1991).

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The influence of Fungal hyphae in the mycorrhizosphere is much greater than previously thought (Tisdall *et al*.1997) with the discovery of ‘glomalin,’ a heat stable glycoprotein that coats hyphae and spore surfaces and accumulates in soil (Wright *et al*. 1996, 1998). Evidence indicates a strong involvement of glomalin in soil aggregate stability; researches found a highly significant correlation between glomalin concentration and soil aggregate stability (Wright and Upadhyaya 1998).

Perhaps the largest obstacle to understanding the biology and ecology of AM fungi is our inability to culture them apart from their plant hosts. The plant provides carbon to the fungus largely via an arbuscule – plant cell plasmalemma interface. It also provides a protected site in root cells where the fungus can live. The external fungal hyphae improve phosphorus acquisition by the plant in soils with low levels of phosphorus (Safir 1987; Smith and Gianinazzi-Pearson 1988; Smith and Read 1970). In soils in which phosphorus levels exceed requirements of the host, however the AM

symbiosis often is inhibited. Under those conditions for certain host-soil interactions, mycorrhizal development can reduce plant growth and thus become pathogenic (Modjo and Hendrix 1986).

In nature, fungal communities are taxonomically complex and rarely, if ever, consist of only one species (Morton 1999). When individual fungi are cultured on plants, host specificity appears to be minimal or absent (Smith and Gianinazzi-Pearson 1988; Brundrett 1991; Smith and Read 1997). Investigators at the International Culture collection of (vesicular) Arbuscular Mycorrhizal Fungi (INVAM) in West Virginia showed that more than 1000 isolates of 98 species of fungi in all genera are able to grow and sporulate on one plant host, sorghum sudanese, or Sudangrass (Morton *et al.* 1993). Roots of Sudan grass accommodate colonization by as many as 10 species of fungi at one time in pot cultures using field soil as inoculum. And those fungi can be members of any genus (Morton *et al*. 1995, Morton 1999). Lack of host specialization may be the result of mutualistic co evolution of the plants and their fungal partners over the 400 million years since their origin (Simon *et al*. 1993; Taylor *et al.* 1995).

A combination of host and environmental factors can differentially influence rates and degrees of colonization and/or sporulation by different AM fungi in a community (Johnson *et al.*1992; Bever *et al*. 1995), which are manifested as changes in species richness and relative abundance in sporulation. In general, those responses represent compatibility adjustments rather than specificity, although Mc Gonigle and Fitter (1990) and Brundett (1991) explained the 'ecological specificity,' respectively. Divergence in physiological and life –history traits within a fungus species and between species is expected in these asexual organisms as a natural response to local and regional selection pressures (Morton 1990, 1999). Members of a small number of plant orders do not form AM associations (Tester *et al.*1987). Many other orders, however include both

mycorrhizal and non-mycorrhizal families or genera. Non mycorrhizal taxa are assumed to have evolved away from the symbiosis, based on evidence from their distributions ,relative to those of their mycorrhizal relatives (Trappe 1987),and from the presence of activated defense mechanisms in chemically induced mycorrhizal-resistant mutants (Peterson and Bradbury 1995). A few plant genera are able to support both arbuscular and fungi and ecto mycorrhizaal fungi one of the most widely studied being Eucalyptus (Lapeyrie and Chilvers 1985).

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Among various isolates of *F. oxysporum* f.sp. *ricini*, variation in virulence has been recorded (Nanda and Prasad,1974). Variability study among twelve isolates of *F. oxysporum* f.sp.*ricini* collected from different castor growing areas of Gujarat and Andhra Pradesh was under taken. Considerable variability was observed in cultural, morphological characters and pathogenic ability among the isolates. These isolates were divided into five groups.

Desai *et al.* (2003) studied the variability in *F. oxysporum* f. sp. *ricini* in respect of cultural, morphological characters and pathogenicity. Six isolates produced thin, flat to slight fluffy pinkish mycelium on potato dextrose agar. (Desai *et al.* 2003).

Gigaspora calospora exerted an inhibitory effect on the development of pigeon pea blight caused by *Phytophthora drechsleri* f. sp. *cajani* (Bisht *et al.,* 1985). Similarly, in Tamil Nadu Agricultural University, India, studies showed that another AM fungus

G. etunicatum induced tolerance to cowpea (*Vigna unguiculata*) against *Macrophomina* root rot. Disease incidence was 16% in inoculated plants as against 33% in uninoculated plants (Ramraj *et al.*, 1988). Rosendahl (1985) observed decrease in disease incidence in peas due to *Aphanomyces euteiehes.* Similar results were observed for soyabean (Zambolim and Scheneck1983) and peanut (Abdalla and Abdel-Fattah, 2000) due to *Fusarium solani*. Krishna and Bagyaraj (1983) observed reduction in disease due to *M. phaseolina* in soyabean. Studies conducted at the University of Bayreuth, Germany, showed that in leachates of AM rhizospheric soil of *Zea mays* and *Trifolium subtrerrneum* fewer sporangia and zoospores were produced by *P. cinnamomi* as compared to non AM plants suggesting that sporangium induced microorganisms have declined or sporangium inhibitors have increased (Meyer and Linderman, 1986).

Pandey and Upadhyay (2000) studied the effect of microbial population in development of pigeon pea in Pusa, Bihar. Screening for resident antagonist was done and mode of myco-parasitism was studied. Dual inoculation with AM endophyte (*G. mosseae*) and *M. phaseolina* significantly restricted the progression of the pathogen in the roots of mungbean (*Vigna radiata*). Disease incidence was reduced from 77.9% in pathogen inoculated to 13.3% in AM + Pathogen inoculated plants (Jalali *et al*., 1990). *G. fasciculatum* reduced the number of sclerotia produced by *S. rolfsii* in peanuts (*Arachis hypogaea*) (Krishna and Bagyaraj, 1983)

The term ‘sustainability’ although very well used catch word in politics, economy and science is not a new ethical concept. It dates back to Indian source from around 300 B.C. In a hymn to the earth says, ‘‘May everything I extract from you, mother earth quickly grow again, Oh! Purifier, may we never injure your vital nerve, nor your heart’’ (Atharva veda).

Arbuscular mycorrhizal (AM) fungus- plant relationships are usually described as

mutually beneficial, because fungi supply mineral nutrients, especially phosphorus (P) to their host plants in return for photosynthate (Smith and Read,1997).

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rates and degrees of colonization and/or sporulation by different AM fungi in a community (Johnson *et al*.1992; Bever *et al*. 1995), which are manifested as changes in species richness and relative abundance in sporulation . In general, those responses represent compatibility adjustments rather than specificity, although Mc Gonigle and Fitter (1990) and Brundett (1991) explained them as 'ecological specificity ,' respectively .Divergence in physiological and life –history traits within a fungus species and between species is expected in these asexual organisms as a natural response to local and regional selection pressures (Morton 1990,1999)

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Soil Dilution Method - Soil samples were collected from the fields, soil was collected from the rhizosphere and non - rhizosphere region and the samples were collected in polyethylene bags. The sample was air dried and passed through 2mm sieve. Soil dilution plate method (Parkinson and Thomas, 1965) was used to isolate various microorganisms. Soil dilution method was used for qualitative and quantitative analysis of microflora in the rhizosphere. The root of the plant was washed in 200 ml of sterile distill water and rinsed thoroughly, then the roots were removed and at least 4 times 1ml of the suspension was diluted and 1 ml of the soil suspension was plated in sterilized petridish having potato dextrose agar medium and then the plates were incubated at 25+ - 2°C and examined after 2 days for bacteria and 7 days for fungi.

Field survey was done to see the mortality rate of the castor plants in agriculture fields of seven different places like **Sama, Ranoli, Ode, Padra, Umretha, Sarsa and Chhota udepur** were visited for three consecutive years and percentage plant mortality was recorded. The wilted plants were collected and also the rhizospheric and the non - rhizospheric soil was sampled and analyzed in lab.

Pathogenecity test (Koch's Postulates)

For pathogenicity tests of *F. oxysporum* and other two sp. Experiment was performed in field and in pots. Fungal cultures of three *Fusaria* were inoculated in to maize meal sand medium (Singh 1977). In 250ml conical flask, 150 g of sand and 4.5 g of crushed maize grains. 20ml of distilled water was added in 150 g sand maize meal mixture gives 65% saturation. Then the flasks were autoclaved for 30 min at 15 *p.s.i* and were then inoculated with agar inoculum discs (Garrett, 1936) with 10 day old cultures of *F oxysporum, F. solani*, and *F. roseum* flasks were incubated for 21 days at room temperature and in between they were shaken so that the fungus gets distributed equally inside the flask. When the fungal mycelium was well grown then it was ready to be

incorporated in the soil. 5 g of upper 10cm inoculum was used per pot and mixed with the pot soil. Then seeds of *R. communis were* sown. The pots were then watered regularly once a day to maintain appropriate moisture. Then the percentage germination and degree of wilting was recorded. When the plant wilted it was brought to the laboratory washed with tap water and the parts *i.e.* stem and roots were dipped in 0.1% mercuric chloride solution and again washed with sterilized water and then they were incubated on P.D.A medium. The fungus obtained in Petri plates was sub cultured and identified with morphological characters to see whether the same fungus is retrieved from the plant, which was grown in sick soil.

Pathogenicity test was also carried out in the field also a plot size of 1m x 1m was made and three such plots were made for each variety of *R. communis.* Seeds were sown along with maize meal, and 2 different species of *Fusarium i.e. Fusarium oxysporum* and *Fusarium solani* was added.

ISOLATION OF SEED MYCOFLORA

Before the seeds of castor were kept for isolation of the seed mycoflora they were tested for their viability. For seed viability test, four replications of 100 seeds were fully immersed in distilled water for 18 hours to start activity of dehydrogenase enzyme and for the facilitation of the penetration of tetrazolium solution (0.1% of TTC was used) for three hours at room temperature i.e. 25 ± 2 ºC in dark. After three hours the solution was decanted and excess reagent was removed, the seeds were then rinsed with water and evaluated. Individually the seeds were assessed for their viability. On the basis of staining of embryo, staining of cotyledon assessment on basis of necrosis, assessment on the basis of necrosis, and on the basis of color intensity was done, (Khare & Bhale 2000).

Isolation of seed mycoflora was done using-

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

Blotter Method

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

Agar Plate Method

In this method 2 different types of media were used which were semi- synthetic media and the synthetic media. In the semi- synthetic media potato was used along with the dextrose and Agar and this medium is known as Potato Dextrose Agar medium. 200 g of potato were peeled and sliced into small pieces. It was then boiled for 40 min in 500ml of distilled water and filtered through a cheese cloth twenty grams of dextrose (D-glucose) was added and the total volume was raised to 1000ml. Agar agar (2%) was used as a solidifying agent.

Synthetic media

Asthana and Hawker's medium 'A'

D Glucose	5.0 g
KNO_3	3.5 g
KH_2pO_4	1.75 g
$MgSO_4.7H_20$	0.75 g
Distilled water	1000ml

Modified Asthana and Hawker's medium 'A'

D- Glucose	10.0 g
KNo_3	3.50 g
KH_2PO_4	1.75 g
$MgSO_4$	0.75 g
Distilled water	1000ml

In Agar plate method, PDA medium and Asthana and Hawker's medium were used. All the contents were mixed and autoclaved and then poured in preautoclaved petriplates in the laminar flow and the medium was allowed to solidify. After that seeds were kept in the petriplates in two different sets treated and untreated seeds. Seeds were treated with NaOCl 1% and then placed on the medium; the petriplates were then kept in the incubator at 25 ± 2 °C and the data were recorded for occurrence of fungi after 7 days. If any fungi were found growing on the seeds it was scrapped with the help of an inoculating needle in the laminar flow and transferred on the P.D.A slants for further identification. After four to five days again it was transferred to culture tubes and the process was repeated to obtain pure cultures. After the pure cultures of the fungi were obtained, slides were made so that the fungi could be identified.

Staining Technique

Some of the fungal mycelium was placed on a slide and stained with cotton blue. Excess stain was removed by lactophenol and a coverslip was placed on the slide and observed under the microscope. Also fresh and pure cultures of fungi were sent.

Changes in biochemical contents of seed after pathogenesis

Seeds of *R. communis* of three different varieties GCH4, AVANI 41 and a local variety were weighed 100gms each and 6 different packets were made out of which 3 packets of 100gm seeds were labeled as infected as they were infested with *Fusarium oxysporum* and were left for 30 days in an incubator. After 30 days the seeds were removed, weighed and the extraction process of oil was started using the soxhlet apparatus (Akpan *et al* 1999)

The castor beans are basically processed for extraction by various means and they are:-

Clearing- If some foreign materials and dirt are present it is separated by hand picking.

Drying- The cleaned beans are sundried in the open till the casing splits and sheds the seeds. The beans are further dried in the oven at 60 degree for seven hours to a constant weight in order to reduce its moisture content.

Winnowing- The separation of the shell from cotyledons was carried out using tray to blow away the cover in order to achieve a very high yield.

Grinding- (Size reduction) Mortar and Pestle is used to crush the beans in to a paste (cake) in order to weaken or rupture the cell walls to release castor fat for extraction.

Determination of Moisture Content of Seeds

40g of the sample was weighed and dried in an oven at 80 degree for seven hours and the weight was taken after every two hours. The procedure was repeated till a constant weight was obtained. After each two hours the sample was removed from the oven and placed in the desiccator for 30 minutes to cool. It was then removed and re-weighed. The percentage moisture in the seed was calculated from the formula-

Moisture = 100(W1- W2) /W2 %

Where **W1 = original weight of the sample before drying**

W2 = Weight of sample after drying

Determination of the percentage of castor oil extracted.

10g of seed sample was placed in the thimble and about 300ml n- hexane was poured in the round bottom flask. The apparatus was heated at 60 degrees and allowed for 3 hours continuous extraction using soxhlet apparatus. At the end the solvent was distilled and percentage of oil was determined. This process was repeated thrice.

Determination of Saponification value.

Indicator method as specified by ISO 3657(1988) was used.

2g of sample was weighed in a conical flask 25ml of 0.1N ethanolic potassium hydroxide was added. The content was continuously stirred and allowed to boil gently for 60 min.

A reflux condenser was placed on the flask containing the mixture. After 1 hour Phenolphthalein indicator was added to the warm solution and then it was titrated with 0.5 M HCl to the end point until the pink color of the indicator just disappeared. The same procedure was used for other samples with healthy and diseased seeds along with a blank solution. The saponification value was calculated as -

$$S.V = 56.1N (VO - V1)/ M$$

Where **VO = Volume of solution used for blank test**
V1= Volume of solution used for determination
N = Actual normality of Hcl used
M= Mass of the sample

Determination of specific gravity

Density bottle was used for determining the density of the oil. A clean and dry bottle of 25ml capacity was weighed (WO) and then filled with the oil, stopper was inserted and reweighed to give (W1). The oil was substituted with water after washing and drying the bottle and weighed to give (W2). The formula for calculating specific gravity is-

$$\text{Sp. gr} = \frac{(W1-W0) \text{ = Mass of Substance}}{(W2-W0) \text{ Mass of an equal volume of water}}$$

Determination of viscosity

A clean, dried viscometer with a flow time above 200 seconds for the fluid to test was elected; the sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid material in the liquid sample. The viscosity meter was charged with the sample and suction force was drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed in to a holder and inserted to a constant temperature bath set at 29 °C .The suction force was then applied to the thinner arm to draw the sample slightly above the upper

timing mark. The afflux time by timing the flow of the sample as it flows freely from the upper timing mark to lower timing mark was recorded.

Determination Of pH value

2g of the sample was poured in to a clean dry 25 ml beaker and 13 ml of hot distilled water was added to the sample in the beaker and stirred slowly. It was then cooled in a cold water bath to 25 °C. The pH electrode was standardized with a buffer solution and the electrode immersed in to the sample and the pH value was recorded.

Control of seed borne fungi by plant extracts

100g of the leaf sample was taken, washed dried and crushed with distilled water, filtered and made 100 ml and then two different concentrations of 10% and 25% was prepared using potato dextrose agar medium, the experiment was set up in triplicates. And the plant extracts were tested on the three *Fusaria i.e*. *F. oxysporum* , *F. solani* and *F. roseum*, readings were taken at an interval of 7 days and 10days respectively.

Effect of different fungal metabolites on the seed germination of three varieties of *Ricinus communis*

Seed samples of three different varieties of *Ricinus communis* were collected from Vadodara and Padra .400 Seeds of each variety along with their control were placed in Petri dishes lined with filter paper and incubated at ± 25 °C in the incubator and the occurrence of fungi was recorded after seven days. The fungi were then sub cultured and identified on the basis of their morphological structure. The seeds were treated with 1% sodium hypochlorite .The culture filtrates of eleven fungi *Fusarium oxysporum, Fusarium solani, Fusarium roseum, Rhizoctonia, Alternaria alternata , Aspergillus flavus, Aspergillus niger, Rhizopus, Trichoderma, Curvularia* and C*olletotrichum* were grown in liquid Modified Asthana and Hawker's medium for 15 days and then filtered using Whatman no.1 filter paper. All the seeds of different variety were first tested for

their viability by soaking overnight in distill water and then in Tetrazolium salt solution. The pink color on the cotyledons showed viable seeds. After this the seeds were soaked separately in each culture filtrate for 12 hours and allowed to germinate in presterilized petriplates containing sterile filter paper. Required moisture was maintained by adding sterile distill water and the percentage germination was recorded after seven days.

Effect of Am fungi on increase in biomass of three varieties of castor

Ninety pots were used for the experiment in which 45 pots were sown with 5 maize seeds per pot along with AM fungi. The maize plants were allowed to grow for 2 months. After 2 months the roots were then cut in to small pieces and mixed with the pot soil. Three different varieties of castor seeds i.e. GCH4, AVANI 41, and a local variety were used for this experiment and were sown in 15 pots each, for each variety 8 seeds were sown per pot. Control sets were also maintained. After every 15 days the plants were screened for their morphological parameters i.e. fresh weight of root and shoot, dry weight of root and shoot, height of plant, length of root, and percentage root colonization till 90 days. To find out the root colonization, the roots of the *Ricinus* plant which were uprooted were washed, and blotted dried, after that cut in to 1 cm pieces and boiled in 10% KOH solution after that it was again washed with distilled water and then stained**.** (Phillips and Hayman's 1970) Phillips and Hayman's method was followed to find out percentage VAM colonization.

Physiological studies on Fusaria

Effect of different culture media on fusaria

For physiological studies first different culture media were prepared to see which culture media suited the fungal mycelium the best. Two different types of media were selected for this purpose-

1. Semi- synthetic medium.

2. Synthetic media.

Semi- synthetic medium (Potato Dextrose medium)

200 gm of potatoes were peeled and sliced into small pieces it was boiled for 40 min in 500 ml of distilled water and then filtered through a cloth. 20 gm of dextrose (D glucose) was added and total volume was raised to 1000 ml.

Synthetic media

Asthana & Hawkers medium 'A'

D – Glucose	: 5 g
KNo_3	: 3.5 g
KH_2Po_4	: 1.75 g
$MgSo_4.7H_2o$	: 0.75 g
Distilled water	: 1000 ml

Modified Asthana and Hawkers medium 'A'

D – Glucose	: 10 g
KNO_3	: 3.5 g
KH_2PO_4	: 1.75 g
$MgSO_4.7H_2O$	: 0.75 g
Distilled water	: 1000 ml

Richard's Medium

Sucrose	: 5 g
KNO_3	: 10 g
KH_2PO_4	: 5 g
$MgSO_4.7H_2O$	: 2.5 g
$FeCl_3$	: 0.02 g
Distilled water	: 1000 ml

Czapek's Medium

Sucrose	: 30 g

$NaNo_3$	: 2 g
KH_2Po_4	: 1 g
Kcl	: 0.5 g
$MgSo_4.7H_2o$	: 0.5 g
$FeSo_4.7H_2o$	: 0.01 g
Distilled water	: 1000 ml

The fungus was grown in all the above media to see which culture media facilitated its growth.

For physiological studies 25 ml of liquid basal medium (Modified Asthana and Hawker) was taken in 150 ml Erlenmeyer conical Flask. Unless otherwise stated the culture media was auto claved at 15 lbs.p.s.i for 15 min. whenever the medium contained complex substances liable to decomposition or de naturalization, fractional sterilization was done which involves exposure to steam for 30 min on three successive days with the help of agar disc method (Garrett 1936) 10-12 days culture was used for inoculating the flask containing different media. Inoculated flasks were incubated at 25 ± 2 º C for 15 days. At the end of incubation period change in pH of the medium and degree of sporulation of the organism was recorded. The degree of sporulation was classified into 5 categories viz. nil, poor, fair and good on the basis of visual and microscopic observation.

In order to access the growth of organism, the fungal mats were harvested at the end of incubation period on previously dry and weighed whatman filter paper no. 42 and several times with distilled water. The filter paper was again dried in an electric oven at 60 °C for 72 hrs and they were cooled in desiccators at least for 24 hrs and finally weighed. The difference between the final and initial weights of the filter paper indicated the dry weight of the fungal mat. Dry weight of the mycelial mat and degree of

sporulation were considered as a measure of response of the organism to different treatments. Each set of the treatment was run in triplicates and only the average dry weight was always taken as standard value for comparison of growth. The dry weight results were statistically analyzed and standard error was calculated by formula

$$\textbf{S.E} = \sqrt{\frac{\text{mean square of the error}}{\text{No. of replicates}}}$$

Critical Difference was calculated by the formula

C.D = S.E x t x √2 , Where 't' represent probability at 5 % level.

Dry weight of the mycelial mats was graded into good, less, very less and poor. The dry weights higher or lower of the moderates have been designated as good or poor respectively except for some preliminary studies dealing with the effect of certain media on growth and sporulation the initial pH was adjusted in subsequent studies by adding adequate quantities of N/10 HCl or NaOH soln. The pH of the medium or filtrate was determined with the help of pH meter.

Effect of different pH

After selecting the suitable basal culture medium for the fusaria, which was Modified Asthana and Hawkers medium, different species of *fusaria* i.e. F*usarium oxysporum, Fusarium solani and Fusarium roseum* were grown on different pH ranging from pH 3 to pH 8. The preparation of the basal culture medium was same as mentioned above, also the fungal mats were harvested at the end of 15 days and standard error and critical difference was calculated in the aforementioned manner. The experiment was carried out in triplicates.

Effect of different Temperature

A wide range of temperature varying from 5, 10, 15, 20, 25, 30, 35, and 40 º C was selected for this particular experiment. The basal medium selected was Modified

Asthana and Hawkers' medium, its preparation and inoculation of fungus, the harvesting of the fungal mats and calculation of standard error and critical difference was similar as mentioned above.

Effect of different sugars and their utilization

Chromatographic studies were conducted in order to know the utilization pattern of different monosaccharide, Oligosaccharides and polysaccharides. The quantity of various sugars was similar to that used in experiment dealing with carbon requirements. Solutions containing monosaccharide's were autoclaved at *p.s.i.* for 15 minutes while fractional sterilization was carried out for disaccharides and polysaccharides These solutions were inoculated with different species of *Fusarium* and the fungal mats were retrieved at the interval of 5, 10, 15 days which were in turn filtered dried and weighed on pre-dried and weighed whatman's filter paper to determine the growth. Change in pH if any was recorded. Drops of known volume (0.0005ml) were taken from the filtrates at an interval of 2,4,6,8,10,12,14 and 15 days and were placed on the chromatograms by a micropipette at a position located for this purpose. The chromatograms were run in n Butanol: acetic acid: water (4:1:5 v/v) (Rajan *et al.* 1955) for about 7 hours and they were subsequently dried at room temperature, while the upper organic layer is used as the developing solvent, the lower aqueous layer is used to saturate the chromatographic chamber. In this solvent system sugars exhibit low R_f values in winter, But in summer high Rf values are seen and therefore it is the recommended solvent for the latter season. After the paper chromatograms were dried a spray agent was prepared. Aniline hydrogen phthalate. 100ml of n butanol is saturated with water by slow addition of small aliquots of water and shaking in between approximately 10ml of water goes into butanol. To 100ml of this solution 0.91ml (9.30mg) of Aniline and 1.6g phthalic acid is added and stirred well. After that this spraying agent is kept for the separation of layers and the

upper layer is used for the spraying purpose and the lower layer is discarded. The chromatogram papers are then sprayed with the spray reagent. The sprayed papers are then heated over hot plate (or in an oven) at 110 ºC for 5-10 min. The sugars are identified by comparing the color with the blank and R_f values of the spots with those of standard sugars.

R_f value was calculated by the formula

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Effect of different nitrogen sources

An experiment was set up to study the effect of different nitrogen sources on the different *Fusaria.* In all ten different nitrogen sources were used for this experiment. Asthana and Hawker's (M) broth medium was used for this experiment. The experiment was carried out in triplicates and although Potassium nitrate was used to the prepare the medium, other Nitrogen sources were also used replacing Potassium nitrate.

Effect of vitamins

An experiment was set up to see the effect of different vitamins on the three *Fusaria.* In all 10 vitamins were used which are as follows:

1 Thymine: used in the concentration of 50ppm, 100ppm, 150ppm, 200ppm

2 Pyridoxine : used in the concentration of 50ppm,100ppm, 150ppm, 200ppm

3 Riboflavin: used in the concentration of 25ppm, 50ppm, 75ppm, 100ppm

4 Ascorbic acid: used in the concentration of 25ppm, 50ppm, 75ppm, 100ppm

5 Nicotinic acid: used in the concentration of 25ppm, 50ppm, 75ppm, 100ppm

6 Folic acid: used in the concentration of 10ppm, 20ppm, 30ppm, 40ppm

7 Biotin: used in the concentration of 5ppm, 10ppn, 15ppm, 20ppm

8 Inositol: used in the concentration of 50ppm, 100ppm, 150ppm, 200ppm

9 Vitamin B12: used in the concentration of 10ppm, 20ppm, 30ppm, 40ppm

10 Niacin: used in the concentration of 10ppm, 20ppm, 30ppm, 40ppm.

For this experiment double distilled water was used and 5g/l activated charcoal was added in a round bottom flask and brought to boil, after which it was filtered and KNO_3 3.5 g, KH_2PO_4 1.75 g, $Mg_So4.7H_2O$ 0.75 g and volume was made 1000ml (double distilled activated charcoal water) then different vitamins according to their different concentrations were made and duly labeled. 25ml of this basal medium was taken in 150ml Erlenmeyer flask. The experiment was set up in triplicates. These flasks were autoclaved and cooled and under pre-sterilized conditions they were inoculated with the three different *Fusaria*, After which they were allowed to grow under a temperature of ± 25 degree for 15 days, After fifteen days the fungal mats were harvested, they were filtered on pre - weighed whatman filter paper, the filtrate was checked to see if there was any difference in the initial pH and the final pH. Also sporulation was examined if any. After filtration the fungal mats were kept at 60 ºC overnight in an oven to dry for 24 hours. Then it was removed and kept in the dessicator for cooling and final dry weight was recorded.

Another experiment contrary to this was set up which consisted of All vitamins -1 vitamin at a time was done to see the effect of the deducted vitamin on the fungus. The difference was calculated by deducting the initial weight of the filter paper from the final

weight of the filter paper and the mean was calculated. Also the sporulation rate was recorded. Along side a control experiment was also set in which none of the vitamins were added and the growth of the fungus was compared using that as standard.

Survey, Disease symptoms and pathogenicity

Castor belonging to the family *Euphorbiaceae*,It is one of the most important non- edible oilseed crop of arid and semi- arid regions of India. Castor oil finds its application in the manufacture of a wide range of ever expanding industrial products, such as nylon fibers, jet engine lubricants, hydraulic fluids, dyes, detergents, soaps, ointment, greases, paints, varnishes, cosmetics and perfumes, etc. (Pathak, 2003).

Castor is grown in tropical and subtropical climates; the major growing countries are India, China and Brazil. India. India occupies about 57% of the world castor acreage, but produces about 62% of world production. The major castor growing states in India are Gujarat, Andhra Pradesh, Tamil Nadu and Orissa. Productivity is highest in Gujarat state because more than 90% of the cultivated area is covered by castor hybrids under irrigation.

India is a major oilseed producer accounting for 6.7% of world production. Though oilseed production in India has more than doubled from 11 tones in 1985- 86 to 23.2 m tonnes in 2000-01, the productivity of 791 kg/ ha is one of the lowest in the world (Damodaran and Hegde, 2002). Lack of sustainability has been a major drawback in oil seed production. In this background, crop protection in a given agro- climatic situation gains importance. The loss due to biotic stresses in oil seed crops is about 19%. The reasons for low yields are primarily due to the cultivation of oil seed crops (76% area) under rain fed conditions, where diseases inflict severe losses, it is essential to know the causal agents, their behavior and means to attack the vulnerable phase.

Perhaps a resource poor oil seed grower would accept an ideal plant type resistant to many rather than to only one disease. Thus, the multiple disease resistance concept needs to be advocated by overcoming the one of single disease resistance. The multiline cultivar concept, so successfully propounded in cereals, with resistance to major

diseases, needs to be imported to major oilseed crops. Identification of sources of resistance to disease caused by *Macrophomina Phaseolina* in groundnut, sesame, sunflower, safflower and castor is required. Horizontal resistance to disease may be preferred for sustainability. There is also need to transfer resistance available in wild species *viz.*, resistance to rape seed – mustard or sunflower *Alternaria* blight from wild related species. Studies on biochemical and physiological basis of diseases resistance in oilseed crops *viz.*, disease or pathogenic stress physiology need to be taken up. There is need for prioritization of diseases in each crop and focus on specific problem rather than dissipated effort on multitude of diseases. Generation of adequate information on bio-ecology, population dynamics, mass multiplication and artificial maintenance techniques, race/ biotype pattern of pathogens, functional genomics of host pathogen interaction, rapid reliable screening and evaluation techniques against specific diseases as per priority needs to be addressed. For this purpose, there is need for creation of national facility for reference (type) cultures to speed up work on genetic enhancement. Immediate confirmation and utilization of sources of resistance or tolerance to diseases to design well thought out crossing programs targeted at resistance to diseases is required. Study of mechanisms and genetics of resistance to key diseases, wherever still unknown, needs attention. Exploitation of modern tools for screening of large number of germplasm lines and for transfer of resistance in background of cultivated and popular varieties should be taken up. Immunization of plants through induced resistance and investigation on molecular and biochemical basis of resistance could be another approach. National crop laboratories may be given specific responsibilities and be commensurately equipped to cater to the assigned task.

From the list of diseases that are known to be important in oil seed crops, it is clear how much of biotic stress the crops face under on farm situation. Many a time more

than one disease occurs on the same crop simultaneously. Sometimes the plant may be harboring the pathogen(s) but may not be infected. However that may also lead to poor yields due to latent stress caused by such deleterious microorganisms. So talking about integrated disease management (IDM) in oil seed crops may not be a productive idea. However, a systems approach in IDM needs to be considered for a better oilseed crop management. Through adaptive research at respective AICORPO centre's in frontline demonstrations, component wise and in whole package, the following measures may be combined for designing location specific packages depending on the demand of the locals.

Choosing the sowing time is very important as they affect the disease incidence in a big way. Deep summer ploughing or preferably soil solarization for six weeks during hot summer months using transparent polyethylene film, after flood irrigation along with use of disease resistant varieties and use of high quality seeds.

Seed treatment with biocontrol agents viz., *T. viride*, *G. virens* or botanicals like neem leaf and *Allium sativum* bulb extract (1% w/v) or carbendazim with Apron 35 SD (6g/kg) and 250 ppm streptomycin should be mandatory. There is a need for mixture of fungicides for avoiding resistance development in pathogens to fungicides. Uses of biocontrol agents are advantageous as they are often effective against a wide range of soil- borne pathogens. Moreover, they are ecofriendly, cost effective and their use avoids the risk of development of resistance in the pathogen towards the control agent.

A survey of different places of Vadodara district (Padra, Ranoli, Sama) was conducted in the months of November 2005, February 2006, December 2007,March 2006, November 2007, December 2005, October 2006 and April 2007. Similarly in Anand District Places visited were Ode, Sarsa, Umretha in the months of November

2005, February 2006, January 2007, November 2005, February 2006, January 2007 and November 2005, February 2006and January 2007. Similarly In Chhota Udepur fields were surveyed in the months of November 2005, March 2006 and December 2007. At every different site three fields each were visited and the total number of plants and their mortality was recorded and the results are summarized in Table:1. It was found that the highest mortality rate was recorded in Anand district in Ode in the month of February 2006, followed by Padra in November 2005, then in Sama April 2007, and Sarsa in the month of February 2006 whereas in Ranoli and Umretha in December 2005 and November 2005. Least mortality was recorded in the district of Chhota Udepur. On the other side largest number of plants (approximately) was recorded in Padra followed by Ranoli, Ode, Sama, Sarsa, Chhota Udepur and then Umretha.

Table 1: Occurrence of mortality of castor plants observed in different field survey

Places visited	Month of survey	Fields visited	Approximate no. of plants	Mortality of plants
Vadodara district Padra	Nov 2005	3	8000	18
	Feb 2006		1500	9
	Dec 2007		1500	11
Ranoli	Dec 2005	3	4000	12
	March 2006		1321	9
	Nov 2007		2752	11
Sama	Dec 2005	3	1900	8
	Oct 2006		936	8
	April 2007		1456	15
Anand district Ode	Nov 2005	3	1210	11
	Feb 2006		5200	22
	Jan 2007		1200	9
Sarsa	Nov 2005		1210	9
	Feb 2006		1408	13
	Jan 2007		1652	13
Umretha	Nov 2005		1100	9
	Feb 2006		900	12
	Jan 2007		1215	13
Chhota Udepur	Nov 2005	3	198	7
	March 2006		234	7
	Dec 2007		1456	11

Also serial soil dilution tests of the fields of the rhizospheric and non-rhizospheric soils revealed the presence of various soil fungi like *F .oxysporum*, *F. solani*, *A. flavus, Colletotrichum* sp., *Chaetomium* sp., *Rhizopus* sp., *Penicillium* sp. and T*richoderma* sp.results summarized in table :2. The occurrence of these fungi was higher in The rhizospheric soil as compared to the non rhizospheric soil. As wilt being the most destructive disease not only in case of castor but also pigeon pea (*Cajanus cajan* (L.) Mill (sp). In the absence of a suitable host , a soil borne plant pathogen like *F. udum* may perennate in soil in competition with other soil microorganisms.(Mukhopadhyay and Sen Gupta, 1991)

Table: 2 Showing Rhizospheric soil fungi in different areas surveyed.

No.	Fungi	Padra	Ranoli	Sama	Ode	Sarsa	Umretha	Chhota Udepur
1	*A. flavus*	3	7	6	8	10	9	3
2	*Chaetomium*	5	3	7	5	3	4	5
3	*Colletotrichum*	9	2	3	4	6	4	4
4	*F. oxysporum*	11	10	4	6	7	8	3
5	*F. solani*	6	12	8	6	7	4	6
6	*Penicillium*	8	3	4	6	5	8	4
7	*Rhizopus*	4	3	1	2	1	4	2
8	*Trichoderma*	6	6	9	4	7	5	8

Pathogenicity test

Ricinus communis or castor is an important oilseed crop of India occupying about six lakh ha primarily in Gujarat and Andhra Pradesh. Wilt complex is one of the major constraints in castor cultivation. Though several organisms have been intercepted from wilt affected castor plants, only recently the primary agents for causing the syndrome viz., *F. oxysporum F.sp. ricini* and or *Rotylenchus reniformis* have been sorted out (Chattopadhyay *et al*., 1996; Chattopadhyay and Reddy, 1995). Some of the varieties (Jwala 48-1) and hybrids (DCH-32) are tolerant to the disease. But field situations with complex etiology present a major hurdle for the farmer in castor culture (Chattopadhyay and Varaprasad, 2002). Seed treatment with carbendazim (0.1% a.i.) and soil application of carbofuran at 3 kg a.i. /ha may be useful. Utility of soil solarization in reclaiming affected soils in seed production plots need to be explored. Wilt complex of castor could be managed in field trials by along with reduction in soil population of *F. oxysporum* and *R. reniformis* besides growth promotion effects and seed yield increment compared to control (Chattopadhyay and Varaprasad, 2001). Gray rot caused by *Botrytis ricini* is another recent problem in the Indian castor scenario. It is related to occurrence of rainfall, cloudy and windy weather for two to three days. Specific epidemiological studies need to be conducted to ascertain the exact conditions favoring the disease to enable the farmers undertake timely control measures. The pathogen is otherwise known to be a weak one. Well directed studies can lead to identification of tolerant sources and framing of sound control strategies.

An experiment was conducted to find out percentage germination and mortality of castor seedlings after 45 days. It is evident from data in table 3. 100% germination was recorded on castor seeds in case of three varieties in control and *F. roseum*. GCH4 and local when placed in sick soil containing *F. oxysporum* and varieties AVANI 41 and,

local when placed in sick soil containing *F. oxysporum* and varieties AVANI 41 and Local when placed in sick plot containing *F. solani*. Percentage mortality was about 80% in case of three varieties each having *F. solani* and *F. roseum.* However in variety GCH4 and LOCAL percentage mortality reduced to 65%.

Above results are indicative of, the three *Fusarium* used in the experiment. *F. oxysporum* showed more pathogenic potential as compared to other two species of *Fusarium.*

Table 3: Mortality rate and percentage seed germination of 3 different varieties of castor plants in different *Fusarium* inoculated pots

Fungus	Seed variety	Germination %	Mortality rate	No. of plants survived
Fusarium oxysporum	GCH4	70	65	5
	AVANI 41	100	82	18
	LOCAL	73	64	9
Fusarium solani	GCH4	100	80	20
	AVANI 41	96	76	20
	LOCAL	90	78	12
Fusarium roseum	GCH4	100	78	22
	AVANI 41	100	78	22
	LOCAL	100	78	22
	CONTROL	98	0	100

AM fungi were incorporated in plots having *F. oxysporum* and *F. solani*. In comparison to sick plots the fresh and dry weight of AM inoculated plants was more as compared to seedlings grown on soil containing *F. oxysporum.* Almost similar results were obtained after 15 days in treatments with *F. solani.* After 60 days of growth seven plants showed wilting as compared to 11% in plants infected with *F. oxysporum* The percentage wilt was less (4% in case of *F. solani* + Am fungi, than 9% in case of *F. solani* treatment without incorporation of Am fungi. It is evident from table 4. that number of leaves increased in both the cases; the control plants as well as AM treated

plants. However the leaves were more in number when AM fungi was inoculated in castor plants growing with *F. oxysporum* (10 leaves) and *F. solani* (13 leaves). Percentage wilt incidence reduced and an increase in height and fresh and dry weight was recorded in presence of endophytic fungi.

Table 4: Pathogenicity test

	F. Oxysporum				***F. Oxysporum* + VAM**				***F. solani***				***F. solani*+VAM**			
Days	Fresh wt	Dry wt	Ht. (cm)	No. of lv	Fresh wt	Dry wt	Ht (cm)	No. of lv	Fresh wt	Dry wt	Ht (cm)	No. of lv	Fresh wt	Dry wt	Height (cm)	No. of lv
15	2.331	0.241	5.4	2	2.474	1.581	11	4	2.56	1.214	5.5	2	3.261	0.987	6.6	4
	2.237	0.18	7.2	2	2.83	1.891	11.3	4	2.891	0.987	6.5	2	2.86	0.43	9	4
	1.681	0.13	6.8	2	2.232	0.987	13	4	2.76	1.68	7.5	2	2.965	0.453	11	4
30	2.73	0.83	15	5	2.12	0.11	21	7	1.157	0.38	17	4	2.567	0.101	23	6
	2.421	0.72	18	5	2.79	0.48	27	7	2.603	0.64	21	4	2.987	0.203	26	6
	2.768	0.768	20	6	2.225	0.615	35	8	2.631	0.78	23	4	3.2	0.21	38	6
45	4	0.448	36	6	4	0.448	53	10	15.2	2.316	34	6	11	1.408	62	10
	5	0.736	42	6	5	0.736	56	9	14.5	2.12	39.5	6	16	2.188	71	12
	3	0.521	39	5	3	0.521	62	9	10	1.511	41	6	20	2.893	78.5	10
60	7	1.155	43	7	7	1.155	71	10	6	0.987	44.5	15	15.5	2.676	83	11
	3	0.559	48	7	3	0.559	98	10	9	2.319	46.5	14	26	4.515	82.5	12
	4	0.74	53	7	4	0.74	110	11	9	1.411	52.5	12	17	2.773	102	11

Ht =Height, No. of lv = Number of leaves

Study of Seed borne diseases of castor

Discoloration: caused by *Aspergillus* and other fungi in seeds and kernels.

Deformation: The seeds may be deformed and viability is lost.

Seed abortion:

Reduction in germination percentage:

Mortality in nursery conditions.

Changes in nutritional compounds and production of toxins. National committee appointed by Government of India estimated food grain losses in this country at 9.3 percent which is increased to 10.0 percent according to current estimates (Dharam Vir, 2002). At this rate, loss has been estimated at about 20 million tones of food grains worth Rs. 9000 crores. Poor storage facilities add substantially to this loss in different parts of the country. Arya and Arya (2002) reported occurrence of fungi in 15-22 % cotyledons of cashew nut in Indian markets. The losses were up to 30% kernels in Nigeria. Recent survey (Cockerel and Rennie, 1995) has shown that diseases such as bunt and loose smut of wheat are present at low levels in a high proportion of seed although seed borne diseases are at low levels but the cost are substantial with cereal growers in the U.K paying an estimated 23 millions per annum for fungicide treatment. U.S.A is the largest producer of soybean (*Glycine max* (L.) *Merr*.) Presently it covers more than 6 million hectares in India. Normally, soybean has to be stored for about 8 to 9 months from its harvest till sowing, but to maintain 70% germination of its seed is difficult. Effect of environmental conditions is one factor while association of seed borne bacteria and viruses is another (Sinclair, 1982). Sinclair, (1982) listed more than 30 seed borne fungi, which infect soybean seed during its formation and ripening stage. *Phomopsis, Cercospora* and *Colletotrichum* are few of these. Studies conducted revealed that 9 different fungi were associated from one year old seeds of 4 different varieties of pigeon

pea (Arya and Mathew, 1991). While 7 different fungi, were associated with freshly harvested seeds.

So far, thirteen species of *Fusarium* colonizing sorghum grain (Sorghum bicolor (L.) Moench have been reported from different parts of the world (Leslie, 2000, Marasas *et al* 2001). Shabbir and Rajasab (2004) reported 8 spp. of *Fusarium* on different regions of seed. Some of these strains are toxigenic producing fumosin group of toxins. The General Agreement of Tariffs and Trade (GATT) negotiations of Uruguay Round successfully persuaded Japan to open ita rice market to foreign supplies and it is supposed to import approximately 10 million tonnes of rice per annum. Thus offers an excellent market opportunity for Indian exporter to enter into Japanese market for the supply of japonica varieties. Koshihikari, a highly popular cultivar generally posess 15-17% amylase content when sown in Japan. If 1 or 2% amylase content was reduced it would be more preferable to the consumers.

A large number of fungal pathogens are transmitted through seeds and vegetative propagating parts. Some of the fungal pathogens transmitted through seeds and vegetative propagating parts. Some of the fungal pathogens transmitted in this way are many cereal smuts. Leaf blight of wheat caused by *Alternaria triticina,* leaf stripe and seedling blight of oats (*Helminthosporium avenae*), leaf stripe of barley (*H. gramineum)* anthracnose of French and summer beans (*Colletotrichum lindemuthianum*), red rot of sugarcane (*C. falcatum*), blight of gram (*Ascochyta rabiei*), ergot of rye (C*laviceps purpurea*). These are some examples where the pathogens are transmitted on the seed surface, inside the seed coat or deeper in the embryo. Incase of *Ustilago segatum*, recent researches have been done on floral infection in the field on the pathway of infection of the seed. It has been found that penetration usually takes place through the ovary wall and not via the stigma as previously supposed (Mehrotra and Aggarwal,2003).The

hyphae cross the cells of pericarp enter the testa (seed coat) and then move towards the ovary. After reaching the ovary the hyphae turn sharply from the testa, enter the scutellum and pass into the growing point of the embryo. Mycelium is intracellular with the pericarp and testa but it becomes intercellular with the tissues of the embryo. There is apparently no effect on grain development and it is not possible from external appearance to identify that the seed is infected.

Physiological alterations or effects in seed: Metabolic products of seed-borne microorganisms may affect the seed itself or sometimes may have other serious consequences such as toxicity to animals and human beings (*Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. Moreover, as it is previously mentioned seed fungi are classified as field and storage fungi. Genera such as *Alternaria, Cladosporium, Fusarium* and *Bipolaris*, invade seeds as they are developing on the plants in the field or after they have matured, but before they are harvested, and, for this reason, they have been designated "field fungi". These fungi require moisture content in equilibrium with a relative humidity of more than 90% to grow and usually do not continue to grow in grains after harvest, since grains and seeds are stored with moisture contents below those required by the field fungi.

The "storage fungi" comprise, mainly, several species of *Aspergillus s*pp. *Penicillium* are encountered at times, usually in lots of grain storage, fungi do not invade grains to any appreciable degree or extent before harvest (Christensen and Kaufmann, 1965).

Seed treatment is the oldest practice in plant protection. Treatment of seeds may be curative but should also protect the seeds from attack by organisms that are present in the soil. It is an affordable and effective way to optimize seed germination, plant establishment, early growth and yield potential.

Seed treatments can be classified as physical, biological or chemical (McGee, 1995). Regardless of type, successful seed treatment practices must satisfy the following biological requirements:

1) Consistently effective

2) Safe to operators during handling and planting

3) Wide safety margin between diseases harmful to the pathogen and that to plants

4) Safe to wild life

5) Compatible with other materials used on seeds

6) Chemical or biological methods should have desirable qualities with respect to application and retention on the seeds.

Perhaps the most widely used seed disease control practice is treatment of seed with fungicides. Chemical application of seed treatment is the traditional one of protecting the germinating seedling against seed and soil- borne fungi in the period immediately after planting (McGee, 1995). Fungicides amount used accounted for 10% of total world market pesticide which represents more than 500 million pounds of active ingredients released to the environment per year (USDA/ NASS, 2011). Among Pesticides used to protect, fungicides were perceived until recently as relatively safe. However, a 1986 National Academy of Sciences (NAS) report on pesticides residues on food indicated that fungicides pose more of a carcinogen risk than herbicides together. Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective. Moreover, the constraint economy of many more small scale farmers together with the problems of health risks and environmental pollution owing to misuse of chemicals, provide strong arguments for carrying out studies on alternative methods. Improved methods for disease management are the most productive step to make the best use of a limited number of fungicides. They have potential roles in strategies for disease

management and can be further exploited. Present activities to find both natural and synthetic fungicides focus on finding compounds that are safe to humans and environment. Among them several plant derived substances have biocidal properties and are considered chemically safe, more readily degradable in the environment by microorganisms and photodecomposition. Recent studies have shown that especially vapor extracted essential oils of many different tropical and temperate plant species are highly potent biocides that in very low efficiency as synthetic anti - microbial compounds (Amadioha,2000;Pandey *et al. 1982)* and as such they can be applied as surface disinfectants or fumigants (Mishra *et al*; 1989; Paster *et al;* 1998).

Mechanism of Disease Control

Any one more mechanisms may be operative in plants imparting them resistance against pathogens.

1) Physical alteration in plant body
2) Physiological changes
3) Biochemical mechanisms

(1) Physical Alteration in Plant Body

According to some scientists AM affects on soil borne plant pathogens on the basis of physical alterations. Lignification of cell wall and production of other polysaccharides has been reported to prevent penetration of mycorrhizal plants by *Fusarium oxysporum* (Dehne and Schonbeck, 1979) and *Phoma terrestris* (Becker, 1976). Mycorrhizal inoculation improves plant growth, Arya (2006) found better growth of neem seedlings after inoculation with three isolates of *G. fasciculatum*. It has also been suggested that a stronger vascular system of the mycorrhizal plants is likely to increase the flow of nutrients, impart greater mechanical strength and diminish the effect of vascular pathogens (Schonbeck, 1979). A few electron opaque structure resembling the deposits

were found in some cells and inter cellular spaces of non-infected mycorrhizal carrot roots but were absent in infected, non-mycorrhizal carrot roots. Restriction of pathogen growth together with an increase in hyphal alteration and accumulation of new plant products in mycorrhizal roots but absent in non-mycorrhizal roots shows that mycorrhizal infection is responsible at least in part for the plant defense system which provides protection against pathogen attack (Benhamon *et al.* 1994)

(2) Physiological Changes

AM fungi can interact directly with the pathogens through phenomenon like antagonism, antibiosis or predation. The studies conducted so far suggest that they indirectly affect host-pathogen relationship through physiological alteration or by competing for space or host resources. Through increased P nutrition, AM fungi enhance root growth, expand the absorptive capacity of the root system for nutrients and water and affect cellular processes in roots (Hussey and Roncadori, 1982; Reid, 1984; Smith and Gianinazzi, 1988). In addition to phosphorus, AM fungi are known to enhance uptake of Ca, Cu, S and Zn (Gerdemann, 1968; Sharma, 1990).*Glomus monosporum* was found effective against *Phytopthora capsici* in black pepper (Sivaprasad *et al*., 2006) they found resistance due to improved nutrient uptake. Host susceptibility to infection by the pathogen and tolerance to disease is influenced by the nutritional status of the host and fertility status of the soil (Wallace, 1973). For example, nematode damaged plants frequently show deficiencies of B, N, Fe, Mg and Zn (Good, 1968). High levels of P fertilization in the absence of AM fungi can interact with minor elements creating deficiency situation which predisposes plants to root knot nematodes (Smith *et al.*, 1986). AM fungi may, therefore, also increase host tolerance to pathogen by increasing uptake of essential nutrients other than P which are other wise deficient in non-mycorrhizal plants. Production of sidrophore can suppress the root pathogens (Sharma

and Johri, 2002). Higher levels of amino acids, especially arginine in combination with root exudates of mycorrhizal plant have been reported to reduce chlamydospore production of *Thielaviopsis basicola* (Baltruschat and Schoenbeck, 1975). Increased levels of phenyl alanine and serine have been observed in tomato roots inoculated with *G. fasciculatum*. High concentrations of orthodihydroxy (O-D) phenols in mycorrhizal suppressed the growth of *S. rolfsii* (Krishna and Bagyaraj, 1983; Goodman *et al.*, 1967). Presence of HCN precursors has been observed in rubber plant infected with *Glomus etunicatum* (Lieberei and Feldmann, 1990).

(3) Biochemical Mechanisms

Production of phytoalexins in AM containing plants has been conclusively demonstrated. Enhanced accumulation of glyceollin I, a highly antifungal phytoalexin has been reported in roots of mycorrhizal soybeans (Morandi *et al.*, 1984). According to Sharma and Johri (2002) it is not clearly understood how do AM fungi induce the productions of phytoalexins and elicitors? It may be a possibility that mycorrhizal fungi perturb root tissues so that the plant elicitors are liberated. Cell damage, which is closely associated with the production of isoflavanoids in legumes (Bailey, 1982) was rarely observed in the mycorrhizal soyabean roots. The concentration of coumenstrol increased in mycorrhizal roots (25μg g^{-1}) and was much greater than that of glyceollin I (Morandi *et al.*, 1984) coumestrol inhibits growth of bacteria and nematodes. According to Chakraborty *et al.*, (2005) induction of disease resistance in pea plants against charcoal stump rot wee associated with accumulation of defense enzymes followed by stimulation of antifungal phenolics.

Blotter and Agar plate method

Storage Fungi

Prolonged and better seed storage is an important aspect of any sound seed programme. Since most of the leguminous trees have hard seed coat. These seeds can be successfully stored at low or sub freezing temperature for long periods. Stored seeds are of little value, if on sowing they fail to produce healthy and vigorous plants. Careful storage to maximize seed vigor and viability is more important than ever. Seed storage is a fundamental component of systems and policies to preserve genetic diversity (Cohen *et al*., 1991).

The relationship between the storage environment and influence of packing over germination and loss of seed viability has been analyzed in detail by Popovska *et al*., (1981). Seed viability is best retained at low temperature and high concentration of CO_2. (Hadidi, 1996). Paul (2002) studied the biodeterioration of French bean (*Phaseolus vulgaris* L.) seeds and found that seed borne fungi belonging to fungi imperfecti group cause deformation and loss in viability of seeds. Production of mycotoxins by fungi in stored grains including legumes have been studied by Nandi *et al.* (1982) and Chandra and Sarbhoy (1997). They found spoilage of grains during storage for two years. Temperature and moisture are two important factors responsible for loss in germination. Loss in viability has been recorded in soybean by Khattra *et al*., (1998), Verma *et al.*, (2003) suggested not to store seeds of Indian mustard for more than two years as it may affect the productivity of crop. On low moisture content and at low temperature storage of *Cinnamomum camphora* seeds showed 60% germination after twelve months (Chien 1999). The potential storage life of seeds varied from species to species (Harrington, 1972; Agarwal, 1980) and within species, from variety to variety (Agarwal, 1977, 1979) under the same storage condition.

Seed borne fungi affect the seed quality adversely. *Aspergillus spp., Alternaria sp. Cladosporium herbarum, Fusarium. sp, Penicillium frequentence, Phoma linga and, P. nebulosa* caused reduction in oil content on artificial inoculation of seeds (Rai and Saxena 1980 and Shivpuri *et al* 1990). Kadian and Saharan (1983) reported decrease in oil content with the increase in disease intensity. Sharma *et al* (1994) reported seed borne pathogens of oil seed crops in India and their effects.

Seed health ensures better crop in the field. Seed storage is a serious problem in tropical countries where high temperature and high humidity reduce the vigor and viability of seeds. The problems become more acute due to frequent occurrence of draught / flood /disease problems. Oil seeds are generally poor storer as compared to cereals and pulses working with cotton and sunflower. It was found seeds having 65 percent germination the best for invigoration and field performances. With the age reduction in germination percentage, germination of seeds of three different varieties were analyzed (in treated and untreated condition) by blotter and Agar plate methods. One of the draw backs of Agar plate method is over growth of fast growing saprophytes such as species of *Aspergillus*, *Mucor and Rhizopus* over other slow growing fungal species, which were masked (Gowda and Sullia 1987). The percentage incidence of dominant mycoflora was calculated and is recorded in table 4, 5 and 6.

It is evident from table 4 that out of the nineteen different fungal organisms obtained from the three different varieties, untreated seeds of varieties GCH4, Avani 41 and treated seeds of varieties Avani 41 and local produced eight different types of fungal colonies. With Blotter method least number of fungal organisms were observed in treated seeds of variety GCH4 as shown in Table 7.

Fungus *Aspergillus awamorii* was present in untreated seeds only. Care of seed for agricultural crops is documented in 'Vraksh Ayurveda.' Treated the seeds with ash or

seed pelleting with jaggery is suggested for leguminous crops like pea, gram or pigeonpea. It may be associated as surface fungi on seeds of castor. Contrary to it most of the fungi were present in both the treatments. However their percentage occurrence varied. Deuteromycetous fungus *Trichoderma viride* was found associated with Avani 41 and local varieties of castor seeds after treatment of seeds with NaOCl.

It is evident from table 4, 5 & 6 that total 19 different fungal organisms were found associated with three different varieties of castor, of these two belonged to Zygomycetes, seven were of Ascomycetes and nine of Deuteromycetes, Black and white non sporulating unidentified fungi were also recorded. Total number of colonies in PDA medium were more as compared to Czapek's and Asthana and Hawker's media. Addition of sodium chloride in the medium helps to provide the conditions for growth of those fungi which are able to survive at low moisture concentration. Water is required by fungi not only as a metabolite and solvent but also to maintain sufficient hydrostatic pressure within hyphae to drive their apical extension. Growth of terrestrial fungi is dependent not on the absolute amount of water present but on water availability. Most fungi are restricted to water potential exceeding 60 to 80 bar (Griffin 1972) Ascomycotina spp. *Monascus bisporus* together with some species within the *Aspergillus glaucus* group are unable to grow at higher water potential and have exceptionally low minima. These fungi are characteristic of dried plant material.

Presence of *Mucor hemalis* was recorded in control sets. After treatment with one percent NaOCl seeds of none of the three varieties showed any presence of fungi. Contrary to it, *Rhizopus stolonifer* was recorded in both the cases. However, the percentage incidence decreased. The number of colonies in case of *A. awamorii* and *A .candida* reduced. *A. awamorii* showed growth in Asthana and Hawker's (A) medium, while *A. candida* showed growth in P.D.A medium contrary to it the untreated seeds of

GCH4 variety showed 5 percent occurrence in Asthana and Hawker's (A) medium, while it increased to 60 percent occurrence in Asthana and Hawker's (M) medium. *F. solani* and *F. oxysporum* showed their growth in treated seeds only. Arya and Chauhan (1995) reported percentage occurrence of fresh and two year old seeds of six different varieties of chick pea (*Cicer* sp.). Jain and Patel (1969) isolated spp. of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Helminthosporium, Hormicium, Memnoniella, Nigrospora, Penicillium, Stachybotrys* and *Stemphylium* . Vaidihi *et al*. (1984) recorded occurrence of 12 fungi on seeds, which included *A. ricini, Aspergillus sp. Mucor varians, Rhizopus nigricans* and *Trichoderma viride*. *Aspergillus sp and Fusarium sp*. caused seed rot, while *Alternaria sp.* including *A. ricini* produced leaf spots. (Jain and Patel (1969)) Saharan and Chand (1988) mentioned that *Alternaria* blight by *A. ricini* may result in the destruction of 50 percent flowers, *Cercospora ricini* causing cercospora leaf spot affects leaves and pods and in severely infected plants, seeds turn black and shriveled (Saharan and Chand 1988). Other seed-borne fungi causing diseases are *Cladosporuim oxysporum* (Capsule rot). *Fusarium oxysporum* fsp. *ricini* (*Fusarial* wilt), *Macrophomina phaseolina* (charcoal rot) (Aulakh, 1970).

Jain and Patel (1969) recommended shell seed dresser for eliminating the mycoflora except *Cladosporium* which can be controlled by pesticides like Harvasan and Thiram. Vaidihi *et al* (1984) considered *A. flavus, A. niger, Mucor varians, R. nigricans* and *Trichoderma viride* inhibitory to *A. ricini*. Topsin M.30 was effective against *Rhizoctonia bataticola* at 1000 ppm (Sarwar and Raju 1985).

Table 5: Percentage Occurrence of seed mycoflora of 3 varieties of *Ricinus communis* in different culture media (Treated/Untreated)

	Fungus Isolated	Untreated (Control)															Treated (1% NaOcl)													
		GCH4					AVANI 41					LOCAL					GCH4					AVANI 41					LOCAL				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
	Zygomycetes																														
1	*R. stolonifer*	-	13	2	-		25	-	22	-	-	-	15	-	-	45	-	20	-	-	1	-	-	-	-	-	-	3	-	-	-
2	*Mucor hiemalis*	30	-	-	-		-	14	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ascomycetes																														
3	*A. awamorii*	-	-	-	-	-	-	-	-	9	23	-	-	-	-	-	10	-	-	-	-	-	14	-	-	-	-	-	-	-	-
4	*A. fumigatus*	18	-	15	29	-	-	-	-	24	2	-	-	-	-	-	-	-	-	16	-	14	-	-	6	3	-	20	-	-	-
5	*A. flavus*	16	26	-	16	17	23	26	22	-	-	-	18	46	-	-	20	-	-	6	8	-	-	18	-	-	16	10	8	-	-
6	*A. niger*	20	5	-	-	11	-	20	13	14	22	30	16	40	14	-	18	60	-	-	10	-	-	-	4	20	-	-	4	8	-
7	*A. ochraceous*	-	-	-	14	-	-	-	-	-	18	-	-	-	14	-	-	-	-	12	-	-	60	-	-	-	-	-	-	11	-
8	*Chaetomium*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	-	-	-	14	-	-	-	-
9	*Penicillium citrinum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-
10	*Monilia sitophila*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
11	*Rhizoctonia solani*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	2	-	-	-	-
	Deutromycetes																														
12	*A. alternata*	-	-	-	21	22	-	-	-	27	-	-	-	24	11	-	6	-	13	11	-	15	6	12	3	-	-	-	18	3	14
13	*A. candida*	-	-	-	-	12	-	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
14	*C. lunata*	-	-	-	12	-	-	-	-	-	-	14	-	-	26	-	-	-	-	-	-	10	-	12	-	-	-	-	-	12	-
15	*F. oxysporum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	3	12	-	10	-	2	-	-	45
16	*F. roseum*	-	-	22	-	-	-	-	-	-	12	12	-	-	10	-	3	-	12	-	-	-	-	-	-	8	20	3	-	8	-
17	*F. solani*	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-	-	-	12	-	12	12	-	-	-	-	-	-	-	-	-
18	*Mycelia sterilia*	-	-	-	-	-	10	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-
19	*T. viride*	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6: Percentage Occurrence of untreated seed mycoflora of *Ricinus communis* in PDA, A&H, Czapek's, 1% & 5% Nacl

Fungi	GCH4					AVANI 41					LOCAL				
	PDA	A&H (M)	1% Nacl	5% Nacl	czapek's	PDA	A&H (M)	1% Nacl	5% Nacl	czapek's	PDA	A&H (M)	1% Nacl	5% Nacl	czapek's
Zygomycetes															
R. stolonifer	-	13	2	-		25	-	22	-	-	-	15	-	-	45
Mucor hiemalis	30	-	-	-		-	14	-	-	-	-	-	14	-	-
Ascomycetes															
A. alternata	-	-	-	21	22	-	-	-	27	-	-	-	24	11	-
A. awamorii	-	-	-	-	-	-	-	-	9	23	-	-	-	-	-
A. candida	-	-	-	-	12	-	-	-	-	-	20	-	-	-	-
A. fumigatus	18	-	15	29	-	-	-	-	24	2	-	-	-	-	-
A. flavus	16	26	-	16	17	23	26	22	-	-	-	18	46	-	-
A. niger	20	5	-	-	11	-	20	13	14	22	30	16	40	14	-
A. ochraceous	-	-	-	14	-	-	-	-	-	18	-	-	-	14	-
Chaetomium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. lunata	-	-	-	12	-	-	-	-	-	-	14	-	-	26	-
Penicilliun	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monilia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18
Rhizoctonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deutromycetes															
F. Oxysporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F.roseum	-	-	22	-	-	-	-	-	-	12	12	-	-	10	-
F. solani	-	-	-	-	-	-	-	13	-	-	-	-	-	-	-
Sterile mycelia	-	-	-	-	-	10	-	-	-	-	-	-	-	-	10
T. viridii	-	-	-	-	-	-	14	-	-	-	-	-	-	-	-

Table 7: Percentage Occurrence of treated seed mycoflora of *Ricinus communis* in PDA, A&H, Czapek's, 1% & 5% Nacl

Fungi	GCH4					AVANI 41					LOCAL				
	PDA	A&H (M)	1% Nacl	5% Nacl	czapek's	PDA	A&H (M)	1% Nacl	5% Nacl	Czapek's	PDA	A&H (M)	1% Nacl	5% Nacl	czapek's
Zygomycetes															
R. stolonifer	-	20	-	-	1	-	-	-	-	-	-	3	-	-	-
Mucor hiemalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ascomycetes															
A. alternata	6	-	13	11	-	15	6	12	3	-	-	-	18	3	14
A. awamorii	10	-	-	-	-	-	14	-	-	-	-	-	-	-	-
A. candida	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
A. fumigatus	-	-	-	16	-	14	-	-	6	3	-	20	-	-	-
A. flavus	20	-	-	6	8	-	-	18	-	-	16	10	8	-	-
A. niger	18	60	-	-	10	-	-	-	4	20	-	-	4	8	-
A. ochraceous	-	-	-	12	-	-	60	-	-	-	-	-	-	11	-
Chaetomium	-	-	-	-	-	-	20	-	-	-	14	-	-	-	-
C. lunata	-	-	-	-	-	10	-	12	-	-	-	-	-	12	-
Penicilliun	-	-	13	-	-	-	-	-	-	-	-	-	-	-	-
Monilia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13
Rhizoctonia	-	-	-	-	7	-	-	-	-	-	2	-	-	-	-
Deutromycetes															
F. oxysporum	-	10	-	-	-	-	3	12	-	10	-	2	-	-	45
F. roseum	3	-	12	-	-	-	-	-	-	8	20	3	-	8	-
F. solani	-	-	12	-	12	12	-	-	-	-	-	-	-	-	-
Sterile mycelia	-	-	-	-	-	-	-	-	-	-	-	-	14	-	-
T. viridii															

Table 8 : Percentage Occurrence of seed mycoflora of *Ricinus communis* in Blotter method

No.	Fung Isolated	Untreated (Control)			Treated (1% NaOCl)		
		GCH4	AVANI 41	LOCAL	GCH4	AVANI 41	LOCAL
	Phycomycetes						
1	*R. . stolonifer*	-	-	13	-	3	8
2	*Mucor*	10	22	3	-	-	4
	Ascomycetes						
3	*A. awamorii*	3	1	15	-	-	-
4	*A. candida*	1	-	5	-	-	-
5	*A. flavus*	40	16	-	3	7	4
6	*A. niger*	30	8	-	2	3	4
	Deutromycetes						
7	*A. alternata*	11	14	26	5	3	6
8	*C. lunata*	-	2	-	-	1	1
9	*F. roseum*	16	12	-	8	2	-
10	*F. solani*	20	10	6	-	1	1
11	*T. viride*	-	-	-	-	1	3

Study on storage Fungi of Castor

Seeds of three different varieties of castor were obtained from local markets in Baroda, since no proper storage methods are known for long term storage of castor an effort was made to study the occurrence of percentage seed mycoflora and their effect on percentage germination of seeds for 1 year. Samples were analyzed (three replicates each at an interval of four months. The seeds were stored at three different temperatures (5, 10, and 25ºC). The incubated seeds were examined under stereo binocular microscope for identification of fungal colonies on the seeds. It is evident from table 8 after incubation of seeds at four month at 5ºC in all the three conditions of storage in glass bottle reduced the chances of fungal infection. From table 8 it is clear that occurrence of fungi in polybag and paper bag resulted into more percentage germination than glass bottle. At 10ºC percentage germination in glass bottle was 70 % in GCH4, 72 % in Avani 41 and 90 % in local variety. When the seeds were stored in poly bag at 25ºC for 4 months the percentage germinations was 92 % in GCH4 and 94 % in local variety while

in case of Avani 41 better germination percentage (92) was observed in castor seeds placed in glass bottles.

Table 9: Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 4 months of incubation.

Storage temp	Container	Name of fungi	GCH 4		Avani 41		Local	
			%occurrence of fungi	Germination %	%occurrence of fungi	Germination %	%occurrence of fungi	Germination %
5 ^{o} c	Glass Bottle	*A. flavus* *A. niger* *F.solani* *A. candida*	1 2 4 -	60	1 4 4 -	60	1 - 8 1	50
	Paper Bag	*A. flavus* *A. niger* *A. candida* *R. stolonifer*	4 2 2 2	64	4 2 4 6	75	4 2 - 3	82
	Poly Bag	*A. flavus* *Chaetomium* *C.lunata* *R. stolonifer*	2 4 2 8	62	*2* - - *6*	70	*6* - - *2*	80
10^{o} c	Glass Bottle	*A. alternata* *A. fumigatus* *A. flavus* *A.. awamorii* *A .niger* *F. solani* *C. lunata*	- - 1 - - 6 1	70	3 2 1 1 4 4 -	72	10 6 - - 10 8 -	90
	Paper Bag	*A .flavus* *A .niger* *A .alternata* *C .lunata* *R. stolonifer* *F. roseum*	1 2 - 1 4 3	65	1 2 1 - 4 1	78	- 1 - - 4 -	86
	Poly bag	*A. flavus* *A. niger* *A. alternata* *C. lunata* *R. stolonifer* *Mucor* *White sterile mycelia*	7 4 3 1 6 - -	84	8 - - 12 3 8 -	72	*12* *17* - - - *8* -	86
25^{o} c	Glass Bottle	*A. flavus* *A. niger* *F.roseum*	4 3 1	70	- 1 1	92	- 1 1	86
	Paper Bag	*A. niger* *A. flavus* *F.roseum* *A. candida* *F.solani*	3 6 2 1 -	81	1 - - - 2	80	- - - -	88

	Poly bag	*A. flavus* *A. niger* *F.roseum*	1 3 6	92	1 1 3	86	1 1 2	94

It is evident from table 9 that after 8 months of storage at 5 to 10ºC the percentage germination reduced drastically in different set of storage conditions. Five different fungi were recovered by blotter method. Germination percentage was comparatively better in Avani 41 variety at 25ºC than the other two cases. Most common occurring fungi were species of *Aspergillus, Mucor,* and *Rhizopus,* Members like *Alternaria, Fusarium* were also present.

Table 10: Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 8 months of incubation.

Storage temp	Container	Name of fungi	GCH 4		Avani 41		Local	
			%occurrence of fungi	Germination %	%occurrence of fungi	Germination %	%occurrence of fungi	Germination %
5 ^{o} c	Glass Bottle	*A.flavus* *A.niger* *R. stolonifer*	4 2 10	14	6 5 15	20	11 4 6	11
	Paper Bag	*A .alternata* *A .flavus* *A .niger* *F .solani* *R .stolonifer*	1 11 6 1 26	11	3 - 14 - -	16	1 2 6 -	12
	Poly bag	*A .flavus* *A .niger* *R .stolonifer*	5 5 10	18	- - *8*	22	- - 10	26
10^{o} c	Glass Bottle	*A .flavus* *A .niger* *R .stolonifer*	11 8 4	14	8 - 2	16	11 4 6	16
	Paper Bag	*A .flavus* *A .niger* *R .stolonifer*	8 8 10	20	- 4 -	18	- 2 -	24
	Poly bag	*A .flavus* *A .niger* *R .stolonifer* *A .awamorii* *Mucor*	3 5 9 - -	17	*2* *5* *14* *4* *1*	21	6 1 - - -	30

25° c	Glass Bottle	*A .flavus* *A .niger* *A .alternata*	6 12 3	50	12 11 1	54	11 6 -	40
	Paper Bag	*A .flavus* *A .niger* *A .candida* *F .roseum* *R .stolonifer*	8 5 1 - 22	48	10 16 - - 18	52	4 14 - 1 30	39
	Poly bag	*A .flavus* *A .niger* *R .stolonifer* *A .candida*	8 10 16 1	53	- *12* *14* -	58	- 6 6 -	38

It is evident from table 10 that at 5, 10 and 25ºC the number of fungi occurring reduced as well as there was reduction in percentage germination of seeds. From the results it can be concluded that glass bottles were better for storage of GCH4 and local variety while it was not true for Avani 41 polybag method was better in this case.

Table 11: Percentage occurrence of fungi and seed germination at different temperature and seeds stored in glass container, paper bags and poly bags (Blotter method) after 12 months of incubation.

Storage temp	Container	Name of fungi	GCH 4		Avani 41		Local	
			%occurrence of fungi	Germination %	%occurrence of fungi	Germination %	%occurrence of fungi	Germination %
5 ° c	Glass Bottle	*A. flavus* *A. niger* *A. alternata* *C .lunata* *R.stolonifer*	6 1 3 1 3	12	4 6 1 - -	8	4 6 - 1 8	6
	Paper Bag	*A.flavus* *A.niger* *Mucor*	8 11 6	14	6 4 -	6	11 15 -	8
	Poly bag	*A.flavus* *A.niger* *A.alternata* *R.stolonifer* *Mucor*	4 6 3 12 15	8	14 3 4 8 25	8	16 8 4 6 10	8
10° c	Glass Bottle	*A.flavus* *A.niger* *A.alternata*	3 1 -	13	- 5 1	7	3 3 3 -	12 8
	Paper Bag	*A.flavus* *A.alternata* *F. roseum* *C .lunata* *A. niger*	1 - 1 -	8	- 1 - 1	9	- - - - 1	8

	Poly bag	*A. flavus* *A. niger* *F. roseum* *R. stolonifer*	5 5 - 10	10	*1* *2* *1* *1*	10	14 6 1 -	6
25° c	Glass Bottle	*A. flavus* *A .niger* *R. stolonifer*	11 17 8	10	- - 12	20	15 - -	38
	Paper Bag	*A. flavus* *A. niger* *F.solani* *R. stolonifer* *Sterile mycelia*	18 - 1 26 1	10	14 - - 17 8	18	14 - - 20 4	19
	Poly bag	*A. flavus* *A. niger* *A .ochraceous* *F. solani* *R. stolonifer*	5 5 - - -	14	20 17 - - 10	16	14 6 - 2 -	16

However at 4ºC, complete loss in seed viability was noticed, which might be due to the chilling sensitivity of seeds. Most of the tree species, which are sensitive to dessication cannot withstand chilling temperature and so lose viability quickly.

Rhizopus oryzae, sclerotinia sclerotiorum and Aspergillus parasiticus caused 68 to 78 percent inhibition in seed germination of sunflower. Similar observations were made by Jhamaria *et al*. (1974) who showed poor seed germination due to association of *Rhizoctonia*, *Rhizopus*, *Aspergillus*, *Alternaria* and *Curvularia* with the seeds.

The seed mycoflora studies indicated that species of *Fusarium*, *Alternaria*, *Stemphylium*, and *Aspergillus* were found to be associated with the seeds of all the varieties which got eliminated during storage and were replaced with a storage fungus, *Rhizopus spp.* in case of onion. (Sharma *et al*. 2002).

A classic is the discovery of aflatoxins, produced by *A. flavus* and *A. parasiticus*, identified as causal agents for turkey x disease in 1960 in U.K (Austwick, 1988). The resulting investigations were largely responsible for the development of an awareness of

how important fungi can be a toxin producer, especially in the light of the carcinogenicity of aflatoxins.

From the seed germination studies it has been concluded that seed germination decreases with an increase in the storage period of seed samples. This could happen due to the association of storage seed mycoflora with stored seeds. This finding was confirmed by earlier reports (Miles, 1961 Bhajbhuje 1989, Barve 1995, Ellis *et al*. 1996). The samples of wheat, barley, corn, sorghum and peas stored at different moisture contents and temperature showed favorable growth of storage fungi. But kept free of fungi, retained the germinability upto 95-100 % for some months. Whereas experiments planned with similar samples inoculated with storage fungi, were reduced to zero or near zero germinability (Christensen, 1965).

Seed treatment is the oldest practice in plant protection. Its origins can be traced to the 18^{th} century with use of brine for control of cereal smuts (Neergaard, 1979). The modern era of seed treatments began with the introduction of organo-mercury fungicides in 1912 which were widely used for several decades. The post-World War II period saw the development of new fungicide chemistry and the first use of seed treatment for insect control. Today, the most widely used application of seed treatment in the traditional one of protecting the germinating seedling against seed-and soil-borne fungi in the period immediately after planting (Mc Gee, 1995). Chemical fungicides can control the plant diseases, but they have bad effects on human health, plants and animals which is harmful to our environment. Besides using conventional seed treatment with synthetic fungicides to kill pathogens, is a practice not allowed in organic production. Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective.

Worldwide ecological awareness requires more natural foods and products which has influenced the improvement and utilization of integrated pest management. In this kind

of control, alternative methods are used to protect seeds to decrease the use of chemical products. Moreover, recent increases in the production and sale of organic seed has heightened the scrutiny of organic seed quality and in particular brought attention to concerns of seed-borne disease contamination.

Changes in Biochemical Contents after Pathogenesis

Lower productivity may result due to poor seed germination and inferior quality of seeds. The seed deterioration may occur due to infestation of fungi and bacteria.

Although seeds of *R. communis* are known to have a toxic substance Ricin, castor oil is termed as a vegetable based oil because it is made from the seeds of castor plant (*Ricinus communis*). Castor oil naturally biodegrades quickly, it is non- toxic and comes from a renewable energy source (plants).The castor seed oil contains unusual hydroxyl fatty acid ricinoleate (ricinoleic acid) and it is known to inhibit the growth of many viruses, bacteria, yeasts and molds. The United States Food and Drug Administration (FDA) has recognized medicinal castor oil and its derivatives are used to make many modern drugs for instance, anticancer drugs, antifungal drugs, heart and blood pressure drugs, HIV drugs, organ transplant drugs etc.

To study the effect of *F. oxysporum* (wilt causing pathogen) on seeds of castor different studies were undertaken like change in weight of seeds, oil extracted, pH, viscocity, acid value, saponification value, specific gravity and iodine value of healthy and diseased seeds were calculated. There was a remarkable difference in the dry weight of healthy and diseased seeds, Dry weight of seeds of variety AVANI 41 was found to be more than the diseased seeds of the same variety followed by local variety and GCH4. Where as diseased seeds yielded less oil in comparison to the healthy seeds. Also there was a drop in the pH levels from 6.9 to 4.5 in GCH4, 6.7 to 4.7 in AVANI 41 and 4 to 4.8 in Local variety. Acid value of GCH4 and AVANI 41decreased from 1.41 to 0.007 and 0.009, and of Local variety decreased from1.128 to 0.003. Also there was a remarkable change in the Saponification value, Specific gravity and Iodine values of seeds (infected) but viscosity of castor oil remained same in healthy and diseased seeds.

Table 12: Physico-chemical properties of Castor oil extracted from healthy and diseased seeds of *Ricinus communis*

	Seed variety	Dry wt seed	oil extracted (ml)	pH	Viscocity	Acid value	Saponi. value	Specific gravity	Iodine value
Healthy seeds	GCH4	49.61	35.33	6.9	900	1.41	221.5	1.728	88.1
	AVANI 41	56.68	21.83	6.7	900	1.41	221.5	2.32	76.8
	LOCAL	55.96	30.33	6.4	900	1.128	207.5	1.673	78.1
Diseased seeds	GCH4	33.73	25	4.5	900	0.007	227.2	0.518	89.9
	AVANI 41	34.76	27.83	4.7	900	0.009	227.2	0.946	78.9
	LOCAL	39	18.5	4.8	900	0.003	205	0.95	79.9

Control of seed borne fungi by plant extracts

To study the effect of antifungal property of five different plants *viz. Azadirachta* indica, C*ocoloba*, *Aegal marmelos, Cretava* and *Ashoka* were selected. Along with these plant extracts, Potato dextrose Agar medium (Normal 100%) and Potato dextrose Agar in the concentration of 10% and 25% was used. The plant extracts were taken in two different concentrations of 10% and 25% respectively. Readings were taken at an interval of 7 and 10 days respectively.

No growth was observed in normal PDA in *F. oxysporum* and *F. solani* where as *F. roseum* showed colony diameter of 3.7 in 7 days and 5.7 in 10 days. In 10% P.D.A *F. oxysporum* and *F. roseum* showed similar and more growth in 7 days as compared to *F. solani* in 10 days followed by *F. oxyspoum* and highest in *F. roseum*. In P.D.A 25% also similar results were observed.

Plant extract of *Azadirachta indica* (10%) was found to be most effective on *F. solani* followed by *F. roseum* and *F. oxysporum*, where as in 25% no growth was observed in *F. solani* and it, was found to be least effective on *F. roseum* followed by *F. oxysporum.* *Cocoloba* plant extract was found to be least effective on *F. roseum*, followed by *F. solani a*nd *F. oxysporum* in 10% and 25%. *Aegel marmelos* was found to be effective in concentration of 10% and 25% in *F .oxysporum, F. solani and F. roseum* in 7 days and least in 10 days in all three *Fusaria.* Ashoka extract was found to have very less impact on all the three *Fusaria.*

Table 13: Antifungal property of different plant extracts on three different F*usaria*

Treatment number	Percentage Concentrations	Conc.	*F. oxysporum* 7 7 Days	*F. oxysporum* 10 Days	*F. solani* 7 Days	*F. solani* 10Days	*F. roseum* 7Days	*F. roseum* 10 Days
1	Normal P.D.A		3.6	-	-	-	3.7	5.7
2	P.D.A	10	3.5	4.6	3.2	3.8	3.5	5.6
		25	2.2	6.4	2.8	3	3.6	4.5

3	*Azadirachta indica*	10	3.56	4.2	2	2.5	3.4	4.9
		25	2.5	3.29	-	-	4.6	7.7
4	*cocoloba*	10	1.9	5.2	3.4	5.2	4.7	7.5
		25	2	3.5	3.4	3.6	4.3	7.7
5	*Aegel marmelos*	10	3	4	3.2	3.85	4.2	6.5
		25	2	3.5	3.2	3.8	2.2	5.2
6	*cretava*	10	1.2	5.9	3.1	4.2	3.3	4.3
		25	3.2	4.7	2.2	3	1.8	4.3
7	*Ashoka*	10	3	4	3.5	4.7	3.9	5.9
		25	2.56	3.2	3.4	7.9	4.2	5.9

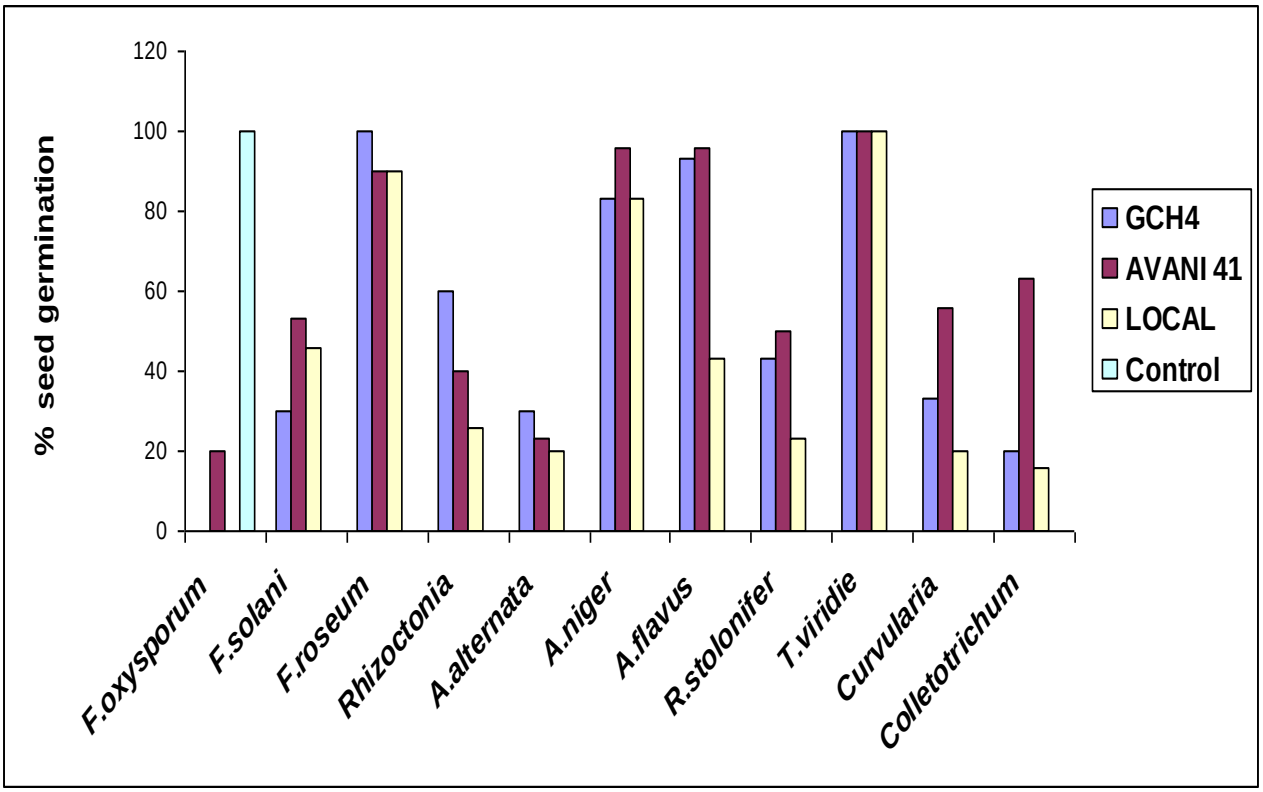
Effect of fungal culture filtrate on seed germination

To study the impact of fungal metabolites on the seed germination of *R. communis* seeds, in all eleven fungal culture filtrates were used. It was found that *F. oxysporum* was the most pathogenic fungi which inhibited seed germination in variety GCH4. Where as in local variety only one seed germinated as compared to control sets and in seed variety AVANI 41 only 20 seeds germinated. *Colletotrichum* sp. was also found to inhibit seed germination in Local variety as seed germination recorded was only 16%,in GCH4 it was 20%,as compared to AVANI 41 it was 63%. *F. solani* and *A. alternata* were found inhibiting seed germinating in GCH4 up to only 30%, In AVANI 41 *F. solani* was not so effective as seed germination was 53% and in local it was 46%. *F. roseum*, *Rhizoctonia* sp. *A. flavus*, A. *niger*, and *T. viride*, were not able to inhibit seed germination as the germination percentage was much higher in seeds soaked with these culture filtrates *i.e.* variety GCH4. The results are summarized in Table no

Table 14: Effect of different fungal metabolites on percentage seed germination

	Percentage seed germination		
Name of Fungus	GCH4	Avani 41	Local
Fusarium oxysporum	0	20	1
Fusarium solani	30	53	46
Fusarium roseum	100	90	90
Rhizoctonia	60	40	26
Alternaria alternata	30	23	20
Aspergillus flavus	83	96	83
Aspergillus niger	93	90	43
Rhizopus stolonifer	43	50	23
Trichoderma viridi	100	100	100
Curvularia lunata	33	56	20
Colletotrichum	20	63	16

Fig 1: Effect of different fungal metabolites on Percentage of seed germination



Effects of AM fungi on change in biomass of three varieties of castor

Gigaspora calospora exerted an inhibitory effect on the development of pigeon pea blight caused by *Phytophthora drechsleri* f. sp. *cajani* (Bisht *et al.,* 1985). Similarly, in Tamil Nadu Agricultural University, India, studies showed that another AM fungus *G. etunicatum* induced tolerance to cowpea (*Vigna unguiculata*) against *Macrophomina* root rot. Disease incidence was 16% in inoculated plants as against 33% in uninoculated plants (Ramraj *et al.,* 1988). Rosendhal (1985) observed decrease in disease incidence in peas due to *Aphanomyces euteiehes.* Similar results were observed for soyabean (Zambolim and Scheneck1983) and peanut (Abdalla and Abdel-Fattah, 2000) due to *Fusarium solani*. Krishna and Bagyaraj (1983) observed reduction in disease due to *M. phaseolina* in soyabean. Studies conducted at the University of Bayreuth, Germany, showed that in leachates of AM rhizospheric soil of *Zea mays* and *Trifolium subtrerrneum* fewer sporangia and zoospores were produced by *P. cinnamomi* as compared to non AM plants suggesting that sporangium induced microorganisms have declined or sporangium inhibitors have increased (Meyer and Linderman, 1986).

Pandey and Upadhyay (2000) studied the effect of microbial population in development of pigeon pea in Pusa, Bihar. Screening for resident antagonist was done and mode of myco-parasitism was studied. Dual inoculation with AM endophyte (*G. mosseae*) and *M. phseolina* significantly restricted the progression of the pathogen in the roots of mungbean (*Vigna radiata*). Disease incidence was reduced from 77.9% in pathogen inoculated to 13.3% in AM + Pathogen inoculated plants (Jalali *et al*., 1990). *G. fasciculatum* reduced the number of sclerotia produced by *S. rolfsii* in peanuts (*Arachis hypogaea*) (Krishna and Bagyaraj, 1983)

Long term sustainability in agriculture and forestry is possible only through the use of low cost farm grown inputs which work in harmony with nature. In contrast to

costly chemicals eco- friendly biofertilizers such as Arbuscular Mycorrhizal Fungi (AMF) certainly hold the key, for increasing plant productivity. An important component of natural ecosystem, fungi form mutualistic association with most plants and enhance plant growth by increased uptake of P, ZN, Cu, S, K and Ca (Cooper,1978; Powell,1979;chandra, 1992)

Arbuscular mycorrhizal fungi are obligate biotrophs and lack host specificity.However screening trials have shown their preference in colonization on a particular species. Efforts have been made to develop Mycorrhizal plant mutanta. Mercy *et al* (1990) found that AMF colonization is a heritable trait. This has opened up new possibilities of tailoring plant – fungus combination for maximum benefit. Some of the trees where AMF association has been reported from our country include Acacia catechu and *A. nilotica* (Chandra and Kehri,1991), *Dalbergia sissoo* (Bisen *et al*. 1996; Chakravarty and Misra,1986), *Grevilia pteridifolia* (Chandra *et al*; 1997), *Leucaena leucocephala* (Chandra and Kehri,1991); Manjunath *et al*.; 1984), *Morus alba* (Katiyar,1985), *Paosopis* 1998), *Tamarindus indica* (Reena and Bagyaraj, 1990a; Vijaya and Srivasuki, 1996) and *Zizyphus mauritiana* (Mathur and Vyas,1996). Association of Glomus spp. With neem roots as been observed by Mohan *et al*. (1995). The present paper deals with changes in roots and shoots biomass after artificial inoculation of *Glomus fasciculatum* in neem seedlings.

Plant communities generally are more diverse in tropical than temperate regions, a similar pattern is not predicted for glomalean fungal communities. Correlations between fungal and plant species richness in a given region are neither strong nor consistent. Moreover, trop. Pot cultures of soil samples from tropical regions (Costa Rica, Kenya, Mexico, Nicaragua) often produce fewer sporulating species than those from temperature regions (Morton). Those results suggest that the complexities of fungal

communities in temperate and tropical climates are likely to be similar. Sampling in tropical climates has been limited to individual localities and even then the number of surveys is low. Species richness in field collected samples was 28 in a tropical dry forest of Costa Rica (Johnson and Wedin 1997) and 41 and 17, respectively in *Terminalia* plantations of Cổte d' Ivoire (Wilson *et al*. 1992) and Cameroon (Mason *et al.* 1992). Insufficient data were available to calculate between diversity between *Terminalia* plantations. We would predict, however, that it would be close to the value obtained in temperature regions because of similar levels of species diversity (in at least two of the study areas).

Perhaps the largest obstacle to understanding the biology and ecology of AM fungi is our inability to culture them apart from their plant hosts. The plant provides carbon to the fungus largely via an arbuscule - plant cell plasmalemna interface. It also provides a protected site in root cells where the fungus can live. The external fungal hyphae improve phosphorus acquisition by the plant in soils with low levels of phosphorus levels exceed requirements of the host, however, the AM symbiosis may some times be inhibited. Under those conditions for certain host – soil interactions, mycorrhizal development can reduce plant growth and thus become pathogenic (Modjo and Hondrix 1986).

A mycorrhiza is initiated by hyphae that emanate from another mycorrhizal root or from germinating spores. As colonization spreads within the root cortex via hyphal extension and branching, other structures, such as 'vesicles' and 'auxiliary cells', develop. Both structures contain lipids and therefore are thought to have a function in carbon storage. Auxiliary cells are much trainstory than vesicles, so their storage functions may be tried closely to developmental events in fungal life cycles. Infectivity of different fungal propagules appears to vary among fungi at the sub ordinal level.

Mycorrhizal roots, detached hyphae, and spores of fungi in the suborder Glomineae generally are highly infective, whereas auxiliary cells and detached hyphae of fungi in the suborder Gigasporineae are not (Biermann and Linderman 1983). Generally, new sporulation commences between 4 and 8 weeks after onset of colonization and plateaus in 12-16 weeks in green house pot cultures (Morton *et al*. 1993). Under field conditions, however, sporulation patterns are more variable and fluctuate with season or host phenology (Gemma *et al*. 1989; An *et al*. 1993). Sporulation does not appear to occur in response to nutrient deprivation or environmental stress as in other fungi. When fungi are cultured on plant hosts in a pot containing sand, soil or some other growth medium Hence forth called pot cultures new sporulation generally commences several weeks after onset of colonization and plateaus in 12-16 weeks (Morton *et al*. 1993). Research currently suggests that spores begin to form after a thresh hold of mycorrhizal biomass is achieved (Gazey *et al.* 1992; Franke and Morton 1994) and sporulation increases dramatically after roots cease to grow but continue to function as a sink for carbon from photosynthesizing shoot tissue of the plant.

Phylogeny of arbuscular endomycorrhizal fungi based on morphological character of the mycelium and reproductive spores (Morton 1990, Morton and Benny 1990; Morton and Redecker 2001). Members of a small number of plant orders do not from AM associations (Tester *et al.,* 1987).

Many other orders, however, include both mycorrhizal and non-mycorrhizal families and genera. Non-mycorrhizal taxa are assumed to have evolved away from the symbiosis, based on evidence from their distributions, relative to those of their mycorrhizal relatives (Trappe 1987) and from the presence of activated defense mechanisms in chemically induced mycorrhiza resistant mutants (Peterson and Bradbury, 1995). A few plant genera are able to support both arbuscular fungi and

ectomycorrhizal fungi one of the most widely studied being *Eucalyptus* (Lapeyrie and Chilvers 1985).

To study the growth of three different varieties of castor an experiment was performed by incorporation of VAM consortium obtained from ACP industries (A.P). The results are depicted in table 14 and fig 2 & 3.

It is evident from table 14 that fresh weight of all the castor plants of varieties tested was more than untreated (control castor plants).The root and shoot dry weight also increased in treated plants. The increase in fresh and dry weight of the root and shoot was observed up to 90 days.

Fig no.3 shows shoot length of three different varieties in control and after incorporation of AM fungi. Local variety having AM fungi produced maximum growth after 90 days. It is evident from the graph that no further increase in shoot length was recorded after 75 days in control and after 60 days in treatment with AM fungi. It is evident from fig 2 that there was gradual increase in root length from 15 days to 90 days. The increase in root length was more as compared to control sets in all the three varieties of castor. As compared to control in three varieties of castor, Am incorporation showed almost 100% increase in root length.

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.

Table15: Growth responses on 3 varieties of *R. communis* by Am fungi

Variety	Days	Fresh wt(g)		Dry wt (g)		Shoot length (cm)	Root length (cm)	No. of leaves
		Shoot	Root	Shoot	Root			
Local (Control)	15	3.2	10	0.265	0.013	19.2	1.7	4
	30	7	1	0.642	0.056	23.7	7.2	6
	45	40	2.9	3.18	0.382	46.2	12.2	7
	60	47.5	3.25	4.45	0.747	61.5	17.2	7
	75	50	4.5	5.863	1.184	66.5	18.5	8
	90	75	4.75	9.994	1.262	83	18.7	8
Local +VAM	15	6.5	100	0.583	0.028	24.5	4.5	6
	30	27.5	2.3	3.699	0.371	37.5	14	8
	45	43	4.5	6.367	0.471	56	20.5	10
	60	1.5	5.2	8.935	1.511	126	27.5	12
	75	97.5	11.5	17.74	1.828	135.2	29.2	14
	90	217.5	8	18.171	1.803	163.7	34.5	16
Avani 41 (Control)	15	3	0.1	0.0336	0.0019	22.2	1.2	5
	30	4.5	0.35	0.0643	0.0098	23.7	4	7
	45	9.25	1.75	1.053	0.0158	27.2	4.2	8
	60	57	3.25	6.014	0.0866	67.5	5.1	8
	75	60	2.7	6.151	1.273	101.5	8.7	8
	90	63	4	7.405	1.191	73.5	11.7	8
Avani 41 VAM	15	3.65	400	0.4	0.019	21.7	3.2	5
	30	25	2.75	3.255	0.295	36.2	13.7	9
	45	57.5	4.75	8.319	0.645	62	19.2	11
	60	91.5	6.25	9.022	0.967	140	30	14
	75	99	7.5	10.448	1.585	86.7	35.5	14
	90	132.5	14	24.166	3.513	113.5	40	14
GCH 4 (Control)	15	2.9	100	0.204	0.016	20.2	1.7	4
	30	7	2.85	0.721	0.097	23.5	5	7
	45	27	3.25	2.752	0.357	38	17	8
	60	30.5	3.9	2.851	0.721	53	19.5	11
	75	41.5	7	3.86	0.733	72.5	19.9	12
	90	104.5	8.5	5.782	1.024	99.5	20.2	12
GCH4 + VAM	15	5	90	0.465	0.019	24.2	6	6
	30	27	2.5	2.934	0.332	47.7	11.7	9
	45	35	4.5	3.646	0.53	67.5	22.7	9
	60	64	5.4	5.351	1.024	76.7	35.0	9
	75	75	7	13.533	1.398	78.5	35.9	11
	90	170	12	20.783	2.028	110	41.3	14

Fig 2: Increase in root length of control and Am inoculated Castor plants

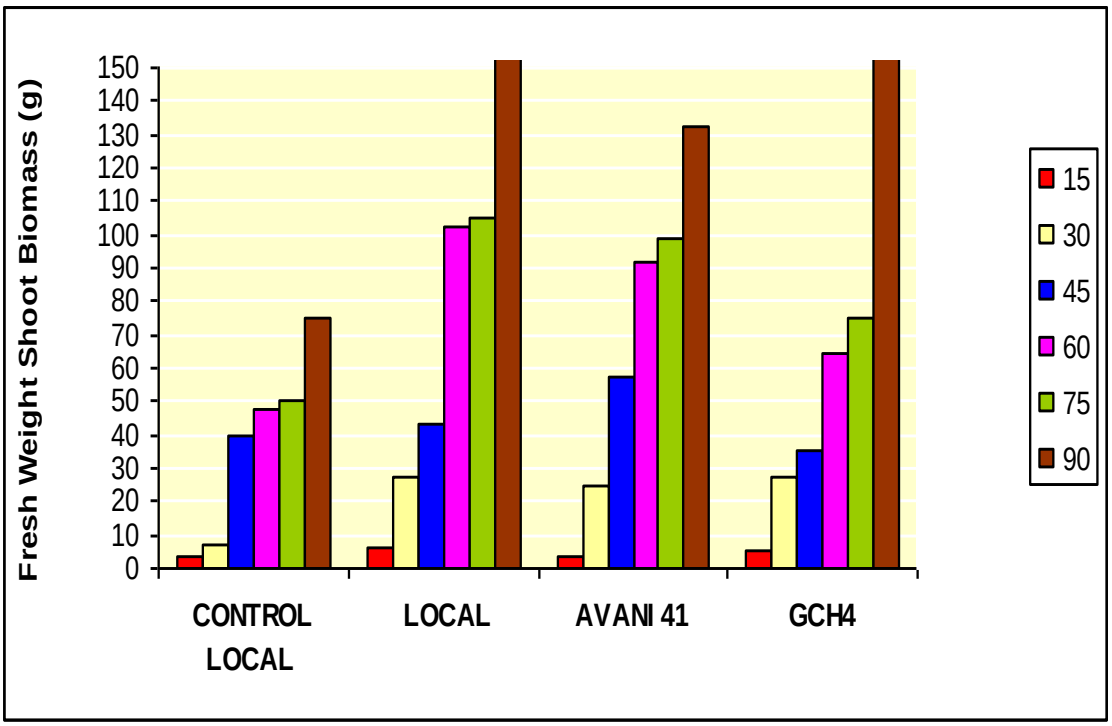


Fig 3: Showing Shoot length (cm) in control and AM inoculated plants

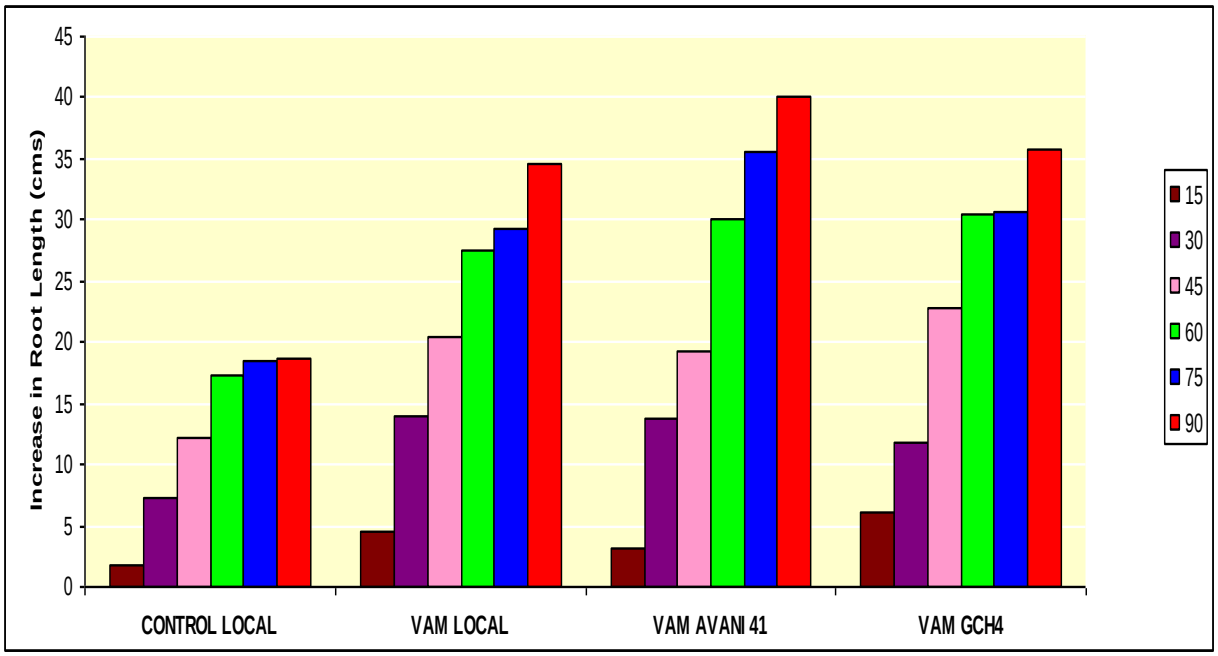
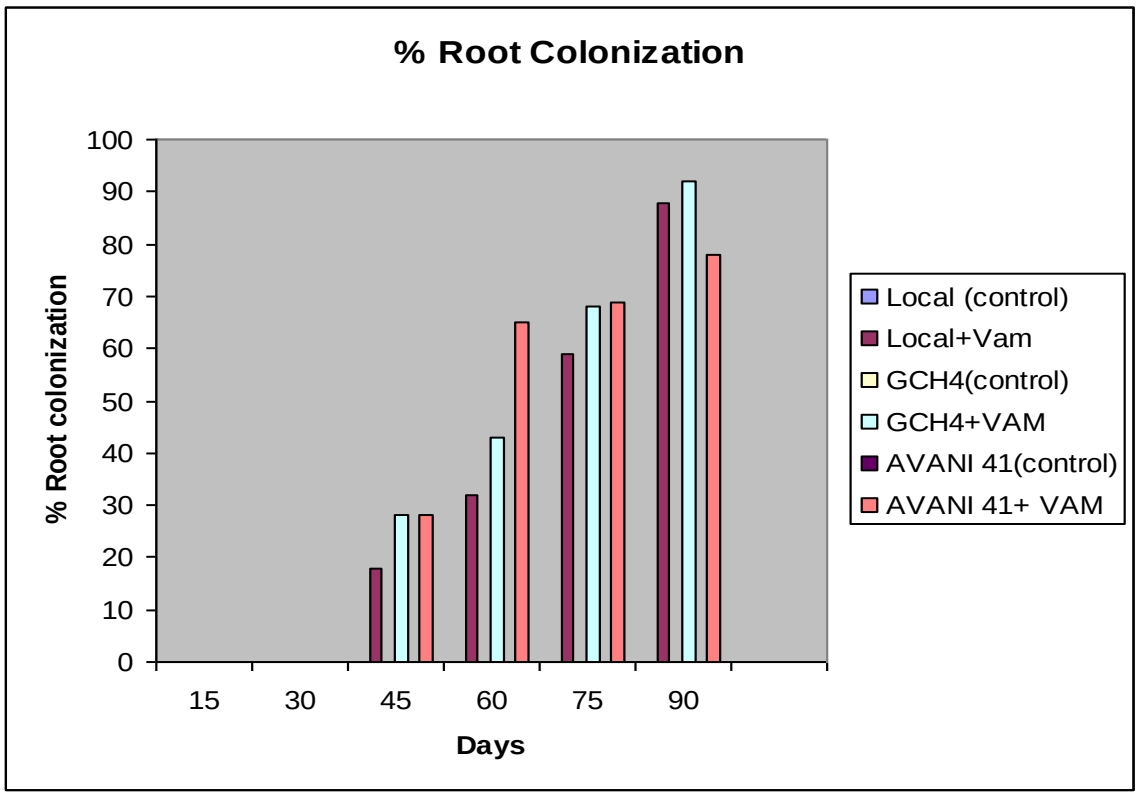


Fig 4: Percentage Root Colonization in 3 varieties of castor (Control and Treated)



Selection of a Suitable Culture Medium

All fungi are heterotrophs. For energy source they rely upon their surrounding environment. For growth of different fungi, elements like Carbon, Nitrogen, Potassium, Calcium, Magnesium, Iron, Manganese, Copper, Zinc etc. are required. Role of vitamins for healthy growth of fungi has been determined long back. Thiamine (Vitamin B1) was the first vitamin shown to be required by a filamentous fungus (Schopfer, 1934). Knowledge of nutrition of the fungi is very essential for culturing them in laboratory or for commercial use in an industry. Earlier natural plant materials were used for the culture of fungi in laboratories. Now a days semi synthetic and synthetic media are used to culture them in laboratories. There is no universal natural substrate or artificial medium upon which all fungi will grow (lilly and Barnett, 1951). Even closely related forms may differ considerably in their nutritional requirements qualitatively as well as quantitatively. The three *Fusarium* spp. under the present investigation were grown on five different culture media and their mycelial growth, sporulation and change in final pH was recorded, so that a suitable basal medium could be selected.

Fig 5 depicts the composition of five different media used in the present study and the results are summarized in Table 15. A perusal of table 15 and fig 6 shows that Richard's medium supported maximum growth of all the three *Fusarium* spp. Similar results were obtained by Mathew, (1993) for *F. udum* and *F. oxysporum*. The growth of *F. oxysporum* was best in Richard's followed by Modified Asthana and Hawker's medium, Potato Dextrose medium, Asthana and Hawker's medium 'A' and Czapek's medium.

Fig. 5: Composition of culture tried

Asthana and Hawker's medium 'A'	
D Glucose	5.0g
KNO_3	3.5g
KH_2PO4	1.75g
$MgSO_4.7H_20$	0.75g
Distilled water	1000ml

Modified Asthana and Hawkers medium 'A'	
D- Glucose	10.0g
KNO_3	3.50 g
KH_2PO_4	1.75 g
$MgSo_4$	0.75 g
Distilled water	1000ml

Potato Dextrose Broth medium	
Peeled Potato	200g
D Glucose	20g
Distilled water	1000ml

Czapek's medium	
Sucrose	30g
$NaNo_3$	2.0g
KH_2PO_4	1.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.1g
Distilled water	1000ml

Richard's medium	
Sucrose	50.0g
KNO_3	10.0g
KH_2PO_4	5.0g
$MgSO_4.7H_2O$	2.5g
$FeCl_3$	0.02g
Distilled water	1000ml

Although the growth was maximum in Richard's medium, excellent sporulation was produced in Modified Asthana and Hawker's medium 'A' and Potato Dextrose medium.

Final pH in all the cases shifted towards alkalinity. However in case of Czapek's medium final pH turned acidic.

Dry weight of *F. solani* was maximum in Richard's, followed by PDA, Asthana and Hawkers modified, Czapek's and Asthana and Hawker's 'A' medium. The sporulation was excellent in PDA and Richard's and fair incase of Asthana and Hawker's (Modified). Final pH in all the cases shifted towards neutral or alkaline side.

Average dry weight of *F. roseum* was maximum in case of Richard's as in other two *Fusaria,* Potato Dextrose broth was found to be second best followed by Asthana and Hawker's (M), Czapek's and Asthana and Hawker's medium. Only Potato Dextrose broth could support excellent sporulation. Like other two fungi a gradual shift towards neutral or basic side was recorded.

Table 16: Average dry weight (in mg), sporulation and change in pH, on different culture media in 3 species of *Fusarium*

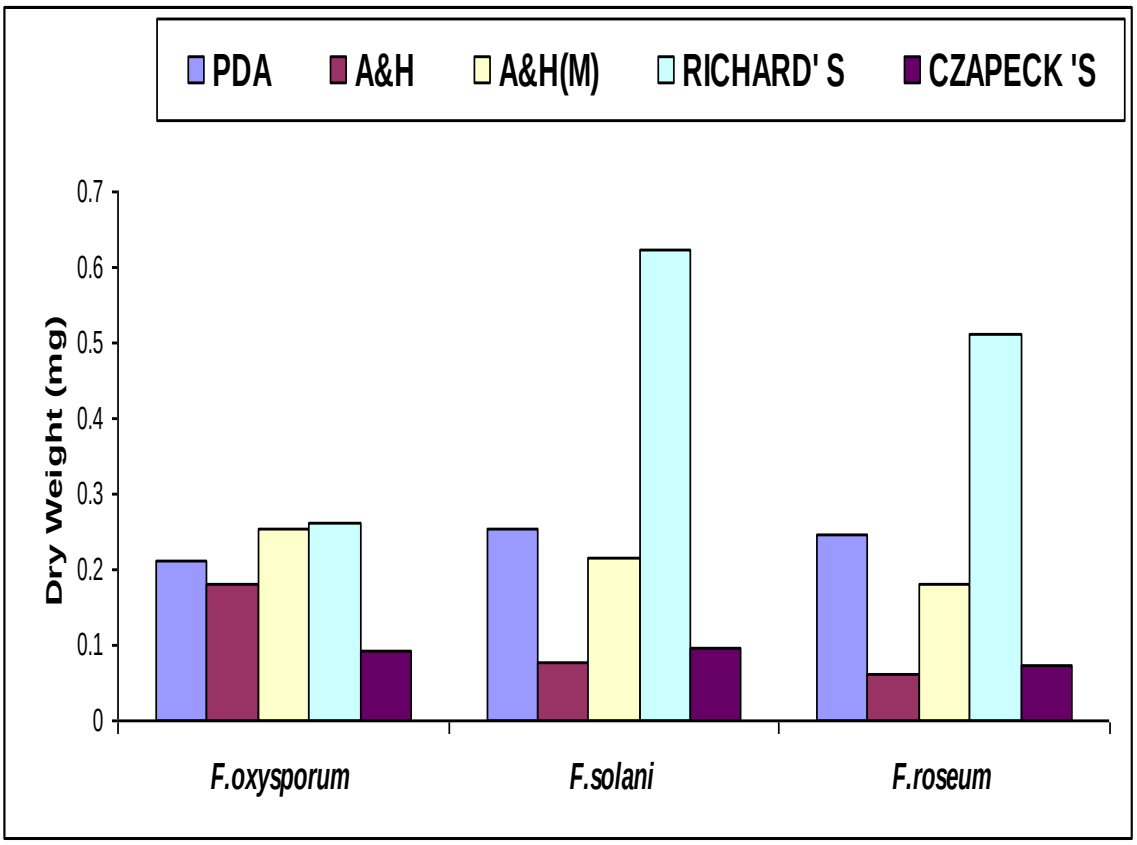
Treat	Medium	*F. oxysporum*			*F. solani*			*F. roseum*		
		Dry Wt (mg)	Sporulation	Final pH	Dry Wt (mg)	Sporulation	Final pH	Dry Wt (mg)	Sporulation	Final pH
1	PDA	213	+++	7.50	254	+++	6.00	246	+++	8.00
2	A&H A'	182	++	8.94	77	+	8.81	6 3	+	9.33
3	A&H(M)	254	+++	8.29	215	++	9.11	182	+	8.39
4	RICHARD's	261	++	9.28	622	+++	9.18	512	+	9.09
5	CZAPEK's	92	+	5.88	97	+	7.95	72	+	7.00

Sporulation grade + = Fair, ++ = Good, +++ = Excellent

Summary of dry weight results and conclusions at 5% level of P.

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non significant	Non significant	Non significant
S.E	68.54	219.61	182.90
C.D	2,645.64	8,476.94	7,059.94
Treatment Nos.	4>3>1>2>5		

Fig 6: Average dry weight (mg) of 3 species of *Fusarium* in different culture media



Suitability of potato dextrose medium was earlier reported by Ramakrishnan and Damodaran (1954), Tandon (1960). Lapis and Deangkinay (1966), Hasija and Chowdhary (1980) for the organisms they studied. This media cannot be considered suitable for further studies as the required chemical alterations will not be possible. Potato being a natural substance forms a semi synthetic medium. Although this medium supported good growth and sporulation for the present three *Fusarium sp.*

Richard's medium was found best. It has been also found to be an excellent medium for *F. pallidoroseum* (Mathew, 1993).Complex sugars may be hydrolyzed during autoclaving of the medium. Acidity of the medium facilitate this phenomenon more. Medium should not have its constituents in high concentration for general use

(Leonian and Lilly, 1940).Cochrane (1958) also suggested use of more dilute media than those often used in the studies of morphology and reproduction.

Although modified Asthana and Hawker's medium 'A' did not support excellent growth it supported sufficient growth and sporulation in all the *Fusarium* species under present study. Moreover, this medium is easy to manipulate with respect to the expected need for modifications and substitutions of its constituents. Hence it was used in all subsequent cultural studies.

pH refers to potentials of hydrogen. This scale was proposed by French scientist Sörenson. Growth of fungi is known to be highly influenced by hydrogen ion concentration of the culture medium. It not only affects permeability of protoplasmic membranes but also has an influence in uptake of minerals, Vitamins and organic acids into the cells. Activity of enzyme systems and synthesis of proteins and other similar life processes are directly related to change in pH of the medium. A pH change can alter the process of pigment formation, vitamin uptake and formation of antibodies etc. The pH may be a determining factor while the immature fruits are not easily colonized by pathogenic fungi. Incipient infection of *Gleosporium psidii* in the young green guava fruits was due to interaction of pH with other factors (Midha and Chohan, 1968).

The organic catalysts have a great influence on growth and sporulation patterns of an organism. It has been found by various phytopathologists that, enzymes have optima between pH 4 and 8. According to Lilly and Barnett (1951). 'The chemical changes in media due to alteration of pH, whether imposed from outside or caused by the fungus, affect metabolic processes. The pH of a culture medium change during the growth of a fungus and these changes may affect the composition of the medium and thus the response of the fungus.

Agarwal (1955) found that pH range for the two strains of *Fusarium coerulum* was between 3.4 and 11.0 and pH 6.4 was optimum for growth and sporulation. Rose (1960) reported that *Fusarium culmarum*, *F. sambucinum* var.*coerulum* and *F. oxysporum* grew well between pH 5 and 8. Bhargava (1962) found the optimum range for the growth of *Fusarium solani* as 4.5 to 6.5. *Malca et al.* (1966) lvaraj (1971) reported that higher pH values up to 10.0 are better tolerated in *Verticillium* and *Fusarium* sp.

Effect of different pH

An experiment was planned and it was decided to study the effect of pH of the medium on growth and sporulation of three species of *Fusarium*. The initial pH values of modified Asthana and Hawker's medium 'A' were adjusted from 3.0 to 8.0 (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0).

Results depicted in table 16 and fig. 7 indicate that 3 *Fusarium* sp. Could grow on a wide range of pH (3- 8). The sporulation was found between pH 4.5 and 7.5. The maximum growth of *F. oxysporum* was obtained at pH 5.5. It was best pH for *F. roseum* while growth of *F. solani* was same at pH 5 and 5.5. Maximum growth of *F. oxysporum* was at pH 5.5 and the sporulation was of good grade. It was excellent at pH6 and 6.5. Final pH in all the cases drifted towards alkalinity. However between pH 5.5 and 6.5 where sporulation was better, pH was either acidic or neutral.

Incase of *F. solani* maximum mycelial weight (150 mg) was recorded at pH 5 and 5.5. Sporulation was excellent at 6. However between 3 and 4 and at pH 8 no sporulation was observed.

Incase of third *Fusarium*, *F. roseum* excellent growth was observed at pH 5.5. The pH did not change in this case and sporulation was fair.

Table 17: Effect of different pH on average dry weight (in mg), final pH and sporulation on 3 different Fusaria

Treat	pH	*F. oxysporum*			*F. solani*			*F. roseum*		
		Dry Wt (mg)	Sporulation	Final pH	Dry Wt (mg)	Sporulation	Final pH	Dry Wt (mg)	Sporulation	Final pH
1	3.0	67	-	8.59	129	-	8.50	112	-	7.50
2	3.5	67	-	8.50	99	-	8.59	73	-	8.55
3	4.0	65	-	8.04	78	-	8.29	70	-	8.06
4	4.5	106	+	8.15	137	+	8.41	88	+	8.10
5	5.0	110	+	8.59	150	+	9.01	116	+	8.89
6	5.5	180	+ +	5.50	150	+	5.50	147	+	5.50
7	6.0	80	+++	6.00	103	+++	6.00	59	++	6.00
8	6.5	76	+++	6.50	100	++	6.50	57	++	6.50
9	7.0	60	++	7.00	80	++	7.00	70	++	7.00
10	7.5	60	+	7.50	86	+	7.50	108	-	7.50
11	8.0	69	-	8.00	74	-	8.00	60	-	8.00

Summary of dry weight results and conclusions at 5% level of P.

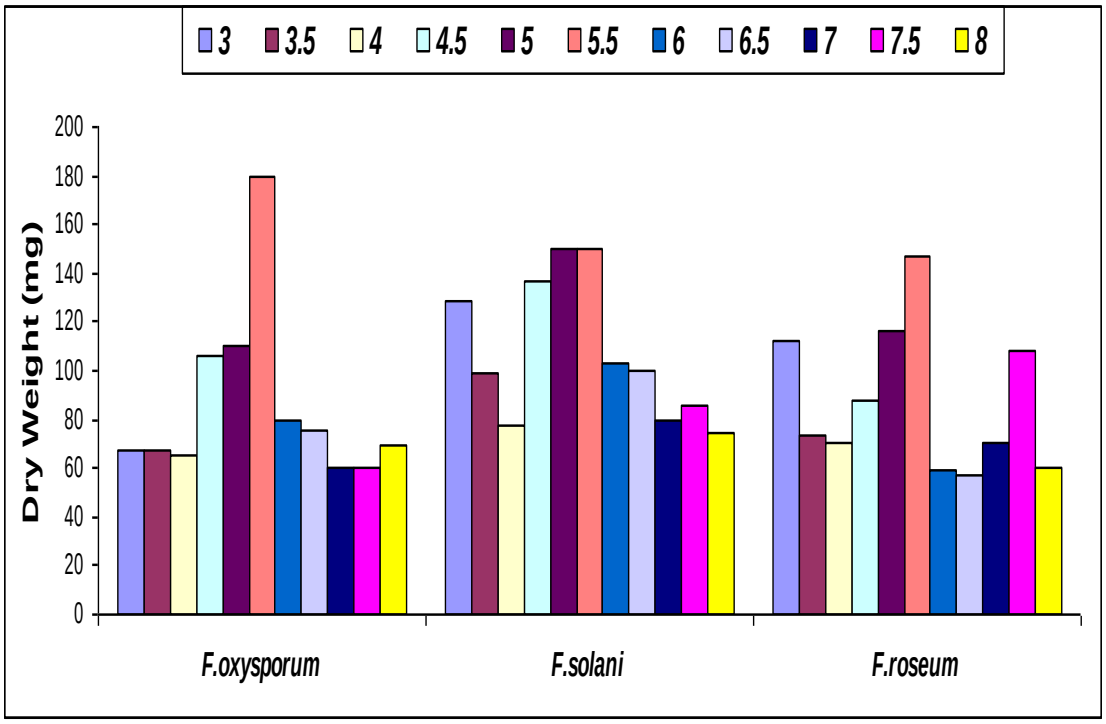
Sporulation grade + = Fair, ++ = Good, +++ = Excellent, - = nil

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non significant	Non significant	Non significant
S.E	35.70	28.81	29.48
C.D	263.4	212.5	216.9

Treatment Nos 6>5>>4>7>8>11>1>2>3>9>10

The maximum dry weight of *F. oxysporum* was attained at pH 5.5 (Mathew 1993). On the acidic side he recorded better growth of *Fusarium* sp. than towards alkaline pH. Yogeshwari (1948) reported that optimum pH for *Fusarium vasinfectum*, *F. udum* and *F. monoliforme* was 5.0. Joffe and Palti (1972) observed that in culture, isolates of *F. solani* and *F. javanicum* grew at pH 7.0 rather 4.2. Cochrane and Cochrane (1971) observed that the proportion of chlamydospore fell linearly in the case of *F. solani* between pH 4.0 and 6.5. Optimum growth and excellent sporulation of all the four organisms under present study were observed at 5.5.

Fig.7: Effect of different pH on average growth dry weight in mg) produced by 3 different *Fusarium*



The change in pH of the medium as a result of a fungal metabolism is a common phenomenon. As stated by Lily and Barnett (1951) 'Four metabolic processes operate in the change of pH of a culture medium; (1) utilization of cations, (2) utilization of anions, (3) Formation of acid from neutral metabolites, especially carbohydrates and (4) Formation of bases, especially anions from amino acids and proteins. The net change in pH is the result of the interaction of all the processes. The rise in pH of the culture medium has been attributed to the metabolic activities during growth resulting in adsorption of anion or production of ammonia from nitrogenous compounds. Lowering of pH in case of media with higher initial pH was, possibly due to absorption of carbon dioxide produced by the fungus during the process of respiration (Singh 1977).

On the basis of present investigation it was found that pH 5.5 supports excellent growth and good sporulation, therefore in all the subsequent experiments the initial pH of Asthana and Hawker's modified was adjusted to 5.5.

Effect of Different Temperatures

Temperature affects various activities of organisms such as mycelial growth, reproduction and spore germination. Thus, temperature determines various metabolic activities of fungi and even distribution of pathogenic fungi around different parts of the world. Disease can be avoided if the crops are grown at temperature in apposite to the pathogen, for example a rust disease will be less severe if the crop is sown a little late in December or early in October. The optimum temperature for fungal growth is between 20º C and 30º C. Below 0ºC and above 40º C usually fungi do not grow, still though, certain fungi are reported to grow even below the freezing conditions. Broad foot and Cormack (1941) as well as Pehrson (1948) reported that some fungi are able to grow slowly at 0º C or slightly less. Even plants under snow may be infected by parasitic 'snow mould' *Fusarium nivale* (Dahl, 1934). The other way round, there are certain organisms which have been found to grow at higher temperatures. La Touche (1948) reported the growth of C*haetomium* sp. on straw at 62 ºC. Bhargava (1962) obtained best growth and sporulation of *F. solani* at 25 ºC. Bhatnagar (1967) observed a considerable variation in the production of micro and macrospore in two isolates of *F. solani* and also reported that, as the temperature increased the production of chlamydospores also increased.

From the above mentioned facts it is evident that, before starting any physiological study, it is indispensable to have a thorough knowledge about the temperature requirement of the organism concerned. Moreover, this would also give an idea about the most suitable environmental conditions for the survival of a pathogen in optimum temperature for the growth and sporulation of the present isolates.

It is evident from table 17 and in fig.8, that maximum mycelial weight of *F. oxysporum* was observed at 25 ºC. Same temperature was found excellent for *F. solani* but it was 20 ºC for *F. roseum*. At pH 5 no sporulation was produced in any of the *Fusaria.* No sporulation and no growth was observed at 40 ºC. When inoculum of *F. oxysporum* was placed on P.DA and incubated at 25 º C the growth appeared while *F. solani* and *F. roseum* failed to do so. At 25 ºC the pH shifted from 5.5 to 8.6. In case of *F. oxysporum* pH changed from 5.5 to 8.5.

Table18: Effect of different temperature on growth of three different Fusaria

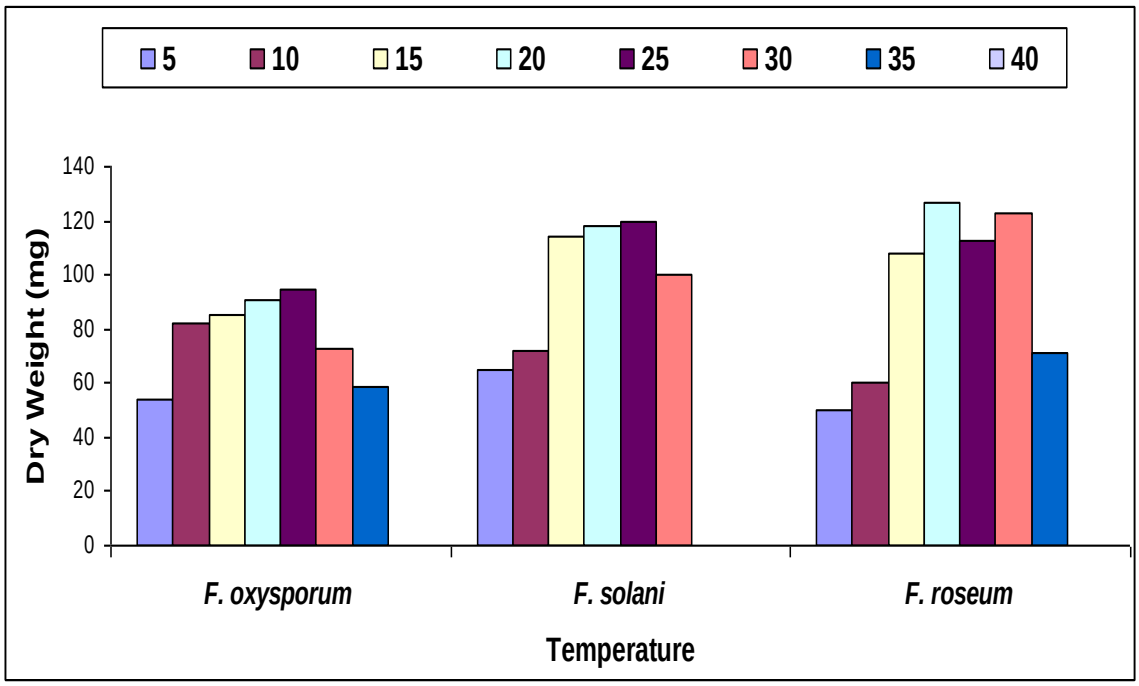
Treat	Temp ºC	*F. oxysporum*			*F. solani*			*F. roseum*		
		Dry wt (mg)	S	Final pH	Dry wt (mg)	S	Final pH	Dry Wt (mg)	S	Final pH
1	5	54	-	6.12	065	-	6.59	50	-	6.79
2	10	82	+	5.79	072	-	6.02	60	-	6.98
3	15	85	+	7.88	114	++	8.05	108	+	7.90
4	20	91	+	8.00	118	++	8.3	127	+	8.40
5	25	95	++	8.50	120	++	8.6	113	+++	8.60
6	30	73	-	8.68	100	++	8.22	123	++	7.59
7	35	59	-	6.65	-	+	8.13	71	-	9.80
8	40	-	-	10.0	-	-	9.08	-	-	10.0

Sporulation grade (S) + = Fair, ++ = Good, +++ = Excellent, - = nil

Summary of dry weight results and conclusions at 5% level of P.

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non significant	Non significant	Non significant
S.E	30.44	49.80	44.22
C.D	301.35	493.02	437.7
Treatment Nos.	5>4>3>2>6>7>1>8		

Fig.8 Effect of different temperatures on growth of 3 different species of *Fusarium*



Effect of Different Sugars and their Utilization

It has been found that fungi are heterotrophic organisms. Thus fungi utilize different sources of sugars and polysaccharides by different rate. Monosaccharides usually are easily assimilable forms of carbohydrate, among which glucose has been reported to be the most efficient source of carbon and energy for most of the fungi (Bilgrami and Verma 1978). Some fungi like *Polychytrium aggregatum* (Agello, 1948) exhibit exclusive choice for this sugar and fails to grow on other hexoses. Incapacity to use glucose is restricted to few fungi like *Fusarium lini* (Tochinai, 1926) and *Pythium aphanidermatum* (Kumar and Grover ,1967). Glucose is also a constituent of different fungal mycelia. Irani and Ganpathy (1960) reported that mycelium of *Penicillium crysogenum* exhibited consistent presence of glucose, which they attributed to the fact that glucose may be formed due to breakdown of polysaccharides and other sugars. Hasija and Wolf (1969) reported that in *A. niger* glucose was always present as a constituent of mycelium irrespective of the carbon source.

Trehalose is called as fungal sugar. Trehalose is made up of two glucose moieties which are held together by α glucosidic linkages. Unlike maltose it is a non- reducing sugar. Arya (1991) studied the effect of different monosaccharides on four different pathogenic species of P*homopsis*. *P. viticola* utilized D- glucose within nine days, while other three fungi utilized it between 11 and 13 days. D- fructose and D- galactose were preferred by *P. viticola* than D-glucose. D- fructose and D- galactose were utilized by *P. viticola* in five and six days respectively. D-xylose (a pentose sugar was also preferred by another fungus *P. gulabia* as it was utilized in five days in comparison to ten days by *P. viticola*. Whittaker (1951) reported that dry mycelium of a fungus contains carbon between 40 and 50%.

Fungi are so specific in utilization of different carbon sources, that a carbon source may be utilized by a fungus while another source of almost similar chemical nature may prove useless for it. Different workers have found dextrorotatory sugar more preferable than levoratatory of same type. Lily and Barnett (1956) reported that *Sporobolomyces salmonicolor* could grow on D- arabinose but not on L- arabinose. Shreemali (1973) reported that not only different species of a genus but different isolates of a species have been shown to be very selective in their choice of carbon compounds. Arya (1991) found that L+ arabinose was preferred source of carbon for *P. viticola* and *P. sidii* than its D (- isomer). Moderate growth of *F. oxysporum* and *F. acuminatum* was observed on arabinose (Singh, 1977). Similar results were reported for *F. roseum* (Lopez and Ferges 1965). According to Wolf *et al.* 1950 arabinose was found to be a poor source for the growth of *F. solani*.

Glucose was found to be a good source for the growth of *F. acuminatum* and moderate growth was observed of *F. oxysporum* and *F. solani* by (Singh, 1977). He also found moderate growth of three species of *Fusarium* on sucrose while the growth was poor in another disaccharide maltose. According to Lilly and Barnett (1951) "Not all carbon sources are equally suitable for fruiting of fungi. Some which are favorable for mycelial growth, do not favor sporulation. '' Another well known fungal physiologist Cochrane (1958) stated that, in general, it is found that oligosaccharides and polysaccharides support more fruiting bodies than the simple hexoses. Considering the different growth and sporulation patterns and the time required to deplete a particular sugar or polysaccharide an effort was made to study one pentose, two hexoses, two disaccharides and two polysaccharides. Three different species of *Fusarium* were grown in broth media containing above substrates and results are recorded in Table: 18

Carbohydrate sources

A Monosaccharides

(i) Pentoses ($C_5 H_{10}O_5$)

D Arabinose

(ii) Hexoses ($C_6H_{12}O_6$)

D glucose, D galactose

B Oligosaccharides

Disaccharides ($C_{12}H_{22}O_{11}. H_2O$)

Sucrose, Maltose

C Polysaccharides ($C_6H_{10}0_5)_n$

Soluble starch, Pectin

Utilization of monosaccharides

D – arabinose (R_f 0.70)

This aldopentose occurs in nature in the form of arabans, as common constituent of plant polysaccharides and various gums specially gum Arabica.

It is evident from the table 18. that D - *arabinose* supported good growth of all the three *Fusarium* sp. Growth was more in case of *F. solani* on this pentose source. *F. oxysporum* was more capable to deplete it very fast. This carbon source was utilized completely by *F. oxysporum* in 10 days while *F. solani* and *F. roseum* took 12 days for its depletion from the medium.

D-glucose (R_f 0.60)

This aldohexose is found in various parts of the plants, This hexose is also a component of complex carbohydrates like starch and cellulose. Sucrose, lactose, maltose, cellobiose and raffinose are made up of glucose moieties. Glycosan the most common reserve carbohydrate of fungi is composed of glucose units.

Table: 18 shows that a gradual increase in dry weight was recorded in culture medium having three *Fusarium* species. *F. oxysporum* and *F. solani* were capable to utilize this hexose sugar within 10 days. Where as *F. roseum* could deplete this sugar only after 12 days. Of all the 3 species *F. oxysporum* produced maximum mycelial dry weight. Final pH in all the three cases remained basic on 15^{th} day.

D-galactose (R_f 0.60).

An aldohexose, which differs from D- glucose with respect to asymmetrical carbon atom 3 and D- mannose with asymmetrical carbon 1 and 3. It is an important constituent of certain other sugars like lactose, melibiose and raffinose. A perusal of table clearly showed that this hexose was assimilated much faster than D-glucose. *F. oxysporum* utilized it in six days, while *F. solani* and *F. roseum* assimilated this sugar in eight days.

In all the three cases maximum mycelial weight of the fungi was recorded after 10 days. The pH shift was towards basic side after 15 days of incubation. This hexose was assimilated by *F. oxysporum*, *F. acuminatum* and *F.solani* within nine, five and six days respectively (Singh, 1977). He also found that there was no difference in the dry weights of these three organisms on sixth and 12^{th} day of incubation.

Sucrose (R_f 0.43)

This disaccharide is commonly present in plants particularly in fruits along with fructose and in stem of *Saccharum officinarum (*sugarcane*)* in roots of *Beta vulgaris* (Beet root). Many workers while their studies related to fungal physiology have found that fungi are capable to hydrolyse sucrose in to glucose and fructose and thus this disaccharide is assimilated through a hydrolytic pathway. However, a few fungi like *Myroethecium verrucria* (Mandels 1954) and seven members of mucorales (Raizada, 1957) were able to consume this sugar through a non- hydrolytic pathway.

It is evident from Table 18 that in all the three fungi presence of sucrose was detected in the medium up to second day.

Presence of hydrolysed glucose was recorded up to two days in case of *F. oxysporum* and *F. solani* as compared to 4 days in *F. roseum*. Fructose was last to be utilized of the two hydrolytic products of sucrose. It was observed up to 8 days in *F. solani* and up to four days in case of *F. roseum*. Preferrential utilization of glucose has been earlier reported by other mycologists. Singh (1977) found that this disaccharide was hydrolysed by *F. acuminatum, F. solani* and *F. oxysporum* on third, fourth and fifth day respectively. He also found preferential utilization of glucose. The fructose was fully utilized on 11^{th} day by *F. acuminatum* and *F. solani* and up to 12^{th} day by *F. oxysporum.*

In case of *F. oxysporum* and *F. roseum* an increase in dry weight was observed up to 10^{th} day.

Final pH of the medium remained towards acidic side.

Maltose (R_f 0.40).

It does not usually occur in the free form in chlorophyllous plants, But this disaccharide is obtained as an intermediate product during the digestion of starch to glucose. It consists of two glucose units which are held together by α- 1,4 glucoside linkage. Maltose is utilized by a majority of fungi through a hydrolytic pathway. It yields two molecules of glucose when hydrolysis is accomplished by the enzyme α glucosidase.

It is evident from Table 18 that maltose was utilized by hydrolytic pathway. It was detected up to two days only on chromatograms. Glucose was utilized completely from the culture medium after four days in case of *F. oxysporum* and *F. roseum. F. solani,* however was more efficient to deplete this breakdown product after two days only. Singh

(1977) found that this disaccharide was completely hydrolyzed on fourth day by *F. acuminatum.*

The experiment showed that mycelial dry weight increased up to 15 days of incubation period incase of *F. solani* and *F. roseum* while mycelial dry weight was more on 10^{th} day incase of *F. oxysporum* as compared to 15^{th} day. The two isolates of *F. solani* and *F. aurentifolia* responsible for lime twig disease were found to utilize carbon from a wide variety of compounds. Monosaccharides prove to be better than other sources tested for growth and sporulation. Maltose exhibited best growth.

Utilization of polysaccharides

The compounds having polymeric structures consisting of large number of monosaccharide units- homopolysaccharides or comprising of two or more different types of sugar units are called heteropolysaccharide. Such carbohydrates are grouped as

1) Structural polysaccharides, which are found mainly in cell walls and other extra protoplasmic inclusions.

2) The reserve or nutrient polysaccharides are stored in the plant tissue, to be used during the period of active metabolism. Cellulose, pectic substances and chitin are structural polysaccharides.While starch dextrin and glycogen are the common reserve polysaccharides. Polysaccharides are not preferable source of nutrition for fungi but they induce good growth and sporulation of various fungal organisms. The utilization of soluble starch and pectin was observed in the present investigation and results are summarized in Table 18.

Starch

Starch consists of glucose residues joined through α glycosidic linkages and thus may be thought of consisting as of repeating units of Maltose. Starch consists of two types of

molecules called amylase and amylopectin. Enzymatic hydrolysis of starch may be represented schematically a follows -

Starch → Dextrin → Maltose → D-glucose

It is evident from Table 18. that starch was depleted from medium of *F. oxysporum* and *F. solani* after eight days. While it was consumed after eight days in *Fusarium roseum* . The mycelial weight was more on 10^{th} day as compared to 15^{th} day of incubation in all the three cases. The pH drifted to wards alkalinity.

Starch could not be completely hydrolysed by *F. oxysporum* in 12 days (Singh,1977). The other two fungi *F. acuminatum* and *F. solani* hydrolysed starch on ninth and tenth day respectively.

Mathew (1993) reported different rate of utilization of starch by four *Fusarium* sp. *F. udum* and *F. pallidoroseum* failed to utilize this polysaccharide within the incubation period of 15 days. *F. oxysporum* utilized it within 12 days and *F. moniliforme* took 14 days for its complete depletion.

Pectin

Pectin is a structural polysaccharide. Calcium and Magnesium peptate forms the middle lamella in plants. Pectin was utilized completely by *F. roseum* in 9 days and 10 and 12 days in case of *F. solani* and *F. oxysporum* respectively.

Table 19: Effects on dry weight, final pH and utilization of carbon source by three different *Fusaria*

Sugar	Days	Name of fungus	Dry Wt (mg)	Rate of growth	Final pH	Presence(Days)
D- Arabinose	5	*F. oxysporum*	40	40	5.0	10
	10		88	48	4.1	
	15		120	32	5.5	
	5	*F. solani*	55	55	5.5	12
	10		100	45	6.0	
	15		130	30	6.2	
	5	*F. roseum*	45	45	6.0	12
	10		85	40	6.2	
	15		122	37	5.5	
D-Glucose	5	*F. oxysporum*	53	53	6.54	10
	10		124	71	8.7	
	15		250	126	8.23	
	5	*F. solani*	350	350	8.03	10
	10		154	-196	8.86	
	15		200	46	8.96	
	5	*F. roseum*	42	42	4.81	12
	10		148	106	8.6	
	15		182	76	8.8	
D Galactose	5	*F. oxysporum*	55	55	6.1	6
	10		174	119	8.86	
	15		94	-80	8.5	
	5	*F. solani*	93	93	6.57	8
	10		231	138	8.89	
	15		117	-114	9	
	5	*F. roseum*	75	-42	5.96	8
	10		233	158	8.26	
	15		137	-76	8.1	
Sucrose	5	*F. oxysporum*	82	82	8.74	S 2
	10		100	18	8.9	G 2
	15		127	27	8.74	F 2
	5	*F. solani*	86	86	7.78	S 2
	10		143	57	8.92	G 2
	15		138	-5	9.14	F 8
	5	*F. roseum*	55	55	7.65	S 2
	10		81	26	8.5	G 4
	15		125	44	8.57	F 4
Maltose	5		78	78	8.57	M 2
	10	*F. oxysporum*	144	66	8.9	G 4
	15		122	-22	9.15	
	5	*F. solani*	97	97	8.59	M 2
	10		165	68	9.11	G 2
	15		181	16	9.24	
	5	*F. roseum*	72	72	8.3	M 2
	10		185	113	0	G 4
	15		78	78	8.57	

Starch	5	*F. oxysporum*	150	150	8.3	8
	10		208	58	8.9	
	15		186	-22	8.5	
	5	*F. solani*	140	140	6.8	8
	10		169	29	7.3	
	15		133	-36	7.4	
	5	*F. roseum*	178	178	6.5	10
	10		206	28	7.8	
	15		203	-3	7.6	
Pectin	5	*F. oxysporum*	40	40	4.5	12
	10		35	-5	4.1	
	15		38	3	4.8	
	5	*F. solani*	36	-2	4.9	10
	10		32	-4	5.6	
	15		34	2	5.8	
	5	*F. roseum*	55	21	6.3	9
	10		42	-13	7.9	
	15		32	-10	7.2	

Effect of different nitrogen sources

Nitrogen is an essential element, and is used by fungi for functional purposes. Chitin the chief component of cell wall in most of the fungi is a linear polymer of D - glucosamine. Similarly proteins, the basis of protoplasm are composed of nitrogenous substances. Purines, pyrimidines, some vitamins and other essential metabolites are also nitrogen containing compounds. Nitrogen content of the mycelium generally lies between 3 and 6%.

According to Lilly and Barnett (1951) with the exception of certain amino acids and ammonia, most nitrogen sources undergo modifications before entering the synthetic metabolic pathways. Nitrates, nitrites and hydroxyl amines are presumably reduced to ammonia before assimilation. Those amino acids which do not directly enter in to the metabolic pathways leading to the synthesis of protein are probably deaminated. According to Cochrane (1958) and Devi and Reddy (2005) no single pattern of nitrogen assimilation can be described to apply to all fungi.

It has been found that presence of VAM in the roots influences the amino acid metabolism of the host by increasing the specific activity of the enzyme concerned. Among various Nitrogen sources, Potassium nitrate has been generally reported to be a good source of nitrogen for inducing growth and sporulation of different fungi (Arya 1982). The literature is full with conflicting claims regarding the comparative superiority of a particular form or a source of nitrogen over the other. Keeping in the view of above findings, it was thought desirable to study the influence of various nitrogen compounds on the growth and sporulation of three different sp. of *Fusarium*. The influence of the following nitrogen sources was studied.

I Inorganic sources

1)Potassium Nitrate ……. ……. KNO_3

2)Calcium Nitrate…….$CaNO_3$

3)Sodium Nitrate ……..$NaNO_3$

4)Ammonium Nitrate……$NH_4(NO_3)_2$

5)Ammonium Chloride…….NH_4Cl

6)Ammonium Molybdate …….....$(NH_4)_6Mo_7O_{24}.4H_2O$

7)Ammonium dihydrogen Ortho Phosphate… $NH_4H_2PO_4$

8)Ammonium Oxalate ……..$(COONH_4)_2.2H_2O$

9)Ferrous Ammonium Sulphate……..$FeSO_4(NH_4)_2SO_4.6H_2O$

II Organic Sources

10) Amine ……..Urea

The results are summarized in Table 19 .It is evident from table 19. that three species of *Fusarium* differ in their ability to utilize various sources of nitrogen. All the three species produced scanty growth in complete absence of the nitrogen. Of the four nitrate sources tried, calcium nitrate was best for *F. oxysporum* followed by Potassium nitrate; same was true for the other two *Fusarium* spp.

Of all the nitrogen sources tried ferrous ammonium sulphate supported excellent growth and good sporulation of all the three *Fusarium* sp. Nitrates were found to be good sources of nitrogen for most of the fungi studied, the sequence in reduction nitrate through ammonia proceeds as follows:

$$NO3 \rightarrow NO2 \rightarrow HNO \rightarrow NH2OH \rightarrow NH3$$

Incase of *F. oxysporum* variety Nicotiani poor growth was recorded (Wolf, 1955). Ammonium chloride supported poor growth of all the three fungi. no growth occurred in medium supplemented with this compound up to 10 days. However the mycelial dry weight of *F. oxysporum* increased to 89 mg after 15 days of incubation period.

Ammonium molybdate also supported poor growth of all the three pathogenic fungi, no growth was observed up to five days incase of *F. roseum* and up to 10 days in other two cases. In case of ammonium dihydrogen ortho phosphate poor growth was recorded in all the cases. The decreased fungal growth may be due to more acidic nature of culture medium. In case of ammonium oxalate also the growth was poor. Ammonium oxalate is a different group of compound because both cation and anion of the salt are utilized as carbon and nitrogen sources. The presence of additional carbon source may be the cause of some growth after 10 and 15 days of incubation. Madan (1978) recorded good growth of *F. moniliforme.*

In case of organic source of nitrogen, urea produced sufficient growth and fair sporulation in case of all the three pathogenic species. of *Fusarium*. In case of *F. solani*, the growth was maximum at 10 days (80 mg) and reduced at 15 days (73 mg), while in other two cases a gradual increase was recorded up to 15 days of incubation. The urea can be utilized as a source of nitrogen but not as a carbon source. Urea is converted in to ammonia and carbon dioxide by enzyme urease (Scazzocchlo 1967).

Species of *F. coeruleum* utilized urea poorly (Tandon 1961). The mycelial growth of *F.roseum* (Rawla and Parmar, 1973), *F. moniliforme* (Madan, 1972), *F. solani* and *F. equisetii* (Agarwal and Sarbhoy) recorded good growth on urea. Final pH of different media used in the experiment is recorded in table 19 in most of the cases. Final pH shifted towards acidic side, however in few cases it was towards basic side.

Table 20: Effect of Nitrogen on dry weight, Final pH and sporulation of three different species of *Fusarium*

Treat	Nitrogen sources	Days	*Fusarium oxysporum*			*Fusarium solani*			*Fusarium roseum*		
			Final pH	Dry wt (mg)	S	Final pH	Dry wt (mg)	S	Final pH	Dry wt (mg)	S
1	Potassium Nitrate	5	4.00	8	--	6.80	36	+	6.90	22	--
		10	4.00	96	+	7.10	37	++	7.20	88	+
		15	9.98	67	+	7.80	98	+	9.80	65	+
2	Calcium nitrate	5	4.00	34	+	4.60	34	--	6.00	11	--
		10	4.20	74	+	5.06	108	++	5.20	119	++
		15	9.60	99	+	6.29	107	++	8.50	122	++
3	Sodium nitrate	5	4.00	28	--	5.50	36	--	6.50	31	--
		10	4.00	49	--	6.10	61	+	7.29	56	--
		15	10.23	59	+	8.20	121	++	9.95	82	+
4	Ammonium nitrate	5	3.50	12	--	5.20	69	+	4.22	49	+
		10	4.99	36	--	5.18	75	+	4.61	62	+
		15	5.76	43	--	5.24	83	+	4.71	72	+
5	Ammonium Chloride	5	2.00	-	--	2.46	-	--	3.01	-	--
		10	2.05	-	--	2.89	-	--	3.11	-	--
		15	3.14	89	+	3.00	16	--	3.33	34	--
6	Ammonium molybdate	5	2.00	-	--	2.67	-	--	2.08	-	--
		10	2.99	-	--	3.42	-	--	3.00	19	--
		15	5.30	23	--	5.25	15	--	5.31	33	--
7	Ammonium Dihydrogen phosphate	5	2.50	-	--	3.15	-	--	3.21	-	--
		10	2.89	-	--	3.56	-	--	3.82	-	--
		15	4.27	31	--	4.29	33	--	3.80	17	--
8	Ammonium oxalate	5	3.21	-	--	3.02	-	--	3.30	-	--
		10	4.99	14	--	3.33	-	--	5.01	21	--
		15	8.06	22	--	4.60	16	--	8.06	47	+
9	Ferrous Ammonium sulphate	5	4.50	53	+	4.99	65	+	3.39	11	--
		10	5.21	98	+	5.10	111	++	4.35	55	+
		15	3.47	117	++	3.50	264	++	3.39	146	+
10	Urea	5	4.50	37	--	4.02	9	--	5.56	40	+
		10	3.99	77	+	4.11	80	+	7.29	70	+
		15	7.06	88	+	5.95	73	+	7.58	83	+
11	Control	5	2.03	-	--	2.55	-	--	2.00	-	--
		10	4.71	55	+	4.59	45	--	4.62	25	--
		15	4.81	57	-	5.01	50	-	5.57	40	+

(S) = sporulation - = Depicts no growth, -- = Sporulation nil, + = Fair, ++ = Good

Summary of dry weight results and conclusions at 5% level of P.

Treatments	Highly significant	Highly significant	Highly significant
Replicates	Non significant	Non significant	Non significant
S.E	36.17	55.01	39.32
C.D	266.9	405.9	290.18

Effect of different vitamins on growth and sporulation of three *Fusaria*

Vitamins though required in smaller amount are essential for growth and sporulation of Fungi .Fungi in their ability to synthesize their vitamin requirement occupy a position in between the totally independent higher plants and completely dependent animals. Following four concentrations in *ppm* of the vitamins were used in order select the most suitable concentration of vitamin for the growth and sporulations of three F*usarium spp.* The results are summarized in Table 20. An effort was made to study the effect of all vitamins added in the basal medium in their optimum concentrations. And subsequent treatments included exclusion of single vitamin from a combination of all the vitamin present in the medium. The results are recorded in table 20.

Thiamine – (Vit B1 or Aneurin) Thiamine molecule consists of two moieties i.e.

(1) 2,5- dimethyl 6 amino pyrimidine

(2) 4- methyl-5- hydroxyethylthiazole.

Thiamine was the first vitamin, which was reported by (Schopfer 1934) that was required by a filamentous fungus.

All the three *Fusarium sp*. showed better growth than the control set. Maximum mycelial dry weight of *F. oxysporum* was obtained at 50 ppm concentration of Thiamine. Growth of *F. solani* and *F. roseum* was however, more at 200 ppm concentration. The sporulation in all the three cases was observed at lower concentration i.e. in 50 and 100 *ppm.*

Riboflavin –Riboflavin is also referred as vitamin B_2, vitamin G or Lactoflavin with its chemical name 6, 7-dimethyl 1-9-(1- D-ribibyl) - isoalloxazine having empirical formula $C_{17}H_{20}N_4O_6$. Maximum mycelial yield of *F. oxysporum* and *F. roseum* was observed at 50 ppm concentrations of riboflavin. In case of *F. roseum* 50 ppm showed lesser

mycelial weight in comparission to concentration 100, 150 and 200 *ppm*. No sporulation was produced by all the three fungi up to 15 days of incubation.

Inositol: Importance of this vitamin as growth factor for fungi was first demonstrated in yeast. It was the first component of the ‘‘Bios complex ’’ to be identified. Inositol is a carbohydrate, with the same empirical formula as the monosaccharide i.e. CH_2O.

Except *F. oxysporum* other two fungi produced maximum mycelial yield at 200 ppm concentration. *F. oxysporum* produced better growth at 150ppm. Sporulation in *F. oxysporum* was present between 50 and 150 ppm, in *F. roseum* and *F. solani* sporulation was present in all the concentrations tried.

Pyridoxine: (Vitamin B_6). Pyridoxine along with its two closely allied derivatives viz., pyridoxal and pyridoxamine are together referred as vitamin B_6 or Adermin, They differ slightly in the presence of either a primary alcohol or an aldehyde or a primary amine group in their molecule.

F. oxysporum produced maximum mycelial weight at 150 ppm followed by 200, 50 and 100 ppm. 150 ppm also supported maximum growth of other two *Fusarium* spp. Sporulation was also present at this concentration in all the three fungi.

L Ascorbic acid: Ascorbic acid may affect indirectly to the growth of different fungi through its effect on oxidation reduction potential. Of the 4 concentrations of Ascorbic acid tried 100 ppm produced maximum mycelial dry weight of *F. oxysporum*, while it was 75 ppm concentration for the other two *Fusarium* sp. Sporulation was present in *F. solani* and *F. roseum* while it was absent in different concentrations in case of *F. oxysporum*.

Nicotinic acid: It is believed that active form of nicotinic acid (An oxidation product of nicotine) is nicotinamide. As a component of NAD and NADP (The coenzymes of various dehydrogenases). The nicotinamide, which is the active biological derivative of

this vitamin, participates in essentially all the oxidation-reduction reactions occurring in the living cells. Niacin – Heterotrophy has been frequently reported both in yeasts and filamentous fungi, and the deficiency appears to be more common among the yeasts (Bilgrami and Verma 1978).

Lower concentration *i.e*. 25 ppm produced maximum mycelial dry weight in all the cases except *F. roseum*. Sporulation was not recorded at this concentration in any of the 3 *Fusaria.* Growth was maximum at 50ppm.Sporulation in case of *F. solani* was recorded at 75 and 100 ppm concentration.

Folic acid: This vitamin was first obtained from the leaves of spinach and was accordingly designated as 'Folic acid' (*L. folium).* It is pteroylglutamic acid. The molecule consists of three different compounds viz. glutamic acid, p- amino benzoic acid and 2- amino- 4-hydroxy-6-methyl-pterin.

F .oxysporum produced maximum growth and sporulation at 20 ppm concentration. However *F. solani* and *F. roseum* produced maximum growth at 10 ppm concentration. In all the cases however the growth was more after incorporation of folic acid. Arya (1996) reported inhibitory effect on coelomycetous fungus *Phomopsis viticola* on folic acid.

Biotin: This vitamin belongs to B-complex family of vitamins and comprises of a single tetrahydrothiophene ring with a side chain of four methyl groups. It has also been referred as vitamin H.

Fungal dry weight of *F. oxysporum* reduced with the increasing concentration of Biotin. Arya (1991) found best growth of *P. psidii* at 5 ug/l of Biotin. In case of *F. solani* maximum mycelial yield was recorded at 15 ppm while it was 20 ppm of *F. roseum.* Sporulation was present in all the cases at 5 and 10 ppm. While in case of *F .solani* it was present in all the cases.

Cyanocobalmine (Vit B_{12} group).. This vitamin consists of four pyrrole rings and is thus porphyrin. The tetrapyrrole ring of this vitamin contains in its centre a single atom of cobalt. In Cynocobalmin the unsatisfied valence of cobalt is filled up by a cyanide group and hence the name cynocobalmine. Vitamin B_{12} is essential for the reduction of one carbon fragements, i.e. reduction of formyl 2 methyl group. Vitamin B_{12} showed a marginal increase in mycelial dry weight of *F. oxysporum* and *F. solani*. A marked increase in mycelial dry weight was observed at 20 and 30 ppm concentration in case of *F. roseum*. All the 3 fungi showed sporulation in all the concentrations tried.

The absence of an individual vitamin was studied by excluding one of them at a time from the mixture of the vitamins. A medium devoid of any vitamin served as control. It is evident that addition of all the vitamins produced maximum mycelial dry weight in the three *Fusarium* sp. under study. Removal of Thiamine, Pyridoxine, Inositol, Cynocobalamine reduced the mycelial dry weight than the control set. In case of *F. solani* the elimination of Thiamine, Pyridoxine, Riboflavin, L-Ascorbic acid, Nicotinic acid, Inositol, Cynocobalamine reduced the mycelial weight than of the control set. In case of *F. roseum* the elimination of folic acid proved to be harmful as the mycelial dry weight reduced in this case. Same was true for Inositol in case of *F. solani*. Response of different vitamins on sporulation of three different *Fusaria* varied.

Therefore, it can be concluded that *F. oxysporum* was deficient in five vitamins, *F. solani* in seven different vitamins and *F. roseum* in one vitamin *i.e.* folic acid. Partial deficiency towards different vitamins is also reported for four different pathogenic species of *Phomopsis* by Arya (1996).

Table 21: Average mycelial dry weight, sporulation and Final pH in different concentration of 9 different vitamins

Treat.	Vitamins	Conc (ppm)	*Fusarium oxysporum*			*Fusarium solani*			*Fusarium roseum*		
			Final pH	Dry Wt (mg)	S	Final pH	Dry Wt (mg)	S	Final pH	Dry Wt (mg)	S
1	Thiamine	50	10.3	81	+	3.6	65	+	5.0	81	+
		100	10.0	52	+	4.1	44	+	5.2	75	+
		150	9.7	60	+	3.4	52	-	4.8	68	-
		200	10.1	75	+	3.1	75	+	4.9	101	-
2	Riboflavin	50	10.2	115	-	10.0	107	-	9.5	76	-
		100	10.3	93	-	9.7	81	-	9.6	96	-
		150	9.9	81	-	10.0	101	+	9.2	97	-
		200	10.3	85	-	10.3	11	-	9.7	96	-
3	Inositol	50	10.3	49	+	9.9	40	+	9.5	49	+
		100	9.8	41	+	9.8	43	+	9.3	47	+
		150	9.8	58	+	9.9	54	+	9.9	42	+
		200	9.7	50	-	9.9	56	+	9.9	58	+
4	Pyrido xine	50	9.8	65	+	7.0	73	+	7.0	72	+
		100	9.4	59	+	3.0	80	-	5.5	64	+
		150	9.3	108	+	4.0	87	+	6.3	98	+
		200	9.2	74	+	2.0	68	-	6.1	75	+
5	L Ascorbic Acid	25	9.4	74	-	9.8	71	-	9.4	92	-
		50	9.8	74	-	9.7	75	-	9.3	85	-
		75	10.0	78	-	9.9	103	+	9.5	126	+
		100	10.0	80	-	10.0	82	+	9.6	120	+
6	Nicotinic Acid	25	7.0	169	-	9.7	133	-	9.0	179	-
		50	8.9	135	-	9.9	112	-	9.4	181	-
		75	7.6	148	-	9.9	116	+	8.5	143	-
		100	8.7	124	-	9.7	116	+	9.5	179	-
7	Folic Acid	10	9.4	74	-	5.6	111	-	7.6	99	-
		20	9.7	85	+	9.7	100	+	7.6	95	-
		30	9.6	68	+	4.5	94	-	5.8	88	-
		40	9.2	80	+	4.4	76	+	8.1	51	-
8	Cynocob almine	10	9.7	48	+	9.6	52	+	9.3	92	+
		20	9.7	46	+	9.3	49	+	9.4	105	+
		30	9.7	48	+	9.6	47	+	9.0	107	+
		40	9.7	46	+	10.1	62	+	9.3	81	+
9	Biotin	5	9.9	84	+	9.9	49	+	9.3	75	+
		10	9.7	64	+	9.7	52	+	9.6	79	+
		15	9.8	70	+	9.8	64	+	10.1	67	-
		20	9.9	37	+	9.7	53	+	9.4	103	-
10	Control		10.4	106	+	9.7	126	+	9.0	143	+

Table 22: Average Dry weight, sporulation and final pH in medium supplemented with different vitamins (-) minus the mentioned vitamin

Vitamin absent from medium	*Fusarium oxysporum*			*Fusarium solani*			*Fusarium roseum*		
	Dry Wt (mg)	Sporulation	pH	Dry wt (mg)	Sporulation	pH	Dry wt (mg)	Sporulation	pH
All vitamin added	109	+	6.7	107	- -	8.3	102	-	8
- Thiamine	7	-	5	42	++	6	65	-	7
- Pyridoxine	14	-	4.6	63	++	6.8	53	++	7
- Riboflavin	55	++	5	58	++	6	81	-	7
L Ascorbic Acid	48	-	5	64	++	6.3	58	-	6.4
- Nicotinic Acid	52	-	5.2	65	++	6.3	78	-	6.8
Folic acid	27	++	5	72	++	6.3	37	-	6.3
-Biotin	55	++	5	71	++	6	85	-	7
-Inositol	42	-	4	45	++	6.3	61	-	6.9
-Cynocobalmin	42	-	5.2	48	++	6.8	61	-	6.4
Control	17	-	5.6	69	++	6.3	41	+++	6.3

- = Absent , + = Poor, ++ = Fair, +++ = Good, ++++ = Excellent

Conclusions

Survey

In the survey conducted of different places in Vadodara district, Anand district and Chhota Udepur it was found that the highest mortality of castor plants was in Anand District in Ode and the largest number of castor production (approximately) was recorded in Padra (Vadodara district), Least mortality was recored in the district of Anand then Chhota udepur. Although Chhota udepur had least number of castor production it was also reported to have the least number of mortality.

Serial soil dilution tests

Serial soil dilution tests of the fields of the rhizospheric and non-rhizospheric soils of castor plants was done to find out various rhizospheric and non – rhizospheric fungi.It was found that the occurrence of *F. oxysporum, F. solani, A. flavus, Colletotrichum* sp., *Chaetomium* sp., *Rhizopus* sp., *Penicillium* sp.and T*richoderma* sp. was higher in The rhizospheric soil as compared to the non rhizospheric soil.

Pathogenicity tests

An experiment was conducted to se that the fungus found from the seed and if infected again produced the same symptoms in the plant and if it is pathogenic or not. So when this experiment was performed it was found that remarkable change in biomass of plants grown with AM AM inoculum as compared to the ones infested with the strains of *F. oxysporum* and *F. solani,* Also less wilting symptoms were reported in AM fungi than *F. oxysporum* and *F. solani*

Mortality

Highest mortality was recorded (80%) in plant infested with *F. Solani* and *F. roseum,* followed by *F. oxysporum* but 100% germination was recorded in control varieties. Highest rate of mortality was recorded in variety AVANI 41 and Local when infested with *F. oxysporum.* Where as in GCH4 and Local variety percent mortality reduced to 65% when infested with F. solani and F.roseum thus proving F. oxysporum is more pathogenic than other two strains.

Storage Fungi

In blotter method least number of fungal organisms were observed in treated seeds of GCH4, where as in treated seeds the number was more . In treated seeds of AVANI 41 and LOCAL it was found that eight different types of fungal colonies were found. Also A. awamorii was found only in untreated seeds. Compared to the blotter method P>D>A maedium had more number of fungal colonies as compared to Czapeck's and Asthana & Hawker's media.

The reason can be that water is required as a metabolite and solvent to maintain sufficient moisture for apical extension of hyphae . But also growth of some terrestrial fungi is dependent on water availability.

I year storage fungi

It was found that when castor seeds were placed in polybags, paperbags and glass bottles Temperatur of 10° C showed germination upto 70% inGCH4, 72% in Avani 41, and 90% in Local variety . Also in polybags it showed 92% in GCH4, 94% in Local but 92% in Avani 41.in glass bottles.

After 8 months of storage at 5 to 10ºC the percentage germination reduced drastically in different set of storage conditions. Germination percentage was comparatively better in

Avani 41 variety at 25ºC . Most common occurring fungi were species of *Aspergillus, Mucor,* and *Rhizopus,* Members like *Alternaria, Fusarium* were also present.

The number of fungi occurring reduced as well as there was reduction in percentage germination of seeds. At 4ºC, complete loss in seed viability was noticed, which might be due to the chilling sensitivity of seeds as some of the plant species, are sensitive to chilling temperature and lose viability quickly.

From the seed germination studies it has been concluded that seed germination decreases with an increase in the storage period of seed samples. This could happen due to the association of storage seed mycoflora with stored seeds.

Biochemical properties of Oil

Due to antifungal property of the castor oil which is very well known it was tried to study the effect of *F. oxysporum* (wilt causing pathogen) on seeds of castor and how the infestation dpletes the properties of oil.Parameters like weight of seeds, oil extracted, pH, viscocity, Acid value, Saponification value, specific gravity and Iodine value of healthy and diseased seeds were studied it was found that there was a remarkable difference in the dry weight of healthy and diseased seeds, Also from diseased seeds lesss oil was extracted in comparision to healthy seeds. pH levels dropped in all the three varieties of castor seeds. Acid value decreased.Similarly saponification value, Specific gravity and Iodine values of seeds (infected) decreased but viscosity of castor oil remained same in healthy and diseased seeds.

Control of seed borne fungi by plant extracts

To study the effect of antifungal property of five different plants *viz. Azadirachta* indica, C*ocoloba*, *Aegal marmelos, Cretava* and *Ashoka* were selected. Along with these plant

extracts, Potato dextrose Agar medium (control) and Potato dextrose Agar in the concentration was used.

It was fond that plant extract of *Azadirachta indica* had more antifungal properties and was found to be most effective on *F. solani* followed by *F. oxysporum*, but at the same time it, was found to be least effective on *F. roseum.* Similarly *Cocoloba* plant extract was found to be least effective on *F. roseum*, followed by *F. solani a*nd *F. oxysporum* . *Aegel marmelos* was found to be effective in *F .oxysporum, F. solani and F. roseum* and Ashoka extract was found to have very less impact on all the three *Fusaria.* It was concluded that out of all the plant extracts tried *Azadirachta indica* had the highest antifungal property.

Effect of fungal culture filtrate on seed germination

To study the impact of fungal metabolites on the seed germination of *R.communis* seeds, l eleven fungal culture filtrates were used.Results revealed that *F. oxysporum* was the most pathogenic fungi which inhibited seed germination in variety GCH4. Local variety compared to control sets with the seed variety AVANI 41 only 20 seeds germinated. *Colletotrichum* sp. was also found to inhibit seed germination in Local variety as seed germination recorded was only 16%,in GCH4 it was 20%,as compared to AVANI 41 it was 63%. *F. solani* and *A. alternata* were found inhibiting seed germinating in GCH4 up to only 30%, F. *solani* was not so effective as seed germination was 53% in Avani 41and in local it was 46%. *F. roseum* , *Rhizoctonia* sp. *A. flavus*, A. *niger*, and *T. viride*, were not able to inhibit seed germination as the germination percentage was much higher in seeds soaked with these culture filtrates

Effects of AM fungi on change in biomass of three varieties of castor

To see the effect of Am fungi on change in biomass of three varieties of castor an experiment was set up an the results revealed increase in fresh and dry weight of the root and shoot was observed up to 90 days after application of AM fungi.

Increase in shoot length was recorded after 75 days in control and after 60 days in treatment with AM fungi. There was gradual increase in root length from 15 days to 90 days. The increase in root length was more as compared to control sets in all the three varieties of castor. As compared to control in three varieties of castor, Am incorporation showed almost 100% increase in root length. It was thus proved that 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.

Culture medium

Different culture mediums were tried and although the growth was maximum in Richard's medium, excellent sporulation was produced in Modified Asthana and Hawkers medium 'A' and Potato Dextrose medium. The final pH in all the cases shifted towards alkalinity but in case of Czapek's medium the final pH turned acidic. Also it was noted that the dry weight of *F. solani* was maximum in Richards, followed by PDA, Asthana and Hawkers modified, Czapek's and Asthana and Hawker's 'A' medium. The sporulation was excellent in PDA and Richard's and fair incase of Asthana and Hawker's (Modified). The final pH in all the cases shifted towards neutral or alkaline side.

Although potato dextrose medium was reported suitable for the organismst this media cannot be considered suitable for further studies because chemical alterations are not

possible as Potato is a natural substance and forms a semi synthetic medium. Although this medium supported good growth and sporulation for the present three *Fusarium sp.* Richard's medium was found best. Also modified Asthana and Hawker's medium 'A' did not support excellent but sufficient growth and sporulation in all the *Fusarium* species under the present study. Moreover, this medium was easy to manipulate to do the expected need for modifications and substitutions of its constituents. Hence it was used in all subsequent cultural studies.

Effect of different pH

To study the effect of pH of the medium on growth and sporulation of three species of *Fusarium*. The initial pH values of modified Asthana and Hawker's medium 'A' were adjusted from 3.0 to 8.0 (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0). Results showed that 3 *Fusarium* sp. grew on a wide range of pH (3- 8). The sporulation was found between pH 4.5 and 7.5. Also the maximum growth of *F. oxysporum, F. roseum* was obtained at pH 5.5. while growth of *F. solani* was same at pH 5 and 5.5.

. On the basis of studies conducted at different pH levels it was found that pH 5.5 supports excellent growth and good sporulation, therefore in all the subsequent experiments the initial pH of Asthana and Hawker's modified was adjusted to 5.5 for further studies.

Effect of Different Temperatures

Temperature affects various activities of organisms such as mycelial growth, reproduction and spore germination. Thus, temperature determines various metabolic activities of fungi and even distribution of pathogenic fungi around different parts of the

world.Also reports have been shown that disease can be avoided if the crops are grown at temperature which is apposite to the pathogen, The optimum temperature for fungal growth is reported between 20º C and 30º C as below 0ºC and above 40º C fungi usuallydo not grow, still certain fungi are reported to grow even below the freezing conditions.

Effect of Different Sugars and their Utilization

As fungi are heterotrophic organisms thus they utilize different sources of sugars and polysaccharides by different rates. Monosaccharides are easily assimilable forms of carbohydrate, among which glucose has been reported to be the most efficient source of carbon and energy for most of the fungi. Fungi are very specific in utilization of different carbon sources, that at a time a carbon source may be utilized by a fungus while another source of almost similar chemical nature may prove useless for it.

It was noted that D- Arabinose Was utilized on the 11^{th} day in F.O, and in F.S and F.R it was presnt till the 12^{th} day. Similarly D-Glucose was present for 10days in F.O and F.S and 12 days in F.R. Sucrose was utilized and break down in to glcose and fructose occurred on 2^{nd} day 8 days in F.S and 4 days in F.R. Maltose was utilized by F.O in 2 days, F.S and F.R in 2 days.Presence of starch in F.O and F.s was uptil 2 days and in F.R in 10 days. Pectin was present till 12 days in F.O and 10 days in F.S and 9 days in F.R

N**ITROGEN**

To study the effect of different nitrogen sources on three fusaria an experiment was set up and it was found that the three species of *Fusarium* differ in their ability to utilize various sources of nitrogen. All the three species produced scanty growth in complete absence of the nitrogen. Of the four nitrate sources tried, calcium nitrate was best for *F.*

oxysporum followed by Potassium nitrate and the same was true for the other two *Fusarium* spp. Ferrous ammonium sulphate supported excellent growth and good sporulation of all the three *Fusarium* sp. Nitrates were found to be good sources of nitrogen for most of the fungi studied poor growth was rcorded in Ammonium molybdate in all the three pathogenic fungi and Ammonium chloride also showed similar results. Ammonium dihydrogen ortho phosphate,also showed poor growth. Ammonium oxalate is a different group of compound because both cation and anion of the salt are utilized as carbon and nitrogen sources. The presence of additional carbon source may be the cause due to which there was some growth after 10 and 15 days of incubation. In case of organic source of nitrogen, urea was used and it was found that it produced sufficient growth and fair sporulation in case of all the three pathogenic species. of *Fusarium* thus urea can be utilized as a source of nitrogen but not as a carbon source. Urea is converted in to ammonia and carbon dioxide by enzyme urease

Effect of different vitamins on growth and sporulation of three *Fusaria*

Maximum mycelial dry weight of *F. oxysporum* was obtained at 50 ppm concentration of Thiamine. Growth of *F. solani* and *F. roseum* was however, more at 200 ppm concentration. sporulation in all the three cases was observed at lower concentration i.e. in 50 and 100 *ppm.* In Riboflavin Maximum mycelial yield of *F. oxysporum* and *F. roseum* was observed at 50 ppm concentrations of riboflavin. In case of *F. roseum* 50 ppm showed lesser mycelial weight in comparission to concentration 100, 150 and 200 *ppm*. No sporulation was produced by all the three fungi up to 15 days of incubation.

In Inositol Except *F. oxysporum* other two fungi produced maximum mycelial yield at 200 ppm concentration. *F. oxysporum* produced better growth at 150ppm. Sporulation in *F. oxysporum* was present between 50 and 150 ppm, in *F. roseum* and *F. solani* sporulation was present in all the concentrations tried. *F. oxysporum* produced maximum mycelial weight at 150 ppm followed by 200, 50 and 100 ppm. 150 ppm also supported maximum growth of other two *Fusarium* spp. Sporulation was also present at this concentration in all the three fungi in Pyridoxine. L Ascorbic acid Of the 4 concentrations of Ascorbic acid tried 100 ppm produced maximum mycelial dry weight of *F. oxysporum*, while it was 75 ppm concentration for the other two *Fusarium* sp. Sporulation was present in *F. solani* and *F. roseum* while it was absent in different concentrations in case of *F. oxysporum.* Lower concentration of Nicotinic acid *i.e*. 25 ppm produced maximum mycelial dry weight in all the cases except *F. roseum*. Sporulation was not recorded at this concentration in any of the 3 Fusaria. Growth was maximum at 50ppm.Sporulation in case of *F. solani* was recorded at 75 and 100 ppm concentration.In Folic acid the *F .oxysporum* produced maximum growth and sporulation at 20 ppm concentration. However *F. solani* and *F. roseum* produced maximum growth at 10 ppm concentration. In all the cases however the growth was more after incorporation of folic acid. Arya (1996) reported inhibitory effect on coelomycetous fungus *Phomopsis viticola* on folic acid. In Biotin the Fungal dry weight of *F. oxysporum* reduced with the increasing concentration of Biotin. Arya (1991) found best growth of *P. psidii* at 5 ug/l of Biotin. In case of *F. solani* maximum mycelial yield was recorded at 15 ppm while it was 20 ppm of *F. roseum.* Sporulation was present in all the cases at 5 and 10 ppm. While in case of *F .solani* it was present in all the cases.

Cyanocobalmine(Vitamin $B_{12)}$ showed a marginal increase in mycelial dry weight of *F. oxysporum* and *F. solani*. A marked increase in mycelial dry weight was observed at 20 and 30 ppm concentration in case of *F. roseum*. All the 3 fungi showed sporulation in all the concentrations tried. The absence of an individual vitamin was studied by excluding one of them at a time from the mixture of the vitamins. A medium devoid of any vitamin served as control. It is evident that addition of all the vitamins produced maximum mycelial dry weight in the three *Fusarium* sp. under study. Removal of Thiamine, Pyridoxine, Inositol, Cynocobalamine reduced the mycelial dry weight than the control set. In case of *F. solani* the elimination of Thiamine, Pyridoxine, Riboflavin, L-Ascorbic acid, Nicotinic acid, Inositol, Cynocobalamine reduced the mycelial weight than of the control set. In case of *F. roseum* the elimination of folic acid proved to be harmful as the mycelial dry weight reduced in this case. Same was true for Inositol in case of *F. solani*. Response of different vitamins on sporulation of three different *Fusaria* varied.

Therefore, it can be concluded that *F. oxysporum* was deficient in five vitamins, *F. solani* in seven different vitamins and *F. roseum* in one vitamin *i.e.* folic acid.

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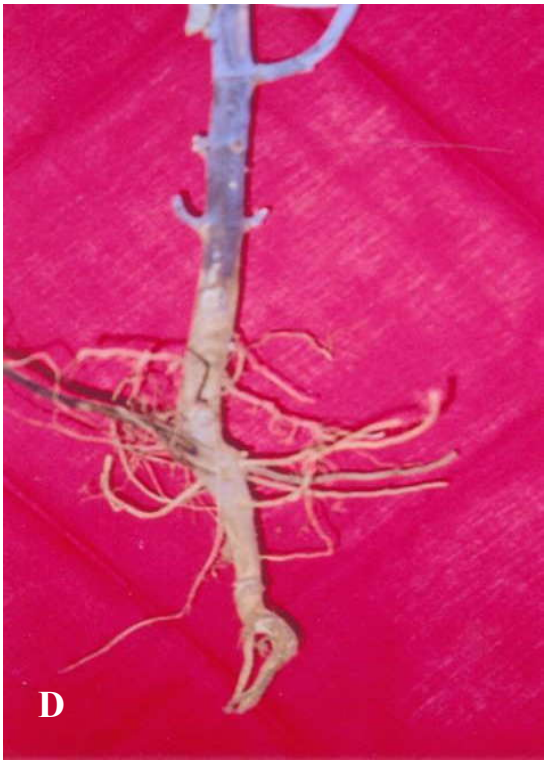
Plate I



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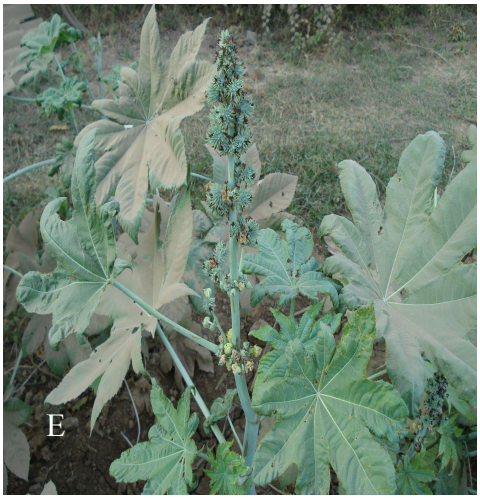
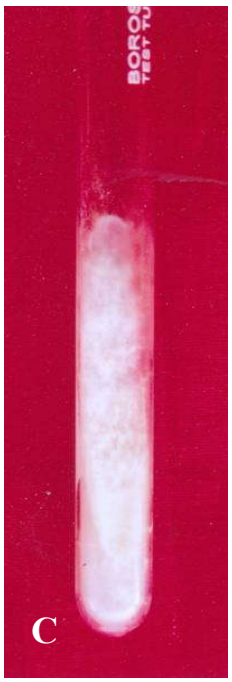


Plate III

Plate IV



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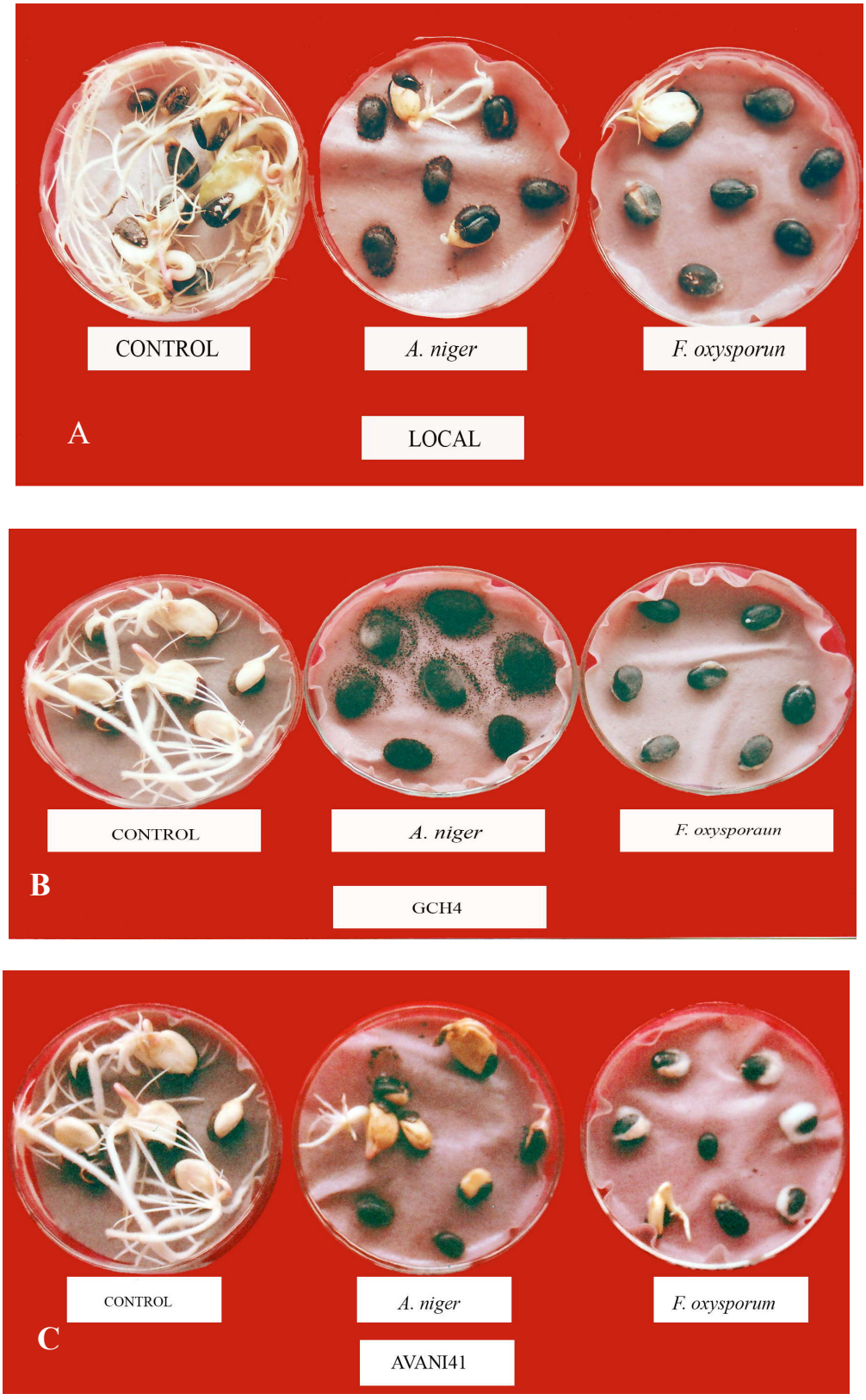
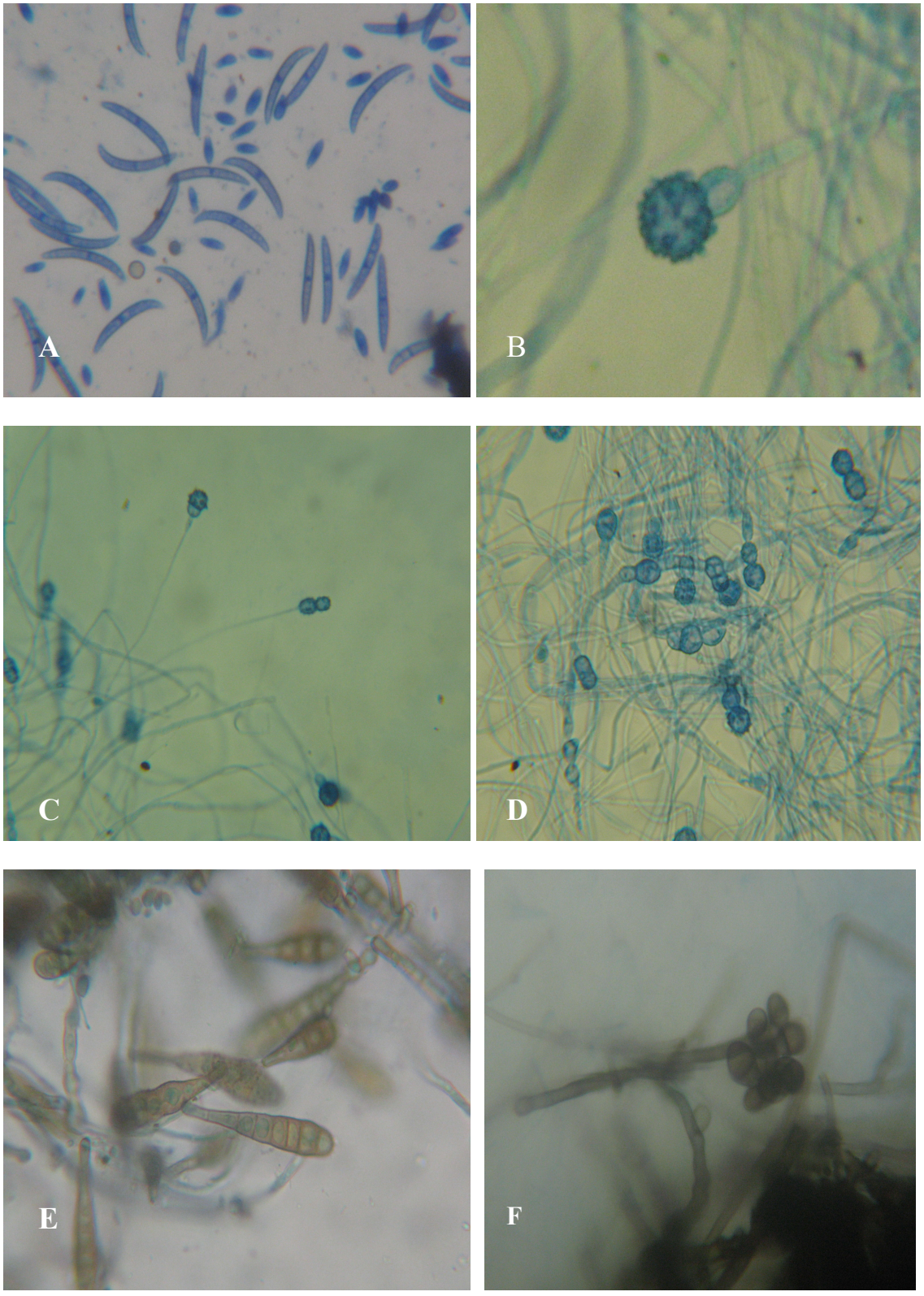

CONTROL
A. niger
F. oxysporun
A
LOCAL
CONTROL
A. niger
F. oxysporaun
B
GCH4
CONTROL
A. niger
F. oxysporum
C
AVANI41

Plate VI



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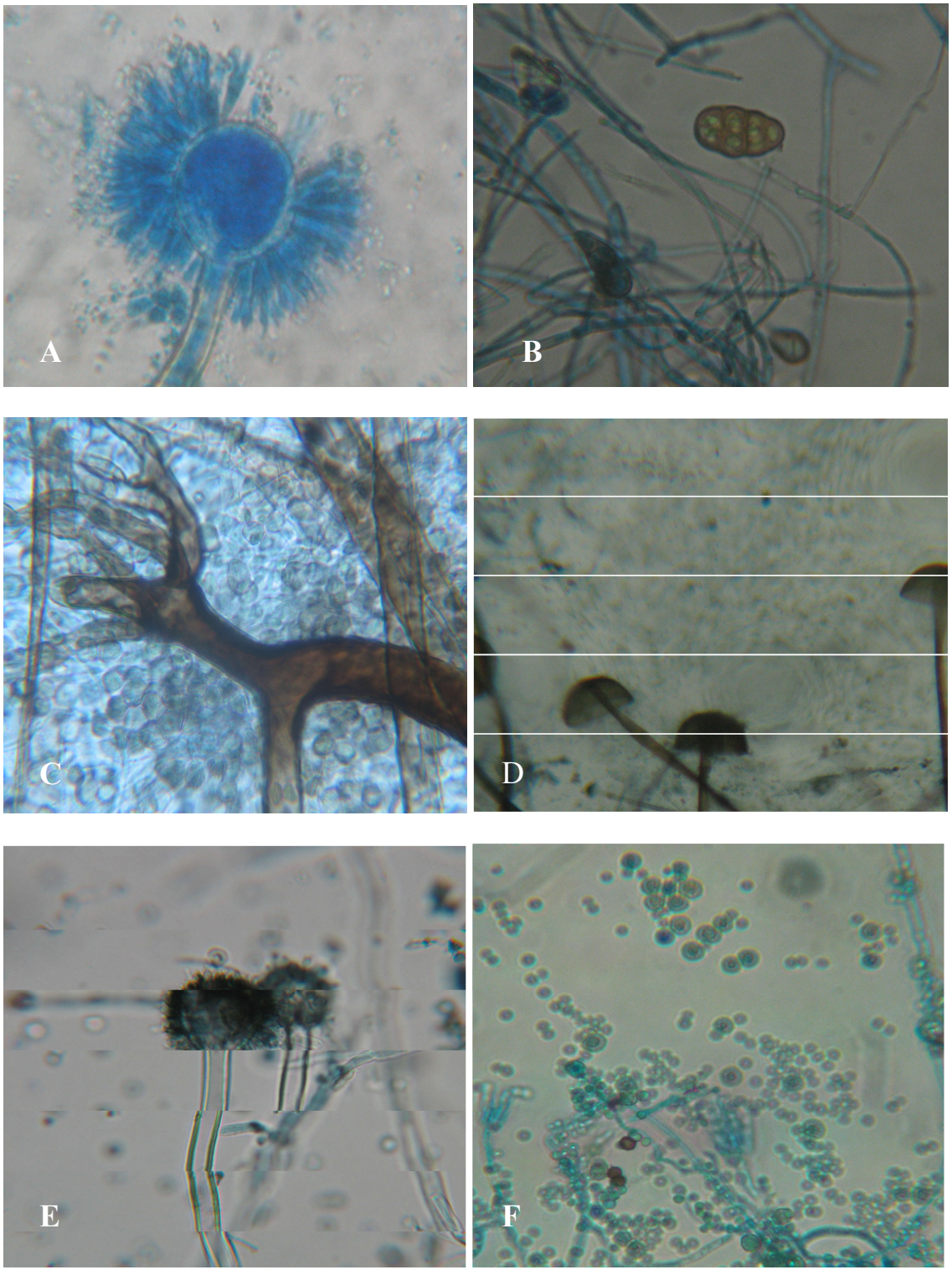
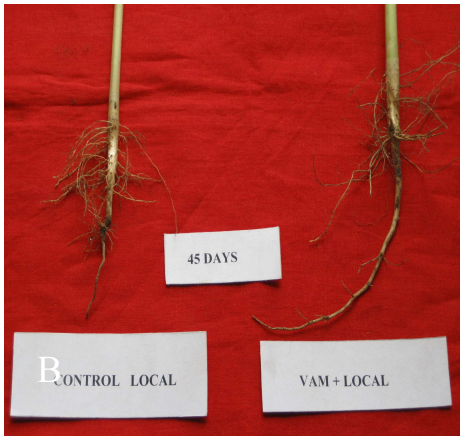


Plate VIII


A


45 DAYS
B
CONTROL LOCAL
VAM + LOCAL


CONTROL GCH4
45 DAYS
VAM GCH4
C


45 DAYS
D


E

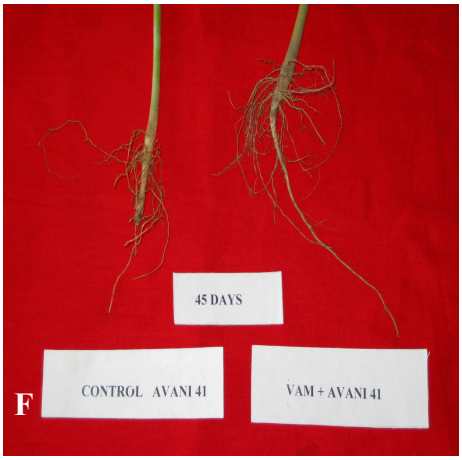
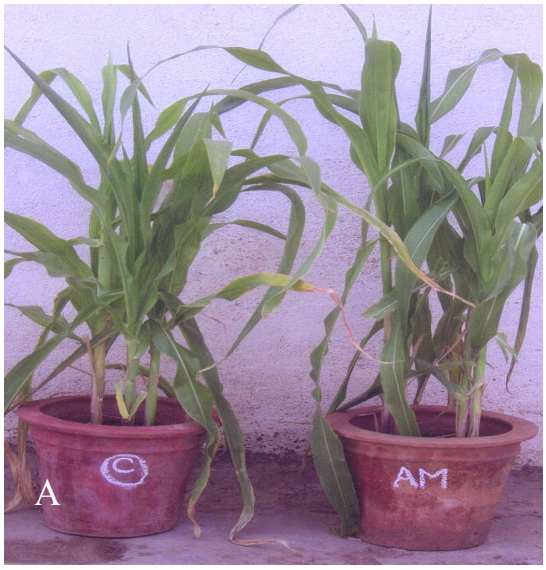
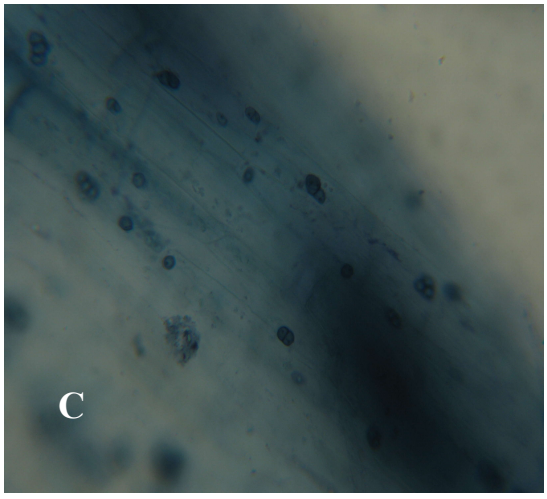

45 DAYS
CONTROL AVANI 41
VAM + AVANI 41
F

Plate IX





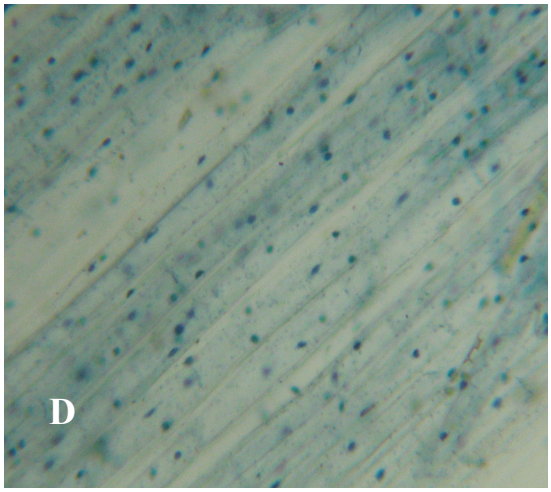
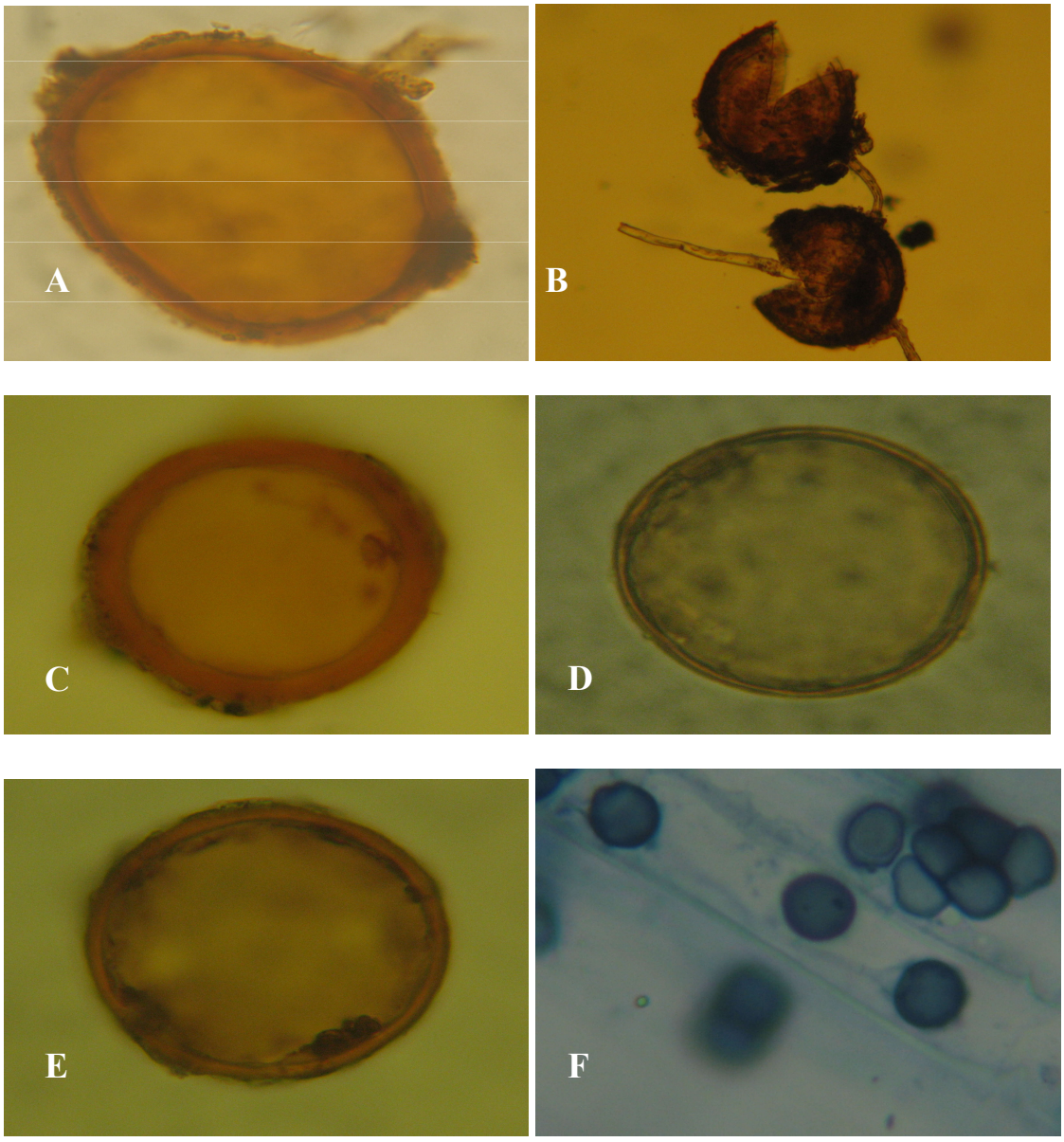


Plate X



ANNEXURE I

2nd Asian Congress of Mycology and Plant Pathology , Dec 19-22, 2007, Osmania University, Hyderabad, India.

Arun Arya, Renu Misra and Chitra Arya

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S-07. P-294. Mycoflora associated with certain medicinal plant parts are affected by fungi. These deplete valuable nutrients and contaminate them with several toxins. Among all the mycotoxins, aflatoxins have been studied extensively with regard to their toxic effects on plants and human beings. Carcinogenic potential of aflatoxins have been reported. These may be harmful to various organisms such as the liver, kidney and gastrointestinal tract. Aflatoxins B_1 and B_2 can be converted to aflatoxins G_1 and G_2. During storage of medicinal plant parts if conditions are moist and temperature ranges between 25 and 30 °C the production of mycotoxins is higher. Ten samples of medicinal plants were analyzed for the percentage incidence of fungal contaminants. The association of fungi producing aflatoxins was determined. Incidence of *Aspergillus flavus* was found higher in *Terminalia chebula*, *Asparagus racemosus* and *Emblica officinalis*. Occurrence of *Aspergillus niger* was found higher in *T. bellarica* followed by *Glycyrhiza glabra, Punica granatum*, *Citrus medica* 9 fruit rind) dried fruits of *E. officinalis*, *Piper longum, T. chebula*, *Piper nigrum*, *Rauwolfia tetraphylla* and *A. racemosus*. The presence of aflatoxin B_1 was detected in all the medicinal plant materials.

ANNEXURE II

Diseases of Fruit Trees: Recent researches and Eco- friendly Management : 2009

Diseases of Papaya and strategies for their effective management

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The papaya (Carica *papaya* L.) is rapidly becoming an important fruit internationally, both as fresh fruit and as processed products. Its latex is used for tenderization of meat . fruits are rich source of sugar and Vit. A. Some of the Indian varieties include Honey dew, Coorg honey dew, Washington, Pusa delicious, Co-1, Co-6. papaya is stacked by various fungal, viral and bacterial pathogens. Fusarial rots and anthracnose fruit rot cause havoc.

Postharvest handling and storage methods are described in detail. Fruits can be stored between 10 and 16 ° c for more than 15 days. Seal – packaging with low density polyethylene retarded the development of peel color and prevented the softening of fruit. Plastic film wraps were more effective than waxing in reducing the waterloss. Hot water treatment alone or in combination with fungicides was recommended. Biological control of Papaya with *Bacillus subtilis* and *Pseudomonas cepacia* are recommended during postharvest phase to prevent the fruit rot in storage.

ANNEXURE III

Management of Fungal Plant Pathogens

Mechanism of Action in arbuscular Mycorrhizal symbionts to control Fungal Diseases

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Currently the world over, especially in developing countries, maintenance of soil fertility and control of plant diseases have become crucial issues in meeting the biomass needs for food, fodder and fuel, as well as preserving a clean environment. An ideal fertile soil is characterized not only by microbiological processes that are maintained in equilibrium. More than 905 of land plants are estimated to form arbuscular mycorrhizal (AM) associations with soilborne fungi in the phylum *Glomeromycota*. They have a wide host range, yet certain host and fungal combinations are more effective from either the perspective of the fungus, i.e. greater spore/ hyphae production, or from that of the host, i.e. enhanced growth, nutrient acquisition or pathogen resistance. Besides improving uptake of phosphorus, Am fungi improve plant health through improved resistance to various botic and abiotic stresses. Of particular importance is the bioprotection conferred to plants against many soil borne pathogens, such as species of *Aphanomyces*, *Cylindrocladium*, *Fusarium*, *Macrophomina*, *Phytophthora*, *Pythium, Rhizoctonia*, *Sclerotium*, *Thielaviopsis* and *verticillium*, as well as various nematodes by AM fungal colonization of the plant roots.

Achieving the effective and sustainable control of plant diseases remains a formidable challenge for all agricultural systems. Despite the continued release of resistant cultivars and pesticides, pathgens still cause crop damages and losses that exceed 12% worldwide. Studies have shown that root rot in wheat caused by S. rolfsii was prevented by the inoculation of *Glomus fasciculatum*. Reduced quantum of lesioned roots was found in take – all diseases caused by *Gaeumannomyces graminis tritici* due to *G. deserticola* in wheat. The association of *G. radiatum* with apple has been studied in the USA. It was found that soil borne fungi, *Cylindrocarpon*, *Pythium* and the parasitic nematode, *Pratylenchus* spp., were common with replant diseases of apple. In this disease, young trees are stunted and develop fewer branches than healthy trees.

The exact mechanisms by which AM fungal colonization confers the protective effect are not completely understood, but a greater understanding of these beneficial interactions is necessary for the exploitation of AM fungi in organic and / or sustainable farming systems. The mechanisms employed by AM fungi in indirectly to suppress plant pathogens include enhanced nutrition to plants; morphological changes in the root; increased lignification; changes in the chemical composition in the mycorrhizosphere. Bioprotection within AM fungal – colonized plants is the outcome of complex interactions between plants, pathogens and AM fungi. In this chapter, the different diseases of cereals, pulses fruits and vegetables and the potential mechanisms by which AM fungi contribute to bioprotection against plant soil borne pathogens are discussed.

ANNEXURE IV

Role of seed borne Fungi on Percentage Germination of Three Varieties of Castor (*Ricinus communis* L.) and their Growth Perfomance by Incorporation of AM fungi

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Aryaarunarya@rediffmail.com

India is one of the leading producers of castor. The oil of castor (*Ricinus communis* L.) is used in pharmaceuticals, medicine and industries related to soap, paint, and lubricants etc. agricultural scientists in the country have developed a large number of hybrid varieties exploiting hybrid vigor. The studies were undertaken to assess the occurrence of seed borne mycoflora at different temperatures, when the seeds were stored for one year in different types of containers in Baroda, India.

Seed health ensures a better crop in the field Seed storage is a serious problem in tropical countries. The experimental results showed association of 19 different fungi with three different varieties of castor. Presence of seed mycoflora reduced percentage seed germination to 10 in GCH4, 20in Avani 41, and 38 in local var. after storage at 25° C. It was 13% in GCH4, while germination reduced to 7 and 12 % in Avani 41 and local var. when stored to one year at 10 °C.

The growth of plants is influenced by Mycorrhizal Symbiosis. AM fungi not only provide nutrients and vital minerals necessary for growth but also provide water balance and protection from phytopathogens. To assess the role of AM fungi on growth enhancement a pot experiment was performed. In which AM consortium was added and increase in plant biomass was recorded in all the treatments. After 90d of growth the numbers of leaves were 9 as compared to 8 in control plants of local var. The leaves were 14 in other two var. after 90 d. five percent wilting was recorded in control plants of local var., in other plants no such symptoms were recorded.