SURVEY AND DETECTION OF SEED BORNE FUNGI OF CLUSTER BEAN (*Cyamopsis tetragonoloba*) AND THEIR CONTROL BY NON- HAZARDOUS METHODS

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CERTIFICATE

I feel great pleasure in certifying the Thesis entitled ***"SURVEY AND DETECTION OF SEED BORNE FUNGI OF CLUSTER BEAN (Cyamopsis tetragonoloba) AND THEIR CONTROL BY NON-HAZARDOUS METHODS"*** *is an original piece of work carried out by* ***Mr. Vikas Pareek*** *under my guidance and supervision for the degree of* ***DOCTOR OF PHILOSOPHY in Botany, Govt. College Kota, Kota (Rajasthan).*** *No part of this thesis has been submitted for any degree or diploma in India or Abroad.*

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TO WHOM-SO-EVER-IT-MAY CONCERN

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NAME OF PAPER	MAX. MARKS	MARKS OBTAINED
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RESULT :	Pass	

It is also certified that Mr. /Ms. **Vikas Pareek** *was assigned the task of Paper II in* ***Ph.D.*** *programme, w.e.f* ***15.02.2011*** *under the supervision of* **Dr Rashmi Verma**

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Shree Ganeshaya Namah

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expertise is who, helps in adversity
here are few lines I am dedicating
to those who made me to step up in my further career."

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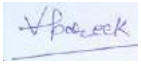

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Dedicated to

My Respected Parents

Mr. Ramavtar Pareek

&

Mrs. Nirmala Pareek

CONTENTS

Chapter – 1

Introduction

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] commonly called guar is an important legume crop belongs to family Fabaceae. It is drought tolerant crop suitable for cultivation under rainfed conditions of kharif season in arid and semi arid regions of India. Cluster bean is grown as green manuring, green fodder and vegetable crop for human consumption since ancient times in India and Pakistan. It is also grown for production of seeds and extraction of guar gum purposes in India. The crop has special importance because of gum content present in its seed (Paroda and Arora, 1978; Patel, Patel and Desai, 2002; Meena, Godara and Gangopadhyay, 2010; Pathak et al., 2010; Pachundkar et al., 2013). The guar seed comprises three parts: the seed coat (14-17%), the endosperm (35-42%), and the germ (43-47%). The guar gum which is the prime marketable product derived from endosperm of guar (Sharma and Gummagolmath, 2011).

India accounted approximately 80% of the world trade of guar gum and guar seed was among the top three traded agricultural commodity on Indian bourses (Mishra, 2008). Guar crop has gained worldwide recognization especially in South Western United States where it was introduced from India around 1906 (Poats, 1960) and in Australia for the glactomannan gum having diversified industrial uses (Hymowitz and Matlock, 1963; Guha, 1963; Whistler and Hymowitz, 1979 and Jackson and Doughton, 1982). The presence of glactomannan gum resulted in to making guar as an important industrial cash crop. Guar meal is the main by-product of guar gum production. It is a mixture of germs and hulls at an approximate ratio of 25 % germ to 75 % hull (Lee et al., 2004).

Guar is known as various names in India such as Gorani (Sanskrit), Guar ki Phalli, Gower (Hindi), Bavachi, Guwar, Gavari (Marathi), Gover (Gujarati), Guara, Guwar (Panjabi), Kothaverai (Malyalam). These vernacular names suggest its wide uses (Menon, 1973).

ORIGIN AND HISTORY

Cyamopsis tetragonoloba belongs to the tribe Indigoferae of Fabaceae. Chevalier (1939) postulated that *C. senegalensis* extended upto Sindh where after domestication a few of its cultigens became cultivated in India. Vavilov (1951) suggested India as the geographic center of variability of guar, though it was not found to occur in wild state in this region. Gillett (1958) pointed tropical Africa as its probable center of origin due to the occurrence of wild species viz. *Cyamopsis tetragonoloba*, *C. senegalensis* and *C. serrata* in that country. Hymowitz (1972) and Ecoport (2010) believes *C. senegalensis* to be the ancestor of *C. tetragonoloba* and that Arab traders were influential in moving the cultigens along the coast of southern India as horse fodder. The major world suppliers are India, Pakistan and the United States with smaller acreages in Australia and Africa. Commercial production of guar in the United States began in the early 1950s and has been concentrated in northern Texas and south western Oklahoma. Unlike the seeds of other legumes, the guar bean has a large endosperm (Undersander et al., 1991).

On cytological basis, Senn (1938) suggest that the genus *Cyamopsis* has been derived from genus *Indigofera* by aneuploidy but Gillett (1958) concluded that although there is close relationship between *Cyamopsis senegalensis* and *Indigofera*, it was preferable to retain *Cyamopsis* as a distinct genus.

DISTRIBUTION AND CULTIVATION:-

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] is an erect, bushy, annual herbaceous legume up to 3 m height with trifoliate leaves up to 10 cm long and white or rose flowers. The pods are straight, hairy, pale shiny green, up to 12 cm long and contain 5 to 12 hard seeds (beans). Guar is extensively grown in the semi arid and subtropical areas of the North and North West of India (notably in Rajasthan) and East and South East of Pakistan since ancient times later spreads to other Asian countries including Indonesia, Malaysia, and the Philippines. It is now grown in many other countries such as Central Africa, Australlia, South

Western United States and Srilanka. It was turned into a gum-producing crop during the second world war in the United States (Wong et al., 1997).

It is grown without irrigation in areas with 250-1000 mm of annual rainfall and most of seed production occurs in areas with less than 800 mm rainfall. Guar responds well to irrigation during dry periods but does not tolerate water logging. Excessive rainfall and humidity affect fertilization, pod development and seed quality of the crop. Guar is adapted to wide range of soil conditions and is tolerant of low fertility, soil salinity and alkalinity. It performs best on fertile, medium-textured and sandy loam alluvial soils but does not tolerate heavy black soils. Hence it is cultivated throughout the India. (Ecocrop, 2010; Ecoport, 2010; Wong et al., 1997).

India is the largest producer of guar seeds with 80% of total production in the world and Rajasthan is leading producer with about 75% of total production of India. The other major states involved in its production are Gujrat, Haryana, Panjab and Uttar Pradesh while in other states viz. Orrisa, Maharashtra, Madhya Pradesh and Karnataka guar is not grown to any appreciable extent (Directorate of economics and statistics, Government of India, New Delhi). Although in Rajasthan guar is grown in all the 33 districts but Alwar, Barmer, Bikaner, Churu, Hanumangarh, Jaipur, Jaisalmer, Jalore,, Jhunjhunu, Jodhpur, Kota, Nagaur, Pali, Shri-Ganganagar and Sikar districts are major producer with 80% of the total production in Rajasthan (Fig. 1). During the year 2011-12 total area under its cultivation was 3444 thousand hactares with a total production of 2218 thousand tones and the yield was 644 kg/ hactares; in 2012-13 total area under its cultivation was 5152 thousand hactares with a total production of 2461 thousand tones and the yield was 478 kg/ hactares and in the year 2013-14 total area under its cultivation was 5070 thousand hactares with a total production of 2862 thousand metric tonnes and the yield was 564 kg/ hactares (Board of Revenue of Rajasthan, Ajmer). The district wise area and production of guar seed in Rajasthan during the year 2013-14 is given in Fig. 1. The crop is grown commercially either as a pure crop or mixed with Bajra, Sorghum, Sesame, Mung and Jower (Menon, 1973; Anonymous, 1976). In India two cultivars, giant and dwarf are generally

recognized (Koccher, 2003). The former bears large, glabrous pods with flat seeds used for vegetables while latter possesses hairy, small sized pods with round seeds.

USES

Cluster bean is multipurpose kharif crop grown for green manuring, green fodder, vegetable, gum and grains. The gum and the water-soluble resin extracted from the seeds are used in industries including paper manufacturing, cosmetics, mining and oil drilling (Wong et al., 1997). Guar is a galactomannan polysaccharide that forms a viscous gel when placed in contact with water and forms a solution that range from slightly acidic to neutral pH. Guar gum forms gels in water even at low concentrations.

Food grade guar gum contains 80% guaran (a galactomannan composed of D-mannose and D-galactose units) with an average molecular weight of 220 kDa. However, guar gum is not a uniform product and its viscosity may vary in proportion to the degree of galactomannan cross-linking but the crop has special importance because of gum content present in its seed. (Gupta, Sagar and Pradhan, 1978; Paroda and Arora, 1978; Anonymous, 1987; Patel, Patel and Desai, 2002; Meena, Godara and Gangopadhyay, 2010).

The green and tender pods are cooked as favorite vegetables in many parts of country including South India. The green pods serve as a nutritious vegetable which contains 82.5% water, 3.7% protein, 9.9% carbohydrate, 0.2% fat, 2.3 % fiber, 1.4% other minerals viz. 0.13% Calcium, 0.25% Phosphorus, 5.8mg/ 100g Iron and 49 mg/ 100g Vitamin (Deore, Sawant and Ilhe, 2004).

During summer and mansoon seasons the crop is grown for green fodder under rainfed conditions hence its cost of production is lower in comparison to other legumes. The green plants at fruiting stage are used as a fodder for cattle containing 16% protein and 17 % mineral contents (Lander and Dharmani, 1942). Guar forage contains hydrocyanic acid (HCN) during the early stage. However its level decreases with the advancement in age of plant and is almost negligible at

maturity hence it is advisable to feed this fodder at fruiting stage (Oke, 1964; Bhan and Prasad, 1967; Vishwakarma et al., 2009). Guar residues are used for seeding camels in the arid regions of India (Saini et al., 2006; Bhakat et al., 2009).

For increase of fertility of soil by the guar plants during the vegetative stage, plants are ploughed down and mix with the soil. By adding organic matter they fix a considerable amount of atmospheric nitrogen (Rewari, Sen and Pandey, 1957).

The major three components of guar seeds are seed coat, endosperm and germ (embryo). Endosperm after mechanical separation from seeds yields 35-42% of gum which is utilized in various industries. The byproduct of gum industries consists of outer seed coat and germ material called guar meal.

Guar meal

The sweet and tender young pods are consumed as a vegetable or snacks in north-western and southern India and the mature seeds can be eaten during food shortages. Young pods, fresh or dry forage are used as livestock feeds. The plant is also used as a green manure and cover crop. Guar yields up to 45 t/ha of green fodder, 6-9 t/ha of green pods and 0.7-3 t/ha of seeds (Undersander et al., 1991; Wong et al., 1997; Ecocrop, 2010; Ecoport, 2010). Guar meal is the main by-product of guar gum production. It is a mixture of germs and hulls at an approximate ratio of 25 % germ to 75 % hull (Lee et al., 2004). A protein-rich material containing about 40 % protein, it is used as a feed ingredient but may require processing to improve palatability and remove antinutritional factors. The removal of the gum from seed enhances the protein contents (51%) of guar meal in comparison to seed. In addition to the regular guar meal ("churi"), certain Indian manufacturers sell a high-protein guar meal ("korma"). Guar meal contains about 12 % gum residue (7 % in the germ fraction and 13% in the hulls) (Lee et al., 2005), which increases viscosity in the intestine, resulting in lower digestibilities and growth performance (Lee et al., 2009). It is also good source of essential amino acids and useful protein supplement for chicks and laying hens.

Guar Gum

Guar gum is a Galactomannan (Galactose + Mannose) known as "guaran". It is a high molecular weight carbohydrate polymer. Guar gum is an extract of the guar bean, where it acts as a food and water store. The guar seeds are dehusked, milled and screened to obtain the guar gum. It is typically produced as a free-flowing, pale, off-white colored, coarse to fine ground powder. Guar gum obtained from the endosperm of the seed of the legume plant *Cyamopsis tetragonoloba*. The crude gum is grayish white powder which contains 78-82% galactomannon, 10-13 water, 4-5% protein, 1.5-2% crude fiber, 0.5-0.9% ash and 0.5-0.75% fat. Guar seeds contain 35-42% gum. Guar gum is a potential raw material derived from endosperm used in paper industry, cosmetics, pharmaceutical, oil well drilling, explosives, icecream processed cheese products, dressings and sauces, beverages, baked goods, pastry icings, as meat binders, canned meat products, textile, mining industry, tobacco and other various industrial applications. (Undersander et al., 1991; Saleem, Shah and Akhtar, 2002).

Food industries and bakery Products

It is used as thicken and stabilize salad dressings, icecreams, backery products, meats , confectionaries, sausages and cheese due to its ability to bind water.

Paper industry

Guar Gum provides better properties compared to substitutes. It gives denser surface to the paper used for printing. Guar Gum imparts improved erasive and writing properties, better bonding strength and increased hardness. Due to improved adhesion, it gives better breaking, mullen and folding strengths. It is added to pulp slurry before the formation of sheet on machine ensuring a regular distribution of pulp- fiber.

Textile industries

Guar Gum gives excellent film forming and thickening properties when used for textile sizing, finishing and printing. It reduces warp breakage, reduces dusting while sizing and gives better efficiency in production.

Oil Field applications

Industrial grade guar gum powder are use in oil well fracturing, oil well stimulation, mud drilling and industrial applications and preparations as a stabilizer, thickener and suspending agent. It is a natural, fast hydrating dispersible guar gum and is diesel slurriable. In the oil field industry, guar gum is used as a surfactant, synthetic polymer and deformer ideally suited for all rheological requirements of water-based and brine-based drilling fluids. High viscosity guar gum products are used as drilling aids in oil well drilling, geological drilling and water drilling. These products are used as viscosifiers to maintain drilling mud viscosities that enable drilling fluids to remove drill waste from deep holes. Guar gum products also reduce friction in the holes, and so minimizing power requirements. Some guar gum products act to minimize water loss should occur in broken geological formations.

Pharmaceuticals and Cosmetics

A high concentration of flavanoids and other phenolics compounds like kaempherol in guar seeds may expand its neutraceutical and pharmaceutical use. Leaves are used in asthma and to cure night blindness where as the pods and seeds are used to cure inflammation, sprains (Khare, 2004), arthritis (Katewa et al, 2004), as anti-oxidant, antibilious, laxatives and in polluting boiling. As per ayurveda the plant is used to reduce fire and can be used as cooling, digestive, tonic, galactogogue, useful in constipation, dyspepsia, anorexia, agalatia, hyetalopia and vitated condition of kapha and pitta. The Plant is also mentioned as aperitif and flatugenic (James, 2002).

Guar gum is used as an appetite depressant and also used as a bulking agent in laxative, in gastric ulcer and asthama treatment. It also reduced blood

cholesterol and glucose levels significantly by using an aqueous extract of pods of the plant at a dose of 250mg/kg of body weight. (Sharma, Dubey and Kaushik, 2011)

Guar gum used as a thickener in the manufacturing of battery electrolytes, printing inks and polishes protective colloid in skin care products, creams and lotions. It also used for easy extruding of toothpaste and shaving cream.

DISEASE STATUS IN CLUSTER BEAN

Guar is known to suffer from many diseases which are responsible for its poor quality and low yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange (Chand and Gandhi, 1978). The major diseases causing low planting value of crop include fungal, bacterial and viral diseases. Few names are sited so as to highlight the problem.

The important fungal diseases are *Alternaria* blight and leaf spot (*Alternaria cucumerina var. cyamopsidis, A. tenuis, A. tenuissima*) , Anthracnose (*Colletotrichum dematium*), dry root rot and charcoal rot(*Rhizoctonia bataticola syn. Macrophomina phaseolina, Rhizoctonia Solani*), *Fusarium* wilt, root rot and damping off (*Fusarium caeruleum, F. oxysporum, F. semitectum, F. solani*), Sothern blight and root rot (*Sclerotium roridum*), Powdery mildew (*Oidiopsis taurica* syn. *Levillula taurica* and *Sphaerotheca fuliginea*), leaf spot (*Curvularia lunata, Choanephora cucurbitarum*), damping off (*Pythium myriotylum*) and vascular wilt (*Neocosmospora vasinfecta*) causes major losses to yield of cluster bean crop. (Shivanna and Shetty, 1988a,b; Undersander et al., 1991; Mathur and Sinha, 1993; Lodha, Sharma and Aggarwal, 2002; Deore, Sawant and Ilhe, 2004; Jaiman and Jain, 2004; Saharan and Saharan, 2004; Jatav and Mathur, 2005; Singh, Chandil and Tripathi, 2005; Wijesekara, Aggarwal and Agarwal, 2005; Mohamed, Baulomy and Ibrahim, 2006; Purkayastha, Kaur, Dilbaghi and Chaudhury, 2006 and Awurum and Uwajimgba, 2013)

The important bacterial diseases of cluster bean are Bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*) and bacterial leaf spot (*Pseudomonas cyamopsicola*) which causes major losses in yield of cluster bean crop. (Kaur, Purkayastha, Dilbaghi and Chaudhury, 2006, Jain and Agrawal, 2011)

The viral disease of cluster bean is top necrosis and new green sterile disease caused by Guar Green Sterile virus (GGSV) **(**Moshe, Rey, Sibara, Gernett and Beck, 1991; Gillaspie, Pappu and Jain, 1998).

Some of these diseases have been reported to be seed borne (Neergaard, 1977; Richardson, 1979). In the book "Seed Pathology" Neergaard (1977) mentioned only two seed borne pathogens on Cluster bean *Alternaria cyamopsidis* and *Cercospora kikkuchii.*

Pursual literature shows that a very little stress has been given by earlier worker on guar concerning seed borne disease. Thus the present study has been carried out with following objectives:-

1. Survey and Detection of seed borne mycoflora associated with *Cyamopsis tetragonoloba* seeds in Rajasthan.
2. Find out the incidence and occurrence of various types of seed symptoms in cluster bean seed samples and fungi associated with them.
3. Histopathology of naturally infected guar seeds to determine invasion and spread of *Fusarium solani*.
4. Study of Phytopathological effects and disease transmission of *Fusarium solani* from seed to seedling/ plant using seeds carrying natural infection.
5. Biochemical analysis of seed, stem and leaves infected with *Fusarium solani*.
6. Control of seed borne inoculam of *F. solani* by using physical (Heated oil), chemical (Systemic fungicides), biological (Plant extract) and by using fungal antagonists (*Trichoderma harzianum* and *Trichoderma viride*).

7. Control of seed and soil borne inoculum by drip irrigation method as a culture practice in cluster bean field.

Hopefully this study will result in some meaningful application in field of seed pathology, agriculture and modern research.

Fig. 1: Map of Rajasthan showing district wise area (hectares) under cultivation and production of cluster bean.

- Coloured regions showed seed samples collected districts.


100 km
60 mi
RAJASTHAN
Shri Ganganagar
498200
544653
Hanumangarh
570776
543852
Bikaner
1133397
504961
Churu
525483
210258
Jhunjhunu
118470
110047
CB 70
Sikar
131655
97944
Alwar
65047
80239
Jaisalmer
579924
53405
Jodhpur
267634
170907
Nagaur
200836
93988
CB 29
Jaipur
69857
70534
Dausa
17634
18663
Bharatpur
33754
43740
Dholpur
11153
2882
1627
Karauli
14593
Ajmer
44540
80026
Tonk
15165
11473
13765
Sawai Madhopur
15547
Barmer
465225
106636
Pali
72162
25064
Bhilwara
58380
36231
Bundi
3867
2183
Kota
1960
1106
Baran
460
260
Jalore
105125
34767
Sirohi
33369
11159
Rajsamand
5457
2851
Chittorgarh
10842
9619
Jhalawar
467
264
Udaipur
13883
13177
180
102
Pratapgarh
Dungarpur
2483
1952
14
12
Banswara
YEAR : 2013-14
AREA (IN HACTARES)
PRODUCTION (IN METRIC TONNES)
DATA ARE BASED ON FOURTH ADVANCED ESTIMATE OF KHARIF 2013 BY BOARD OF REVANUE OF RAJASTHAN, AIMER UNDER AGRICULTURE MINISTRY, RAJASTHAN.

Fig. 1

Chapter –2

Review of Literature

The guar or cluster bean (*Cyamopsis tetragonoloba*) is an important annual legume crop and the source of guar gum. It is also known as gavar, guwar or guvar bean. Guar as a plant has a multitude of different functions for human and animal nutrition but its gelling agent containing seeds (guar gum) have the most important and worldwide use. The yield and quality is reduced due to many diseases. These diseases attack the crop in field as in storage. The most common diseases of guar are wilt, anthracnose**,** powdery mildew, leaf spots and charcoal rot. Post harvest or seed storage fungi as well as viral and bacterial diseases have also been reported from time to time. A critical comprehensive review of literature is inevitable for any scientific investigation. Menon (1973); Chand and Gandhi (1978) and Richardson (1979) observed and listed about few seed borne pathogens and disease of cluster bean. Here, an attempt is made to give a brief account on diseases of major and minor importance including seed borne mycoflora, biochemical analysis and non hazardous control in seed due to fungal infections. Since the literature of seed borne diseases on cluster bean is limited; relevant literature on other crop is also reviewed.

Wilt Disease

Fusarium wilt infected seedlings, collapse and lie flat on ground, retaining their pale- green colour. Adult plants of chickpea showed typical wilt symptoms of drooping of petioles, rachis and leaflets. The roots of these plants do not show any external rotting but when split open vertically, dark brown discoloration of cortex is seen (Nene et al., 1991). Various species of *Fusarium* viz., *F.moniliformae* (Sheld.) Emend by Snyder and Hansen (1941), *Fusarium solani* (Mart.) Sacc. and *F. caeruleum* (Lib.) have been reported to cause wilt disease in cluster bean. (Singh 1951; Desai and Prasad, 1955; Satyaprasad and Ramarao, 1981 and Dwivedi, Dubey and Dwivedi, 1991).

Singh (1951) reported typical wilt symptoms caused by *F.caeruleum* in cluster bean. In the field they observed sudden drooping of the plant. During severe infection, copious mycelial growth was observed around base of the stem. Split half of infected stem and root showed presence of mycelium in cortical

region. Pathogenicity was established by artificially inoculating the soil which also produced typical wilt symptoms. (Singh, 1951)

According to Singh (1954) wilt of guar (*Cyamopsis psoralioides*), caused by *Fusarium caeruleum* are common in Uttar Pradesh and had shown certain degree of periodicity in virulence. The total mortality of plants is lowest in crops sown during 1st and 2d week of May and highest in crops sown during July. Early sowing has proved better than the sowing carried out during July in respect of the disease incidence. This advantage is restricted to irrigated tracts.

Satyavir (1968) from New Delhi made extensive studies on wilt of guar. Satyavir and Grewal (1972) was observed wilt due to *F. caeruleum* on the variety "Sadabahar" at IARI, New Delhi causing heavy losses. Kuniyasu and Kishi (1977) studied seed transmission of *Fusarium* wilt of bottle gourd caused by *F. oxysporum*. They were observed pale yellow discolouration of stem and at the later stages fruit and seeds are also show wilt symptoms. Haware et. al. (1978) reported the fungus *Fusarium oxysporum f. sp. ciceris* caused wilt in chickpea is soil borne pathogen however, it can be transmitted through seeds also (Pande, Rao and Sharma, 2007). If the wilt (*F. oxysporum*) disease occurs in the vegetative and reproductive stages of the chickpea, the seed setting is very poor (Haware and Nene, 1980; Haware et. al., 1990; Halila and Strange, 1996; Nava et. al., 2000). The disease was menifests as mortality of young seedlings within 25 to 30 days after sowing in chickpea plants. Pande, Rao and Sharma (2007) observed wilt (*Fusarium oxysporum*) is the most destructive disease in India. It is seed borne as well as soil borne pathogen.

According to Palodhi and Sen (1983) bottle gourd seed yielded *Fusarium sp.* caused wilt mostly from micropylar end which is the region of placental attachment. Hyphal strands were also produced in abundance from the setures. They also reported seed infection was confined to testa and tegmen in asymptomatic seeds but heavily infected seed had inoculum in embryo also.

Shivanna and Shetty (1992) found carbendazim i.e. 50% as most effective against the spp. of *Fusarium* viz. *F.solani*, *F. equiseti* and *F.moniliformae* in

cluster bean when used singly. Mathur (1992) and Sharma (1992) studied about phytopathological and physiological effects on some seed disorder in soyabean. They observed redish brown discolouration in *Fusarium oxysporum* infected soybean seeds.

Bhatia (1995) reported wilt disease caused by *Fusarium oxysporum* in cluster bean seeds. According to them the heavily infected seeds carried inter as well as intracelluar mycelium in all components. Epidermal and hypodermal cells were disintegrated due to heavy aggregation of mycelium and chlamydospores. Patel et al. (1998) reported wilt disease of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] caused by *Neocosmospora vasinfecta*.

Gupta, Dubey and Singh (2011) studied seed borne nature of *Fusarium semitectum* caused wilt disease in *Dalbergia sisso.* Infected seeds showed either dark brown discoloration with compressed hilar region or covered with white mycelial crust. On incubation these seeds yielded pure growth of the pathogen. For the study seeds were characterized as asymptomatic and symptomatic (weakly and heavily infected) on the basis of severity of infection. Cleared whole mount preparations of seeds and their sections observed mycelium in various seed components, in asymptomatic it is restricted to layers of seed coat and it invades endosperm and embryonal axis in symptomatic seeds. The fungus gained entry in the seed either through hilar region or indirectly through the pores in seed coat. The pathogen is both externally and internally seed-borne. The internal inoculum affects seed germination and viability and caused high total (pre-and post emergence) losses, pathogen is transmitted from seed to seedling causing heavy losses to the tree plantation. Singh (2013) reported brown discolouaration with or without mycelial growth in lentil seeds infected with *Fusarium oxysporum*, symptoms appeared as pale brown discolouration on hypocotyl region.

For the control of wilt pathogen (*F. caeruleum*), Satyavir and Grewal (1972) tested 22 fungicide drench methods, found that ethyl mercury chloride was not only more effective but had better persistence with no phytotoxicity at minimum effective dosage of 25 ppm.

Root rot

Singh (1951) observed a serious root rot in 1947 at Kanpur for the first time in cluster bean. *Fusarium caeruleum, Rhizoctonia Solani, Sclerotium rolfsii* and *Neocosmospora vasinfecta* were isolated from diseased plants.

Singh (1954) reported root rot disease of cluster bean caused by *Rhizoctonia solani*. According to Ahmed and Ahmed (1969) cultural characteristics and growth rates of eight different jute isolates of *Macrophomina phaseolina* appeared to be related to their pathogenicity.

According to Nemec (1978) the fibrous root decay and epidermal and cortical sloughing were common on blight trees in two citrus groves with severe symptoms. These symptoms were present to varying extent on blight trees in other groves, and some fibrous root decline also was present on apparently healthy rough lemon trees. *Fusarium solani* was recorded from the roots of diseased and healthy rough lemon (*Citrus limon*) trees.

Sheikh and Ghaffar (1979) found that *Macrophomina phaseolina* (Tassi) Goid, [*Rhizoctonia bataticola* (Taub.) Butl.] is known to produce seedling blight, charcoal root rot, stem rot and pod rot. The fungus is believed to persist in soil in the form of small, black sclerotia, which are produced in large numbers on infected host tissues. Sclerotial density of *Macrophomina phaseolina* is different at various soil moisture levels on disease incidence in black gram, guar, okra and cotton.

Satyaprasad and Ramarao (1981) reported root rot of guar caused by *F.solani* from Hyderabad. The infected plants turn yellow, leaves droop and roots show black lesions at soil surface. The diseased plants wilt and die prematurely. An internal brown discolouration is observed when roots are spilt open.

Ramarao (1983**)** tested thirty varieties of guar infected with *Fusarium solani*. They were reported their disease reaction in pot culture and in field conditions against the pathogen caused root rot in guar.

Satyaprasad and Ramarao (1984) and Mathur and Shekhawat (1988) studied competitive saprophytic colonization by *Fusarium solani* caused root rot in *Cyamopsis tetragonoloba* grown in loam soils.

Lodha and Burman (2000) conducted an experiment at Jodhpur on a loamy sand soil during 1995-97 for the disease suppressive characteristics of composts in cluster bean and cowpea. The efficacy of three composts prepared from residues of pearl millet (*Pennisetum glaucum*), neem (*Azadirachta indica*) and weeds was ascertained on nitrogen fixation, dry root rot caused by *Macrophomina phaseolina* and seed yield of cluster bean (*Cyamopsis tetragonoloba*) and cowpea (*Vigna unguiculata*). Amendment of soil with pearl millet and weed composts for two consecutive year's reduced resident population of *M. phaseolina* by 20-40% compared with the non-amended plots.

According to Varma, Singh and Singh (1989, 1992b,c) and Kalim et al. (2003) severity of root rot were caused by *Rhizoctonia solani* and *Rhizoctonia bataticola* in cowpea and moth bean.

Purkayastha et al. (2006) selected 10 different isolates of *Macrophomia phaseolina* from cluster bean (*Cyamopsis tetragonoloba*) on the basis of wide geographical distance and potassium chlorate sensitivity, showed remarkable variation in virulence on the susceptible cluster bean genotype.

Yadav *et al* (2007) isolated *Rhizoctonia bataticola, R. solani, Fusarium oxysporum* and *Sclerotinia sclerotiorum* caused root rot of cluster bean (*Cyamopsis tetragonoloba*).

Iqbal and Mukhtar (2014) found *Macrophomina phaseolina* is a serious pathogen of mung bean crops. They obtained 65 isolates of *Macrophomina phaseolina* from different agro ecological regions of Punjab and Khyber Pakhtunkhwa provinces of Pakistan were analyzed for morphological and pathogenic variability. They observed significant differences among 65 isolates in their radial growth, sclerotial size, and weight as well as in pathogenicity (Riaz, Khan, Iqbal, and Shoaib, 2007). Earlier Atiq et al (2001) also reported pathogenic

and cultural variation of *Macrophomina phaseolina* in sunflower caused charcoal rot. Beas- Fernandez et al. (2006) revealed that pathogenicity of *Macrophomina phaseolina* has no relation with size and weight of sclerotia (Dhingra and Sinclair, 1977).

Seed treatment with oil seed cakes like Cotton cake, Mustard cake (Black and yellow) and Taramera cake alone or in combination with *Trichoderma harzianum* and *Trichoderma resei* significantly reduced colonization of roots by charcoal rot fungus (*Macrophomina phaseolina*) and significantly increased growth of sunflower (*Helianthus annus* L.) plants. Highest reduction in charcoal rot of sunflower was observed when seeds of sunflower were coated with cotton cake and *Trichoderma resei* followed by Taramera cake and *T. harzianum* and Mustard cake in combination with *Trichoderma harzianum* (Muhammad and Dawar, 2010).

Singh (2010) studied the effects of inoculation of three arbuscular mycorrhizal (AM) fungi namely *Glomus mosseae, Glomus sinuosum*, and *Scutellospora erythropa* in addition to *Pseudomonas fluorescens* and treatment with mustard oil cake on root-rot disease of *Cyamopsis tetragonoloba* L. taub. plants caused by *Macrophomina phaseolina* were evaluated under polyhouse conditions for 2 years. Inoculations of an arbuscular mycorrhizal fungus (AMF) in combination with *P. fluorescens* and mustard oil cake showed best supporting biocontrol system against the root-rot disease besides increasing the plant height, weight, and yield.

Lodha and Singh (2011) were developed eco-friendly management options in order to avoid dependence on expensive and hazardous chemical means of control. In rain fed agriculture, amendment of soil with composts prepared from residues of wastes was found effective in reducing incidence of *Macrophomina phaseolina* induced charcoal rot on legumes. The best control of this fungus and yield of crop were observed in compost amended plots compared to in non amended control. They were also observed similiar reduction of *Fusarium oxysporum* f. sp. *Cumini* pathogen.

Preeti et al. (2013) were observed leaf essay for resistance against *Macrophomina phaseolina* the causal agent of charcoal root rot of chickpea (*Cicer arietinum*).

Damping off

Damping off of guar caused by the seed borne inoculum of *Fusarium solani*. The pathogen from the stored guar seeds showing brownish discolouration. The infection was observed as blackish brown water soaked areas on the collar region and the seedlings drooped down (Dwivedi, Dubey and Dwivedi, 1991).

The severe infection occurred at the seedling, pre flowering and fruit maturation stages. The seeds obtained from infected pods were flat, deformed, discoloured and light in weight. The pathogen survives as a saprophyte when the crop is not in the field and remained viable for 4 months in plant debris. Dormant fungal propagules like thick – walled hyphae and chlamydospores like structure which are capable to withstand unfavorable conditions during high temperatures act as primary source of inoculum (Shivanna and Shetty, 1987, 1988a). They observed symptoms on seedling as dark brown circular spots on cotyledons, irregular black patches on hypocotyl and root region. Severely infected seedlings showed symptoms of blight and damping off.

Mohamed et al. (2006) observed efficacy of different natural products in Egypt as safe management of damping off disease in cluster bean. They were isolated *Fusarium oxysporum* Schlect.; *Macrophomina phaseolina* (Tassi) Goid.; *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc; from infected roots and basal stem parts of guar plants. According to them Topsin M and Vitavax/Thiram as well as clove essential oil (4000 ppm) have completely inhibited the mycelial growth of the four fungi. Chauhan et al. (2012) studied potential of *Azotobacter spp.* as biocontrol agents against *Rhizoctonia solani* and *Fusarium oxysporum* in cotton (*Gossypium hirsutum*) and guar (*Cyamopsis tetragonoloba*).

Antharacnose

Anthracnose is an important leaf and stem disease caused by seed-borne *Colletotricum dematium* (pers. ex. Fr.) Grove f. sp., *C. truncatum* (Schw.) Arxsyn and *Collectotrichum capsici* in cluster bean (Sowell 1965b). In georgia U.S.A. severe damage to guar crop was observed due to *C. dematium* f. *truncate* during 1960 (Sowell, 1963 and 1965b). The pathogen was reported to be seed borne inoculum of cluster bean (Sowell, 1965b; Shetty, 1988). The pathogen is located in seed coat and is seed borne. Shivanna and Shetty (1988a) reported the presence of mycelium in the inner layer of seed coat by using artificially inoculated and incubated seeds of cluster bean infected with *Colletotrichum dematium*. Shivanna and Shetty (1988b) also reported the conidia of the *Colletotrichum dematium* fungus germinated on seed surface of cluster bean producing appressoria and infection pegs which pierced the seed coat and invaded inner parts. The Inoculum was transmitted to plant causing systemic infection. They suggested extra embryonal infection followed by systemic type of disease transmission.

Shivanna and Shetty (1992) tested captan; zineb; carbendazim; maneb; quintozene and thiram individually and in combination with carbendazim and observed that the individual application both of captan and zineb i.e. 75% significantly reduced the incidence of *C. dematium*. Varma, Singh and Singh (1992a) revealed seed borne infection of *Colletotrichum dematium* in moth bean (*Vigna aconitifolia*). They observed black discolouration on infected seeds. Hyaline, pale brown to dark brown, septate mycelium present in all seed parts of moth bean. Sadda and Varma (2010) reported anthracnose disease of smooth gourd caused by *Colletotrichum orbiculare*. They observed black discoloration in infected smooth gourd seeds and symptoms appears as black pin head like acervuli in seeds. Disease is more severe in high rainfall subtropical to temperate areas than in tropical areas. The fungus is seed borne and symptoms may start as early as in seedling stage. The most characteristic symptoms of the disease are black, sunken, crater like cankers on the pods, stem or cotyledons. The lesions remain isolated by yellow -orange margins. Diseased areas give out dull salmon colored ooze from the centre when humidity is very high (Varma, Singh and

Singh, 1992a). According to Wijesekara, Aggarwal and Agarwal (2005) management of the *C. dematium* is possible through the use of healthy seed. Avoid excess watering; giving wider spacing; use of hot water treated seed ; spraying fungicides like Dithane M -45 or Dithane Z- 78 at the rate of 2 kg in l000 litres of water per hectare.

Powdery mildew

Powdery mildew of guar is caused by *Oidium spp*., *Odiopsis taurica* (Lev.) salm. Syn., *Levillula taurica* (Lev.) Arn and *Sphaerotheca fuliginea* (Schlecht ex Fr.) poll. *Levillula taurica* (Lev.) Arn is an important pathogen of guar in India and Pakistan (Rao and Rao,1954; Khan, 1958; Mihail and Alcorn, 1984 and Chandra and Saxena, 1990).

The first report of powdery mildew caused by *Levillula taurica* in North America comes from Arizona in cluster bean seeds (Mihail and Alcorn, 1984).The infection was characterized by copious production of conidiophores and conidia on both the leaf surfaces. The affected areas were showed slight chlorosis followed by defoliation.

Chandra and Saxena (1990) noticed a new pathogen *Sphaerotheca fuliginea* causing powdery mildew in cluster bean around Aligarh (Uttar Pradesh). The disease appears as small on both the surface of leaves. Infection gradually spreads on stem and fruits (pods) covering whole plant. Shivanna and Shetty (1991b) recorded infection of *Levillula taurica* in October-November sown cluster bean crops with maximum infection during January to March.

Rao and Rao (1954) observed that sprays of copper sulphate solution , copper oxychloride , cuprous oxide, calcium cynamide, bordeaux mixture , burgandy mixture, dusting sulpher and cerason on both the surface of leaves resulted in control of disease caused by *Levillula taurica* to a large extent in cluster bean but bordeaux mixture was most effective. Benlate 0.025% @ 1ml/g and sultaf, thiovit, cosan and sulphur compounds are higly effective against *L. taurica* (Mathur and Batrice, 1971; Reddy and Rao, 1971).

Sharma (1984) tried 0.05% tridemorph sprays against *Levillula taurica* infection in cluster bean at 12 day interval from the onset of infection grown in different cropping seasons and observed increase in the yield of green pods with the increase in number of tridemorph sprays. Ratnam, Pandit and Rao (1985) showed bavistin to give a control of the pathogen *Levillula taurica* in cluster bean.

Jani, Dange, Desai and Patel (1991) tested 11 fungicides/ chemicals for three years and observed minimum disease intensity 28.44% with 0.025% tridemefon. Based on incremental cost benefit ratio they recommended 2 sprays of 0.2% wettable sulphur at 15 days interval (Jani et al. 1991).

Alternaria leaf or target spot or blight

The disease is caused by *Alternaria brassicae* (Berk.) Sacc., *A. cucumerina* (Ell. & Ev.)Ellict. var *cyamopsidis*(Rangaswami and Rao), *A. tennussima* (Fr. Wiltsh) and *A. tenuis* Nees in cluster bean. (Luttrell, 1951; Rangaswami and Rao, 1957; Sowell, 1965a; Chand and Verma, 1967).

Leaf spots of *Alternaria* in cluster bean were first reported by Streets (1948) from Arizona and later from Georgia, U.S.A. by Luttrell (1951) and Sowell (1965a). Sowell (1965a) reported the fungus to be seed borne. Symptoms on leaves were characterized by large lesions with concentric rings and small greyish – olive green centre. Prasad and Desai (1951) observed blight disease caused by *F. moniliforme* in cluster bean. The infection was noticed as thin black streaks on the stem that extended both upwards and downwards reaching the leaf pedicels. *Alternaria alternata* causes shriveling and discoloration in seeds, loss in seed germination and seedling blight in guar. (Rangaswami and Roa, 1957), in chilli (Chitkara et. al., 1986a, b) and in cumin (Rastogi, 1993) were studied.

Rangaswami and Rao (1957) observed a severe blight of guar in 1953 at Coimbatore. The symptoms appeared mainly on leaf blade as dark brown, round to irregular spots of 2-10 mm diameter. At an early stage of infection water soaked spots appeared on leaf blade that turned grayish dark brown with concentric zonations, demarked with light brown lines inside the spots. During

severe infection several spots coalesce involving a major portion of leaf blade, leaflets become chlorotic and drop off. The plants did not produce flower in case of early infection. Pathogenicity of the fungus was tested by inoculating young leaves of 15 days – 3 months old plants which produced typical symptoms of the pathogen after 5 days of inoculation. Chand and Verma (1967) reported new *Alternaria* leaf spots in cluster bean caused by *A. tennuisima* causing dark brown, round irregular spots of 2-10mm in diameter. The spots may merge together causing severe defoliation. According to Gupta (1986) *Alternaria* leaf spot caused by *Alternaria cucumerina* var. *cyamopsidis* is a common disease in guar [*Cyamopsis tetragonoloba* (L.)Taub.] growing areas of western India and Pakistan. The most prominent symptoms are round to irregular spots varying from 2 to 10 mm in diameter on leaf lamina. Infection starts as water-soaked lesions on the leaf blades. As they mature, the lesions turn grayish to dark brown with concentric zonations, demarcated with light brown lines. In severe infections, several spots merge and give the leaves a blighted look, resulting in defoliation and considerable loss in seed.

According to Undersander et al. (1991) the causal organism of *Alternaria* leaf spot disease in cluster bean is *Alternaria cucumerina* var. *cyamopsidis.* This fungal disease may become severe during periods of heavy dew and high humidity. It causes a brown target-like lesion on the leaf between bloom and pod set. As the disease progresses, lesions enlarge, join and cause leaf drop. Similar observations were reported in cluster bean (Singh 1953; Mihali and Alcorn, 1986; Bhatia, 1995), in chilli (Panwar and Vyas, 1974; Bhale et. al., 2000), in cucurbits (Maholay, 1989; Maholay and Sohi 1985; Chandi and Maheshwari, 1992) and in moth bean and cow pea (Varma, Singh and Singh, 1990). While the evaluating 8 cluster bean seeds varieties for resistant to *Alternaria cyamopsidis* infection at the pre flowering and fruit set stage in pot and field trials Shivanna and Shetty (1991a) showed variety HG182 carrying lowest percentage infection to the fungus *Alternaria cyamopsidis* in cluster bean. Shivanna and Shetty (1991b) observed that the infection of *A. cyamopsidis* occurred throughout the year and was most severe during the Vegitative stages in cluster bean seeds.

According to Joshi, Gupta and Kumar (2004) *Alternaria* blight disease of cluster bean is caused by *Alternaria cucumerina* var. *cyamopsidis*. The disease appears year after year in mild to severe from to cause yield losses, as the pathogen is seed borne in nature.

Saharan and Saharan (2004) observed progression of *Alternaria* blight of cluster bean ranging in their susceptibilities to the disease. The disease progressed faster on the most susceptible variety (FS-277). Their study revealed that there was a significantly positive correlation between the disease severity and certain weather parameters.

Singh and Prasada (1973) tried 5 fungicides at 50,100,200,400,500 and 1000 ppm concentrations and found defoliation to be the best as it inhibited the growth of *A. cyamopsidis* in cluster bean even at 50 ppm followed by cumin, vitavax, antracol and blitox-50.The disease was more prevalent in irrigated regions than the unirrigated ones in cluster bean (Gaur and Ahmed 1983). Gaur and Ahmed (1983) tested dithane M-45 0.15%, difolatan 0.3%, bavistin 0.1% and benlate 0.1%. They observed dithane M-45 is the best for control of *Alternaria* leaf spot. Gaur, Ahmed and Bhari (1983) studied the influence of fertilizers on the incidence of *Alternaria* leaf spot disease in cluster bean. Sharma (1983) observed best control by 4 sprays of dithane M-45 at 10 days intervals starting with onset of *Alternaria* leaf spot disease in the cluster bean field.

Shivanna and Shetty (1992) using captan ,carbendazim, maneb, quintozene, thiram and ziram individually and in combination with carbendazim as seed dressers against *Alternaria* leaf spot disease of cluster bean found that the incidence of *A. cyamopsidis* and *A. alternata* reduced significantly with individual application of captan and zineb each with 75%.

Bacterial blight

Occurrence of bacterial blight and leaf spot of cluster bean caused by *Xanthomonas axonopodis* pv. *cyamopsidis* (XAC) has been reported by Bisht et

al. (1992) and Jain and Agrawal (2011). Decrease in pods/plant ranging from 16.85 to 25.15% has been reported due to increase in the incidence of the disease.

Seed-borne bacterial blight disease of guar can cause loss of plants from the seedling stage until maturity. Symptoms include large angular necrotic lesions at the tips of leaves, which cause defoliation and black streaking of the stems. This is potentially the greatest disease hazard to guar (Undersander et al., 1991).

Jain and Agrawal (2011) reported bacterial blight disease of cluster bean is characterized by irregular, sunken, red to brown leaf spots surrounded by a narrow yellowish halo. Several spots coalesce to from irregular patches. The spots may also develop on pods.

Viral disease

A symptomless, seedborne potyvirus was isolated from guar (*Cyamopsis tetragonoloba*) germ plasm in Griffin, Georgia. The host range and serology were similar to those reported for guar green-sterile virus (GGSV) and guar symptomless virus. Biological, serological, and molecular comparisons of the Georgia isolate and the South African GGSV indicate they are similar and are closely related to bean common mosaic potyvirus (BCMV).The Georgia isolate is seed-transmitted at a rate of up to 94% in guar **(**Gillaspie et.al.1998).

Seed Borne Mycoflora

Jain and Patel (1969) isolated 8 fungi from cluster bean viz. *Alternaria, Aspergillus, Curvularia, Helminthosporium, Fusarium, Penicillium, Rhizopus* and *Cephalosporium* apart from unidentified fungi with septate mycelium. Singh and Chauhan (1973) collected the seed samples of guar from grain market of Panjab and subjected them to moist blotter, agar plate and washing test and isolated 9 fungi. They reported *Fusarium sp.* caused rotting of collar region with the production of wire – loop type roots. Singh and Solanki (1974) isolated 5 fungi viz. *Aspergillus, Fusarium, Penicillium, Rhizopus* and *Rhizoctonia* from guar seeds collected from Pali districts of Rajasthan. Karwasra and Singh (1982)

isolated 9 fungi from cluster bean seeds viz. *Aspergillus, Alternaria cyamopsidis, Chetomium spp., Curvularia spp., Helminthosporium spp., Fusarium spp., Penicillium spp., Rhizopus spp.* and unidentified fungi with septate mycelium along with *Xanthomonas cyamosidis*. Varma, Singh and Singh (1990) studied on seed borne mycoflora and disease of moth bean and cow pea and reported 64 species belonging to 31 genera and 42 species of 23 genera in moth and cowpea seeds respectively in incubation test. *Alternaria alternata, Colletotrichum dematium, Drechslera tetramera, Fusarium moniliforme, F.oxysporum, Macrophomina phaseolina, Myrothecium leucotrichum, Rhizoctonia bataticola* and *Rhizoctonia solani* were important.

Dwivedi and Tondon (1976) reported 9 fungal species on Bottle Gourd viz. *Aspergillus flavus, A. niger, Alternaria alternata, Fusarium oxysporum* and *Rhizopus nigricans* associated with seed coat and cotyledons.12 fungi were isolated from seeds of bottle gourd among these *Alternaria alternata, Aspergillus niger, Fusarium oxysporum, Fusarium moniliforme [Gibberella fujikuroi] and Rhizopus stolonifer* were important (Chandiram and Maheshwari, 1992). Dwivedi and Dubey (1992) isolated a total of 19 and 17 fungal species from unsterilized and sterilized stored cluster bean seeds, respectively. More fungi were isolated by the agar plate method than by the blotter method. *Aspergillus flavus, A. fumigatus* and *A. niger* predominated.

Shakir and Mirza (1992) collected seeds from grain market in Faisalabad, Pakistan and reported 12 seed borne fungi of bottle gourd. *Macrophomina phaseolina* was the most pathogenic in germination trials.

Sharma (1992) reported 52 and 40 fungal species belongs to 35 and 27 genera in SBM and PDA respectively in soyabean seeds. Out of these *Aspergillus candidus, A. flavus, A. niger, penicillium spp.* and *Rhizopus nigricans* were dominant.

Dwivedi and Dwivedi (1994) studied post-harvest association of fungi with Cluster bean seeds and recorded 25 fungal species detected on guar seeds using the agar plate and blotter methods, *Aspergillus flavus, Aspergillus niger,*

Aspergillus fumigatus, Trichoderma viride and *Aspergillus terreus* predominated. Bhatia (1995) isolated 44 fungal species belonging to 26 genra in SBM and 34 species belonging 22 genra in PDA of cluster bean. Out of these *Alternaria alternata, Aspergillus flavus, Colletotrichum dematium, Fusarium oxysporum, Phoma sp.* and *Rhizoctonia bataticola* were dominant. Nagerabi and Elshafie (2001) were studied the incidence of seed borne and aflatoxin producing fungi in seeds of guar (*Cyamopsis tetragonoloba*). Sharma et al. (2007) collected ninety isolates of *Colletotrichum lindemuthianum* from commercial bean (*Phaseolus vulgaris* L.) growing areas of Himachal Pradesh. Sadda (2012) collected 120 samples of sponge gourd from Kota district of Rajasthan and recorded 29 fungal species in SBM and 21 fungal species in PDA. Out of these *Actinomycetes, Alternaria alternata, Aspergillus flavus, A. niger, Colletotrichum orbiculare, Curvularia lunata, Drechslera halodes, Fusarium oxysporum* and *Rhizoctonia solani* were important and showed high percentage and occurrence.

Sharma, Jain, Jain and Sharma (2013) observed and identified 32 fungal species on Okra seeds from Jaipur, Rajasthan viz. *Actinomycetes, Arthrobotrys supberba, Aspergillus fumigates, Cladosporium oxysporum, Drechslera sp., Fusarium moniliforme, Stachyobotrys spp., Verticillium alboatrum* and 3 bacterial species namely *Ralstonia solanacearum* (Smith) Yabuuchi et. al., *Pseudomonas syringae* van Hall and *Xanthomonas axonopodis var. malvacearum* (Smith) Vauterin were reported. The seeds of infected fruits containing seeds with white crust, water soaked symptoms were observed and such seeds on incubation yielded bacteria.

Singh (2013) revealed 26 fungal species belonging to 16 genera in lentil seeds during incubation test. They reported *Fusarium oxysporum* and *Rhizoctonia solani* as an important pathogen of lentil crop. Agrawal (2000) and Kaur et al. (2007) studied on seed borne and post harvest diseases of okra (*Abelmoscus esculentus*) and wheat respectively. They observed mycelium restricted to layers of seed coat in asymptomatic seeds and it invades endosperm and embryonal axis in symptomatic seeds and all components were infected with fungal mycelium in heavily infected symptomatic seeds.

Biochemical studies:

Fungi associated with seeds cause deterioration of its food reserves and other constituents. Storage fungi change the basic constituents of oil seeds. The role of storage fungi in quality loss of seeds has been studied by the Christensen and Kaufmann (1969).

Effect of mycoflora on groundnut seeds has been reported (Gupta and Chauhan, 1970; Kamble and Gangawane, 1987). Cherry (1983) and St. Angelo and Ory (1983) have shown that many seed enzymes with activities similar to those of pathogenic fungi, actively contribute to seed deterioration.

Singh (2013) studied biochemical changes in lentil plant parts infected with *Fusarium oxysporum* and *Rhizoctonia solani*. According to them total soluble protein in *F. oxysporum* infected leaf, stem and seed were lower than the healthy leaves, stem and seeds respectively and their related enzyme activity were higher in infected plant parts than healthy plant parts whereas Sadda (2012) reported higher protein content in sponge gourd leaves, stem, seeds and fruits infected with *Colletotrichum orbiculare* than the normal counterparts and enzyme activity were higher in healthy leaves, stem, seeds and fruits than the infected counter plant parts.

A complex series of biochemical reactions proceeds in an orderly and highly integrated manner with the development of disease in plants. In infected plants the metabolic changes can be observed. Either cell enlargement or cell division or both are affected due to infection and it subsequently altered from stimulus of parasite infection. The fungi associated with seeds caused deterioration of food reserves like total protein, carbohydrates phenols and related enzymes.

Starch content and amylase activity:

Starch the primary storage material in most seeds, has been studied for its deterioration by mycoflora in many crops like *Cajanus* seeds (Sinha, Singh and

Prasad, 1981), wheat (Agrawal, Thakur and Awasthi, 1982) and ground nut (Kamble and Gangawane, 1987), soyabean (Sharma,1992), smooth gourd (Sadda, 2012) and *Lens culinaris* (Singh and Varma, 2010).

Sharma (1992) reported higher starch content in soyabean seeds infected with *Fusarium oxysporum* than the healthy seeds while α and β- amylase was observed higher in asymptomatic seeds than the symptomatic seeds. Afiukwa et al. (2009) studied about determination of amylase activity of crude extracts from germinated mango seeds. The study revealed that amylase activity of crude extract from partially germinated mango seeds (*Mangifera oraphila*) was determined by using Carway-somogyi iodine/potassium iodide (IKI) method. The effects of varied pH and temperature were also investigated. The result showed the presence of amylase activity in the extract, depicted by its ability to gradually decrease the concentration of the starch solution used as substrate. The optimum pH and temperature of the crude enzyme were about 6.0 and 60°C respectively. This study demonstrated that the abundant waste mango seeds in the south-eastern Nigeria, particularly Ebonyi state, could be exploited for production of amylase.

Singh and Varma (2010) revealed decrease in both seed and stem tissue of diseased lentil parts infected with *Fusarium oxysporum*. Total soluble sugar and α- amylase activity were obtained higher in normal plant parts than the diseased plant parts. Meena et al. (2011) studied biochemical changes in constituents of Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] due to *Alternaria blight* revealed that total sugars was maximum in *Alternaria* blight infected leaves in resistant followed by susceptible and was lowest in moderately resistant. The similar trend was also found in non reducing sugar. But in case of reducing sugar, the maximum decrease was observed in susceptible as compared to healthy leaves in all three varieties of cluster bean. Similar results were obtained by Meena et al. (2012). Saada (2012) reported higher starch contents in healthy smooth gourd plant parts than the *Rhizoctonia solani* and *Colletotrichum orbiculare* infected counterparts while α- amylase activity were higher in infected plant parts than the normal plant parts.

Total soluble proteins and Protease Activity

Studies on protease activity in disease tissue have been studied by Dubey (1984). They revealed that activity of protease showed initial enhancement in both asymptomatic and symptomatic seeds which later on declined continuously. Meena *et al*. (2011) studied biochemical changes in constituents of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] due to *Alternaria blight* revealed that **t**he soluble protein in healthy resistant RGC-986 was highest followed by moderately resistant RGC-1003 while it was lowest in susceptible cv. RGC-936. Maximum decrease in soluble protein due to infection was observed in susceptible followed by resistant and moderately resistant cultivar.

Akhtaruzzaman et al. (2012) were isolate and characterize the proteases from seven leguminous seeds namely soybean, lentil, black gram, green gram, bengal gram, groundnut and pea bean. They revealed that groundnut was highest protein concentration (2.40 mg/ml) and lentil accounted was lowest concentration (2.21 mg/m.). Bengal gram showed highest specific activity (0.007659 u/mg of Protein) at pH 7.5 but lowest activity was observed in pea bean (0.001681 u/mg of protein) at pH 9.0. The temperature vs. specific and catalytic activity of all proteases relationship demonstrated a symmetrical distribution with one main peak and optimum at 37°C except black gram which showed two main peaks at 37°C and 70°C degree respectively. The paper concludes that leguminous seeds can be source of proteases for industrial purposes. Sadda and Varma (2011) were reported high protein contents in *Colletotrichum orbiculare* infected *Luffa cylindrica* plant parts than the healthy counterparts viz. seed ,stem, leaves and fruits while protease enzyme activity was higher in case of normal plant parts than its diseased counterparts. Singh (2013) were estimated total soluble protein and protease enzyme activity in lentil plant parts infected with *Fusarium oxysporum* and *Rhizoctonia solani.* She observed higher protein content in healthy plant parts than the infected plant parts while protease enzyme activity was observed higher in infected lentil plant parts than the healthy counterparts.

Phenols and Polyphenol oxidase

Mendgen (1975) showed that peroxidase is synthesized in endoplasmic reticulum, when bean leaves are inoculated with *Uromyces phaseoli*. According to Kumari and Nair (1981) infected hypocotyls of germinating seeds of *Hydrangea hartensis* contained more phenols than healthy but in necrotic portion there is decrease in total phenol. Park, Yoo and Lee (1982) showed that peroxidase activity was higher in soybean seeds infected with *Cercospora kikuchii*.

Studies of Singh, Nagra and Mehrotra (1982) found phenol have no relation with resistance. Singh and Srivastava (1988) showed that total phenolic contents increase with germinating seedlings of moth bean. Kocacaliskan et al. (1995) was studied about Polyphenol oxidase (PPO) activities during seed germination of six different plants namely bean, chickpea, barley, wheat, soyabean and corn by using four different substrates (dopa, catechol, caffeic acid, tyrosine).

Saharan et al (2001) observed phenolic compounds and oxidative enzymes in cluster bean leaves infected with *Alternaria* blight. According to their observation the amount of polyphenoloxidase (PPO) and total phenolic compounds increased with the increase in intensity of *Alternaria* blight up to 50 percent as compared to their respective healthy leaves. At later stage, with the increase in disease intensity enzymes activity started to decline.

Joshi, Gupta and Singh (2003), grown cluster bean genotypes 116565 (moderately resistant) and 116676 (highly susceptible) in pots and inoculated with *Macrophomina phaseolina*. At 65 days after sowing, leaves and roots of inoculated and uninoculated plants were collected and analyzed biochemical parameters. They observed that peroxidase, polyphenol oxidase [catechol oxidase], phenylalanine ammonia-lyase and tyrosine ammonia-lyase [phenylalanine ammonia-lyase] activity increased in leaves and roots of both genotypes due to pathogen inoculation. The activity of these enzymes was higher in moderately resistant genotypes than in highly susceptible genotype. Total phenol, O-dihydric phenol and flavanol contents increased with pathogen

inoculation being higher in moderately resistant genotype than in highly susceptible genotype. According to Kalim et al. (2003) reduction in disease incidence caused by *Rhizoctonia solani* and *Rhizoctonia bataticola* in cowpea increased levels of polyphenol oxidase (PPO), peroxidase (PO) and total phenols. PO activity was several times more as compared with PPO-specific activity and increased markedly after infection either with *R. solani* or *R. bataticola*. Contrary to PPO and PO, the specific activity of catalase declined sharply.

Joshi, Gupta and Kumar (2004) were studied about role of Biochemical factors in *Alternaria* blight resistance in cluster bean. They were used highly susceptible (IC116835) and moderately resistant (IC 116903) genotypes for their study. The observation revealed that catalase activity decreased with the increase in disease intensity in both genotypes. Activity of peroxidase (PO), polyphenol oxidase(PPO), phenylalanine ammonia lyase (PAL). tyrosine ammonia lyase (TAL) and quantity of phenols, flavnols and lignin increased with the increase in disease intensity , indicated thereby that these enzymes play important roles in defense mechanism against *Alternaria* blight in cluster bean.

Singh and Varma (2010) in lentil infected with *Fusarium oxysporum* and Sadda (2012) in smooth gourd infected with *Colletotrichum orbiculare* estimated total phenolics and their related enzymes activity. Different plant parts showed variation in their phenolic contents and related enzymes viz. poly phenol oxidase. The phenolic contents were more in infected plant parts as compared to normal counterparts. The increase in quantity of total phenols might be attributed to defense mechanism while poly phenol oxidase activity was higher in healthy plant parts than the infected plant parts.

Meena et al. (2011) studied biochemical changes in constituents of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] due to *Alternaria blight* revealed that total phenols were higher in healthy leaves of resistant followed by moderately resistant. The total phenolic contents were highest in diseased leaves.

Sharma, Joshi and Sharma (2012) were studied about defense mechanism in cluster bean. The study revealed that cluster bean in semi-arid regions suffers

severely from blight and root rot diseases caused by *Macrophomina phaseolina.* They were observed significant increase in poly phenol activities due to induction of fungal infection. They also showed that the elevated levels of biochemicals viz. phenolic acids and enzymes may play a major role in plant defense.

Physical Control by Oil treatment

Essential oils with botanical toxicants have shown promising results in control of pathogenic fungi in potato (Easton et al., 1978), in vegetable crops (Ghaffar, 1995) and in lentil (Singh, 2013). Essential oils are known to contain a natural cocktail of monoterpens, diterpenes and hydrocarbons with a variety of functional groups showing antimicrobial (Hammer et al., 1990; Cowan, 1999) and antifungal (Daferera et al., 2000) activities. Dwivedi et al. (1991) were tested essential oils from leaves and seeds of some angiosperms from different localities of Varanasi as well as from local markets against the mycelial growth of *Aspergillus flavus* in cluster bean. Amongst them the volatile oil from seeds of *Daucus carota* L. exhibited absolute toxicity against the test fungus. The minimum inhibitory concentration of the oil at which it exhibited fungistasis was 2000 ppm when it was not phytotoxic on seed germination and seedling growth of guar, *Cyamopsis tetragonoloba* L.(Taub.) and demonstrated that essential oils obtained from the leaves and seeds of *Daucus carota* L. exhibited a broad spectrum toxicity against several storage fungi including *A. flavus*. The oil was more effective in inhibiting the growth of the micro-organisms.

Dwivedi et al. (1991) also reported that the growth of *A. flavus* in guar can be inhibited by oil obtained from a *Cuminum cyminum* applied at 1000 ppm. Dwivedi et al. (1993) tested essential oils isolated from leaves and seeds of seven umbelliferous plants were tested against the growth *of Aspergillus flavus* in cluster bean. *Trachyspermum amni, Cuminum cyminum, Carum carvi, Daucus carota* and from leaves of *Anthem* graveolense exhibited antifungal activity against the test fungus. Amongst these, oil from seeds of *Trachyspermum amni* was most toxic. Its minimum inhibitory concentration was 300 ppm, at which it exhibited fungi static but not phototoxic properties, when tested at 200,300 and 400ppm, the

fungi toxic potency or *Trachyspermum amni* seed oil remained unchanged after a long storage period and at high inoculum density of the test fungus. The oil was thermo stable and was more efficacious than the fungicides. Locke (1995) reported that in field *Alternaria alternata*, *Aspergillus niger* and *Fusarium oxysporum* has been completely controlled by using 2-10% neem oil.

Dill seed oil showed antifungal activity against *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, *Helminthosporium sp*. and *Fusarium solani* (Rizki et al., 1997). Inhibitory effect of *A. indica* (neem), *B. campestris* (mustard), *Butea frondosa* (palas) and *R. communis* (castor) oils at different storage period on growth of mycoflora without any adverse effect on seed germination and seedlings growth of bajra and cumin is reported by Jain et al. (1998) and Anonymous (2001a). Kumar (2000) were studied on seed borne microorganism of pigeon pea and reported sesame, Musturd and linseed oil most effective against seed borne inoculum of *F. oxysporum* in pigeon pea at 60 degree- 70 degree. Dill seed oil revealed high effectiveness against the mold *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* (Jirovelz et al., 2003). Sridhar et al. (2003) examined the inhibitory effect of dill seed oil on the growth of 20 fungal species including *Drechslera oryzae*, which was found to be more potent. Fungicide benlate did not show significant reduction when compared with neem seed oil and dill seed oil.

Hall and Fernandez (2004) also studied inhibitory effect of Dill seed oil at 10µl doses on *Penicillium digitatum*. Neem oil at 0.025% concentration has been found to inhibit the growth of *Aspergillus species* (Niaz and Kazmi, 2005). Dry neem seed extracts gave 100% inhibition of mycelial growth of *Fusarium oxysporum* caused wilt in tomato (Agbenin and Marley, 2006). Jaiman, Jain and Sharma (2006) reported that treatment of *Macrophomina phaseolina* infected cluster bean seeds with edible and non-edible oils under test were found significantly better over the control as they minimized the mean per cent incidence of *M. phaseolina* at each storage period. Among these, incidence of *M. phaseolina* was observed to be less in seeds treated with neem oil followed by

mustard oils after 0, 2, 4, and 6 mo of storage. Seed germination was also highest in seeds treated with neem and mustard oils.

Niaz, Sitara and Qadri (2008) revealed effect of neem (*Azdirachta indica*) seed oil and dill (*Anethum graveolens*) and benlate fungicides at 0.1, 0.01, 0.001 and 1.0% concentration against *Drechslera sps.* Neem seed oil and dill seed oil caused significant reduction in the growth of *Drechslera rostrata*, *D. papendorfii*, *D. hawaiiensis* and *D. specifera*. The rate of growth inhibition was directly proportional to the concentration of tested oils in the medium. *D. specifera* and *D. papendorfii* were more susceptible to the neem seed oil at 0.1% and 1.0% concentration whereas *D.rostrata* and *D. hawaiiensis* showed greater suppression at 1.0% concentration. All fungi revealed moderate effect at 0.001% dose. Among 4 species of *Drechslera*, *D. papendorfii* showed more sensitivity towards all concentration of neem seed oil. Dill seed oil exhibited inhibition of mycelial growth of the tested fungi at all dose level whereas 0.1% and 1.0% concentration showed strong fungicidal effect. According to them Dill seed oil 1% was most effective against *D. rostrata* and *D. papendorfii* whereas 1% neem seed oil inhibited the growth of *D. specifera* and *D.* hawaiiensis. All treatments significantly inhibited the growth of all tested fungi; however dill seed oil showed greater suppression at all dose level followed by neem seed oil and benlate fungicides. Singh (2013) examined the effect of seven heated oils viz. coconut, groundnut, sunflower, sesame, neem, castor and mustard at 60ºC, 70ºC and 80 ºC against *Fusarium oxysporum* and *Rhizoctonia solani* in *Lens culinaris* for 2, 5 and 10 min. The maximum control of *Fusarium oxysporum* were observe in mustard oil treatment at 60° C for 2 min whereas the maximum control of *Rhizoctonia solani* were observed in seed treatment with neem oil at 70º C for 2 min.

Systemic Fungicides control

According to Maude (1977) the role of conventional fungicides in the treatment of vegetable disease are mainly for the protection than cure. Shivanna et al. (1992) reported Thiram the most effective fungicide in controlling seed borne storage fungi of guar. Ganeshan (1997) screened eleven fungicides in vitro

condition against *Sclerotium rolfsii* Sacc. which causes basal stem rot in cluster bean, *Cyamopsis tetragonoloba* (L.) Taub. Cv. Pusa Naubahar. Brassicol (0.2%) Dithane M-45 (0.1%), Foltaf (0.2%) and Thiride (0.2%) were effective in completely inhibiting the fungal growth. Among the different methods of fungicidal application, seed treatment by soaking seeds overnight in 0.2% solution of dithane M-45 produced a final crop stand of 43%, dithane M-45 and foltaf when administered as soil dry mix controlled up to 75 and 77% respectively. Brassicol applied as soil drench twice, four days prior to swing of seeds at an interval of 5 days controlled the disease to the extent of 73%.

According to Mohammed et al. (2006) Topsin M and Vitavax/Thiram as well as clove essential oil (4000 ppm) have completely inhibited the mycelial growth of *Fusarium oxysporum* shelct., *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* kuhn and *Sclerotum rolfsii* Sacc. in cluster bean plants collected from different localities in Egypt. In contrast, bauhina, damisis, lemon grass and marjoram wastes (3g/l Media) were the least effective treatment in this respect. Moreover, the two tested fungicides, as well as lemon grass and clove oils (4000 ppm) were the superior treatments against sclerotial formation of *M. phaseolina*, *R. solani* and *S. rolfsii*.

Mohammed, Bauimoy and Ibrahhim (2006) were also observed in greenhouse trials. Fungicides and essential oils were applied as seed dressers, completely reduced percentages of damping-off in soil infested with *M. phaseolina*. Whereas, Topsin M and clove oil (*F. oxysporum*) as well as Topsin M and Vitavax/Thiram (*R. solani*) were superior against pre-and post- emergence, respectively. Bauhinia and damsis wastes recorded (62.7% and 69%) and 100% reduction in pre-and post-emergence caused by *F. oxysporum* respectively. All treatments increased percentages of healthy survival seedlings for all fungi compared with their check. Similar results were also observed under field conditions and reduced fungal incidence.

Maude et al. (2008) was tested a method of soaking seeds in an 0.2% aqueous suspension of thiram for 24 h at 30°C against 13 seed borne pathogens.

The thiram soak treatment was much more effective than dust treatments. It was also generally more effective and less damaging than hot water treatment.

Jaiman et al. (2009) observed severe losses in cluster bean yields due to root rot. They tested three experiments seed dressing fungicides, bioagents and soil amendment with oilcakes to control root rot disease. Seed dressing fungicides found to be effective in reducing the pre-emergence seed rot, post-emergence seedling rot, disease incidence resulted in increased grain yields. Effective fungicides were carbendazim 50WP, thiophanate 70WP, thiram 70WP and captan 50SP.

According to Meena, Godara and Gangopadhyaya (2010) *Alternaria* blight caused by *Alternaria cucumenrina* var. *Cyamposidis* is a serious foliar disease of cluster bean in northern India. They observed disease control efficacy of nine fungicides viz. mancozed (Dithane M-45 75WP 0.2 %), zineb (Diathane Z-78 75WP 0.2%), chorothalonil (Kavach 75WP 0.2%), copper oxychloride (Blue copper 50WP 0.2%), carbendazim 2% + mancozeb 63% (Companian 65WP 0.2%), propineb (Antracol 70WP 0.2%), propiconazole (Tilt 25EC 0.1%), hexaconazole (Controll 5EC 0.1%) and difenconazole (Score 25EC 0.1%) were evaluated against *Alternaria* blight of cluster bean variety RGC-936 during kharif seasons of 2006 and 2007. These fungicides were sprayed twice at 45 and 60 days after sowing.

Patel et al. (2010) suggested the use of inorganic fertilizer, biofertilizer and organic manure that enhanced the growth and seed yield of cluster bean. Higher yields and yield attributes as well as nutrient uptake and protein content were recorded high in the seed treatments.

Kumar, Tapwal and Borah (2012) were used chemical fungicides to control *Verticillium* wilt disease of *Parkia roxburghii*. They screened 4 fungicides viz. Bavistin 50% WP, Dithane M-45, Dithane Z-78 and Fytolan 50% WP against the pathogen at different concentrations using poison food technique in vitro and then in the field conditions using soil drenching technique. They observed that Bavistin 50% WP was most effective at 75 ppm with 100% growth inhibition

followed by Dithane M-45, Dithane Z-78 and minimum efficacy was observed by Fytolan 50% WP with 31.27% growth inhibition in laboratory conditions. All selected fungicides were evaluated against pathogen in field conditions at 0.1% and 0.2% concentrations. Bavistin 50% WP was reported to be the most effective at 0.2% concentration. This observation recommends soil drenching with Bavistin 50% WP at a minimal concentration of 0.2% for management of the disease. In vitro screening of the fungicides had inhibited the growth of pathogen and efficacy of inhibition increased with the concentration. Sadda (2013) were used four fungicides viz. bavistin, mancozeb, captan and thiram at .1%, .2% and .3% conc. against seed borne infection of *Rhizoctonia solani* and *Colletotrichum orbiculare* in *Luffa* seeds. Thiram gave the best percent germination and control the pathogens. Singh (2013) reported best control of *Fusarium oxysporum* in lentil seeds with bavistin at 500ppm.

Biological control by bacterial isolates

Chauhan et al. (2012) were tested 51 bacterial isolates/mutants of *Azotobacter chroococcum*, *Azospirillum spp.* and *Gluconacetobacter diazotrophicus* for antifungal activity against three fungal pathogens, namely *Rhizoctonia solani* in cotton, *Rhizoctonia solani* in rice and *Fusarium oxysporum* in tomato using a dual-culture technique under laboratory conditions, isolates/mutants of *A. chroococcum* were found to be effective biocontrol agents against *R. solani* in cotton and *R. solani* in rice whereas in guar, the crop disease index was 2.5% against *Rhizoctonia solani* in cotton, 13.3% against *R. solani* in rice and 0% against *F. oxysporum* in tomato crop compared with their respective checks.

Biocontrol by Plant extracts

Use of neem leaf powder has also been reported to be effective in controlling storage fungi of sorghum (Meena and Mariappan, 1994), *Luffa* (Sadda, 2012) and lentil (Singh, 2013). According to Jaiman, Jain and Sharma (2006) traditional seed treatments of infected cluster bean seeds were found significantly better over the control as they minimized the mean percent incidence of

Macrophomina phaseolina at each storage period. Among traditional seed treatments, incidence of *M. phaseolina* was observed to be less and percent seed germination was observed highest in seeds treated with neem leaf powder followed by turmeric powder at 0, 2, 4, and 6 mo of storage and significant difference in percentage of seed germination was observed in all treatments including control. Neem leaf powder found economical and safe.

Agrawal, Jain and Sharma (2008) evaluated efficacy of aqueous root extract of *Tinospora cordifolia* against four seed borne fungal pathogens viz. *Aspergillus niger, A. flavus, Fusarium oxysporum* and *Dreschlera tetramera* and two bacterial pathogens *viz. Xanthomonas axonopodis* pv. *Cyamopsidis* and *Ralstonia solanacearum* isolated from cluster bean seeds. The root extract revealed maximum antifungal and antibacterial activities at its 100% concentration. The activity of the root extract was found increasing with increasing concentration.

Bareja et al. (2010) were tested five composts (4 ton ha-1) prepared from residues of *Calotropis procera, Prosopis juliflora, Azadirachta indica, Acacia nilotica* and on-farm weeds to determine their effectiveness in limiting the severity of charcoal rot caused by *M. phaseolina* in cowpea (*Vigna unguiculata*). They observed that soil amendment with the composts significantly reduced plant mortality due to charcoal rot. The lowest mortality was recorded in plants amended with *A. nilotica* compost (5.5%) followed by *P. juliflora* compost , while the highest plant mortality (11.5%) from charcoal rot occurred in the unamended control on the basis of the pooled average of two years. Composts also had a beneficial effect on yield, with a 28.3% increase in seed yield in *P. juliflora* compost-amended plots. These results suggest that in resource-deficient farming, certain on-farm wastes can be effectively utilized for managing soil-borne pathogens, as well as for enhancing crop productivity.

Kaur et al. (2010) was made an investigation to search for plant extracts possessing antibacterial property against *Xanthomonas axonopodis* pv. *Cyamopsidis* in cluster bean seeds. Aqueous and methanolic extracts of fresh leaves of *Emblica officinalis* (aonla), *Cassia fistula* (amaltas), *Tagetes sp.*

(marigold), *Withania somnifera*, *Eucalyptus citriodora*, *Catharanthus roseus*, *Rauvolfia serpentina*, *Glycyrrhiza glabra*, *Andrographis paniculata* and *Syzygium cumini* and extracts of garlic cloves (*Allium sativum*) and kernels of *Syzygium cumini* were tested. Aonla leaf extract exhibited maximum inhibition zone followed by *Eucalyptus*. However, none of the methanolic plant extracts tested exhibited any antibacterial activity against the test pathogen.

Meena et al. (2010) were tested plant extracts viz. *Azadirachta indica* (neem leaf for seed kernel 10%), Nimbecidine (Azadirachtin 0.03%), and leaf extracts (10%) of *Withania sommifera*, *Aloe barbadensis*, *Calotropis gigantia*, *Ocimum sanctum*, *Nerium odorum* and *Vinca rosea* against *Alternaria* blight disease in cluster bean seeds. The disease intensity was significantly reduced by fungicidal sprays. The disease severity was low in treatments with difenconazole and chlorothalonil (<20%) compared to that in control (74%). Nimbecidine (Azadirachitin) spray treatment provided a good level of *Alternaria* blight disease control (59%). Treatment with aqueous leaf extract of *Calotropis* and *Azadirachta* as well as its seed kernel extract were also effective in controlling blight. The highest and significantly enhanced grain yield was with difenconazole and the next best was with cholorothalonil. Azadirachtin, *Calotropis* leaf or *Azadirachta* seed kernel and leaf extracts also enhanced grain yields in Cluster bean.

Kiran et al. (2012) were tested *Millingtonia hortensis* Linn against eight fungal species of maize at 10,20,30,40 and 50%. Concentration showed maximum activity against *A. flavus* at 50% concentration followed by *F. Oxysporum* (90.2), *F. solani* (89.5%), *F. moniliforme* (87.7%), *A. candidus* (78.9%), *A. niger* (78.0%), *A. flavipes* (73.2%) and *F. graminearum* (52.1%) at 50% concentration. Moderate activity was also observed in 20, 30 and 40% concentration and least activity was observed in 10% concentration tested. Compared to synthetic fungicide bavistin and thiram, both the fungicide recorded 100% inhibition.

According to Tongbram and Chhetry (2012) plant extracts showed significant effect against mycelial growth of *F. udam* causing wilt of pigeonpea. They observed that *Allium sativum* showed complete inhibition of radial growth of *F. udam* followed by *Azadirachta indica* (79.4%), *Spilanthes acmella* (68.8%),

Adhatoda vasica (58.8%), *Gynura angulosa* (55.9%), *Aloe vera* (55.9%) and *Eryngium foetidum* (50%). The other test extracts showed less than 50% inhibition of mycelial growth. Highest inhibition of spore germination was observed in *A. sativum* extracts followed by *A. indica* (60.7%), *S. acmella* and *A. vasica* showed more or less similar inhibitory effect. The other tested plant extracts showed less inhibitory effect. Under field conditions, among the four plants, maximum control of disease was observed with *A. sativum*(35.3%), followed by *A. indica* (25.9%), *S. acmella* (14.2%) and *A. vasica*(10.2%). On the basis of these results proved that application of bulb extracts of *A. sativum* could be biopesticidal and ecofriendly substitute for chemical fungicides.

Sadda (2012) used 12 plant extracts against seed borne infection of *Colletotrichum orbiculare* in *Luffa* seeds caused anthracnose. They observed maximum control with the treatment of *Nerium indicum* leaf extracts.

Awurum and Uwajimgba (2013), screened three varieties of groundnut RMP 12, Mbaise Large, and Samnut 23 as well as leaf extracts of *Denettia tripetala*, *Spondias mombin*, *benomyl* and sterile distilled water for their effects against *Fusarium oxysporium* casual agent of wilt of groundnut (*Arachis hypogaea* L.) in the greenhouse. RMP 12 was observed the most tolerant varieties to the disease than other varieties. However, *Dennettia tripetala* and *benomyl* treated seedlings significantly reduced the severity of the wilt disease better than *Spondias mombin*. Singh (2013) were tried 12 plant extracts against *Fusarium oxysporum* in lentil seeds. The maximum inhibition was showed by seed treatment with the leaf extracts of *Catharanthus roseus*.

Biocontrol by Biological Antagonists

Deore et al. (2004) were carried out an investigation to find out the possible use of culture filtrates of five *Trichoderma spp.* namely *T. viride*, *T. harzianum, T. hamatum*, *T. longiforum* and *T. koningii* for the management of powdery mildew of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] caused by *Leveillula taurica* (Lev.) Arn. The culture filtrates of *Trichoderma spp.* either alone or in combination were found effective against powdery mildew. They had

recorded beneficial effect on growth parameters and disease control. The treatment with combination of *T. viride* + *T. harzianum* + *T. hamatum* has recorded highest per cent increase in yield (75.92%) and highest % control (78.71%) of powdery mildew in Cluster bean at 90th day followed by the combination treatment of *T. harzianum* + *T. hamatum.*

Jatav and Mathur (2005) were multiplied and applied 8 biocontrol agents including *Trichoderma spp.* (5 isolates), *Bacillus subtilis* (2 isolates) and *Streptmyces sp.* (1 isolates) as seed treatments of cluster bean seeds infected with *R. solani* and *F. solani*. They also applied four neem based formulations NF1 (neem seed extracts); NF2 (neem oil); NF3 (Azadiractin 10%) and NF4 (achook) at 0.2%. A fungicide carbendazim (bavistin) at 0.1% was also used for comparison. The highest germination was in carbendazim (93.3%) followed by *T. harzianum* Jh 2 (92.5%) and *B. subtilis* Ch-G- b-3 (89.1%). Highest population density among the biocontrol agents, of *Trichoderma viride* ITCC 1433 (21.8 × 106) and *Bacillus subtilis* G-Ud-1 (2.3 × 109) in cluster bean. They were observed maximum suppression of *Rhizoctonia solani* with *Trichoderma harzianum* ICR 23, while maximum suppression of *Fusarium solani* was recorded with *B. subtilis* G-Ud-1. The results showed that biocontrol agents were more effective for *R. solani* and bacterial antagonists were effective for *Fusarium solani*.

Pseudomonas maltophila PM-4, an antagonist of pathogenic fungi and observed characterization of this antagonist against *Rhizoctonia bataticola, R. solani, Fusarium oxysporum* and *Sclerotinia sclerotiorum* associated with root rot of cluster bean (*Cyamopsis tetragonoloba*) (Yadav et al., 2007).

Gomaa et al. (2007) carried out two field experiments at the New Salheyia Region, Sharkia Governorate, Egypt during the two successive seasons 2003 and 2004 to investigate the impact of certain bio-organic treatments consisted of farmyard manure (FYM), *Rhizobium* and/or soil yeast (*Rhodotorula glutinis*) on guar root nodules formation, forage yield, yield and its components as well as seeds nutrient content in comparison with the recommended traditional chemical treatment of NPK as a positive control. Treatment with FYM/fed. + *Rhizobium* +

Rhodotorula combination were obtained highest results for positive control in all characters.

377 *Cyamopsis tetragonoloba* rhizobacteria from cultivated soils of north-west India (Thar desert) and observed their antifungal activity against *Macrophomina phaseolina* (strains of groundnut, mung bean and guar) and *Fusarium oxysporum* (strains of chickpea and cumin) (Shweta et al, 2008).

Trichoderma viride, *T. harziamum* and *Gliocladium virens* mix with neem or neem cake showed reduction in the pre-emergence seed rot, post-emergence seedling rot and root rot incidence caused by *Macrophomina phaseolina* in cluster bean and increasing grain yields (Jaiman, Jain and Sharma, 2009). Sadda (2012) examined the effect of fungal antagonist viz. *Thrichoderma harzianum* and *Trichoderma viride* at .25%, .5% and 1% concentrations against the *Colletotrichum orbiculare*. *Trichoderma harzianum* gave the best result against the pathogen at 1% concentration. Singh (2013) used pure culture suspension of *Trichoderma viride* and *T. harzianum* for the control of *Fusarium oxysporum* in lentil seeds and observed better control of the pathogen with the treatment of *Trichoderma viride* (71.15%) than the *T. harzianum* (56%).

Drip Irrigation

Guar is known for its drought tolerance and grows without irrigation even in areas with as little as 250 mm of annual rainfall. So Drip Irrigation system is used in guar crop only in limited areas. The drip irrigation system mainly used in vegetable crops but it is also used in drought tolerant crops like guar. The review of literature regarding Drip Irrigation system is following:

According to Jain and Kumar (1973) and Aggarwal (1980) water requirement by Drip/ Micro Irrigation is only about 30-40 percent of the surface method of irrigation. Same results were also observed by Singh (1992). Dingar and Prasad (1987) observed the availability of adequate, timely and assured supply of water is an important determinant of agricultural productivity. Irrigation raises cropping intensity and crop yields besides facilitating shifts in cropping patterns. The

Irrigation alone contributed 60 percent to growth in agricultural productivity. Kumar (1999) suggested that drip irrigation is an efficient method of providing irrigation water, fertilizers and pesticides directly into root zones of plants and it permits the irrigation to limit the watering closely to crop water requirement. The system applied water at low rate and under pressure to keep the soil moisture within desired range for plant growth.

Breazeale et al. (2000) observed during their study on Alfalfa crop that due to increased competition for limited water resources, subsurface drip irrigation (SDI) is receiving more attention as an alternative irrigation method for alfalfa hay production. The concern is that the increased competition for water will force farmers to increase water use efficiency.

In a vast country like India with a geographical area of 328 million hectares, less than 45 percent of the area cultivated. Out of cultivated area only 65 million hectares (35 %) gets irrigation. Even after harnessing the entire potential of water resources, not more than 50 percent of cultivated land is irrigated (Anonymous, 2001b).

Malik and Luhach (2002) studied about economic dimensions of drip irrigation in fruit crops viz. grapes, ber and citrus. The study revealed that the economic viability of fruit crops under surface and drip irrigation system calculated acts as a guide for the cultivators to adopt particular irrigation method on the farm. The discounted returns and earning power of drip irrigation was higher in case of drip irrigation as compared to surface irrigation.

Hebbar et al. (2004) studied about the effect of fertigation with sources and levels of fertilizer and methods of fertilizer application on growth, yield and fertilizer use efficiency of hybrid tomato in red sandy loam soil.

Goel et al. (2005) studied about Feasibility of drip irrigation in sugar cane crop. The revealed that increase in yield of sugarcane occurred after use of drip irrigation system.

Drip irrigation is successfully practiced on large commercial scales in the arid and semi-arid regions around the world, such as in Australia, Israel, Jordan, Mexico, South Africa, and USA. Water in such large scale systems is distributed much more efficiently with drip irrigation than conventional flood and sprinkler systems, reducing the total amount of water required to grow a crop (Maisiri et al., 2005; Polak and Yoder, 2006). Namara et al. (2005) reported drip irrigation in the two Indian states of Gujarat and Maharashtra.

Farmers who carry water to their gardens in buckets or watering cans, any interventions that save water and reduce household labor demands have great promise. Drip irrigation kits that operate on small plots of 10-200 m have been successfully adopted by thousands of smallholder farmers in Bangladesh, India and Nepal (Polak and Yoder, 2006).

The general perception is that micro irrigation adoption leads to increase in yield (kg/ha), water saving; increase in area under irrigation due to reduction in water requirement per unit area. But, most of these perceptions are based on research on drip irrigated farms of orchards and cash crops. (Kumar et al., 2008). Narayanamoorthy (2008) was done study on economics of drip irrigation in cotton. They reported 114% increase in yield and 45% reduction in applied water by using drip irrigated in cotton field.

Rao and Shahid (2011) used twenty three accessions of cowpea (*Vigna unguiculata*) and ten accessions of guar for their study. They studied these crops under low salinity water using the drip irrigation system.

Soomro et al. (2012) revealed that decrease in water loss and increase in yield of Okra (*Abelmoschus esculentus*) by the use of Drip Irrigation. Soomro et al. (2013), water is the scarce resource and is available in inadequate amount so its optimal use is essential through drip irrigation which saves water and increases the yield. The proper timing and amount of water to be applied is essential for efficient irrigation. Reduced irrigation cause plant water stress and can reduce crop yield. Where over-irrigation means wastage of water, energy and may lead to

leaching of nutrient from root zone, surface soil erosion and lower air content of soil.

Kumar and Jos (2013), the real water saving through the use of micro irrigation systems is a function of the type of crop, the soil type, type of micro irrigation technology, the climate and geo-hydrology. Therefore, applied water saving also would be a function of the first three factors. In situations like north Gujarat, the most perceptible impact of adoption of Micro-irrigation system is likely to be applied water saving, as it would be high in semi-arid and arid climate, sandy soils, and for row crops. The saving would be more for drip irrigated row crops due to the reduction in non-beneficial soil evaporation (Kumar et al., 2008; Kumar and Jos, 2013).

Sadda (2012), studied use of drip irrigation method to overcome disease caused by *Colletotrichum orbiculare* and *Rhizoctonia solani* on *Luffa* seeds in field condition. She also studied comparative observation between surface irrigation and drip Irrigation and thier study revealed that drip irrigation is more effective method than surface irrigation to obtain high yield. The rate of infection is higher in surface irrigation. Energy, fertilizer, pesticide cost and labour cost can also be reduced by using drip irrigation in field.

Chapter –3

Materials and Methods

SEED SAMPLES

Six field surveys were carried out for collection of seed samples and study the occurrence of disease in Cluster bean crop in 11 districts of Rajasthan during the year 2011-12, 2012-13 and 2013-14 (Fig. 2 A & B). Total One hundred twenty seed samples of Cluster bean from 11 districts of Rajasthan namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar were collected (Table 1). Entry of these collected seed samples were made in laboratory stock register and given an accession number to each sample. They were packed in paper envelops, labelled and kept them in polyethylene bags and stored under cool and dry conditions in steel almirahs for screening of seed samples, seed-borne mycoflora and testing of seed health procedures as prescribed by International Seed Testing Association(ISTA) were followed (Anonymous, 1985) (Table 1 & 2).

DRY SEED EXAMINATION

All the 120 seed samples of cluster Bean (*Cyamopsis tetragonoloba*) collected from Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar were subjected to dry seed examination. 100 seeds per sample were drawn at randomly and examined by naked eyes as well as under steriobinocular microscope -Nikon-SMZ (10-40X). On the basis of external features seeds were categorized in to several groups such as Bold healthy looking seeds, Seeds with black streaks, Grey coloured seeds with white mycelial growth, Shriveled seeds, Broken and insect damaged seeds and Debris and inert matter (Table 3).

INCUBATION TEST

Standard Blotter Method (SBM)

All the 120 Seed samples of *Cyamopsis tetragonoloba* (Cluster bean) collected from experimental fields of 11 districts of Rajasthan were studied. 200 seeds per sample, 100 untreated and 100 pretreated with aqueous solution of

sodium hypochlorite were tested. In preliminary experiments several concentrations of aqueous solution of sodium hypochlorite with 0.25, 0.5, 0.75 and 1.0 % available chlorine for 1, 2, 3 and 4 min were tested. The pretreatment of seed samples with 0.5% available chlorine for 2 min was found to be the most suitable treatment. This concentration used throughout the experiment. 20 seeds were spaced per sterilized petriplate containing three well moistened blotter papers and incubated at 26 ±2ºC under 12 hr. of alternating cycles of artificial day light from Phillips fluorescent tubes fitted at a distance of 60 cm apart and darkness for seven days. Percentage of seed germination, seed-borne mycoflora, its percent incidence, fungal growth pattern, effects on seed germination, symptoms on seedlings and other abnormalities were recorded on the 8th day of incubation. In few cases incubation was prolonged and observations were made on 14th day.

The R.P.O. (Relative Percent Occurrence) of various fungi were calculated by following formula:-

$$\text{R.P.O. of fungi} = \frac{\text{No. of seed samples infected by a particular Fungi}}{\text{Total no of seeds}} \times 100$$

Potato Dextrose Agar Plate (PDA) Method

35 samples belonging to 11 districts of Rajasthan respectively were studied (Table 1). 100 seeds pretreated with 0.5% available chlorine from aqueous solution of sodium hypochlorite for 2 min. were aseptically plated on petriplates (20 seeds/plate) containing 15-20 ml of potato dextrose agar (PDA). PDA was prepared by autoclaving the mixture of extract of 200gm peeled potatoes, 20 gm dextrose (BDH) and 20 gm of agar- agar (BDH) in 1 liter of distilled water at 151b pressure for 10 min. 15-20 ml autoclaved PDA was transferred aseptically in each plate. Plates were already sterilized in oven at 60º C for about one hour. Streptomycin (200ppm/l) was added to PDA medium just before pouring it into the plates to check the bacterial growth. The plates were exposed to UV light for half an hour to check the growth of contaminants. The plates were incubated for 7 days at 28±2ºC in 12/12h alternating cycles of artificial light and darkness.

Percent infection of mycoflora, seed germination and abnormalities of seedling were examined by naked eye as well as under stereobinocular microscope on 8th day of incubation.

HISTOPATHOLOGY OF NATURALLY INFECTED SEEDS

Histopathology of naturally infected seeds were carried out for the location of the pathogen. For this study ac. nos. CB29 (Jaipur) and CB70 (Sikar) of cluster bean seeds infected with *F. solani* were used. Seeds of selected seed samples were categorized into (1) asymptomatic seeds (control) and (ii) symptomatic seeds. Symptomatic seeds were further categorized into (i) weakly (ii) moderately and (iii) heavily infected on the basis of severity of symptoms. Each category was handled separately. The component plating, cleared wholemount preparation and microtome sectioning of seeds were employed for the identification of actual location of fungal infection in the seed components (Johansen, 1940).

Component plating

20 seeds of cluster bean per category per sample were washed individually 2-3 times with sterile distilled water later seeds were soaked in distilled water for 4-6 hrs and dissected aseptically with the help of sterilized forceps and a pair of needles under the stereo binocular microscope to separate seed coat, cotyledons, endosperm, hilar region and hypocotyl shoot root axis. Each component was surface sterilized in aqueous solution of NaOCl (0.5% available chlorine) and tested by Standard Blotter Method. Observations were taken after 8th day of incubation.

Cleared wholemount preparation

20 seeds per category of each sample infected with *Fusarium solani* were boiled individually (one seed per test tube) in distilled water and 10% KOH solution for 5-10 min to clear the tissue, cooled and dissected to separate the components. Each component boiled separately in 10% aqueous solution of potassium hydroxide (KOH) for 5-10 min and washed in distilled water. Then the separated components were boiled in lectophenol and cotton blue (1:1, v/v) for 15

min and kept overnight then mounted in polyvinyl alcohol (Omar, Bolland and Heather, 1979). All the dissected seed components were pressed gently under the cover-slip on the glass slide till the cells spread uniformly and prepared slides were kept in oven at 60º C for drying and observed them under stereobinocular microscope (10 X 40).

Microtome sectioning

5 seeds per category from each sample were soaked in sterile distilled water, kept overnight in Oven at 60º C until they soften and small incision were made at 3-4 places for proper infiltration and embedding. The seeds later fixed in 70% alcohol for 48 hrs in vials. Then dehydrated through tertiary butyl alcohol (TBA) series, infiltrated and embedded in paraffin wax(BDH). The embedded materials was cut into blocks, for further softening the seed embedded wax blocks were chopped from one side to expose the cotyledons and kept in 1% aqueous solution of sodium lauryl sulphate for 24 hrs. The blocks were washed thoroughly under tap water and transferred to acetoglycerine (mixture of glycerin and acetic acid in 1:1) for 7 days. The blocks were washed again with water and serial microtome sections were cut at 15µ thickness. The slides were stained with saffranine and light green combination after deparaffinization and mounted in DPX (Johansen, 1940). The seed sections were also stained in cotton blue/tryphan blue after deparaffinization and mounted in PVA. Prepared slides were observed under stereobinocular microscope (Nikon SMZ 10 X 40) for identify the exact location of infection of *Fusarium solani* in the seed components.

PHYTOPATHOLOGICAL EFFECTS AND DISEASE TRANSMISSION

Studies on phytopathological effects and disease transmission of *Fusarium solani* were carried out by using seeds with natural field infection.

For the study of disease transmission and phytopathological effects of seeds Ac.nos. CB29(Jaipur) and CB70(Sikar) for *Fusarium solani* were used (Table 2). Asymptomatic seeds of the same samples were used as control. The

study was carried out by applying Petriplate method, Water agar seedling symptom test, Pot experiment and Field experiment.

Data on seed germination, symptom due to fungal infection, rotting, survival and mortality of seedling or plants were recorded at 24 hrs intervals in the petriplate methods and water agar seedling test whereas in case of pot experiment observations were made at weekly intervals for isolation and presence of pathogen in different parts of seedlings/plants. Symptomatic and healthy looking seedlings/plants were uprooted at regular intervals, washed in running water and split longitudinally into two halves. One half surface sterilized with 0.5% available chlorine was sown on moistened blotters and incubated for 7 days while other half was cleared by boiling for 5-10 min in 10% aqueous solution of KOH, washed with distilled water, stained with cotton blue and mounted in PVA. Hand cut sections of root and stems were also made and stained with cotton blue.

Petriplate method

Two replicates of 100 seeds per category per sample pretreated with aqueous solution of sodium hypochlorite with 0.5% available chlorine were spaced on moistened blotters. 20 seeds per petriplate were incubated at 26±2ºC under 12hrs of alternating cycles of light and darkness for 7 days. Observations were made at 24hrs interval up to 8th day.

Water agar seedling symptom test

50 Seeds per category per sample treated with 0.5% available chlorine were sown on 1% sterilized water agar medium in test tubes (1 seed/test tube) under aseptic conditions and incubated at 26 ±2ºC for 12h/12h alternating cycles of light and darkness. The observations were taken daily up to 15 days and data were recorded at regular interval.

Pot experiment

Ac. nos. CB29 (Jaipur) and CB70 (Sikar) of infected cluster bean from *Fusarium solani* was used for the experiment. 100 seeds per category per sample

(5 seeds/ pot) were sown in 12 inch earthen pots containing sterilized soil in the month of April. The pots were watered on every alternate day. The seeds were treated with 0.5% aqueous chlorine solution for 2 minutes. Data were recorded weekly to 15 days intervals upto the maturity.

Field experiment

Seed samples of cluster bean infected with *Fusarium solani* were used for field experiment. The 0.5% chlorine treated seeds were sown in field area containing sterilized soil in the month of April. 200 seeds per category per sample (20 seeds per row) were used for experiment. The field was watered every alternate day. Observations were taken regularly at weekly intervals up to the maturity of plant. Data was recorded on seedling emergence, seedling survival, mortality and transmission of disease into various plant parts.

BIOCHEMICAL ANALYSIS

Biochemical analysis of bold normal looking (Asymptomatic) and disease infected (Symptomatic) above ground plant parts viz. leaves, stem and seeds were carried out by standard techniques to observe the altered state of metabolites and enzymes in vivo condition. Estimation of starch, protein and phenol and their enzyme essays for α-amylase, protease and polyphenol oxidase were carried out in the sample ac no. CB-70 of Sikar (Table 2).

In present study, the following metabolites and enzymes were studied in normal looking and disease infected plant parts with *Fusarium solani* of *Cyamopsis tetragonoloba* :-

A. Total protein contents and protease enzyme activity

B. Starch content and α-amylase enzyme activity

C. Total phenol content and Poly phenol oxidase enzyme activity

(A) Total Protein Contents

Introduction

The proteins are the structural and functional unit of life and compounds of 20 essential amino acids. The term "protein" is derived from a Greek word 'proteios' meaning holding the first palace. Berzelius (Swedish Chemist) suggested the name of protein to group of organic compounds that are the most important for life. A dutch chemist Moleler used the term proteins for the high molecular weight, nitrogen rich and most abundant substances present in animals and plants. In present research normal and infected tissue of guar were comparatively analyzed for total protein contents.

Principle

In present study, estimation of total protein contents in cluster bean samples done by employing Lowry's method (1951). Protein reacts with folin ciocalteau reagent to give a coloured complex. This colour is produced by the reduction of phosphomolybdate by tyrosine and tryptophan of protein by the action of alkaline of copper.

The colour intensity depends upon the amount of these aromatic amino acids and varies for different proteins.

Requirements

Reagent A - Alkaline sodium carbonate solution - (20% Na_2CO_3 in 0.1% mg/1 NaOH)

Reagent B - Copper Sulphate-Sodium Potassium tartrate solution (0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartrate) was prepared fresh.

Reagent C - Alkaline Copper reagent- 50 ml of reagent A was mixed with reagent B just before use.

Reagent D- Folin ciocalteau reagent.

Standard protein solution (Stock Standard)

Standard protein solution was made by dissolving 50 mg of BSA (Bovine serum albumin) in 0.1 N NaOH and final volume was made up to 50 ml.

Methods

(i) Extraction of Protein

For protein extraction 500 mg of normal and diseased infected seeds samples were grind in pestle and mortar in 10 ml of 80% ethanol. It was centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded and residue was suspended in 5% perchloric acid to remove sugars and soluble nitrogen fractions. Again centrifugation was done at 2000 rpm for 20 minutes. In order to remove acid soluble frictions and lipids the residues collected were resuspended in a mixture of ethanol, ether and chloroform (2:1:1). The residue was dissolved in 1.0 ml of 1N NaOH. 0.1 ml of solution was taken and subsequently made up 1.0 ml by adding distilled water.

(ii) Estimation of Protein contents

To 1 ml of dissolved residue, 5.0 ml of alkaline copper reagent was added and allowed to stand for 10 minutes. To this, 0.5 ml of Folin- Ciocalteaus's reagent (diluted with equal volume of distilled water) was rapidly added, mixed thoroughly and incubated at room temperature for 30 minutes, which resulted in development of blue colour. The optical density was measured at 750 nm in a UV spectrophotometer (UV-vis-systronics 118). For blank ethanol was used. The amount of protein in sample was calculated from a standard curve prepared from BSA (Bovine Serum Albumin). Protein content was expressed in terms of mg/gm fresh weight of the tissue.

Protease enzyme activity

Material Requirements

Reagent A - Casein solution

Reagent B - 0.1 M Phosphate buffer pH 7.0

Reagent C - 1 ml TCA

Reagent D - 1 ml Folin Ciocalteau's reagent

Reagent E - 2 ml of 20% Sodium carbonate

Methods

2 ml casein solution (1% Casein dissolved in Phosphate buffer pH 7) was mixed with 1 ml of 0.1 M phosphate buffer pH 7.0 and 1 ml of enzyme extract. The mixture was incubated and kept at 30ºC in water bath for 1/2 hr. 1 ml enzyme substrate mixture was taken in centrifuge tube and 1 ml TCA was added. Then it was allowed to stand at room temperature for an hour and then centrifuged at 2000 rpm for 20 minutes. 1 ml of supernatant was pipetted and add 1 ml of folin ciocalteau's reagent and 2 ml of 20% sodium carbonate. Then the test tube was placed for 1 minute in boiling water bath cooled under tap water and made upto 10 ml with distilled water. Estimation of protease activity was done and absorbance was read at 650 nm (Balasubramanian, 1972).

(B) Starch Contents

Estimation of starch was carried out by the method of McCready et al. (1950).

Material Required

Reagent A - 80% Ethyl alcohol

Reagent B - 52% Percholoric acid

Reagent C - Standard glucose solution

Methods

(i) Extraction of starch

500 mg of fresh healthy and disease infected plant parts of cluster bean viz. stem leaves and seeds were homogenized with 10.0 ml of 80% ethanol than grind in pestle and mortar. The samples were centrifuged at 2000 rpm for 20 minutes. After centrifugation the supernatant was discarded and residue was collected.

(ii) Estimation of Starch

Residue was used for the estimation of starch, suspended in 5.0 ml of water and subsequently 6.5 ml of 52% perchloric acid was added to the residue. Stir the mixture and the contents were centrifuged at 2000 rpm for 20 min. The supernatant was decantant and collected and the whole procedure was repeated thrice. Supernatants of each step were then pooled together and the total volume was made up to 100 ml with distilled water. The mixture was then filtered through whatmann filter paper (No. 42); 1 ml of aliquot of this filtrate was taken for estimating starch content. Optical density was measured using UV spectrophotometer at 490 nm after setting for 100% transmission against the blank. The quantity of starch was calculated from standard curve prepared from different concentrations of starch. The quantity of starch was expressed in terms of mg/gm fresh weight of tissue.

α- Amylase activity

α- amylase (α-1-4-glucan, 4 glucanhydrolase) hydrolyze the interior bonds of linear polysaccharide (amylase) in a random manner to yield a mixture of glucose and maltose. It is an endo amylase. The branched polysaccharide (amylopectin) is hydrolyzed by α-amylase into branched and unbranched oligosaccharides.

Estimation of α-amylase activity is done by Bernfeld (1955) assay method. The action of α- amylase produces the reducing sugar which reacts with dinitrosalicylic acid reducing it to a brown coloured product dinitroaminosalicylic acid.

Materials

Reagent A - 0.1 M sodium acetate buffer (pH=4.7)

Reagent B - 1% starch solution (freshly prepared) (1 gm starch dissolved in 100 ml acetate buffer)

Reagent C - Dinitrosalicylic acid reagent (500 ml of 60% sodium potassium tartrate solution with 200 ml of 2 N NaOH)

Reagent D - 40% potassium sodium tartrate (Rochelle salt solution)

Reagent E - maltose solution (50 gm maltose dissolved in 50 ml distilled water) in a flask and stored in a refrigerator

Reagent F - Incubation of 1 ml of enzyme extract with 1 ml of substrate (prepared by dissolving 10 ml of soluble starch in 100 ml of 0.02 M phosphate buffer pH = 6.9 containing 0.0065 N NaCl for 30 min at 30º C. At the end of the reaction add 1 ml of dinitrosalicylic acid)

Reagent G - 0.02 M phosphate buffer (pH= 6.9)

Method

(i) Extraction of α- amylase

1 gm fresh weight of normal looking and disease infected cluster bean sample each was crushed with 1.0 ml of 1 M phosphate buffer pH=7.0. The homogenate was centrifuged at 3000 rpm for 10-15 min and supernatant was collected which served as enzyme extract.

(ii) Estimation of α-amylase activity

1 ml of enzyme extract which mixed with 1.0 ml of substrate (Prepared by dissolving 10 mg soluble starch in 100 ml of 0.02 M phosphate buffer PH=6.9 containing 0.0065 M NaCl) forms the reaction mixture. Incubate the reaction mixture at 30º C for 15-20 min and end the reaction by adding 2.0 ml of dinitrosalicylic acid reagent, and solution of was kept in water bath for 5 min then 1.0 ml of potassium sodium tartrate was added, after this cool the test tubes solution in running tap water and made up the volume to 10 ml by adding 6 ml of distilled water. Read the optical density of the yellow coloured solution developed at 560 nm against blank. The activity was expressed in terms of mg starch hydrolysed/sec/mg fresh weight of tissue.

(C) Phenolics and enzymes involved in phenol metabolism

Phenolics Contents

Phenolic compounds and related oxidative enzymes are one of the important disease resistance biochemical parameters. In higher plants they are involved in disease resistance mechanism. Total phenolic contents were estimated by method of Bray and Thorpe (1954).

Material Required

Reagent A - 20% sodium carbonate (Na_2CO_3):- (20 gm Na_2CO_3 dissolved in 100 ml distill water)

Reagent B - Folin ciocalteau phenol reagent

Reagent C - 80% Ethanol

Reagent D - Standard phenol solution: 50 mg of catechol was dissolved in 50 ml of distilled water. 10 ml of this standard stock was diluted to 100 ml to prepare the working standard solution.

Method

(i) Extraction of total phenol contents

500 mg of dried plant tissue sample was homogenized in 10 ml of 80% ethanol. Centrifugation of the homogenate at 1000-3000 rpm for 20 min, collect the supernatant and the residue was reextracted with 80% ethanol and recentrifuged after supernatant was collected.

(ii) Estimation of total phenol content

Estimation of total phenol content by assay method using ethanolic extract (Bray and Thorpe, 1954). Total phenolic contents were estimated by the 1.0 ml Folin Ciocalteau Phenol reagent (diluted with equal volume of distilled water before use) was added to 1 ml of alcohol extract in a test tube followed by 2.0 ml of 20% sodium carbonate solution. Heat the mixture in a boiling water bath for 1 minute. Blue colour is obtained which was diluted with 25.0 ml of distilled water and absorbance was recorded in UV spectrophotometer (Systronics UV - VIS-118). 80% ethanol was used to prepare the blank. Standard curve prepared from different concentrations of catechol used to calculate total phenols. Total phenols were expressed as mg/gm fresh weight of tissue.

Poly Phenol Oxidase

The enzyme catalyze of monophenols and dehydrogenation of orthodiphenols (Mayor and Haral, 1979). In plants, it appears to catalyze the formation of intermediates in biosynthetic system which produces flower pigments, related flavonoids, the simple and polymeric tannins and their esters, the phenolic alkaloids, the aminones, tropolones and simple plant melanins and lignins (Mason, 1955).

Principle

The poly phenol oxidase activity was assayed by the method of Shinshi and Noguchi (1975).

Enzyme reaction

Catechol (Colourless) ⟹ **Quinone** (Yellow coloured)

Catechol oxidized by enzyme polyphenol oxidase into a yellow coloured quinone compounds.

Materials Required

Reagent A - Catechol solution (0.01 ml)

Reagent B- 1 M phosphate buffer pH= 6.0, 12.3 ml of Na_2HPO_4 and 87.2 ml of NaH_2PO_4 were mixed for preparation of phosphate buffer pH = 6.0

Method

(i) Extraction of Poly Phenol Oxidase

Crush 1 gm of plant tissue samples in 10 ml of chilled 1 M phosphate buffer PH-6.0. Refrigerated centrifugation of homogenate done at 3000 rpm for 15 minutes at 0ºC. The supernatant was collected as enzyme extract and made upto 10 ml with buffer solution.

(ii) Estimation of Poly Phenol Oxidase Enzyme

Polyphenol oxidase activity was assayed by the method of Shinshi and Noguchi (1975). 1.0 ml of enzyme extract and 3.0 ml of buffered catechol (Freshly prepared) [buffered catechol = 0.022 gm/20 ml phosphate buffer pH= 6.0] constitute the reaction mixture. The increase in optical density was recorded at 15 second intervals at 470 nm after mixing enzyme and substrate. A unit of enzyme activity was chosen as change in absorbance 0.001 per second the reaction mixture without the substrate was used as blank, enzyme activity was expressed in terms of units/sec/mg fresh weight of tissue.

CONTROL OF SEED-BORNE INFECTION

For the control of seed-borne infection of *Fusarium solani* asymptomatic (control) and *Fusarium solani* infected seeds sample ac. nos. CB29 (Jaipur) and CB70 (Sikar) were used. Heated oil treatment, chemical (systemic fungicides), biological methods (fungal antagonists and plant extraction method) and culture practice (drip irrigation) were tried to study their effect on reduction of pathogen incidence and seedling growth. Untreated bold healthy looking seeds were used as control. Five replicates of 20 seeds for each treatment were sown on moistened blotters. Observations on seed germination, incidence of fungi and seedling infection were recorded at regular interval (Table 2).

For plant extracts experiments, leaf extracts, bulb extracts and Latex of twelve plants viz. *Azadirachta indica, Dalbergia sisso, Eucalyptus rudis, Lantana camera, Parthenium officinalis, Ricinus communis, Saracca indica* (leaf extracts) *Allium cepa*, *Allium sativam* (Bulb extracts), *Calotropis procera, Datura innoxia* and *Ficus religiosa* (Latex) were used for overcome infection of *Fusarium solani* on cluster bean seeds.

For chemical fungicides treatment systemic fungicides viz. Bavistin, Kitazin-P, Topas and Systhane were used. For biological control fungal antagonists, *Trichoderma viride* and *Trichoderma harzianum* were used. Five replicates of 20 seeds (20 seeds/petriplate) for each treatment were sown on moistened blotters by Standard Blotter Method (SBM). Observation on percent seed germination, percent control of seedling infection and incidence of pathogens were recorded on 8th day of incubation and the data was analyzed statically by Completely Randomized Design (CRD) Method. Percent reduction (control) was calculated by the following formula:

$$\text{Percent reduction} = \frac{\text{Incidence in control (C) - Incidence in treatment(T)}}{\text{Incidence in control (C)}} \times 100$$

PHYSICAL CONTROL

Oil Thermotherapy

Eight oils viz. mustured, coconut, groundnut, sesame, mahua, neem, linseed and castor oil and five replicates of 20 seeds per petriplate of each sample were used for each treatment (Table 2). The seeds tied in cheese cloth were kept in beaker containing oil heated at 50º and 70º C on a temperature controlled hot plate for 5 min and 10 min. The seeds were allowed to cool at room temperature and washed in 70% ethanol to remove excess of oil. These were air dried and plated on blotter (20 seeds/ plate). Untreated seeds served as control. Observations on seed germination, incidence of pathogen and seedling infection were taken on 8th day of incubation.

CHEMICAL CONTROL

Systemic Fungicides

Naturally infected seed samples (Table 2) were used for the systemic fungicidal treatment. Four fungicides Bavistin, Kitazin-P, Systhane and Topas were tested at four concentrations (2000 ppm, 1000 ppm, 500 ppm and 250 ppm). Five replicates of 20 seeds were used for each treatment. Seeds were soaked for 2 hrs in aqueous solution of each fungicide and incubated on moistened blotter papers. Seeds subjected to distilled water surved as control. Data on seed germination, seedling infection and incidence of pathogen was recorded on 8th day of incubation.

BIOLOGICAL CONTROL

Plant Extracts

Leaf extracts of 7 plants viz. *Azadirachta indica, Dalbergia sisso, Eucalyptus rudis, Lantana camera, Parthenium officinalis, Ricinus communis, Saracca indica*; Bulb extracts of 2 plants viz. *Allium cepa; Allium sativam* and three latex yielding plants viz. *Calotropis procera, Datura innoxia, Ficus religiosa* were used for their antifungal properties. 10g leaves of each plant were

taken, sterilized with 0. 5% (Sodium hypochlorite) NaOCl solution, washed and crushed in 10ml of distilled water using Pristle and Mortar and filtered with double layered cheese cloth. Infected seeds were taken (five replicates of 20 seeds each) and treated separately with each of the 12 extracts for 4 hour. For the treatment sample carrying natural infection of *F. solani* were used (Table 2). Seed were incubated on moistened blotters in sterilized petriplates under artificial cycles of light and darkness. Seeds soaked in sterile distilled water served as control. Observations on seed germination, incidence of pathogen and infected seedling were recorded after every 24 hour interval up to 8th day and following formula was applied for calculation of percent seed germination.

$$\text{Percent Seed Germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Fungal Antagonists

Trichoderma viride and *T. harzianum* were used as biological agents. Their pure cultures were obtained from National Center of Fungal Taxonomy, IARI, New Delhi and raised on PDA for seed treatment. Seed samples infected with *F. solani* were used (Table 2). For treating the seeds, 5ml of distilled water was added to 15 day old culture plate and the suspension was diluted to 10ml. Considering 10ml as stock solution (1:1, 1:2, 1:4 and 1:8) 20,40,80 and 120 ml concentrations were made by adding water. 100 seeds per concentration for *F. solani*(naturally infected) were taken at random, surface sterilized with 0.5% chlorine solution soaked in suspension of *T.viride* and *T.harzianum* separately for 5h and sown in petriplates (20 seeds/petriplate) for 8 days. Observations were taken on seed germination and pathogen incidence along with infected seedlings.

CULTURAL PRACTICE

DRIP IRRIGATION

The progress of *Fusarium solani* in relation to the prevailing environmental conditions and irrigation method was studied in selected seed samples (Table 2). A field trial was conducted with local cultivar in experimental

cluster bean sown field (15000sq ft) in tapiplya village situated near the Reengus station of Sikar district of Rajasthan. The drip lines were spread in April to July till the arrival of mansoon. The progress of disease was recorded which were influence due to changes in weather conditions. Through good management of micro irrigation systems or drip irrigation systems the root zone moisture content can be maintained near field capacity throughout the season providing a level of water and air balance close to optimum for plant growth. In addition, nutrient levels which are applied with water through the system can be spread.

The observations were taken at the time of sowing seeds in field in April-August and at the time of maturation of crop in September and November.

TABLE -1 : AREAWISE NUMBER OF SEED SAMPLES OF CLUSTER BEAN COLLECTED AND STUDIED IN DRY SEED EXAMINATION AND INCUBATION TESTS (SBM) IN VARIOUS DISTRICTS OF RAJASTHAN

Districts	No. Of Samples Collected	Dry Seed Examination	SBM	PDA
ALWAR	07	07	07	02
BIKANER	06	06	06	03
CHURU	10	10	10	03
JAIPUR	20	20	20	06
JALORE	05	05	05	02
JHUNJHUNU	14	14	14	03
JODHPUR	09	09	09	02
KOTA	05	05	05	01
NAGAUR	10	10	10	03
SHRI GANGANAGAR	08	08	08	03
SIKAR	26	26	26	07
TOTAL	**120**	**120**	**120**	**35**

TABLE -2 : DETAILS OF SEED SAMPLES (Ac. Nos.) USED FOR HISTOPATHOLOGY, DISAESAE TRANSMISSION, BIOCHEMICAL TEST, THEIR CONTROL AND DRIP IRRIGATION

Name of the Crop	Pathogen	Histopathology	Disease Transmission	Biochemical Test	Control				
			Naturally Infected		Physical	Chemical	Biological		Drip Irrigation
							plant extracts	*fungal antagonist*	
Cluster Bean	***Fusarium solani***	CB-29 CB-70	CB-29 CB-70	CB-70	CB-29 CB-70	CB-29 CB-70	CB-29 CB-70	CB-29 CB-70	CB-70

Fig. 2 (A-B): View of Experimental cluster bean fields

A. Healthy field

B. Infected field showing wilting of plants

Fig. 2

Chapter – 4

Results

SURVEY AND DETECTION OF SEED BORNE MYCOFLORA

DRY SEED EXAMINATION

Total 120 seed samples of cluster bean collected from 11 districts namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar of Rajasthan revealed both asymptomatic (Fig.3F) and symptomatic seeds when observed under stereobinocular microscope for dry seed examination. Symptomatic seeds showed various types of disorders like seeds with black streaks; grey colour seeds with white mycelial growth; shriveled seed; broken and insect damaged seeds and debris and inert matter. (Fig. 3A-F; Table 3 & 4)

Seeds with black streaks

34 seeds samples belonging to 10 districts viz. Alwar (2), Bikaner(3) , Churu (3), Jaipur (5), Jhunjhunu(4), Jodhpur (2), Kota (1), Nagaur(3), Shri Ganganagar (4) and Sikar (7) carried 0.25-12.5% incidence of seeds with black streaks. The streaks varied from few to many.

On incubation such seeds revealed mainly the presence of *Colletotrichum dematium, Curvularia lunata, Rhizoctonia bataticola, R.solani, Macrophomina phaseolina and Phoma betae*. (Fig. 3A; Table 3 & 4)

Grey colour seeds with white mycelial growth

43 samples belonging to all 11 districts namely Alwar (2), Bikaner (3), Churu (2), Jaipur (10), Jalore (2), Jhunjhunu (2), Jodhpur (1), Kota (2), Nagaur (3), Shri Ganganagar (4) and Sikar (12) revealed grey to black discolouration with white colour mycelial growth on seed surface. The incidence varied from 0.25-

40.5%. Maximum incidence was found in 12 seed samples collected from Sikar district (0.25-40.5%) followed by 10 seed samples collected from Jaipur district (0.5-35.5%) of Rajasthan. On incubation such seeds yielded infection of *Fusarium moniliforme*, *Fusarium oxysporum* and *Fusarium solani*. Among these fungi *F. solani* is dominant and show white mycelial growth on seeds. (Fig. 3B; Table 3 & 4)

Shrivelled seeds

Shrivelled seeds were observed in 80 samples from all the 11 districts studied namely Alwar (5), Bikaner (2), Churu (6), Jaipur (13), Jalore (4), Jhunjhunu (7) , Jodhpur (8) , Kota (5), Nagaur (8), Shri Ganganagar (6) and Sikar (16) revealed 0.25-25% incidence. On incubation such seeds yielded a group of fungi and bacteria viz. *Alternaria alternata*, *Aspergillus flavus, A. niger*, *Curvularia lunata, Chaetomium sps., Drechslera tetramera, Fusarium moniliforme*, *F. oxysporum, Penicillium sps., Rhizoctonia solani, Myrothecium roridum* and Bacterial ooze. (Fig. 3C; Table 3 & 4)

Broken and insect damaged seeds

Broken seeds included seeds with cracked seed coat, splitted seeds and insect damaged seeds. These were observed in 75 seed samples collected from 9 districts namely Bikaner (3), Churu(5), Jaipur (15), Jhunjhunu (10), Jodhpur (9), Kota (2), Nagaur (6), Shri Ganganagar (7) and Sikar (18) revealed 0.25-50% incidence. On incubation these seeds yielded *Alternaria alternata*, *Aspergillus flavus, Curvularia clavata, C. lunata, Drechslera tetramera, Rhizoctonia bataticola, Chaetomium spp., Rhizopus sps. and Trichothecium roseum.* (Fig. 3D; Table 3 & 4)

Debris and inert matter

It comprised of soil clods, gravel, stone pieces, broken seed coats and plant debris especially the pod debris showing brown black discolorations. These seeds were observed in 34 seed samples collected from 9 districts namely Bikaner (1), Churu (3), Jaipur (7), Jhunjhunu (3), Jodhpur (2), Kota (2), Nagaur (3), Shri Ganganagar (4) and Sikar (9) revealed 0.25-20% incidence. On incubation these seeds yielded mainly *Aspergillus fumigatus, A. niger, Curvularia pallescens, Drechslera rostrata, Fusarium monaliforme, F.oxysporum, F. solani, Mucor sps., Phoma betae, Rhizopus nigricans* and *Trichothecium roseum.* (Fig. 3E; Table 3 & 4)

INCUBATION TESTS (Fig. 8A-C)

120 & 35 seed samples of cluster bean were tested by SBM (Standered Blotter Method) and PDA (Potato Dextrose Agar plate method). A total of 53 fungal species of 28 genera (SBM) and 40 fungal species of 23 genera (PDA), both saprophytic as well as parasitic were encountered (Table 5, 6, 7). The fungi encountered in PDA test were common to those observed in SBM. These were *Alternaria alternata* (Fr.) Keissler; *A. brassicola* (Schw.) Wilts.; *A. tenuissima* (Fr.)Wiltsh; *Aspergillus candidus* Link ex Fries; *A. flavus* Link ex Fries; *A. fumigatus* Fresen; *A. niger* van Tieghem; *A. oryzae* (Ahlburg) E. Cohn; *A. sulphureus* (Fres.) Thom and Church; *A. sydowi* (Bainer and Sartory) Thom and Church; *Botryodiplodia theobromae* Patouillard; *Cephaliophora tropica* Thaxt.; *Chaetomium globosum* Kunze ex Fr.; *C. indicum* Corda; *C. megalocarpum* Bainier, *C. murorum* Corda; *C. spinosum* Chivers; *Choanophora cucurbitarum* Currey; *Cladosporium oxysporum* Berk and Curt.; *Colletotrichum dematium* (Pers. ex Fr.) Grove; *Curvularia clavata* Jain, *C. lunata* (Wakker) Boedijn; *C. pallescens* Boedijn; *Drechslera halodes* (Drechsler) Subram. and Jain; *D. maydis* (Nisikado) Subram. & Jain; *D. rostrata* (Drechs.) Richardson & Fraser; *D. tetramera* (Mc Kinney) Subram. and Jain; *Eurotium amstelodami* L. Mangin; *Fusarium moniliforme* Sheldon; *F. oxysporum* Schlecht. ex Fr.; *F. pallidoroseum*

(Cooke) Sacc.; *F. solani* (Mart.) Sacc.; *Graphium sp.; Macrophomina phaseolina* (Tassi) Goid.; *Melanospora zamiae* Corda; *Memnoniella echinata* (Rivolta) Galloway; *M. levispora* Subram.; *Mucor sp*.; *Myrothecium roridum* Tode ex Fr.; *M. verrucaria* (Alb. & Schw.) Ditm. ex Fr.; *Neocosmospora vasinfecta* E. F. Sm.; *Paecilomyces varioti* Bainier; *Penicillium spp*. Link; *Phoma betae* Frank; *Rhizoctonia bataticola* (Taub.) Butler; *R. solani* Julius Kuhn; *Rhizopus nigricans* Ehrenb.; *R. stolonifer* (Ehrenb. ex Fr.) Vuill.; *Stachybotrys atra* Corda; *S. chartarum*(Ehrenb.) S. Hughes; *Thielavia terricola* (J.C. Gilman & E.V. Abbott) C.W. Emmons; *Trichothecium roseum* (Pers.) Link ex Fr. and *Trichurus spiralis* Hasselbring. Out of these thirteen fungi viz., *Alternaria brassicola*; *A. tenuissima; Curvularia pallescens; Drechslera halodes; D. maydis; Fusarium moniliforme; Graphium sp.; Memnoniella levispora; Mucor sp.; Myrothecium verrucaria; Penicillium spp.; Thielavia terricola* and *Trichurus spiralis* were not recorded in PDA method. (Table 7)

For each fungus, the percent incidence range and occurrence on Cluster bean seeds have been described. The relative percent occurrence (RPO) has been given in the order of the untreated, pre treated seeds in SBM and PDA test methods (Fig. 4, 5, 6 & 7; Table 5, 6, 7 & 8).

***Alternaria alternata* (Fr.) Keissler**

Colonies usually black or olivaceous black; conidiophores in groups, simple or branched; conidia pale to golden brown, long or branched chains, usually smooth walled , obovoid muriform often with a short conicolor cylindrical beak. (Fig. 4A)

The fungus identified in 36, 33 and 11 seed samples in untreated (0.5-12.5%) and pre treated (0.5- 12.5%) in SBM and PDA (0.5-14.5%) tests respectively from all the districts. 30, 5, 1 ; 29, 3, 1; 7, 3, 1 samples occurred in the range of 0.5-5%, 5.5-10%, 10.5-25% in untreated , pre treated seeds (SBM) and PDA respectively. RPO calculated was 30, 27.5, and 31.43.

***Alternaria brassicola* (Schw.) Wilts.**

Colonies dark olivaceous brown to blackish brown, velvety; conidiophores were arising either singly or in groups of 2-12, simple erect or curved, often slightly swollen at the base ; conidia in chains of up to 20 or more. (Fig. 4B)

The fungus identified in only 2 samples from Jhunjhunu in untreated (4.5%) as well as pre treated seeds (2.5%) in SBM. In PDA test these fungus were not recorded. RPO calculated was 1.67 in both untreated and pretreated seeds in SBM.

***Alternaria tenuissima* (Fr.)Wiltsh**

Conidiophores and conidia were either solitary or in groups. Conidia were in short chains, straight or curved, obclavate, black up to half of the conidium length , smooth or minutely verruculose, 4-7 transversely septate. (Fig. 4C)

The fungus identified in only those 2 samples which was collected from Jhunjhunu (1) and Jodhpur (1). These samples were infected with *Alternaria tenuissima*. The incidence was 2.5 in untreated and 1, 1.5% in pretreated seeds. The RPO calculated was 1.67 in both untreated and pretreated seeds in SBM.

***Aspergillus candidus* Link ex Fries**

White or creamy white, globose head; conidiophores macronematous, erect, colourless and short phialides in two series, heads were large; conidia colourless and smooth. (Fig. 4D)

The fungus identified in 6, 8, and 5 samples in untreated (1.5-5%) , Pre treated (1-5%) in SBM and PDA (3.5-7%) test respectively. The sample belongs to Bikaner, Churu, Jaipur , Jalore, Shriganganagar and Sikar. 6, 0; 8,0; and 3,2

samples were found in the range of 0.5-5% and 5.5-10% in untreated , pretreated (SBM) and PDA tests Respectively. RPO was 5, 6.67 and 14.28.

***Aspergillus flavus* Link ex Fries**

The colonies of this fungus were spreading fast and yellowish green, darkening with age; conidiophores were macronematous, heads were loosely radiate. (Fig. 4E)

37, 31 and 11 samples collected from all 11 districts revealed the fungus in untreated, pretreated (SBM) and PDA with an incidence of 1.5-21.75%; 1-30.25% and 2-18.75% respectively. The samples occurring in the range of 0.5-5 %, 5.5-10%, 10.5-25%, 25.5-100% in untreated, pretreated seeds in SBM and PDA were 23, 11, 3, 0; 21, 7, 2, 1; 7, 3, 1, 0 respectively. RPO was 30.83, 25.83 and 31.43.

***Aspergillus fumigatus* Fresen**

The colonies of this fungus were usually dark smoky green, more or less velvety; the conidiophores were macronematous, short, often greenish, conidial heads columnar alters in length. (Fig. 4F)

It was recorded in 4, 4 and 3 samples in untreated , pretreated (SBM) and PDA with an incidence range of 2-4% , 1-4% and 5-8% respectively. The infected samples belonged to Jodhpur and Nagaur. Among the infected samples 4, 0; 4, 0; and 2, 1 was found in range of 0.5-5% and 5.5-10% respectively.

***Aspergillus niger* van Tieghem**

The colonies of this fungus were spreading very rapidly; conidiophores were macronematous up to several mm long, head globose, blackish- brown to carbonaceous black colour. (Fig. 4G)

This fungus identified in 38, 34 and 10 samples in untreated (0.5-17.5%), pretreated (1-16.5%) seeds in SBM and PDA (2.5-16.75%) respectively. Samples collected from the Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar showed its higher occurrence. Out of infected samples 30, 5, 3; 28, 4, 2; 6, 2, 2 samples in untreated , pretreated seeds (SBM and PDA)were in the range of 0.5-5%, 5.5-10%, 10.5-25% respectively. The RPO was 31.67, 28.33 and 28.57.

***Aspergillus sulphureus* (Fres.) Thom and Church**

This fungus had typically sulphur colour; the conidiophores of this fungus were arising from aerial hypae; heads loose, column of conidial chains rarely radiate; phialides in 2 series; conidia were looking globose, thick walled, smooth. (Fig. 4I)

10, 10 and 4 cluster bean samples revealed this fungus in untreated, pretreated (SBM) and PDA with an incidence of 1-7%, 1-3%, and 4-8.5% respectively from Bikaner, Jaipur, Jodhpur, Kota and Shri Ganganagar. 8, 2; 10, 0; 3, 1 samples occurred in the range of 0.5-5%, 5.5-10% in untreated, pretreated and PDA respectively. RPO value was 8.33, 8.33 and 11.42.

***Aspergillus sydowi* (Bainer and Sartory) Thom and Church**

The colonies were bluish green; conidiophores were colourless, smooth and thick walled, heads were radiated; phialides radiate in 2 series; conidia were globose and spinulose. (Fig. 4J)

The fungus identified in 14, 12 and 6 samples in untreated (1-9.5%), pretreated (0.5-4.5%) seeds in SBM and PDA (4-8%) respectively collected from all districts. 13,1; 12,0; 4,2 samples were in the range of 0.5-5% and 5.5-10%.. the RPO value of these was 11.67, 10.0 and 17.14.

***Botryodiplodia theobromae* Patouillard**

The pycnidia were black, borne in hairy stroma, either submerged or superficial, neck were absent or very small when submerged; conidiophores were simple and short; conidia dark and two celled at maturity, ovoid to elongate. (Fig. 4K)

The fungus identified in 3, 3 and 2 samples of cluster bean seeds collected from Churu , Jhunjhunu and Nagaur in untreated (1-2.5%) and pretreated (0.5-3%) seeds in SBM and PDA (3-3.5%) respectively. The RPO was 2.5, 2.5 and 5.71.

***Chaetomium globosum* Kunze ex Fr.**

The perithecia of this fungus were dark with greenish brown or grey colour hairs, subglobose or ellipsoidal; lateral hairs flexuous; terminal hairs numerous undulate or coiled; ascospores pale-brown subspherical to ellipsoidal. (Fig. 5A)

The fungus identified in 4, 2 and 2 samples in untreated (1-4%), pretreated (2.5, 3%) in SBM and PDA (2, 3.5%) respectively in the samples collected from Alwar, Bikaner, Churu, Jaipur and Jhunjhunu. RPO was 3.33, 1.67 and 5.71.

***Chaetomium indicum* Corda**

Perthecia small dark with hairs almost black globose, lateral hairs few, straight; terminal hairs many, branched dichotomously and repeatedly at a wide angle; asci club-shaped, 8 spored and spores hyaline. (Fig. 5B)

The fungus was identified and recorded in 1, 2 and 2 samples in untreated (2%), pretreated (1, 2.5%) in SBM and PDA (1.5, 2.5%) respectively in the

samples collected from Jalore and Jhunjhunu. The RPO calculated was 0.83, 1.67 and 5.71.

***Chaetomium murorum* Corda**

Perithecia black, globose to subglobose with small simple ostiole, terminal hairs dark, long undulate with graceful arches, loosely coiled with circinate blunt tip. (Fig. 5D)

The fungus identified in 2, 3 and 2 samples in untreated (2.5, 3%), pretreated (2, 3.5%) in SBM and PDA (1, 5%) revealed its presence in samples collected from Jhunjhunu, Nagaur and Shri Ganganagar. The RPO calculated was 1.67, 2.5 and 5.71.

***Chaetomium spinosum* Chivers**

The perithecia were black, subglobose to ovoid with pointed base; lateral hairs black, straight, stiff and unbranched.

The fungus was identified and recorded in 3, 4 and 5 samples Collected from Alwar, Jhunjhunu, and Jodhpur with an incidence range of 2-3.5%, 1.5-2% and 1.5-10.5% in untreated , pretreated seeds in SBM and PDA respectively. 3, 0, 0; 4, 0, 0; 2, 2, 1 samples occurred in the range of 0.5-5%, 5.5-10% and 10.5-25% in untreated, pretreated and PDA respectively. The RPO calculated was 2.5, 3.33 and 14.29.

***Choanophora cucurbitarum* Currey**

The mycelium were white, extensive and growing rapidly in culture; conidiophores were long, enlarged and branched at the apex, each branch bearing

a head of conidia and the conidia were single celled brown or purple and ellipsoidal. (Fig. 5E)

The fungus was identified and recorded in 7, 4 and 2 samples revealed its presence in untreated (0.5-3%), pretreated (0.5-2%) in SBM and PDA (1.5%,) respectively. The samples collected from Bikaner, Jhunjhunu and Jaipur. RPO was 05.83, 03.33 and 05.71.

***Cladosporium oxysporum* Berk and Curt.**

Colonies of this fungus were effuse, grayish brown in colour, thin hairs present on seed surface; conidiophores of this fungus were macronematous and brown coloured with swellings bearing chains of conidia; conidia either cylindrical or subspherical and pale olivaceous brown. (Fig. 5F)

The fungus identified from 29, 29 and 5 samples in untreated (0.5-7%), pretreated (0.5-7%) seeds in SBM and PDA (4-7%) respectively. The samples collected from all districts except Alwar were infected from this fungus. The number of samples having infections in the range of 0.5-5%, 5.5-10% was 25, 4; 27, 2; and 3, 2 in Untreated, Pretreated in SBM and PDA respectively. The RPO was 24.17, 24.17 and 14.29.

***Colletotrichum dematium* (Pers. ex Fr.) Grove**

Acervuli of this fungus were either present in single or in groups, blackish brown to dark black, longer than conidial mass, setae were numerous, trichi form, septate; conidial mass were white to dull white, pale orange or bright orange, mycelium absent; conidia hyaline, fusoid ends rounded or slightly tapering. (Fig. 5G)

The fungus was identified and detected in 6, 5 and 2 samples from Bikaner, Jodhpur, Nagaur and Shri Ganganagar in untreated, pretreated in SBM and PDA with 0.5-7.5%, 0.5-2.5% and 4, 4.5% respectively. The RPO calculated was 5.0, 4.17 and 05.71.

***Curvularia lunata* (Wakker) Boedijn**

Conidiophores of this fungus were macronematous; conidia acropleurogenous or acrogenously arranged, small, straight or curved, broad towards the apical tip pale olivaceous to black, curvature at the region of the bigger cell towards the apical tip, three celled septate, the third cell from base was larger and darkest. (Fig. 5I)

Except Kota district this fungus was identified in 28, 32 and 4 samples collected from all districts in untreated (1.5-7%), pretreated (0.5-7%) seeds in SBM and PDA (2-8.5%) respectively. The RPO was calculated 23.33, 26.67 and 11.42.

***Curvularia pallescens* Boedijn**

The fungus conidia acropleurogenous or acrogenous, pale olivaceous to black, small, either slender straight or slightly curved, nearly cylindrical or clavate, rounded or acute at tip but compact arrangement of conidia make the indistinct shape, septa visible due to pale colour of the conidia and cells paler than intermediate cell; the conidiophores of this fungus were macronematous. (Fig. 5J)

The fungus was isolated and identified from 4 and 3 samples collected from Jaipur and Bikaner in untreated and pretreated seeds in SBM with an incidence of 2-3.5% and 2-5% respectively. This fungus was not revealed in PDA test.

***Drechslera halodes* (Drechsler) Subram. and Jain**

Colonies shining black; conidia cylindrical to ellipsoidal, pseudoseptate; conidiophores macronematous straight, flexuous with geniculate ends. (Fig. 5K)

6 and 7 samples collected from Jaipur, Jhunjhunu, Jodhpur and Nagaur revealed the fungus in untreated (1.5-3%) and pretreated (1-3%) seeds in SBM. The calculated RPO was 05.00 and 05.83. This fungus was not revealed in PDA test.

***Drechslera maydis* (Nisikado) Subram. & Jain**

Colonies hairy, pale brown; conidia curved, fusiform pale to golden brown, smoothened, pseudoseptate; conidiophores erect, geniculate and brown in colour.

The fungal growth was observed in 2, 2 samples collected from Alwar and Jhunjhunu in untreated (1.5, 2%) and pretreated (2, 2.5%) seeds in SBM. This fungus was not revealed in PDA test.

***Drechslera rostrata* (Drechs.) Richardson & Fraser**

Conidia straight or slightly curved, rostrate, broadest in the middle, 6-10 pseudoseptate, distinct protuberant hilum. Conidiophores solitary dark brown, colonies blackish brown and effuse. (Fig. 5L)

The fungus identified in 9, 10 and 2 samples collected from Alwar, Churu, Jaipur, Jhunjhunu, Jodhpur, and Nagaur. The incidence of this fungus recorded varied from 0.5-5%, 1-2.5% and 1.5% in untreated, pretreated in SBM and PDA respectively. RPO was calculated and recorded 7.5, 8.33 and 5.71.

***Drechslera tetramera* (Mc Kinney) Subram. and Jain**

Colonies black, hairy; conidiophores erect single or in clusters of 2-3, flexuous and repeatedly geniculate with numerous well defined scars; conidia acropleurogenous at short intervals, appearing in cluster, short, light to dark brown, 3-pseudoseptate ellipsoidal to almost cylindrical with rounded ends, always perpendicular to the axis. (Fig. 6A)

This fungus was identified in 9, 10 and 3 samples in untreated , pretreated SBM and PDA with an incidence of 1-3.5%, 1-3.5% and 2-3.5% respectively. The samples collected from all districts except Jodhpur, Kota and Sikar revealed the fungal infection. The RPO was recorded 7.5, 8.33 and 8.57.

***Fusarium moniliforme* Sheldon**

Mycelium scanty or well developed and woolly, forming white or peach white colonies on seed surface; either short or long chains of small false heads of microconidia or short simple sterigmata, microconidia in chains, unicellular, oval but microconidia present rarely. (Fig. 6C)

3 and 1 samples revealed the infection of this fungus in untreated (5%) and pretreated seeds (3%) in SBM collected from Bikaner, Churu and Sikar. The RPO was 2.5 and 0.83 in SBM. This fungus was not recorded in PDA test.

***Fusarium oxysporum* Schlecht. ex Fr.**

Colonies pale white colour; mycelium cottony straite, felted to floccose; microconidia in chains, unicellular sometime it becomes bicellular, ellipsoidal; macroconidia falcate, 3-5 septate. Chlamydospores were terminal and intercalary, often solitary, occasionally in chains. (Fig. 6D)

45, 43 and 13 samples collected from all 11 districts of Rajasthan revealed the infection in untreated (1-30.75%), pretreated (1-27.25%) seeds in SBM and PDA (1-21.75%).

Higher incidence of this fungus was recorded in samples collected from Jaipur and Sikar districts of Rajasthan. The no. of samples having infections in the range of 0.5-5%, 5.5-10%, 10.5-25 and 25.5-100 was 31, 7, 4, 3; 29, 10, 2, 2 and 5, 4, 3, 1 in Untreated, Pretreated in SBM and PDA respectively. The RPO calculated was 37.5, 35.83 and 37.14 respectively.

***Fusarium solani* (Mart.) Sacc.**

Dull and white colony with very loose mycelial growth on seed surface; mycelium were also with numerous shiny, milky white, oval droplets microconidia present on simple erect strigmeta; microconidia oval shaped, irregular and formed from lateral phialide; slimy white pionnotes containing numerous macroconidia occurred below the mycelium. Chlamydospores are hyaline, globose, smooth to rough walled borne singly or in pairs on short lateral hyphal branches. (Fig. 6F, G & H)

Macroconidia becomes unequal laterally fusoid with many of the cells having widest diameter in the penultimate cell, well marked foot cells, apical cells is pointed and somewhat beaked. (Fig. 6I)

57, 53 and 15 samples collected from all 11 districts of Rajasthan revealed the infection in untreated (0.25-62%), pretreated (0.25-35%) in SBM and PDA (0.25-30%).

Higher incidence of this fungus was recorded in samples collected from Sikar (18, 15, 5) Ac. No. CB70 followed by Jaipur (13, 12, 3) Ac. No. CB29 in untreated, pretreated seeds in SBM and PDA. Out of infected samples 35, 12, 7, 3;

33, 13, 4, 3; 7, 4, 2, 2 samples in untreated , pretreated were in the range of 0.5-5%, 5.5-10%, 10.5-25% and 25.5-100% in SBM and PDA respectively. The RPO was 47.5, 44.17 and 42.86.

Graphium sp.

Colonies of this fungus olivaceous brown or black, erect synnemata capped by slimy head; conidiophores macronematous; conidia aggregated in slimy heads in long chains, acrogenous, simple either straight or curved, colourless or pale or pinkish olivaceous brown colour and smooth. (Fig. 6J)

This fungus was identified in 2, 2 samples collected from Jaipur in untreated (1.5%) and pretreated (1.5%) seeds in SBM. The RPO calculated was 1.67 in both untreated and pretreated seed samples of cluster bean.

***Macrophomina phaseolina* (Tassi) Goid.**

The pycnidia of this fungus black, superficial globose, smooth walled, psedoparenchymatous; pycniospores of this fungus become single celled hyaline, elongated cylindrical and 16-30 x 5-10 µ size. (Fig. 6K)

The fungus was identified in 12, 20 and 4 samples in untreated (0.5-8.5%), pretreated (1.5-5%) seeds in SBM and PDA (0.5-3%) respectively collected from all district. 10, 2; 19, 1; 4, 0 samples were present in the range of 0.5-5% and 5.5-10% respectively. The RPO was calculated 10, 16.67 and 11.42.

***Melanospora zamiae* Corda**

Perithecia of this fungus either scattered or gregarious, light coloured springly clothed with pale or pinkish brown hairs, nearly globose tip of beak covered completely with hyaline hairs, entire perithecia of this fungus becomes

completely black at the time of maturity; asci broad clavate; ascospores elliptical to fusoid, at first hyaline becoming dark brown. (Fig. 6L)

The fungus was identified and observed in 4, 5 and 7 samples collected from Churu, Jaipur, Jalore, Nagaur and Shri Ganganagar in untreated 1.5-3%, pretreated 1-4.5% seeds in SBM and PDA 2.5-4% respectively. The calculated RPO was 3.33, 4.17 and 20.

***Memnoniella echinata* (Rivolta) Galloway**

The fungal colonies black coloured, effuse, velvety or powdery; conidiophores erect, septate with dark pigment and warted dark granules, the tip of the conidiophores slightly enlarged into rudimentary vesicles from which phialides are developed in groups of 4-8; conidia warted, spherical with black pigment and single celled.

This fungus was identified in 9, 8 and 2 samples in untreated 1.5-3.5%, pretreated 1-3% seeds in SBM and PDA 2-4% respectively collected from Churu, Jalore Jhunjhunu, Jodhpur and Kota. The RPO was calculated 7.5, 6.67 and 5.71.

***Memnoniella levispora* Subram.**

The fungal colonies black coloured, velvety or powdery; conidiophores erect, septate, dark grey to black coloured with scattered dark granules, the tip of the conidiophores enlarged into rudimentary vesicles; phialides are developed from this vesicle; conidia of this fungus smooth, hemispherical and single celled.

Only one samples collected from Bikaner revealed the presence of this fungus in untreated 4.5% and pretreated 3% seeds in SBM. This fungus was not revealed in PDA test.

Mucor sp.

The sporangiophores of this fungus unbranched, erect, turf of this 1 cm high; sporangia spherical, grey coloured, wall leaving a collorette; columella free either spherical or oval colourless; spores unequal, elongate ellipsoidal or kidney-shaped, smooth and hyaline. (Fig. 7A)

1 and 2 Samples collected from Jaipur and Shri Ganganagar revealed 2% infection of the fungus in untreated and pretreated Seeds in SBM. This fungus was not recorded in PDA test.

***Myrothecium roridum* Tode ex Fr.**

Sporodochia of this fungus sessile, discoid, circular or irregular in surface view, often coalescing to form larger masses with a white colour rim, sporodochia margin woolly, composed of loosely intertwined, single celled, contorted hyphae; conidiophores erect once or twice branched, septate, hyaline, with tapering phialides forming a closely packed hymenium like layer; conidia cylindrical or very slightly tapering with rounded ends and pale or pinkish olive green to black in mass. (Fig. 7B)

The growth of this fungus was identified and recorded in 18, 14 and 4 samples collected from all 11 districts of Rajasthan in untreated, pretreated seeds in SBM and PDA with percentage incidence range of 1-4%, 0.5-5.5% and 2-5.5% respectively. The RPO was 15, 11.67 and 11.42 respectively.

***Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr.**

The fungal sporodochia sessile, circular or irregular in surface view but rough-walled hyphae in large number, discoid in shape; conidia navicular, either limeniform or broadly ellipsoidal, slightly protuberant and truncate at the base.

Two samples revealed its presence in both untreated (1.5, 3%) and pretreated seeds (1.5, 2%) in SBM collected from Bikaner and Shri Ganganagar. This fungus was not observed in PDA test.

***Paecilomyces varioti* Bainier**

Colonies effuse; conidiophores arising from the mycelium singly, branched near the apex, branches were divergent; conidia in dry basipetal chains, unicellular, ovoid to fusoid and hyaline. (Fig. 7D)

It was observed in 1, 1 and 3 samples in untreated (2, 2.5%), pretreated (3.5%) and PDA (1-7%).

***Penicillium spp*. Link**

Colonies of this fungus have various shades, vegetative hyphae creeping septate and branched; conidia borne in chains and form typical brush- like head, dry conidia are either ovate or elliptical globose and either smooth or rough. Conidiophores seldom branched, at the apex with vertical or erect primary branches each with a verticil of secondary (metulae) and sometimes tertiary bearing cells known as phialides borne directly on the slightly inflated apex of the conidiophores of the fungus. (Fig. 7E)

This fungus was identified In 3 and 2 samples in untreated and pretreated seeds In SBM with an percentage incidence range of 1.5-3.5% and 0.5, 1.5% respectively from Bikaner, Jhunjhunu and Shri Ganganagar. This fungus was not revealed in PDA test.

***Phoma betae* Frank**

The fungal pycnidia globose, superficially formed, provided with a distinct neck and protruding ostiole, cirrus dry, light brown, conidia single celled, ellipsoidal to cylindrical. (Fig. 7F)

This fungus was observed and identified in 8, 7 and 1 seed samples in untreated 0.5-1.5%, pretreated 0.5-1.5% in SBM and PDA 2% respectively collected from Bikaner, Jaipur , Jalore, Jhunjhunu and Shri Ganganagar. RPO calculated was 6.67, 5.83 and 2.85 respectively.

***Rhizoctonia bataticola* (Taub.) Butler**

The sclerotia small, pinhead like, brown to black colour, inter-connnected by brown to black mycelial threads, sclerotia made up of interwoven and interlocked mycelium. Spores were not produced by this fungus. (Fig. 7G)

This fungus was identified in 12, 09 and 05 samples in untreated 1-7.5%, pretreated 1-5.5% seeds in SBM and PDA 0.5-7% respectively from Bikaner, Jaipur, Jalore, Jodhpur and Sikar. 9, 3; 8, 1 and 3, 2 samples were in the range of 0.5-5% and 5.5-10% respectively in SBM and PDA. The RPO recorded was 10, 7.5 and 14.28 respectively.

***Rhizoctonia solani* Julius Kuhn**

Scelrotia of the fungus was brown to black in colour, closely appressed to the host surface, variable in form, frequently small and loosely formed among and connected by mycelial threads; hyphae of mycelium brown coloured and constricted at point of origin. (Fig. 7H)

This fungus was identified in 30, 31 and 11 samples collected from all districts except Kota revealed its presence in untreated and pretreated SBM and PDA with an Percentage incidence of infection was 1-22.5%, 1-22.5% and 0.5-21.25% respectively. 21, 7, 2; 21, 7, 3 and 7, 3, 1 samples were in the range of 0.5-5% and 5.5-10% and 10.5-25% respectively in SBM and PDA.

***Rhizopus nigricans* Ehrenb.**

Sporangia is hemispherical; columella broad, hemispherical; sporangiophores oval, irregular round, unequal in size, 1-4 mm long and grey blue in colour. Stolons creeping, attached to seed surface by brown coloured rhizoids. (Fig. 7I)

The fungus identified in 26, 20 and 9 seed samples in untreated 1-20% pretreated 0.5-7.5% seeds in SBM and PDA 1-11.5% respectively from all the districts except Jalore. The RPO was calculated 21.67, 16.67 and 25.71 respectively. 22, 3, 1; 19, 1, 0 and 4, 3, 2 samples were in the range of 0.5-5% and 5.5-10% and 10.5-25% respectively in SBM and PDA.

***Rhizopus stolonifer* (Ehrenb. ex Fr.) Vuill.**

Colonies were very fast growing and often over 2 cm high , reddish grey brown and stolons were hyaline to brown, 13-20 µm wide, abundantly branched rhizoids 300-350 µm long and whorls of sporangiophores produced terminally. Sporangiophores were pale to dark brown, usually straight, mostly 1.5-3 mm tall and 13-29µm wide. Sporangia were black, mostly 100-200 µm diameter; collumella subglobose to oval, pale brown, mostly 70-120 µm diameter. (Fig. 7J)

Sporangiophores were subglobose, biconical to oval, ridged and 7-12 X 6-8.5 µm size.

5, 5 and 7 samples collected from Alwar, Jaipur, Jodhpur, Nagaur and Sikar districts of Rajasthan revealed the infection in untreated (0.5, 2%), pretreated (0.5, 2%) seeds in SBM and PDA (2, 5.5%).Out of infected samples 5,0; 5,0 and 6, 1 samples in untreated , pretreated were in the range of 0.5-5% and 5.5-10% in SBM and PDA respectively. The RPO was 4.17, 4.17 and 20 respectively.

***Stachybotrys atra* Corda**

Dark brown coloured conidiophores having upper portion roughened and darker in colour, phialides dark coloured obovoid; conidia present in the form of compact balls held together by mucilage, oval to cylindrical with rounded ends, dark brown verrucose or echinulate. (Fig. 7K)

6,6 and 2 seed samples were identified and observed in untreated 0.5-3.5%, pretreated 0.5-4% seeds in SBM and PDA 2, 4% respectively collected from Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, and Nagaur. The RPO was calculated 5, 5 and 5.71 respectively.

***Trichothecium roseum* (Pers.) Link ex Fr.**

The fungal colonies powdery, pink peach coloured, shining like dew drops; conidiophores long slender hyaline, erect, unbranched and slightly swollen at the tip; conidia smooth, hyaline, obovoid, single, septate, apical cell large and basal cell slightly protuberant. (Fig. 7N)

This fungus was identified in 10, 10 and 3 seed samples in untreated 1-8.5%, pretreated 1-7% seeds in SBM and PDA 3-4% respectively collected from all districts except Kota and Shri Ganganagar. The RPO was calculated 8.33, 8.33 and 8.57 in SBM and PDA test respectively.

***Trichurus spiralis* Hasselbring**

The synnemata dark, stalk slender, spore bearing portion expanded; long black, simple and branched hairs or spines present among the conidiophores; conidia dark and unicellular, ovoid and catenulate. (Fig. 7O)

This fungus was identified and recorded in 3 samples in both untreated 1-2.5% and pretreated seeds 1-1.5% in SBM collected from Churu, Jaipur and Shri Ganganagar. The calculated RPO was 2.5 in both untreated and pretreated seeds.

Other Fungi Associated with *Cyamopsis tetragonoloba*

Aspergillus oryzae (Ahlburg) E. Cohn (Fig. 4H) 7(1-4.5%) untreated, 9(1-3.5%) pretreated and 6(1.5-6.5%) PDA; *Cephaliophora tropica* Thaxt. (Fig. 4L) 14(1-7.5%) untreated, 11(0.5-5.5%) pretreated and 10(1-8%)PDA*; Chetomium megalocarpum* Bainier (Fig. 5C) 10(0.5-4%) untreated, 10(4.5%) pretreated and 4(1-4.5%)PDA*; Curvularia clavata* Jain (Fig. 5H) 9(1-8.25%) untreated, 9(0.5-5%) pretreated and 2(5.5%) PDA*; Eurotium amstelodami* L. Mangin (Fig. 6B) 5(1-2%) untreated, 5(0.5-6%) pretreated and 3(2-4.5%) PDA*; Fusarium pallidoroseum* (Cooke) Sacc. (Fig. 6E) 10(4.5%) untreated, 7(0.5-1.5%) pretreated and 4(1-7.5%) PDA*; Neocosmospora vasinfecta* E. F. Sm. (Fig. 7C) 3(1.5%) untreated, 3(0.5-2%) pretreated and 4 (1.5-6.5%) PDA*; Stachybotrys chartarum* (Ehrenb.) S. Hughes (Fig. 7L) 3(3, 3.5%) untreated, 5(1.5-5.5%) pretreated and 6(1, 5.5%) PDA and *Thielavia terricola* (J.C. Gilman & E.V. Abbott) C.W. Emmons (Fig. 7M) 7(1-5.5%) untreated, 4(1-4.5%) pretreated in SBM were also recorded in incubation test in cluster bean samples collected from various districts of Rajasthan. These fungi revealed low occurrence as well as incidence. (Table 5, 6, 7 & 8)

EFFECT OF SODIUM HYPOCHLORITE PRETREATMENT ON SEED-BORNE FUNGI IN BLOTTER METHOD

In standard blotter method both untreated and pretreated seeds with sodium hypochlorite were used. 0.5% concentration of available chlorine was found to increase the seed germination without affecting the incidence of the pathogenic seed borne fungi. The chlorine pretreatment in general either completely eliminated or reduced the growth of both parasitic and saprophytic fungi and their growth on seed surface was also sparse.

The fungi which occurred in untreated seeds were commonly associated with pretreated seeds (Table 5 & 6). A total 53 fungi of 28 genera were recorded in both untreated and chlorine pretreated seeds. One-five percent chlorine pretreatment was relatively phyto-toxic causing arrested radicle emergence and seedling abnormality. Hence, 0.5% chlorine treatment was used throughout the study.

PHYTOPATHOLOGICAL EFFECTS OF SEED BORNE FUNGI

Seed borne infection of various fungi caused an adverse effect on the seed germination, and resulted in symptomatic seedling. The common fungi hindering seed germination were *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium oxysporum, Curvularia lunata, Fusarium oxysporum, F. solani, Rhizoctonia solani* and *Rhizopus nigricans.* Besides affecting seed germination these fungi caused various symptoms and seedling abnormalities.

Infection of *Alternaria alternata* resulted in the production of swollen hypocotyl with black streaks and brown radicle. *Aspergillus flavus* infection produced brown-black lesions on hypocotyls and base of shoot along with radicle rotting. *Aspergillus niger* infected seedlings produced yellow brown colour of radicle and cause rotting. Heavy infection resulted in rotting and mortality of seedlings. *Curvularia lunata and Drechslera halodes* caused brown spots on

seedling. *Fusarium moliliforme* and *F. oxysporum* mostly hampered seed germination and produce symptomatic seedling with weak radicle, hypocotyl showing discrete black streaks and cotyledonary leaves with necrotic spots.

Fusarium solani was the major fungi adversely affecting the crop causing wilt disease. It is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. The margins of the cotyledonary leaves curl downward and inward. The stem near the soil line or collar region may be slightly thickened and brittle. Yellow-brown to black discolouration appears on stem affected with wilt disease. Pods became flattened and poorly filled. In pods seed setting was very poor and some seeds showed white mycelial growth on seed coat.

The seedling infected with *Macrophomina phaseolina* and *Rhizoctonia bataticola* showed pink to brown water soaked areas on radicle starting from the tip progressing upwards and mostly caused seed rot and rotting of radicle.

The degree of infection was directly correlated with the amount of fungal growth on seed. Heavy growth of the fungi resulted in complete failure of seed germination while sparse and moderate growth produce symptomatic seedling.

TABLE- 3 : DISTRICTWISE NUMBER OF SEED SAMPLES OF *Cyamopsis tetragonoloba* STUDIED IN DRY SEED EXAMINATION

Districts	No. of samples	Seeds with black streaks	Grey colour seeds with white mycelial growth	Shrivelled	Broken and insect damaged seed	Debris and inert matter
ALWAR	7	0.75-5.5 (2)	0.25-3 (2)	0.75-9.5(5)	-	-
BIKANER	6	0.75-4.75 (3)	0.5-10.5 (3)	1.5-9.5 (2)	4-14.5 (3)	0.5-1.5(1)
CHURU	10	0.5-6 (3)	0.75-12.5 (2)	5.75-14.75 (6)	0.25-7 (5)	1.5-5.75 (3)
JAIPUR	20	0.75-10.5 (5)	0.5-35.5 (10)	1.25-19.25 (13)	1.5-45 (15)	0.25-15.75(7)
JALORE	5	-	0.25-7.75 (2)	4.75-12 (4)	-	-
JHUNJHUNU	14	0.5-7.5 (4)	3-25.25 (2)	2-8.75 (7)	0.5-12(10)	0.5-2.5 (3)
JODHPUR	9	0.25-3.75 (2)	0.5-2.5 (1)	0.25-8 (8)	0.75-12.5 (9)	1-3.75 (2)
KOTA	5	0.5 (1)	1-7.75 (2)	4.25-8.75 (5)	0.5-3.5 (2)	1.5-3 (2)
NAGAUR	10	0.5-5.5 (3)	0.25-8.5 (3)	1.25-14.75 (8)	1.25-12 (6)	1.25-5.75(3)
SHRI GANGANAGAR	8	0.5-8.5 (4)	0.5-8.75 (4)	2-16.5 (6)	0.25 -3.5 (7)	0.25-12.5 (4)
SIKAR	26	0.25-12.5 (7)	0.25-40.50 (12)	0.25-25(16)	0.5-50 (18)	0.25-20 (9)
TOTAL	**120**	**0.25-12.5 (34)**	**0.25-40.5 (43)**	**0.25-25 (80)**	**0.25-50 (75)**	**0.25-20 (34)**

No. of seed samples given in parenthesis.

TABLE- 4 : OCCURANCE AND PERCENT RANGE OF VARIOUS SEED DISORDERS IN SEED SAMPLES SHOWED THE FUNGAL ASSOCIATION AND SEEDLING SYMPTOMS

Type of seed disorder	Percent range	Fungi associated with seeds	Seedling symptoms
Seeds with black streaks	0.25-12.5(34)	*Colletotrichum dematium, Curvularia lunata, Rhizoctonia bataticola, R.solani, Macrophomina phaseolina, Phoma betae*	Pale-yellow coloured seedling. Blackening at the base of stem. Necrosis on the root region. Brown spots on seedlings. Pink to brown water soaked areas on radical starting from tip progressing upwards.
Grey coloured seeds with mycelial growth	0.25-40.5% (43)	*Fusarium moniliforme, Fusarium oxysporum, Fusarium solani*	Yellowing of stem bases, drooping of leaves and wilting of seedling. Premature leaf fall. Blighting of leaves. Stunted growth of plant.
Shrivelled seed	0.25-25% (80)	*Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Chaetomium sps., Drechslera tetramera, Fusarium moniliforme, F. oxysporum, Penicillium sps., Rhizoctonia solani, Myrothecium roridum* and Bacterial ooze	Browning of leaves and basal part of shoot. Crinkling of leaves. Brown –black lesions on infected seedling. Light brown spots on leaf and rotting of the radicle Brown streaks on the margins of leaves.
Broken and insect damaged seed	0.25-50% (75)	*Alternaria alternata, Aspergillus flavus,Curvularia clavata, C. lunata, Drechslera tetramera, Rhizoctonia bataticola, Chaetomium spp., Rhizopus sps. and Trichothecium roseum*	Browning and rotting of seedling. Rotting of seedlings. Blightening of leaves and premature leaf fall.
Debris and inert matter	0.25-20% (34)	*Aspergillus fumigatus, A. niger, Curvularia pallescens, Drechslera rostrata, Fusarium monaliforme, F.oxysporum, F. solani, Mucor sps., Phoma betae, Rhizopus nigricans* and *Trichothecium roseum*	Light brown spots on seeddling. Seedling with weak radicle, hypocotyl showing discrete black streaks. Necrotic streaks and spots on radicle and basal part of shoot.

TABLE- 5: OCCURRENCE, RELATIVE PERCENT OCCURRENCE (RPO) AND PERCENT RANGE OF FUNGI IN UNTREATED SEED SAMPLES OF VARIOUS DISTRICTS OF RAJASTHAN IN SBM

FUNGI	ALWAR	BIKANER	CHURU	JAIPUR	JALORE	JHUNJHUNU	JODHPUR	KOTA	NAGAUR	SHRIGANGANAGAR	SIKAR	TOTAL	RPO	% RANGE OF FUNGI
Alternaria alternata	2	2	3	5	5	4	4	-	3	2	6	36	30	0.5- 12.5
A. brassicola	-	-	-	-	-	2	-	-	-	-	-	2	1.67	4.5
A. tenuissima	-	-	-	-	-	1	1	-	-	-	-	2	1.67	2.5
Aspergillus candidus	-	3	1	2	-	-	-	-	-	-	-	6	5	1.5-5
A. flavus	2	-	4	7	2	3	1	2	4	3	9	37	30.83	1.5-21.75
A. fumigatus	-	-	-	-	-	-	3	-	1	-	-	4	3.33	2-4
A. niger	1	-	3	8	2	3	3	2	4	3	9	38	31.67	0.5-17.5
A. oryzae	-	2	-	1	-	-	2	-	1	1	-	7	5.83	1-4.5
A. sulphureus	-	3	-	1	-	-	4	1	-	1	-	10	8.33	1-7
A. sydowi	1	2	1	1	-	1	4	1	1	1	1	14	11.67	1-9.5
Botryodiplodia theobromae	-	-	3	-	-	-	-	-	-	-	-	3	2.5	1-2.5
Cephaliophora tropica	2	-	1	1	-	6	-	1	1	-	2	14	11.67	1-7.5
Chaetomium globosum	1	-	2	-	-	1	-	-	-	-	-	4	3.33	1-4
C. indicum	-	-	-	-	1	-	-	-	-	-	-	1	0.83	2
***C.* megalocarpum**	-	1	2	-	1	-	3	1	-	-	2	10	8.33	0.5-4
C. murorum	-	-	-	-	-	1	-	-	1	-	-	2	1.67	2.5, 3
C. spinosum	1	-	-	-	-	1	1	-	-	-	-	3	2.5	2-3.5
Choanophora cucurbitarum	-	3	-	1	-	3	-	-	-	-	-	7	5.83	0.5-3
Cladosporium oxysporum	-	3	2	6	2	4	2	-	3	1	6	29	24.17	0.5-7
Colletotrichum dematium	-	2	-	-	-	-	2	-	1	1	-	6	5	0.5-7.5
Curvularia clavata	-	-	1	4	-	-	2	-	-	2	-	9	7.5	1-8.25
C. lunata	2	1	2	5	2	2	3	-	5	2	4	28	23.33	1.5-7
C. pallescens	-	1	-	3	-	-	-	-	-	-	-	4	3.33	2-3.5
Drechslera halodes	-	-	-	2	-	2	1	-	1	-	-	6	5	1.5-3
D. maydis	1	-	-	-	-	1	-	-	-	-	-	2	1.67	1.5 , 2
D. rostrata	1	-	2	1	-	1	2	-	2	-	-	9	7.5	0.5-5
D. tetramera	1	1	2	1	1	1	-	-	1	1	-	9	7.5	1-3.5
Eurotium amstelodami	1	-	-	2	-	-	-	1	-	-	1	5	4.17	1-2
Fusarium moniliforme	-	1	1	-	-	-	-	-	-	-	1	3	2.5	5
F. oxysporum	2	5	5	5	3	5	3	2	5	1	9	45	37.5	1-30.75
F. pallidoroseum	-	2	-	3	1	-	2	-	1	-	1	10	8.33	4.5
F. solani	2	4	2	13	3	4	1	2	3	5	18	57	47.5	0.25-62
Graphium sp.	-	-	-	2	-	-	-	-	-	-	-	2	1.67	1.5
Macrophomina phaseolina	1	2	2	2	1	2	1	1	-	-	-	12	10	0.5-8.5
Melanospora zamiae	-	-	-	3	1	-	-	-	-	-	-	4	3.33	1.5-3
Memnoniella echinata	-	-	2	-	-	3	4	-	-	-	-	9	7.5	1.5-3.5
M. levispora	-	1	-	-	-	-	-	-	-	-	-	1	0.83	4.5
Mucor sp.	-	-	-	1	-	-	-	-	-	-	-	1	0.83	2
Myrothecium roridum	-	2	1	3	1	2	4	2	2	1	-	18	15	1-4
M. verrucaria	-	1	-	-	-	-	-	-	-	1	-	2	1.67	1.5 , 3
Neocosmospora vasinfecta	1	-	-	-	-	1	-	-	-	-	1	3	2.5	1.5
Paecilomyces varioti	-	-	1	-	-	-	-	-	-	-	-	1	0.83	2, 2.5
Penicillium spp.	-	1	-	-	-	1	-	-	-	1	-	3	2.5	1.5-3.5
Phoma betae	-	1	-	3	1	2	-	-	-	1	-	8	6.67	0.5-1.5
Rhizoctonia bataticola	-	1	-	4	-	-	2	-	-	-	5	12	10	1-7.5
R. solani	2	2	4	5	3	4	-	-	3	2	5	30	25	1-22.5
Rhizopus nigricans	3	5	4	2	-	1	4	1	4	2	-	26	21.67	1-20
R. stolonifer	1	-	-	1	-	-	2	-	-	-	1	5	4.17	0.5,2
Stachybotrys atra	-	1	1	1	-	-	2	-	1	-	-	6	5	0.5-3.5
S. chartarum	-	-	2	-	1	-	-	-	-	-	-	3	2.5	3,3.5
Thielavia terricola	-	-	-	-	2	-	2	1	-	-	2	7	5.83	1-5.5
Trichothecium roseum	1	1	1	2	2	2	1	-	-	-	-	10	8.33	1-8.5
Trichurus spiralis	-	-	1	1	-	-	-	-	-	1	-	3	2.5	1-2.5

TABLE-6: OCCURRENCE, RELATIVE PERCENT OCCURRENCE (RPO) AND PERCENT RANGE OF FUNGI IN CHLORINE PRETREATED SEED SAMPLES OF VARIOUS DISTRICTS OF RAJASTHAN IN SBM

FUNGI	ALWAR	BIKANER	CHURU	JAIPUR	JALORE	JHUNJHUNU	JODHPUR	KOTA	NAGAUR	SHRIGANGANAGAR	SIKAR	TOTAL	RPO	% RANGE OF FUNGI
Alternaria alternata	2	4	4	5	-	4	2	1	3	2	6	33	27.5	0.5- 12.5
A. brassicola	-	-	-	-	-	2	-	-	-	-	-	2	1.67	2.5
A. tenuissima	-	-	-	-	-	1	1	-	-	-	-	2	1.67	1, 1.5
Aspergillus candidus	-	3	1	2	1	-	-	-	-	1	-	8	6.67	1-5
A. flavus	1	3	2	5	-	-	4	3	-	4	9	31	25.83	1-30.25
A. fumigatus	-	-	-	-	-	-	3	-	1	-	-	4	3.33	1-4
A. niger	-	2	3	6	-	3	4	2	3	3	8	34	28.33	1 -16.5
A. oryzae	-	2	-	2	-	-	1	-	2	2	-	9	7.5	1-3.5
A. sulphureus	-	3	-	1	-	-	4	1	-	1	-	10	8.33	1-3
A. sydowi	-	2	1	1	-	1	1	-	1	1	4	12	10	0.5 -4.5
Botryodiplodia theobromae	-	-	3	-	-	-	-	-	-	-	-	3	2.5	0.5 -3
Cephaliophora tropica	2	-	1	1	-	5	-	-	-	1	1	11	9.17	0.5-5.5
Chaetomium globosum	-	-	-	1	-	1	-	-	-	-	-	2	1.67	2.5, 3
C. indicum	-	-	-	-	2	-	-	-	-	-	-	2	1.67	1 , 2.5
***C.* megalocarpum**	-	-	2	-	1	-	3	1	-	-	3	10	8.33	4.5
C. murorum	-	-	-	-	-	1	-	-	1	1	-	3	2.5	2 -3.5
C. spinosum	2	-	-	-	-	1	1	-	-	-	-	4	3.33	1.5 -2
Choanophora cucurbitarum	-	2	-	-	-	2	-	-	-	-	-	4	3.33	0.5 -2
Cladosporium oxysporum	-	2	2	6	2	4	2	-	3	1	7	29	24.17	0.5 - 7
Colletotrichum dematium	-	1	-	-	-	-	2	-	1	1	-	5	4.17	0.5 -2.5
Curvularia clavata	-	-	1	4	-	-	2	-	-	2	-	9	7.5	0.5-5
C. lunata	2	2	3	5	3	2	3	-	4	3	5	32	26.67	0.5 – 7
C. pallescens	-	-	-	3	-	-	-	-	-	-	-	3	2.5	2-5
Drechslera halodes	-	-	-	2	-	2	2	-	1	-	-	7	5.83	1-3
D. maydis	1	-	-	-	-	1	-	-	-	-	-	2	1.67	2, 2.5
D. rostrata	2	-	2	1	-	1	2	-	2	-	-	10	8.33	1-2.5
D. tetramera	1	1	2	2	1	1	-	-	1	1	-	10	8.33	1 -3.5
Eurotium amstelodami	1	-	-	2	-	-	-	1	-	-	1	5	4.17	0.5-6
Fusarium moniliforme	-	-	-	-	-	-	-	-	-	-	1	1	0.83	3
F. oxysporum	2	3	4	6	3	4	4	2	4	2	9	43	35.83	1-27.25
F. pallidoroseum	-	2	-	2	1	-	-	1	1	-	-	7	5.83	0.5-1.5
F. solani	2	3	1	12	4	3	2	3	2	6	15	53	44.17	0.25-35
Graphium sp.	-	-	-	2	-	-	-	-	-	-	-	2	1.67	1.5
Macrophomina phaseolina	1	2	3	3	1	-	2	1	2	1	4	20	16.67	1-5.5
Melanospora zamiae	-	-	-	3	1	-	-	-	-	1	-	5	4.17	1 -4.5
Memnoniella echinata	-	-	1	-	3	4	-	-	-	-	-	8	6.67	1-3
M. levispora	-	1	-	-	-	-	-	-	-	-	-	1	0.83	3
Mucor sp.	-	-	-	-	-	-	-	-	-	2	-	2	1.67	2
Myrothecium roridum	-	1	1	2	1	1	4	1	1	1	1	14	11.67	0.5 -5.5
M. verrucaria	-	1	-	-	-	-	-	-	-	1	-	2	1.67	1.5, 2
Neocosmospora vasinfecta	1	-	-	-	-	1	-	-	-	-	1	3	2.5	0.5-2
Paecilomyces varioti	-	-	-	1	-	-	-	-	-	-	-	1	0.83	3.5
Penicillium spp.	-	1	-	-	-	1	-	-	-	-	-	2	1.67	0.5, 1.5
Phoma betae	-	1	-	3	-	1	-	-	-	2	-	7	5.83	0.5-1.5
Rhizoctonia bataticola	-	1	-	2	-	-	3	-	-	-	3	9	7.5	1-5.5
R. solani	3	2	4	4	4	4	-	-	3	2	5	31	25.83	1-22.5
Rhizopus nigricans	3	1	3	1	-	1	-	1	5	1	4	20	16.67	0.5 - 7.5
R. stolonifer	1	-	-	1	-	-	2	-	-	-	1	5	4.17	0.5,2
Stachybotrys atra	-	1	1	1	-	-	2	-	1	-	-	6	5	0.5 – 4
S. chartarum	-	-	2	-	1	-	-	1	-	-	1	5	4.17	1.5-5.5
Thielavia terricola	-	-	-	1	-	-	-	2	-	-	1	4	3.33	1-4.5
Trichothecium roseum	1	1	1	2	1	2	-	-	-	-	2	10	8.33	1-7
Trichurus spiralis	-	-	1	1	-	-	-	-	-	1	-	3	2.5	1 -1.5

TABLE -7: OCCURRENCE, RELATIVE PERCENT OCCURRENCE (RPO) AND PERCENT RANGE OF FUNGI IN CHLORINE PRETREATED SEED SAMPLES OF VARIOUS DISTRICTS OF RAJASTHAN IN PDA

FUNGI	ALWAR	BIKANER	CHURU	JAIPUR	JALORE	JHUNJHUNU	JODHPUR	KOTA	NAGAUR	SHRIGANGANAGAR	SIKAR	TOTAL	RPO	% RANGE OF FUNGI
Alternaria alternata	1	2	1	3	-	-	1	-	-	1	2	11	31.43	0.5-14.5
Aspergillus candidus	-	-	-	-	1	1	-	-	-	1	2	5	14.28	3.5-7
A. flavus	-	2	2	3	1	1	-	-	2	-	-	11	31.43	2-18.75
A. fumigatus	-	-	-	-	-	-	1	-	1	-	1	3	8.57	5-8
A. niger	1	-	2	2	-	1	-	-	1	1	2	10	28.57	2.5-16.75
A. oryzae	1	-	-	1	-	-	2	-	1	-	1	6	17.64	1.5-6.5
A. sulphureus	-	-	-	2	1	1	-	-	-	-	-	4	11.42	4 -8.5
A. sydowi	-	-	-	2	1	-	1	-	-	-	2	6	17.14	4-8
Botryodiplodia theobromae	-	-	-	-	-	1	-	-	1	-	-	2	5.71	3, 3.5
Cephaliophora tropica	1	-	1	-	2	-	1	-	2	-	3	10	28.57	1-8
Chaetomium globosum	-	2	-	-	-	-	-	-	-	-	-	2	5.71	2, 3.5
C. indicum	-	-	-	-	1	1	-	-	-	-	-	2	5.71	1.5, 2.5
***C.* megalocarpum**	1	-	-	1	-	-	1	-	-	-	1	4	11.42	1,4.5
C. murorum	-	-	-	-	-	2	-	-	-	-	-	2	5.71	1 , 5
C. spinosum	-	-	-	-	-	4	1	-	-	-	-	5	14.29	1.5 -10.5
Choanophora cucurbitarum	-	-	-	-	-	2	-	-	-	-	-	2	5.71	1.5
Cladosporium oxysporum	-	-	-	-	1	1	1	-	2	-	-	5	14.29	4-7
Colletotrichum dematium	-	-	-	-	-	-	1	-	-	1	-	2	5.71	4, 4.5
Curvularia clavata	-	-	-	-	-	-	-	-	1	-	1	2	5.71	5.5
C. lunata	-	2	-	1	-	-	1	-	-	-	-	4	11.42	2 - 8.5
Drechslera rostrata	-	-	-	2	-	-	-	-	-	-	-	2	5.71	1.5
D. tetramera	-	-	-	1	-	1	-	-	1	-	-	3	8.57	2 - 3.5
Eurotium amstelodami	-	2	-	1	-	-	-	-	-	-	-	3	8.57	2-4.5
Fusarium oxysporum	-	1	1	2	1	1	1	1	-	2	3	13	37.14	1-21.75
F. pallidoroseum	2	-	-	-	-	-	1	-	-	-	1	4	11.42	1-7.5
F. solani	1	1	2	3	-	1	-	1	-	1	5	15	42.86	0.25-30
Macrophomina phaseolina	-	-	2	2	-	-	-	-	-	-	-	4	11.42	0.5-3
Melanospora zamiae	-	-	2	2	1	-	-	-	2	-	-	7	20	2.5 -4
Memnoniella echinata	-	-	-	-	1	-	-	1	-	-	-	2	5.71	2, 4
Myrothecium roridum	2	-	-	-	-	-	-	1	-	1	-	4	11.42	2 -5.5
Neocosmospora vasinfecta	1	-	1	-	-	-	-	-	1	-	1	4	11.42	1.5-6.5
Paecilomyces varioti	-	-	-	1	-	2	-	-	-	-	-	3	8.57	1-7
Phoma betae	-	1	-	-	-	-	-	-	-	-	-	1	2.85	2
Rhizoctonia bataticola	-	2	-	2	1	-	-	-	-	-	-	5	14.28	0.5-7
R. solani	-	1	1	2	1	-	1	1	-	1	3	11	31.43	0.5-21.25
Rhizopus nigricans	-	3	2	-	-	-	1	-	3	-	-	9	25.71	1-11.5
R. stolonifer	1	-	-	3	-	-	-	-	1	-	2	7	20	2,5.5
Stachybotrys atra	-	-	-	-	1	1	-	-	-	-	-	2	5.71	2 , 4
S. chartarum	-	1	-	2	-	-	1	-	2	-	-	6	17.14	1,5.5
Trichothecium roseum	-	1	-	-	-	-	1	-	1	-	-	3	8.57	3-4

TABLE-8: NUMBER OF SEED SAMPLES IN DIFFERENT PERCENTAGE RANGE OF FUNGAL INFECTION IN UNTREATED AND PRETREATED SEEDS IN SBM AND PDA TESTS

FUNGI	Untreated					Pretreated					PDA				
	0.5-5	5.5-10	10.5-25	25.5-100	Total	0.5-5	5.5-10	10.5-25	25.5-100	Total	0.5-5	5.5-10	10.5-25	25.5-100	Total
Alternaria alternate	30	5	1	-	36	29	3	1	-	33	7	3	1	-	11
A. brassicola	2	-	-	-	2	2	-	-	-	2	-	-	-	-	-
A. tenuissima	2	-	-	-	2	2	-	-	-	2	-	-	-	-	-
Aspergillus candidus	6	-	-	-	6	8	-	-	-	8	3	2	-	-	5
A. flavus	23	11	3	-	37	21	7	2	1	31	7	3	1	-	11
A. fumigatus	4	-	-	-	4	4	-	-	-	4	2	1	-	-	3
A. niger	30	5	3	-	38	28	4	2	-	34	6	2	2	-	10
A. oryzae	7	-	-	-	7	9	-	-	-	9	4	2	-	-	6
A. sulphureus	8	2	-	-	10	10	-	-	-	10	3	1	-	-	4
A. sydowi	13	1	-	-	14	12	-	-	-	12	4	2	-	-	6
Botryodiplodia theobromae	3	-	-	-	3	3	-	-	-	3	2	-	-	-	2
Cephaliophora tropica	10	4	-	-	14	8	3	-	-	11	7	3	-	-	10
Chaetomium globosum	4	-	-	-	4	2	-	-	-	2	2	-	-	-	2
C. indicum	1	-	-	-	1	2	-	-	-	2	2	-	-	-	2
***C.* megalocarpum**	10	-	-	-	10	10	-	-	-	10	4	-	-	-	4
C. murorum	2	-	-	-	2	3	-	-	-	3	2	-	-	-	2
C. spinosum	3	-	-	-	3	4	-	-	-	4	2	2	1	-	5
Choanophora cucurbitarum	7	-	-	-	7	4	-	-	-	4	2	-	-	-	2
Cladosporium oxysporum	25	4	-	-	29	27	2	-	-	29	3	2	-	-	5
Colletotrichum dematium	5	1	-	-	6	5	-	-	-	5	2	-	-	-	2
Curvularia clavata	7	2	-	-	9	9	-	-	-	9	-	2	-	-	2
C. lunata	27	1	-	-	28	30	2	-	-	32	3	1	-	-	4
C. pallescens	4	-	-	-	4	3	-	-	-	3	-	-	-	-	-
Drechslera halodes	6	-	-	-	6	7	-	-	-	7	-	-	-	-	-
D. maydis	2	-	-	-	2	2	-	-	-	2	-	-	-	-	-
D. rostrata	9	-	-	-	9	10	-	-	-	10	2	-	-	-	2
D. tetramera	9	-	-	-	9	10	-	-	-	10	3	-	-	-	3
Eurotium amstelodami	5	-	-	-	5	4	1	-	-	5	3	-	-	-	3
Fusarium moniliforme	3	-	-	-	3	1	-	-	-	1	-	-	-	-	-
F. oxysporum	31	7	4	3	45	29	10	2	2	43	5	4	3	1	13
F. pallidoroseum	10	-	-	-	10	7	-	-	-	7	3	1	-	-	4
F. solani	35	12	7	3	57	33	13	4	3	53	7	4	2	2	15
Graphium sp.	2	-	-	-	2	2	-	-	-	2	-	-	-	-	-
Macrophomina phaseolina	10	2	-	-	12	19	1	-	-	20	4	-	-	-	4
Melanospora zamiae	4	-	-	-	4	5	-	-	-	5	7	-	-	-	7
Memnoniella echinata	9	-	-	-	9	8	-	-	-	8	2	-	-	-	2
M. levispora	1	-	-	-	1	1	-	-	-	1	-	-	-	-	-
Mucor sp.	1	-	-	-	1	2	-	-	-	2	-	-	-	-	-
Myrothecium roridum	18	-	-	-	18	13	1	-	-	14	2	2	-	-	4
M. verrucaria	2	-	-	-	2	2	-	-	-	2	-	-	-	-	-
Neocosmospora vasinfecta	3	-	-	-	3	3	-	-	-	3	3	1	-	-	4
Paecilomyces varioti	1	-	-	-	1	1	-	-	-	1	2	1	-	-	3
Penicillium spp.	3	-	-	-	3	2	-	-	-	2	-	-	-	-	-
Phoma betae	8	-	-	-	8	7	-	-	-	7	1	-	-	-	1
Rhizoctonia bataticola	9	3	-	-	12	8	1	-	-	9	3	2	-	-	5
R. solani	21	7	2	-	30	21	7	3	-	31	7	3	1	-	11
Rhizopus nigricans	22	3	1	-	26	19	1	-	-	20	4	3	2	-	9
R. stolonifer	5	-	-	-	5	5	-	-	-	5	6	1	-	-	7
Stachybotrys atra	6	-	-	-	6	6	-	-	-	6	2	-	-	-	2
S. chartarum	3	-	-	-	3	3	2	-	-	5	4	2	-	-	6
Thielavia terricola	6	1	-	-	7	4	-	-	-	4	-	-	-	-	-
Trichothecium roseum	9	1	-	-	10	9	1	-	-	10	3	-	-	-	3
Trichurus spiralis	3	-	-	-	3	3	-	-	-	3	-	-	-	-	-

Fig. 3 (A-F): Dry seed examination of cluster bean seeds

A. Seeds with Black streaks 12X

B. Grey colour seeds with white mycelial growth 22X

C. Shrivelled seeds 12X

D. Broken and insect damaged seeds 12X

E. Debris and Inert matter 12X

F. Bold Healthy looking seeds 22X

Fig. 3

Fig. 4 (A-L): Fungi in incubation test

A. *Alternaria alternata 250X*

B. *A. brassicola 500X*

C. *A. tenuissima 700 X*

D. *Aspergillus candidus 250X*

E. *A. flavus 250X*

F. *A. fumigatus 500X*

G. *A. niger 250X*

H. *A. oryzae 250X*

I. *A. sulphureus 250X*

J. *A. sydowi 700X*

K. *Botryodiplodia theobromae 500X*

L. *Cephaliophora tropica 250X*

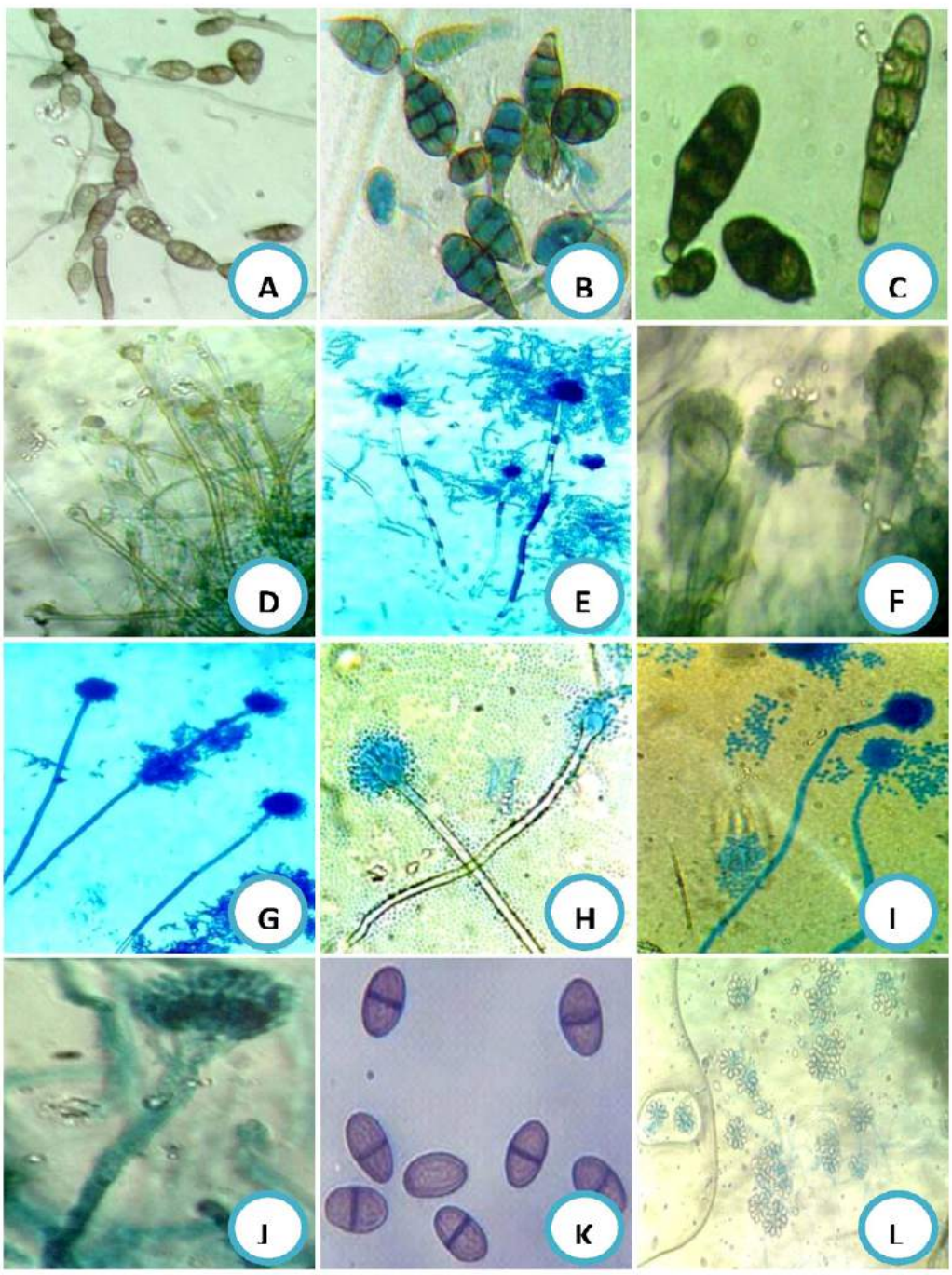


Fig. 4

Fig. 5 (A-L): Fungi in incubation test

A. *Chetomium globosum 250X*

B. *C. indicum 500X*

C. *C. megalocarpum 700X*

D. *C. murorum 700X*

E. *Choanephora cucurbitarum 500X*

F. *Cladosporium oxysporum 250X*

G. *Colletotrichum dematium 250X*

H. *Curvularia clavata 500X*

I. *C. lunata 500X*

J. *C. palliscens 250X*

K. *Drechslera halodes 700X*

L. *D. rostrata 500X*

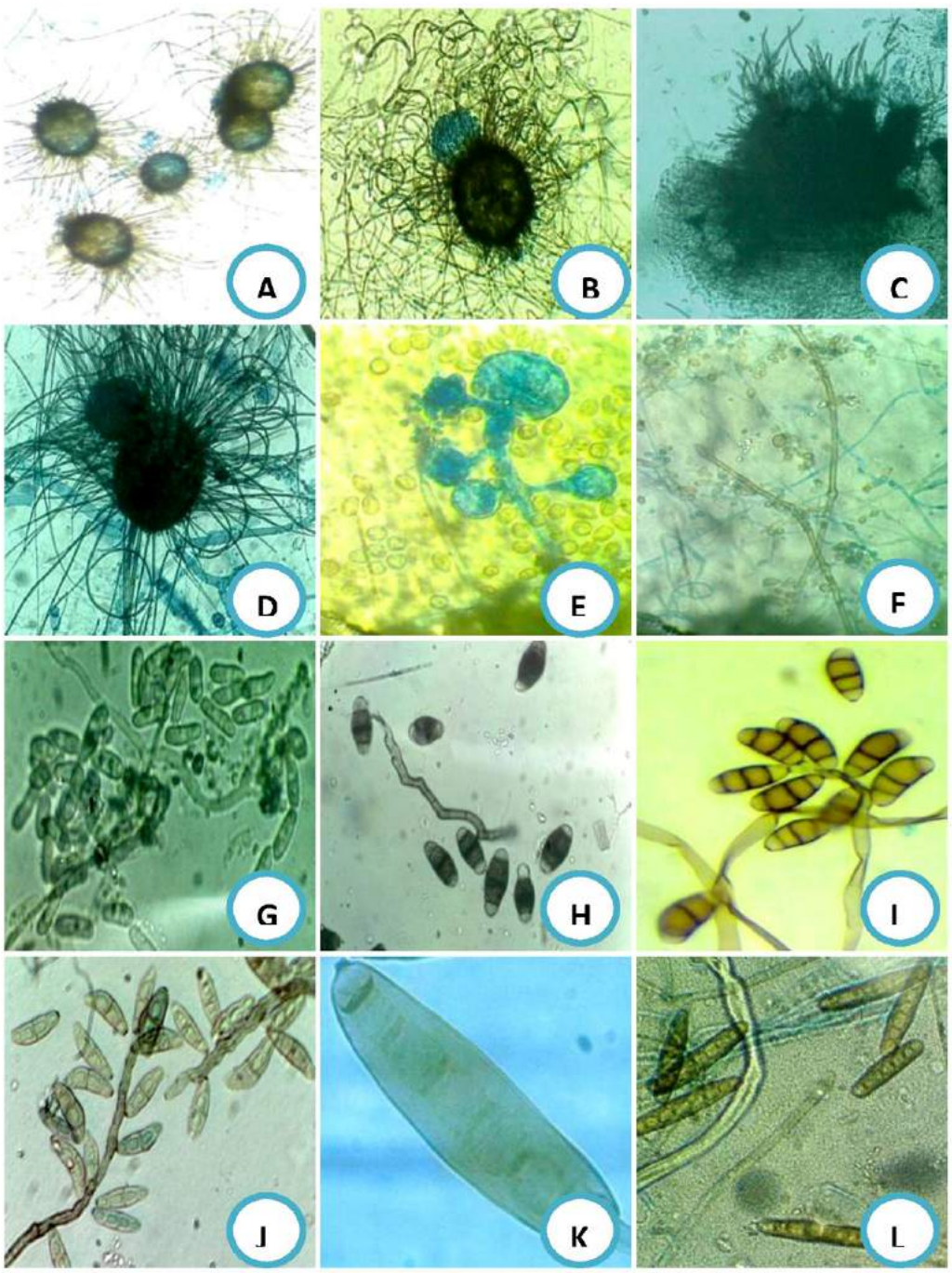


Fig. 5

Fig. 6 (A-L): Fungi in incubation test

A. *Drechslera tetramera 250X*

B. *Eurotium amstelodami 700X*

C. *Fusarium moniliforme 250X*

D. *F. oxysporum 250X*

E. *F. pallidoroseum 250X*

F. Polyphialides of *F. solani 250X*

G. Simple phialide and microconidia of *F. solani 500X*

H. Macroconidia and Chlamydospore of *F. solani 700X*

I. Macroconidia of *F. solani 250X*

J. *Graphium sp. 500X*

K. *Macrophomina phaseolina 500X*

L. *Melanospora zamiae700X*

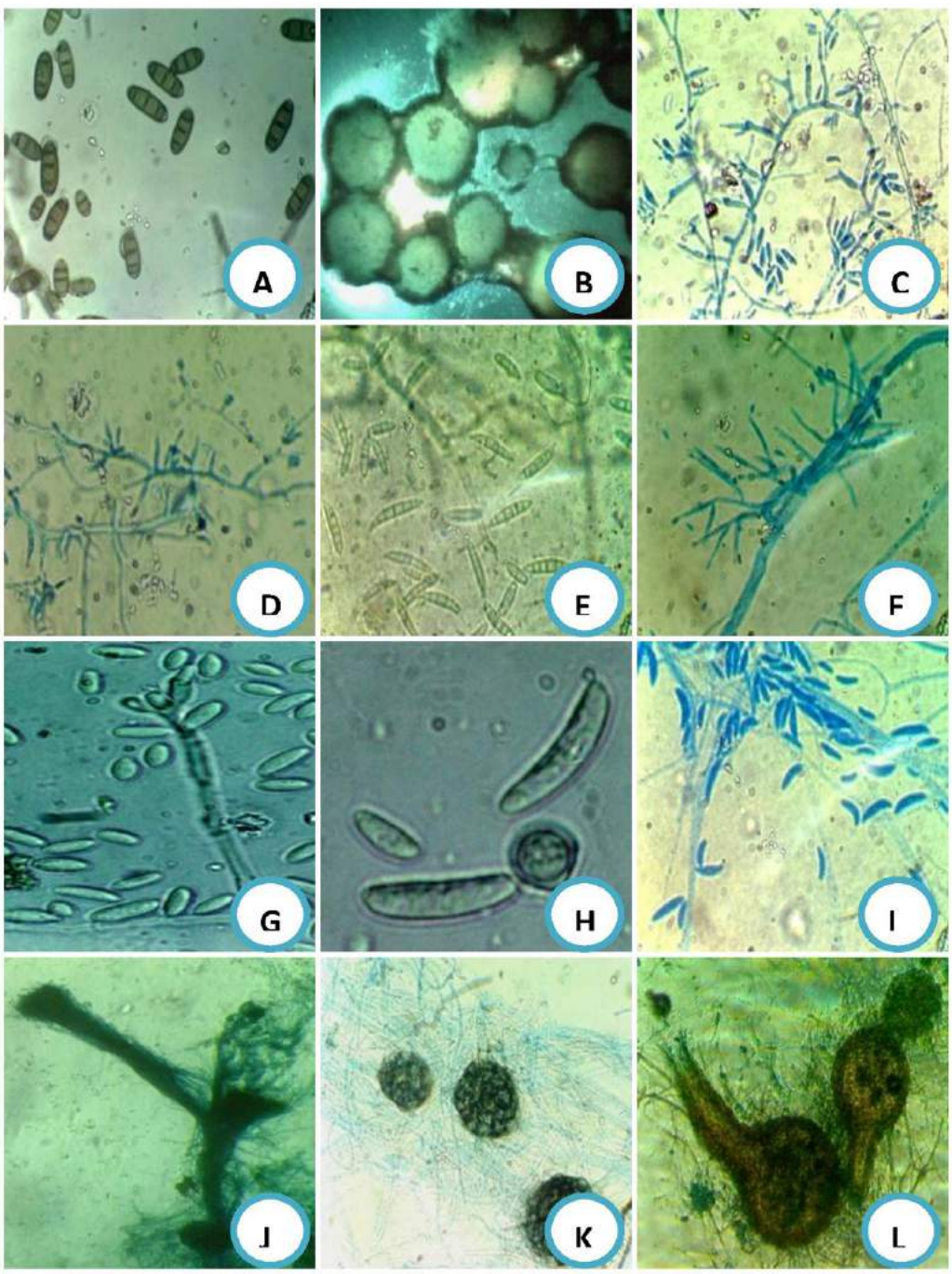


Fig. 6

Fig.7 (A-O): Fungi in incubation test

A. *Mucor sp. 250X*

B. *Myrothecium roridum 250X*

C. *Neocosmospora vasinfecta 250X*

D. *Paecilomyces varioti 250X*

E. *Penicillium citrinum 250X*

F. *Phoma betae 700X*

G. *Rhizoctonia bataticola 500X*

H. *R. solani 500X*

I. *Rhizopus nigricans 250X*

J. *R. stolonifer 250X*

K. *Stachybotrys atra 250X*

L. *S. chartarum 250X*

M. *Thielavia terricola 500X*

N. *Trichothecium roseum 500X*

O. *Trichurus spiralis 250X*

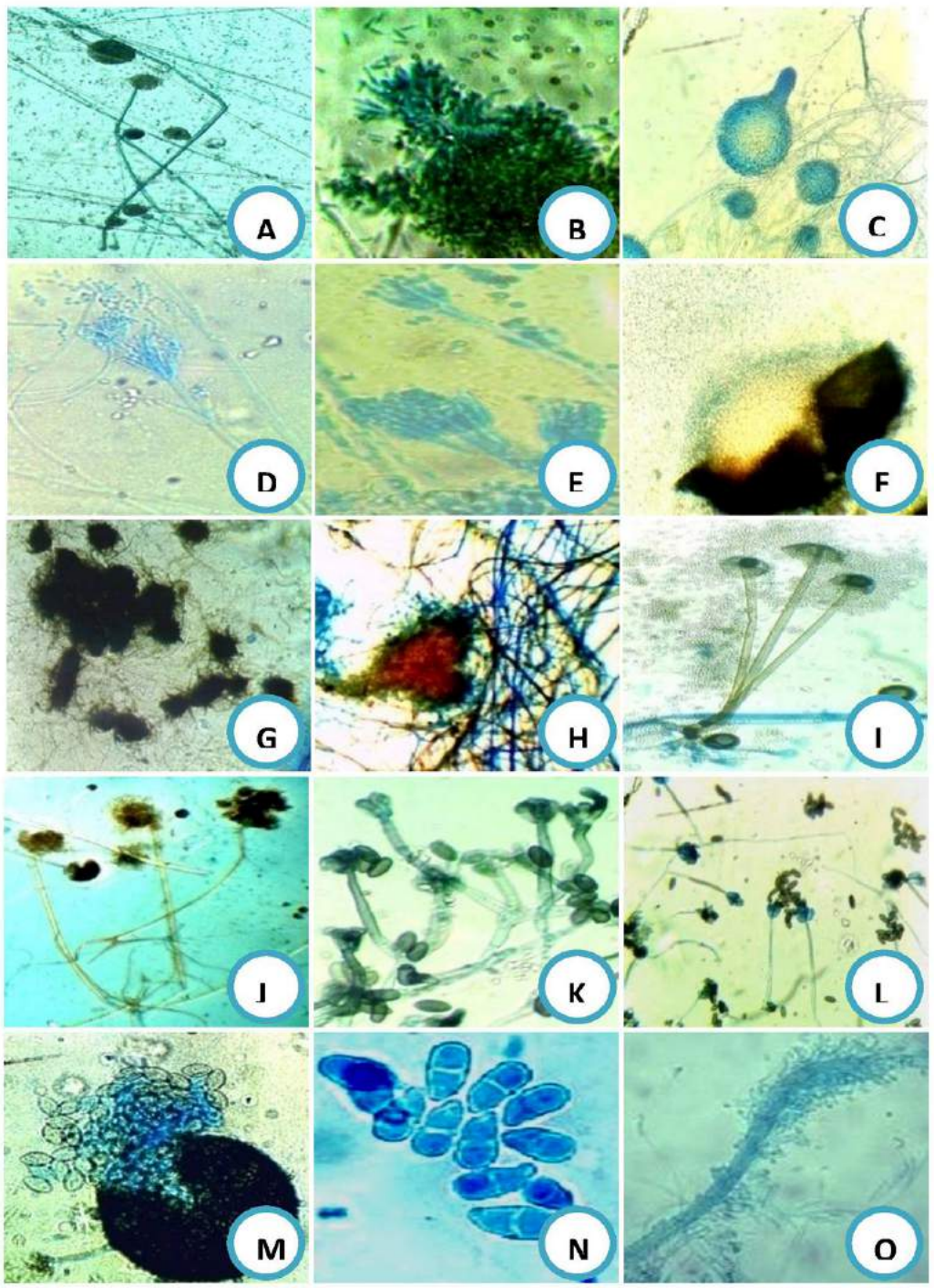


Fig. 7

Fig. 8 (A-C): Fungal spores during incubation test

A. Petriplates showing healthy and *Fusarium solani* infected seeds in SBM during incubation test 12X

B. Enlarged view of fungal mycelium with spores of *Fusarium solani* during incubation test 300X

C. Fungal spores of *Fusarium solani* 150X

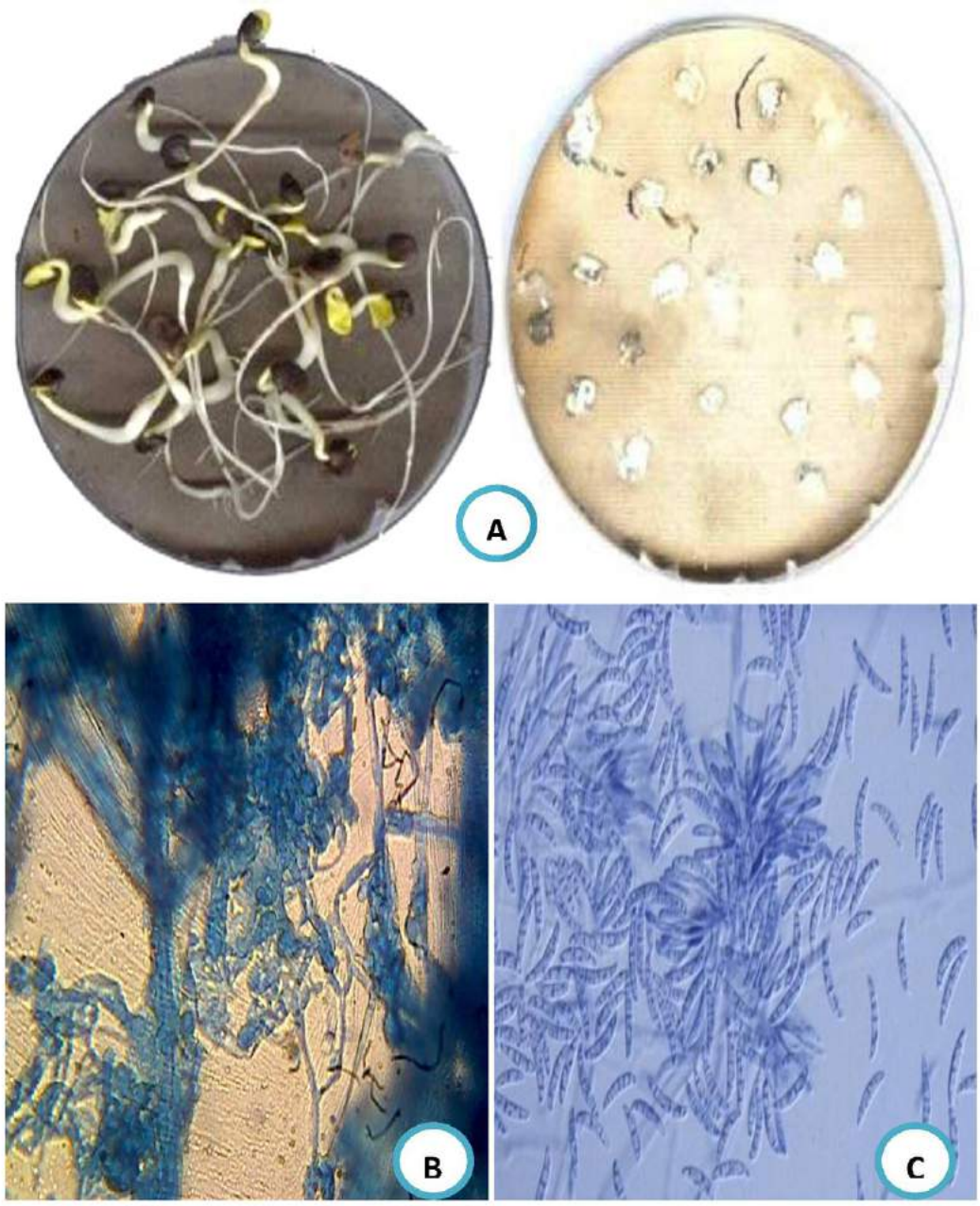


Fig. 8

INFECTION OF *Fusarium solani*

DRY SEED EXAMINATION

Out of 120 seed samples of cluster bean belonging to 11 districts namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar of Rajasthan , 43 samples carried seeds with dull and white cottony growth with loose mycelium on seed surface. *Fusarium solani* was the major fungi adversely affecting the crop causing wilt disease. Incidence of *Fusarium solani* infected seeds varied from 0.25-40.5%. Infected seeds were recorded in samples of all the districts but their higher incidence was observed in 12 seed samples collected from Sikar (0.25-40.5%) followed by 10 seed samples collected from Jaipur (0.5-35.5%) districts of Rajasthan. The Ac. Nos. given to seed samples was CB29 collected from Jaipur district and CB70 collected from Sikar district (Fig. 8B).

White mycelial growth of *F. solani* was distributed throughout the seed surface. Some seeds were completely distorted and carried infection of fungus in inner tissue of seeds also. Heavily infected seeds were completely covered with white mycelial growth of fungus. (Table 9)

INCIDENCE

120 and 35 seed samples of cluster bean were tested by SBM and PDA plate method respectively. *Fusarium solani* was isolated in 57, 53 and 15 samples in untreated, pretreated seeds in SBM and PDA respectively. The pathogen was obtained from all the districts in untreated and pretreated seeds in SBM but on PDA plates method, it was observed in samples collected from Alwar, Bikaner, Churu, Jaipur, Jhunjhunu, Kota, Shri Ganganagar and Sikar. The incidence of fungus was 0.25-62% in untreated seed samples, 0.25-35% in pretreated seed samples and 0.25-30% in PDA tests. The pathogen covered entire seed surface

with milky white peach colored mycelial growth of fungus. The highest percent incidence was observed in samples collected from Sikar CB-70 was 18 (0.25-62%) in untreated, 15(0.5-35%) in pretreated seeds in SBM and 5(0.25-30%) in PDA test followed by samples collected from Jaipur CB-29 was 13 (0.5-58%) in untreated, 12(0.5-34%) in pretreated seeds in SBM and 3(0.5-29%) in PDA.

On the basis of above observation the present study deals with wilt disease caused by *Fusarium solani* infection in seed samples collected from Jaipur CB29 and Sikar CB70. (Table 9)

HISTOPATHOLOGICAL STUDIES:-

STRUCTURE OF NORMAL SEEDS

To understand the actual location and penetration of pathogen in different components of seed and host parasite relationship it is imperative to know the structure of normal seed. A brief description of structure of normal or healthy cluster bean seed has been provided by Saber, Ahmed and Darwish (1956). The details of normal seed structure described here are based on the present study.

Seeds of cluster bean (*Cyamopsis tetragonoloba*) are round, rectangular or squarish in shape measuring 3.0x4.0 (LXB) mm. the colour of seeds varies from cream, dull white to grey, dull grey and black. Externally, surface of seed exhibits granular appearance with a protruding radical ridge and hilum on its lateral side. Histologically seed consists of seed coat, endosperm and a dicotyledonous embryo.

Seed Coat

Seed coat is organized into 3 distinct zones viz. outermost pallisade layer (Epidermis), hourglass (Hypodermis) and innermost parenchymatous layer.

(i) The epidermis is composed of pallisade layer consisting of highly lignified radially elongated, closely packed cells. The thickening material is uniformly deposited on the inner walls leaving empty lumen.

(ii) Hypodermis consists of single layer of hourglass cells which are bordered towards the base and narrow at the top enclosing wide intercellular spaces.

(iii) Parenchymatous layer constitutes the innermost layer of seed coat. It is 4-5 layered and consists of thin parenchymatous cells.

Hilum

It is laterally situated and is lined by a single layered counter pallisade representing head of funiculus. Transactions of the seed showed a median groove in hilar and counter pallisade region leading to ovate tracheidal bar. Sub hilar region consists of stellate parenchyma having large air spaces.

Endosperm

It is commonly absorbed during embryo development in most legumes but persists in the mature cluster bean seeds (Green, Sparks and Postiethwait, 1964). The endosperm is most comprised of two zones, the outermost endosperm made up of small thick walled while the inner endosperm of large thin walled, loosely arranged cells. In both the zones number of cell layers vary from 3-5. Glactomannan gum is stored in this region.

Embryo

It is of pleurorhizal type having radical lying against the margin and plumule in between the two large and fleshy cotyledons. Each cotyledon is delimated from the endosperm by a single layered epidermis covered with thin

cuticle. The cells of cotyledons are very rich in food materials and are supplemented with a well developed vascular supply.

The cells of embryonal axis are thin walled parenchymatous and rich in food materials.

HISTOPATHOLOGY OF NATURALLY INFECTED SEEDS

Component plating (Fig. 9A-D; Table 10A)

Seed samples were categorized as asymptomatic and symptomatic seeds. On the basis of severity of infection the symptomatic seeds further divided in 3 categories viz. weakly, moderately and heavily infected seeds.

In both asymptomatic as well as symptomatic seed samples (weakly and moderately infected seeds) could be easily separated various components like seed coat, cotyledons, endosperm, hilar region and hypocotyls shoot root axis than the heavily infected symptomatic seeds.

In Asymptomatic and weakly infected seeds fungal mycelium was observed in outer layer of seeds whereas in the moderately to heavily symptomatic seeds mycelium and conidia of fungus were recorded in all parts of categorized seeds. Peach- white, fluffy growth of pathogen was observed in symptomatic seeds (Fig. 9A). In some seeds, seed coat was completely covered with peach-white mycelial growth (Fig. 9B). Other component viz. cotyledon (Fig. 9C) and hypocotyls shoot root axis (Fig. 9D), endosperm and hilar region also showed presence of white fungal mycelium in symptomatic seeds. Maximum infection of fungus was observed in seed coat. Asymptomatic seedlings showed normal germination and the infection in seed coat and hilar region only. The pathogen gained the entry in the seed either through the hilar region or through the pores on the surface of seed coat.

In Asymptomatic seeds mycelium 5, 7% infection was observed in seed coat and cotyledons, endosperm, hypocotyl shoot root axis and hilar region are free from infection. Whereas in weakly infected seeds pathogen was observed 57, 50% in seed coat; 15, 35% in cotyledons; 26, 20% in endosperm; 14,24% in hypocotyl shoot root axis and 12, 17% in hilar region of both the samples respectively.

In moderately infected seeds, it was 84, 72% in seed coat; 72, 64% in cotyledons; 77, 62% in endosperm; 67, 60% in hypocotyl shoot root axis and 60, 58% in hilar region and in heavily infected seed components, it was 99, 97% in seed coat; 98, 95% in cotyledons; 99, 97% in endosperm; 98, 94% in hypocotyl shoot root axis and 95, 94% in hilar region of both the samples respectively.

Highest infection of fungal mycelium was observed in heavily infected seeds than the asymptomatic and weakly infected seeds in both the seed samples (CB29 and CB70).

Cleared wholemount preparations (Fig. 10A-F; Table 10B)

Cleared preparations of both the seed samples showed that the mycelium and conidia confined only to outer seed coat (10, 08%) and hilar region (2, 2%) in asymptomatic seeds whereas in weakly infected seeds the fungal infection was 59, 46% in seed coat; 57, 46% in endosperm; 13, 34% in cotyledons; 12, 30% in hypocotyl shoot root axis and 16, 14% in hilar region in both samples respectively. In moderately infected seeds the infection was 75, 71% in seed coat; 73, 71% in endosperm; 61, 67% in cotyledons; 57, 63% in hypocotyl shoot root axis and 59, 60% in hilar region in both samples respectively. In heavily infected seeds the infection was 99, 97% in seed coat; 99, 97% in endosperm; 98, 97% in cotyledons; 98, 96% in hypocotyl shoot root axis and 95, 92% in hilar region in both samples respectively.

Thin, hyaline branched, septate, inter and intracellular mycelium of pathogen was observed in cleared wholemount preparations of seed coat, cotyledons, endosperm, hilar region and hypocotyl shoot root axis of both CB29 (Jaipur) and CB70 (Sikar) seed samples (Fig. 10A-F). In asymptomatic seeds fungal infection only confined to the seed coat and hilar region. The hilar region of the seed is the first to become infected and from there it spreads to the other components viz. seed coat, cotyledon, endosperm and hypocotyl shoot root axis.

The mycelium net work was dense in all the components of heavily infected seeds of both the seed samples. Occasionally chlamydospores were also observed in the seed coat. In moderately to heavily infected seeds the heavy aggregation of mycelium was observed in all the seed components. The cells of endosperm had abundant inter and intracellular mycelium and depleted food contents. The endosperm also carried clumps or aggregation of fungal hyphae. The cells of the palisade and hourglass layers were loosely arranged and withered (Fig. 10A). A thick mycelial mat was found in the parenchyma layer. The cells were indistinguishable. Profuse and characteristic mycelium was seen in all the parts of the seed-coat, cotyledons (Fig. 10B & D), endosperm, hypocotyl-shoot-root-axis (Fig. 10E & F) and space between two cotyledons (Fig. 10C). The deterioration of cells of seed components also observed due to heavy aggregation of fungal mycelium.

Microtome sectioning

Microtome sections revealed the exact distribution and expanses of mycelium of *Fusarium solani* and its deleterious effect on the tissues of seed coat (Fig. 11A-H), cotyledons (Fig. 12A-F) and embryonal axis (Fig. 13A-H) of categorized seeds. The descriptions for each category are provided separately.

Asymptomatic seeds

Asymptomatic seeds revealed the presence of mycelium in 2 out of 5 seeds of both the samples. Mycelium was mostly observed or confined in the soft tissues

of the seed coat (hourglass and parenchyma layers) and in hilar stellate parenchyma. Both inter and intra cellular mycelium was observed in the palisade and hourglass cells. It rarely occurred in the parenchyma layers of seed coat. The remaining components viz. outer and inner endosperm, cotyledons, hypocotyls shoot root axis and hilar region were free of infection. The cells of cotyledons and hypocotyls shoot root axis were well formed and rich in food contents.

Symptomatic seeds

(A) Weakly infected seeds

The weakly infected seeds showed the presence of thin hyaline, branched, septate mycelial bits in the seed coat (Fig. 11A). The lumen of palisade and hourglass cells showed shortly septate with beaded hyphae (Fig. 11B). Inter and intracellular mycelium was found in palisade, hourglass and parenchyma layers of seed coat. Hilar tracheids and stellate parenchyma also showed the presence of thin and septate mycelium. The cells of outer thick walled endosperm showed intercellular mycelium while in inner thin walled endosperm, fine network of thin, hyaline hyphae were seen. Chlamydospores were also observed in this layer (1 seed). In 2 seeds, localized infection of fungus was observed in cotyledons. However infection does not reaches up to the inner cotyledonary tissues. Thus the amount of reserve food material remains unaffected. The fungal infection appeared to induce irregular cell division imparting an undulated appearance to the cotyledons. Such cotyledons showed discontinuous but distinct patches of uninfected and infected cells. The cell division in infected region resulted in an increase in the number of cells which were smaller, irregular, vacuolated with very little cell content and weakly stained with cotton blue as compared to the uninfected cells. The cell division mostly occurred in the marginal cells of cotyledons which progressed in deeper tissues. The cells of embryonal axis also showed cell division as a result of fungal invasion. Here also the cell division was irregular as in cotyledons, the infected cell were small, vacuolated and weakly stained.

(B) Moderately infected seeds

The mycelium colonized all the parts namely seed coat, cotyledon, embryonal axis, hilar region and the space between the coatyledons and hypocotyl shoot root axis. In seed coat mycelial bits were observed in palisade layer. Dense growth and mycelial bits were observed in hourglass layer and parenchyma (Fig. 11D, E & F), with the result that the former was rendered unrecognizable and the latter got compressed. Fungus also invaded the hilar region including the tracheidal bar. In some seeds localized infection was observed in cotyledons and the outer surface of cotyledons revealed both inter as well as intracellular mycelium (Fig. 12A). The cell walls of the cotyledons were either intact or bounded by hyphae and the cell contents were depleted and develop necrotic areas. In one seed, only one of the cotyledons received the pathogen while the other remained free of infection. The uninfected parts of the cotyledon comprised normal cells with starch grains. Radicle and plumule were also infected. Plumule was deformed and showed the presence of mycelium (Fig. 13E).

(C) Heavily infected seeds

Dense inter and intracellular mycelium was observed in all the seed components (5 seeds) viz. seed coat (Fig. 11G & H), cotyledons (Fig. 12B), endosperm, hilar region and embryonal axis (Fig. 13A & B). Aggregation of mycelium and chlamydospores was found all around the seed surface. Mycelium aggregates and penetrates the epidermal cells and the conidiophores produce conidia which accumulate in a slimy white mass. Mycelium also fills in the space between two cotyledons which leads to disintegration and depletion of cell contents. The palisade and hourglass cells were highly deformed and disintegrated (Fig. 11C). At some places palisade cells became loose and hyphae could be easily observed. A thick mycelial mat was formed in the parenchyma layer of seed coat. The cells were greatly compressed and indistinguishable. The hilar region showed profuse mycelium in counter pallisade, tracheidal bar and in stellate parenchyma (Fig. 12D). Endosperm layers carried abundant mycelium and appeared almost disintegrated. The infection was deep seated in the cells of cotyledons; its cells appeared under stress, vacuolated with poor cell contents (Fig. 12F). The epidermis of cotyledon carried intracellular mycelium and at

places showed signs of disintegration (Fig. 12C). Although infection was present throughout the cotyledon, but maximum infection was localized towards proximal and distal ends (Fig. 12E). Mycelium was also observed in the space between two cotyledons and hypocotyls shoot root axis. The vascular region of embryonal axis carried inter as well as intracellular mycelium (Fig. 13C). The epidermis and peripheral layers of hypocotyls shoot root axis carried intracellular mycelium which resulted in the breakdown of epidermal cells. Remaining cells of embryonal axis were loose, vacuolated and poor in cell contents. Heavy aggregation of fungal mycelium were also observed in plumule and radicle region (Fig. 13F & G) of embryonal axis and caused lysis and disintegration of cells (Fig. 13D & H)

TABLE -9 : NUMBER OF INFECTED SEED SAMPLES AND PERCENT RANGE OF *Fusarium solani* IN DRY SEED EXAMINATION, SBM AND PDA TESTS

Districts	No. Of Seed Samples	Dry Seed Examination	SBM		PDA
			Untreated	Pretreated	
ALWAR	7	2 (0.25-3)	2(0.5-12.5)	2(1-11.65)	1(1-2.5)
BIKANER	6	3 (0.5-10.5)	4(1-19.75)	3(0.5-17.75)	1(0.25-11.25)
CHURU	10	2(0.75-12.5)	2 (0.5-13.75)	1(0.25-5.8)	2(0.5-3.75)
JAIPUR	20	10(0.5-35.5)	13 (0.5-58)	12(0.5-34)	3(0.5-29)
JALORE	5	2 (0.25-7.75)	3(0.5-15.6)	4(1-9.5)	-
JHUNJHUNU	14	2 (3-25.25)	4(1-24.75)	3(1.5-18.75)	1(1-2.5)
JODHPUR	9	1 (0.5-2.5)	1(0.25-5.8)	2(1-7.25)	-
KOTA	5	2 (1-7.75)	2(0.5-13.75)	3(0.5-10.25)	1(1-8.5)
NAGAUR	10	3 (0.25-8.5)	3(0.5-15.6)	2(0.25-13.75)	-
SHRI GANGANAGAR	8	4(0.5-8.75)	5(1.5-20.50)	6(1-21.75)	1(1.5-18.75)
SIKAR	26	12 (0.25-40.50)	18(0.25-62)	15(0.5-35)	5(0.25-30)
TOTAL	120	43 (0.25-40.5)	57(0.25-62)	53(0.25-35)	15(0.25-30)

TABLE -10 : **PERCENT INFECTION OF *Fusarium solani* IN DIFFERENT PARTS OF ASYMPTOMATIC AND SYMPTOMATIC (WEAKLY, MODERATELY AND HEAVILY INFECTED) SEEDS OF CLUSTER BEAN IN COMPONENT PLATINGAND CLEARED WHOLE MOUNT PREPARATION**

A. Component Plating

AC. No.	Asymptomatic seeds					Symptomatic Seeds														
						Weakly					Moderately					Heavily				
	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR
CB-29 JAIPUR	5	0	0	0	0	57	26	15	14	12	84	77	72	67	60	99	99	98	98	95
CB-70 SIKAR	7	0	0	0	0	50	20	35	24	17	72	62	64	60	58	97	97	95	94	94

B. Cleared Wholemount Preparation

AC. No.	Asymptomatic seeds					Symptomatic Seeds														
						Weakly					Moderately					Heavily				
	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR
CB-29 JAIPUR	10	0	0	0	2	59	57	13	12	16	75	73	61	57	59	99	99	98	98	95
CB-70 SIKAR	8	0	0	0	2	46	46	34	30	14	71	71	67	63	60	97	97	97	96	92

SC = Seed Coat **ENDO** = Endosperm **COT** = Cotyledon
HSRA = Hypocotyl-shoot-root axis **HR** = Hilar region

Fig. 9 (A-D) : Component plating of cluster bean seeds infected with *Fusarium solani*

A. Incubated seed covered with white fungal mycelium of *F. solani* 12X

B. Seed coat covered with white mycelium of *Fusarium solani* 12X

C. Disrupted cotyledons with white fungal mycelium 22X

D. Embryonal axis infected with white mycelial growth of *Fusarium solani* 22X

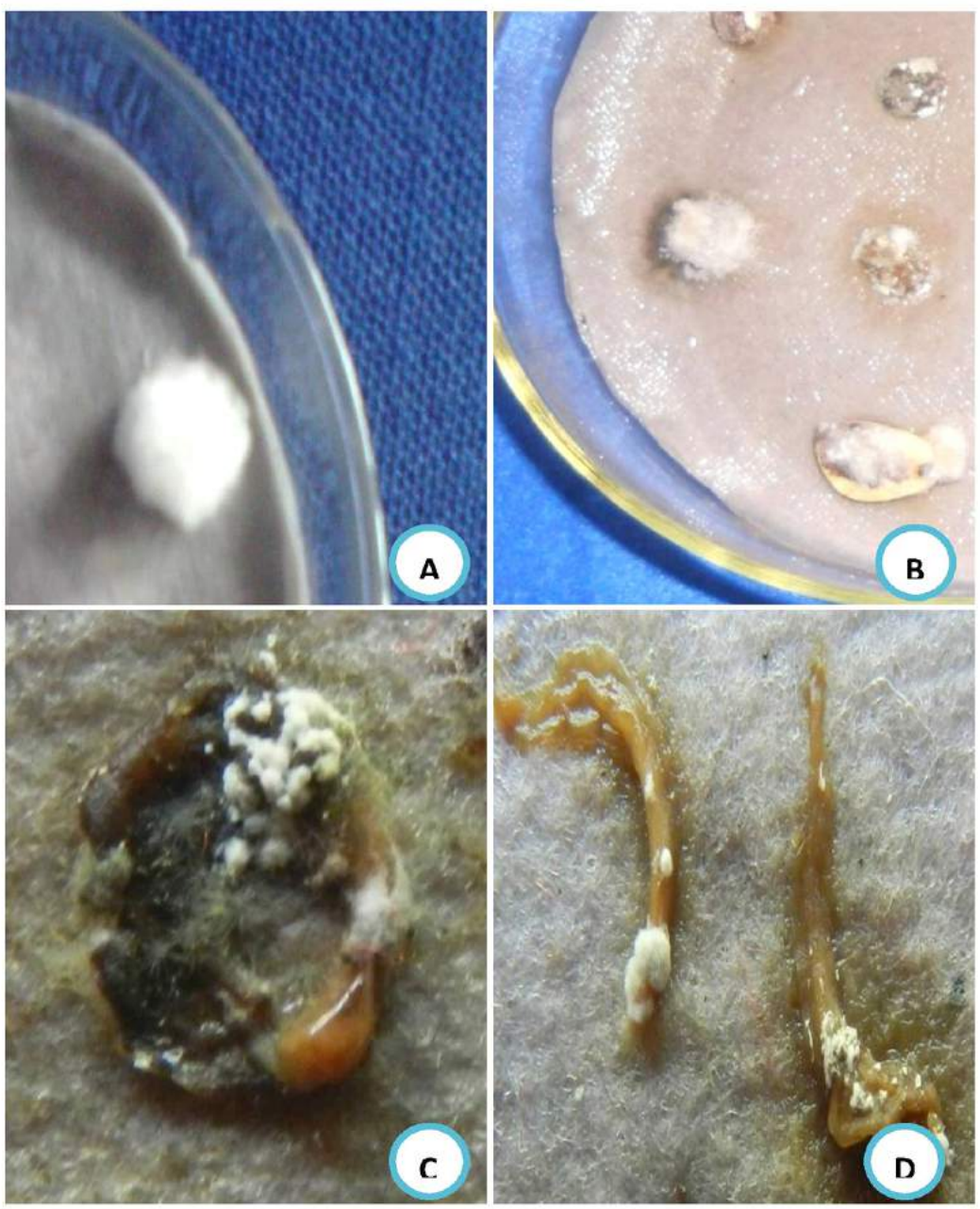


Fig. 9

Fig. 10 (A-F): Cleared wholemount preparation of seeds infected with *Fusarium solani*

A. Dense inter and intracellular mycelium in hourglass layer of seed coat X 150

B. Characteristic mycelium in outer region of cotyledon and aleurone layer X 250

C. Space between two cotyledons showing fungal mycelium X 250

D. Inter and intracellular mycelium in storage region of cotyledon X 500

E. Embryonal axis showing fungal mycelium in plumule region X 250

F. Embryonal axis showing fungal mycelium in radicle region X 250

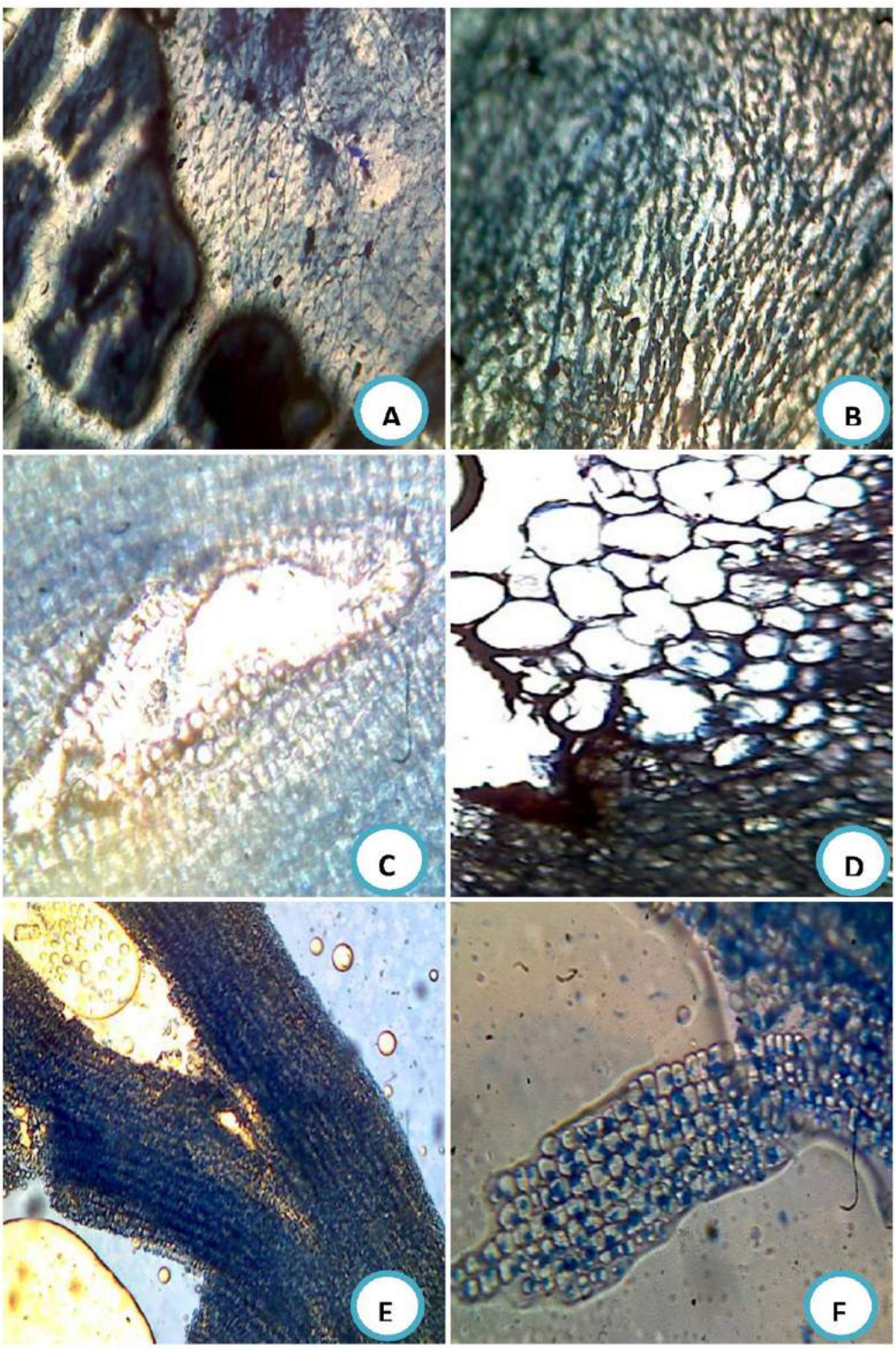


Fig. 10

Fig. 11 (A-H): Microtome sectioning of seed coat infected with *Fusarium solani*

A. T.S. seed showing fungal mycelium in seed coat region X 150

B. T.S. showing withered seed coat layers X 250

C. Disintegration and lysis of hourglass cells of seed coat due to heavy fungal infecetion X 150

D. Mycelial bits in hourglass cells of seed coat X 250

E. Enlarged view of a portion of seed coat showing hyphae of fungal mycelium X 500

F. Hourglass cells of seed coat with mycelial bits X 500

G-H. Dense inter and intracellular mycelium in seed coat layers X 500

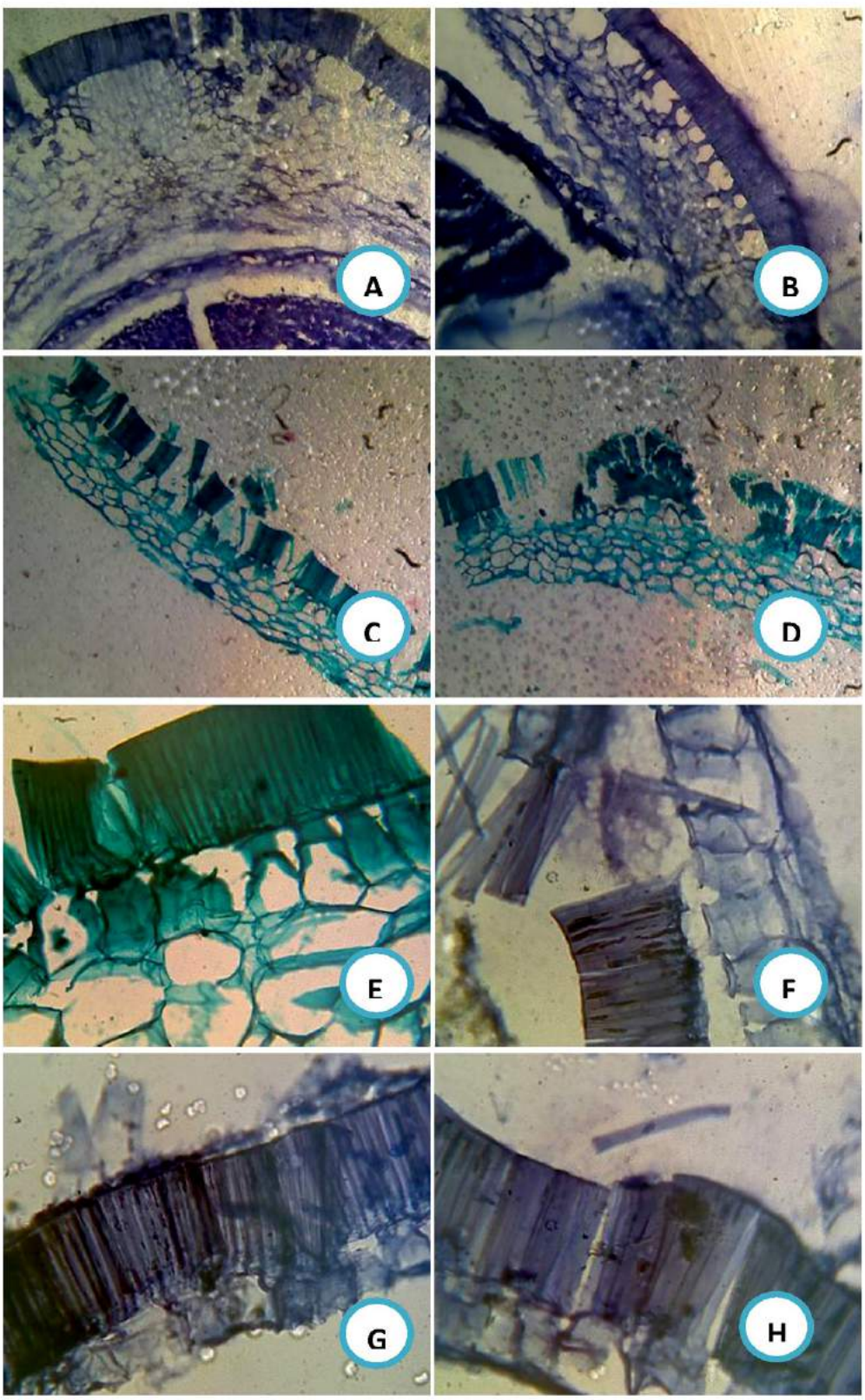


Fig. 11

Fig. 12 (A-F): Microtome sectioning of infected cotyledons with *Fusarium solani*

A. Fungal mycelium in outer surface of cotyledon X 250

B. Inter and intracellular mycelium in inner surface of cotyledon X 500

C. Disintegrated cells of storage region X 700

D. Profuse growth of fungal mycelium X 250

E. Proximal end of cotyledons showing infection X 250

F. Lysis of cells of storage region due to heavy aggregation of fungal infection X 500

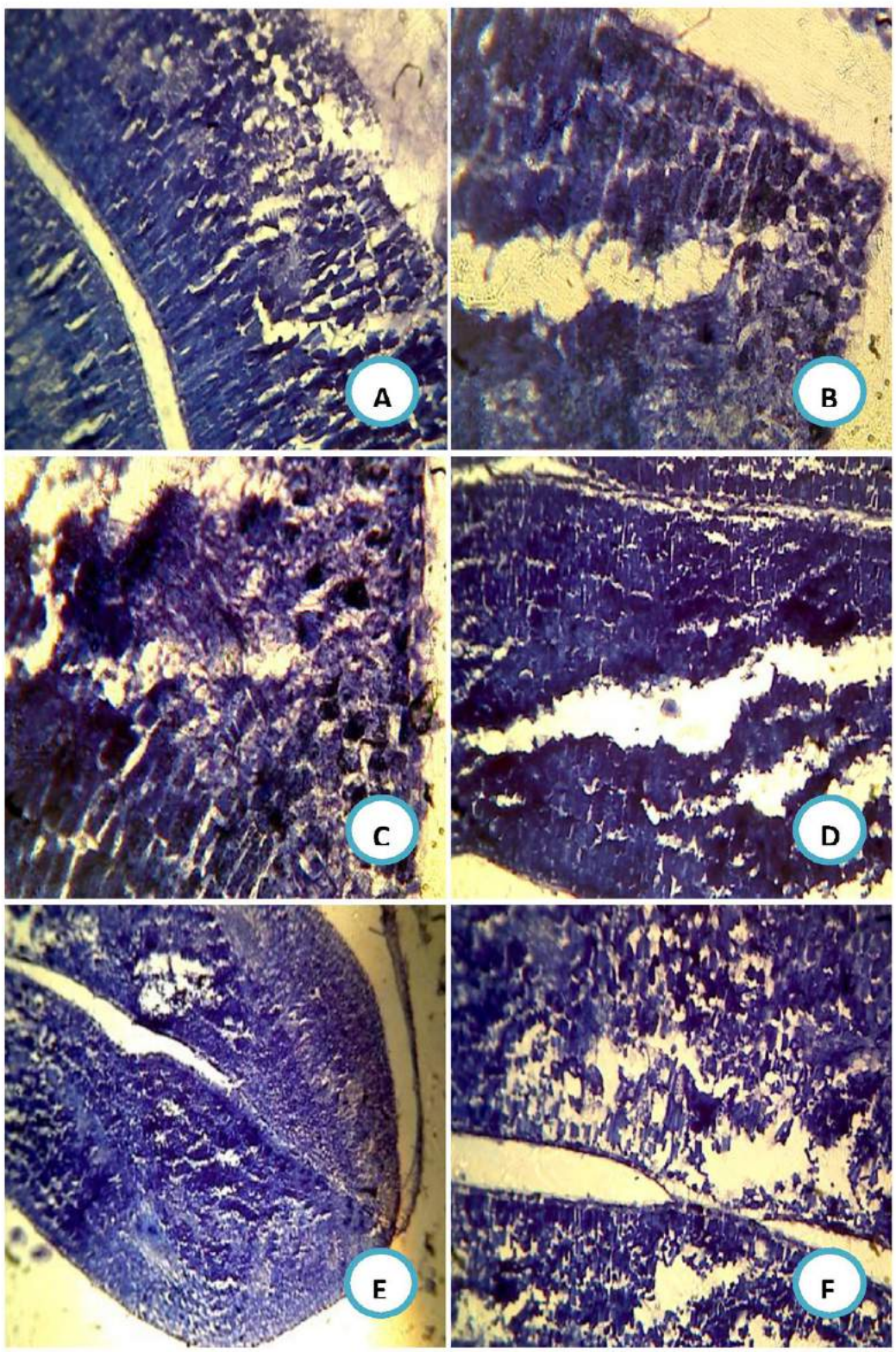


Fig. 12

Fig. 13 (A-H): Microtome sectioning of infected embryonal axis with *Fusarium solani*

A-B. Inter and intracellular mycelium X 500

C. Plumule region of embryonal axis showing infection in vascular region X 250

D. Lysis of cells in plumule region X 700

E. Plumule region showing fungal mycelium X 250

F-G. Radicular region showing heavy aggregation of fungal mycelium X 250

H. Lysis of cells in radicular region X 500

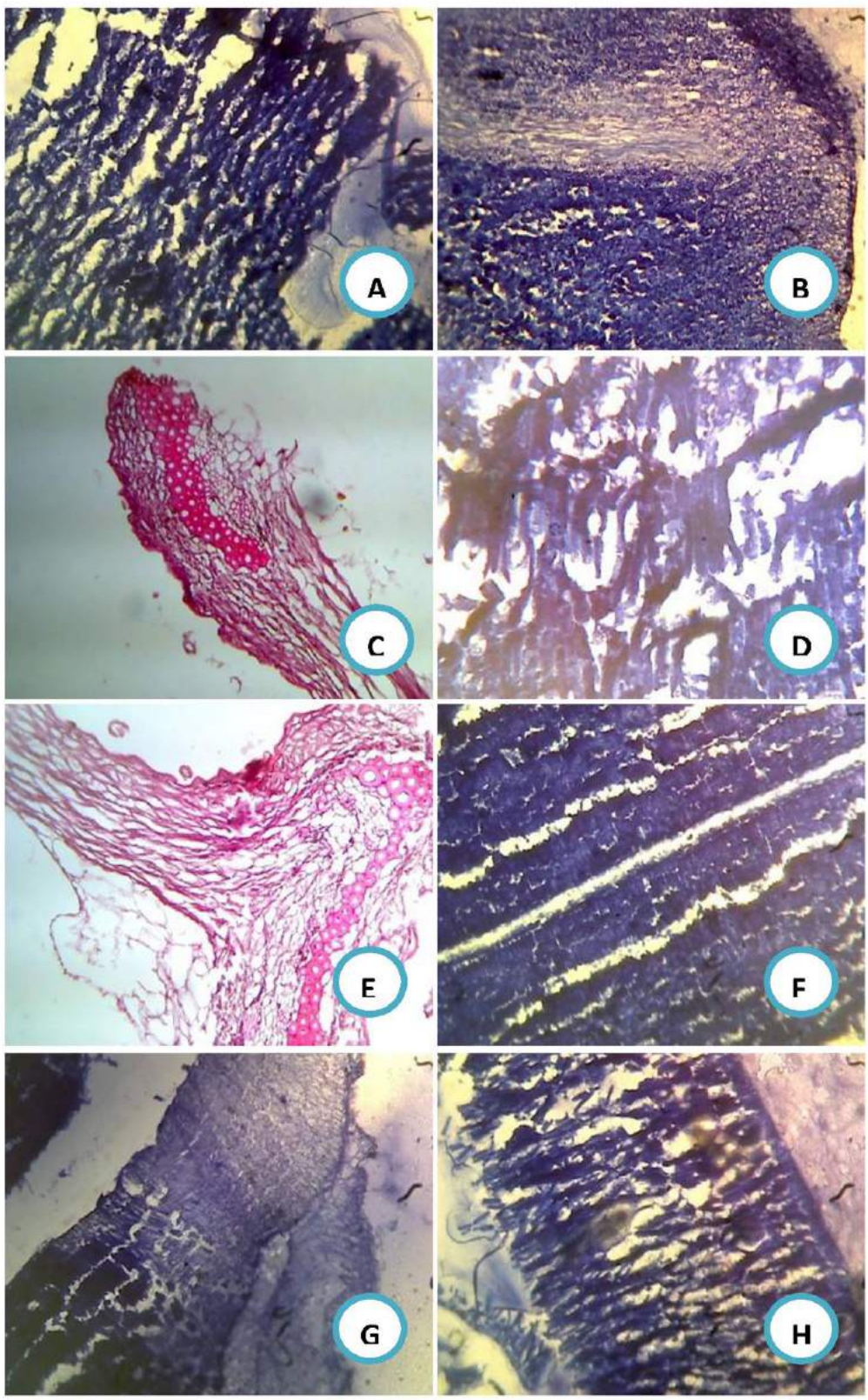


Fig. 13

PHYTOPATHOLOGICAL EFFECTS AND DISEASE TRANSMISSION

The performance of naturally infected seed samples with *Fusarium solani* and the transmission of disease from seed to seedling were studied using Petriplate seedling method or SBM, Test tube seedling Symptom test or Water agar seedling test, Pot and Field Experiments. Regarding the performance of seed samples, effect of the fungus on germination and seedling survival were determined. Symptoms on seedling were observed in all experiments and isolation of pathogen was made from infected as well as healthy looking seedlings. Sequential observations were made on the percentage germination, seedling infection, seedling survival and incidence of the pathogen. Seeds were categorized as asymptomatic and symptomatic. The symptomatic seed samples further categorized into weakly, moderately and heavily on the basis of severity of seed infection of pathogen and intensity of symptoms on seed coat of cluster bean seeds.

Asymptomatic and symptomatic seed samples of ac. nos. CB29 (Jaipur) and CB70 (Sikar) were used for all the phytopathological and disease transmission experiments.

SEEDS CARRYING NATURAL INFECTION

Standered Blotter Method

Germination starts after 24h of sowing and maximum germination was 94 and 97% on 8^{th} day in asymptomatic seeds, whereas in symptomatic seeds (weakly, moderately and heavily infected), it was 27,18,7% and 35, 30, 22% in ac no. CB29 (Jaipur) and CB70 (Sikar) respectively.

The ungerminated seeds in both the samples were covered with peach-white coloured mycelium of *Fusarium solani* and showed seed rot due to dense growth of fungus on seed surface (Fig. 14A & B and Fig. 16A & B; Table 11).

The initial disease symptoms appeared as pale to brown on radicle region on 3rd to 5th day of sowing. Dark brown patches on root shoot transition zone which progressed up towards to hypocotyl and downwards to radicle region. The appearance of brown radicle was soon followed by small, discrete, irregular black streaks on hypocotyl which became more prominent after 72 hours. Later the symptoms increased rapidly and spread to the cotyledons in the form of irregular necrotic brown colored spots. Severity of these symptoms caused mortality of seedlings (Fig. 16C). Microconidia formation was also observed on the hypocotyls and cotyledonary leaves. The various degree of infection also observed in both asymptomatic and symptomatic (weakly, moderately and heavily) seeds (Fig. 16D).

Asymptomatic ungerminated seeds with pathogen were 4, 2% in CB29 and CB70 respectively. It was 62, 65% in weakly infected; 75, 65% in moderately and 78, 61% in heavily infected seeds.

Ungerminated seeds without pathogen were 2, 0% in asymptomatic seeds whereas in symptomatic seeds it was 11, 12% in weakly; 7, 22% in moderately and 15, 22% in heavily infected seeds.

Percent seedling survived with symptoms, seedling mortality, incidence of pathogen and percent normal seedling were 3, 8%; 11, 11%; 18, 14% and 80, 85% in asymptomatic seeds of both the samples respectively whereas in symptomatic seeds, it were 3, 2%; 7, 8%; 72, 69%; 23, 23% in weakly; 3, 11%; 7, 13%; 85, 79%; 8, 19% in moderately and 0, 7% ; 7, 21%; 85, 79%; 0, 4% in heavily infected seeds of both the samples respectively.

The intensity of symptoms and number of infected seedlings increased gradually. Total loss of yield were 6, 3% in asymptomatic seeds whereas it were 73, 65% in weakly infected seeds; 82, 70% in moderately and 93, 78% in heavily infected seeds of symptomatic seeds in both the seed samples respectively.

Water agar seedling symptom test

Seed germination started after 24h of sowing and maximum germination was 89 and 87% on 15th day in asymptomatic seeds; whereas in symptomatic seeds (weakly, moderately and heavily infected), it was 34, 26, 9% and 37, 35, 22% in ac no CB29 and CB70 respectively. The seeds which showed failure of germination were fully covered with fungal growth. The symptoms occurred on 3rd to 5th day and were observed as brown patches on radicle and pale-black discontinuous streaks on hypocotyl region. At a later stage, these streaks spread to cotyledons as necrotic spots. The ungerminated seeds in both the samples were covered with *Fusarium solani* and showed seed rot. The infected seeds showed various degree of infection in both symptomatic and asymptomatic seed samples (Fig. 14C & D and Fig. 17A, B & C; Table 12).

Percent ungerminated seeds with and without pathogen were 9, 9%; 14, 8% in asymptomatic seeds of both the samples respectively whereas it was 57, 43%; 13, 21% in weakly; 65, 43%; 24, 26% in moderately and 75, 63%; 13, 23% in heavily infected seeds of both the samples respectively.

Percent seedling survived with symptoms and percent seedling mortality were 6, 2%; 9, 4% in asymptomatic seeds of both the samples whereas it were 11, 8%; 13, 11% in weakly; 6, 6%; 11, 11% in moderately and 1, 5 %; 11, 09% in heavily infected seeds of both the samples respectively.

Percent incidence of pathogen and normal seedlings in asymptomatic seeds were 15, 14%; 80, 83% and in symptomatic seeds 72, 61%; 17, 20% in weakly; 71, 61%; 8, 19% in moderately and 83, 73%; 0, 7% in heavily infected seeds of both the samples respectively.

Total loss were recorded 11, 13% in asymptomatic seeds; 66, 63% in symptomatic weakly infected; 74, 65% in symptomatic moderately and 91, 78% in heavily infected seeds of both seed samples respectively.

Pot experiment

For growing on test the seeds of two samples (ac.nos.CB29, CB70) were categorized as asymptomatic and symptomatic (weakly, moderately and heavily) were used in pot experiments (Fig. 15A & B and Fig. 18A-H).

Seed germination started after 72 hrs of sown seeds in pot, on 8th day it were observed maximum. Observations were taken at regular interval and data were recorded after 30-40 days of sowing seeds. The seed germination of cluster bean was recorded 96, 93% in asymptomatic seeds samples and 73, 69% in weakly; 57, 46% in moderately and 25, 18% in heavily symptomatic seeds samples of both the seed samples respectively. Fungal infection affects the seedling emergence and growth of cluster bean seeds. It was 89, 91% in asymptomatic and 65, 68% (weakly); 46, 50% (moderately) and 37, 40% (heavily) infected seeds in symptomatic seeds.

The percentage seedling mortality were 5, 7% in asymptomatic seeds; 27, 35% in symptomatic weakly; 41, 46% moderately and 77, 83% in heavily infected seeds respectively.

The observations were recorded after 30-40 days of sowing in pot experiment. Total losses were recorded 4, 7% in asymptomatic seeds; 27, 31% in weakly; 43, 54% in moderately and 75, 82% in heavily infected seeds of symptomatic seeds. The heavily infected seeds remain small, shrivelled and not able to grow normally than the other seeds.

Disease symptoms and their effect on host

In 30-40 days old seedlings the margin of cotyledonary leaves rolled inwards and fall off, entire seedling appeared dried and collapsed. This was observed as initial stage of wilting (Fig. 18B). These symptoms caused mortality of seedlings within one month. The symptomatic seedling developed

characteristic symptoms and fungal growth on plumule and radicle region. Four week old plant showed ill- defined, yellow- brownish patches on leaves followed by their shriveling, dryness and drooping ultimately resulting in the death of such plants (Fig. 18C). The infected root showed brown or black discolouration. The surviving plants which grew to their full height showed black spots/patches on basal part of stem near the collar region (Fig. 18D). Some patches showed partial wilting. White mycelial growth of fungus was also observed on *F. solani* infected stem (Fig. 18E). Pods and stem of infected plants also showed fungal growth and dryness (Fig. 18F, G & H). Seeds from these pods are abortive and shrivelled. Seed setting is very less in the infected pods. The harvested seeds were covered with white mycelial growth. Split half of the stem and root showed the presence of fungal hyphae. The unaffected branches produced symptomless pods. The seeds obtained from these pods did not show mycelial growth. Entire plant seedling produced by symptomatic seeds appeared dried and collapsed after a short period of emergence.

Isolation and Establishment of pathogen in host

30-40 days old seedlings showed symptoms on all parts including radicle (47, 85%), hypocotyls (61, 93%) and cotyledons (5, 18%) of asymptomatic and weakly symptomatic seeds.

Field experiments (Fig. 15C & D; Fig. 19A-H and Fig. 20A-H)

In field experiment seed germination was observed maximum in asymptomatic seeds of both seed samples were 94, 91%, while it was 45, 62% in case of infected seeds or symptomatic seeds after 8^{th} day of sowing. Seedling survival with pathogen was observed 5, 3% in asymptomatic and 9, 6% in symptomatic seeds. Mortality of seedling was observed minimum in asymptomatic seeds (3, 0%) and maximum in symptomatic seeds (22, 47%). Total losses were 6, 9% and 55, 38% in asymptomatic and symptomatic seeds respectively (Fig. 15C & D).

The symptoms were observed similar to pot experiments. The infected and uninfected plant samples were collected directly from experimental field. Pale brown patches were observed in split half of the infected stem. Mycelial bits were also observed in split half of infected stem. Infected root showed black or brown discoloration (Fig. 19D) and brown blackened spots/patches appear on basal part of infected stem near the collar region (Fig. 19B). Yellow brownish necrotic patches were observed on the infected leaves (Fig. 19C). The heavy fungal infection also causes the necrosis on infected leaves. The pod harvested from the infected plants had black spots/patches (Fig. 19E & F and Fig. 20A-D) and produced seeds covered with fungal mycelium and some with white cottony growth (Fig. 19H and Fig. 20E-H). Normal pods and seeds were produced by asymptomatic plants (Fig. 19G). The degree of infections was also seen in plants samples collected from experimental field (Fig. 19A).

Clearing and Hand cut Sections **(Fig. 21A-H)**

Such preparations revealed thin, hyaline mycelium traversing the vascular region of radicle, hypocotyl and cotyledons. The mycelium was mostly intercellular in the cortical cells of radicle and hypocotyl. The presence of mycelium was sparse in cotyledons but dark, necrotic cells with depleted cell contents were common.

The cleared wholemount preparation of plant parts viz. root, stem, leaves and pods also showed thin, hyaline, branched, septate fungal mycelium. Transverse hand cut sections of root (Fig. 21E & F), stem (Fig. 21C & D), leaves (Fig. 21A & B) and pod (Fig. 21G & H) revealed inter as well as intracellular mycelium in epidermis, cortex, xylem vessels and in pith tissues. In leaf the hyphae was mostly localized in mesophyll cells. The fungus was also detected from cortical region of the stem and root.

TABLE -11 : PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* DURING GERMINATION DETERMINED IN SBM (100 SEEDS/CATEGORY/SAMPLE)

AC. NO. CB-29

Categories	Asymptomatic				Symptomatic											
					Weakly				Moderately				Heavily			
Days	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**
Germination	77	94	94	94	25	27	27	27	18	18	18	18	7	7	7	7
Ungerminated seeds without pathogen	23	2	2	2	75	12	11	11	82	7	7	7	15	15	15	15
Ungerminated seeds with pathogen	0	4	4	4	0	61	62	62	0	75	75	75	78	78	78	78
Surviving seedling with symptom	0	4	7	3	0	1	3	3	0	3	5	3	0	3	3	0
Seedling mortality	0	3	5	11	0	0	1	7	0	3	3	7	0	2	2	7
Incidence of pathogen	0	11	16	18	0	62	66	72	0	81	83	85	0	81	82	85
Normal seedling	77	87	82	80	25	26	23	23	18	12	10	8	7	4	2	0

AC. NO. CB-70

Categories	Asymptomatic				Symptomatic											
					Weakly				Moderately				Heavily			
Days	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**
Germination	68	97	97	97	32	35	35	35	27	30	30	30	19	21	21	22
Ungerminated seeds without pathogen	36	9	0	0	75	12	12	12	70	22	22	22	89	35	23	22
Ungerminated seeds with pathogen	0	2	2	2	0	65	65	65	0	69	65	65	0	69	65	61
Surviving seedling with symptoms	0	5	9	8	0	8	7	2	0	15	13	11	0	10	7	7
Seedling mortality	0	0	3	11	0	2	3	8	0	0	10	13	0	13	17	21
Incidence of pathogen	0	2	7	14	0	70	68	69	0	73	77	79	88	77	71	79
Normal seedling	68	97	86	85	32	27	25	23	27	21	19	19	19	13	7	4

TABLE -12 : PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* DURING GERMINATION DETERMINED IN WATER AGAR SEEDLING TEST (50 SEEDS/CATEGORY/SAMPLE)

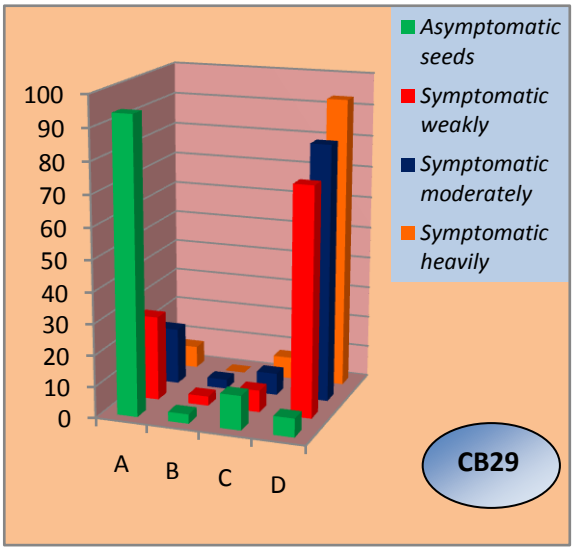
AC. NO. CB-29

Categories	Asymptomatic					Symptomatic														
						Weakly					Moderately					Heavily				
Days	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15
Germination	57	89	89	89	89	31	34	34	34	34	23	26	26	26	26	-	5	9	9	9
Ungerminated seeds without pathogen	45	17	14	14	14	74	17	13	13	13	29	27	24	24	24	18	14	13	13	13
Ungerminated seeds with pathogen	3	7	9	9	9	2	57	57	57	57	51	60	65	65	65	73	75	75	75	75
Surviving seedling with symptom	0	5	5	5	6	0	5	7	9	11	3	5	6	6	6	5	7	7	5	1
Seedling mortality	0	4	5	7	9	0	3	9	10	13	2	5	5	7	11	2	5	5	7	11
Incidence of pathogen	0	3	11	15	15	0	63	69	72	72	51	66	68	68	71	74	81	83	83	83
Normal seedling	57	89	83	80	80	31	27	25	17	17	23	18	12	8	8	7	5	3	3	0

AC. NO. CB-70

Categories	Asymptomatic					Symptomatic														
						Weakly					Moderately					Heavily				
Days	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15	2	4	6	8	15
Germination	62	75	87	87	87	35	37	37	37	37	30	34	35	35	35	18	22	22	22	22
Ungerminated seeds without pathogen	36	19	8	8	8	61	23	21	21	21	71	26	26	26	26	80	23	23	23	23
Ungerminated seeds with pathogen	0	7	9	9	9	47	43	43	43	43	0	41	43	43	43	61	63	63	63	63
Surviving seedling with symptom	0	0	4	4	2	0	4	2	8	8	0	4	4	4	6	2	3	5	5	5
Seedling mortality	0	0	2	4	4	0	2	5	7	11	0	2	5	7	11	0	3	5	9	9
Incidence of pathogen	8	12	12	14	14	45	49	53	56	61	49	1	55	55	61	63	67	71	73	73
Normal seedling	63	73	85	85	83	37	33	29	20	20	30	28	24	19	19	20	13	10	10	7

A-B : PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* ON 8th DAY IN SBM (100 SEEDS/CATEGORY/SAMPLES)



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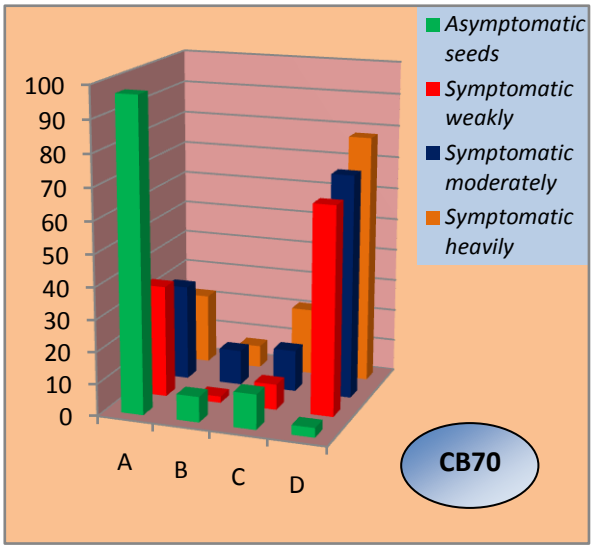
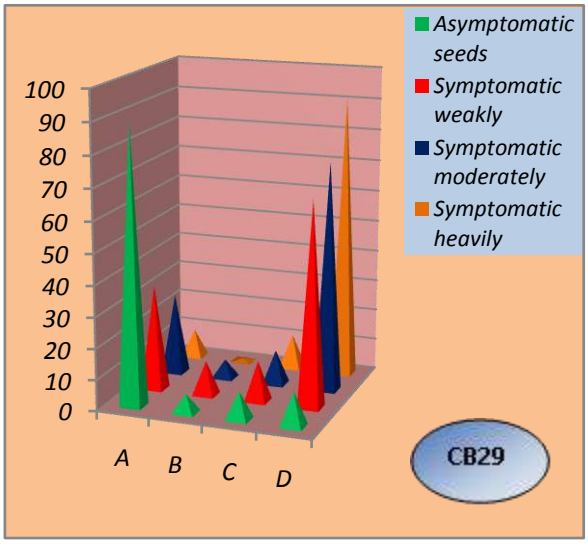


B

C-D : PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* ON 15th DAY IN WATER AGAR SEEDLING SYMPTOM TEST (50 SEEDS/CATEGORY/ SAMPLES)



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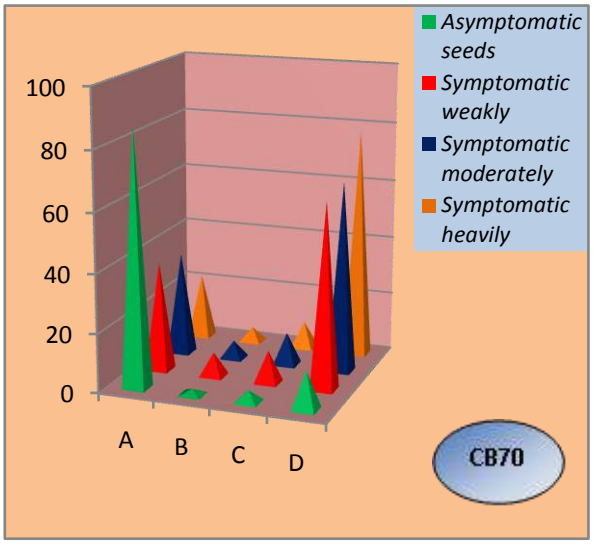


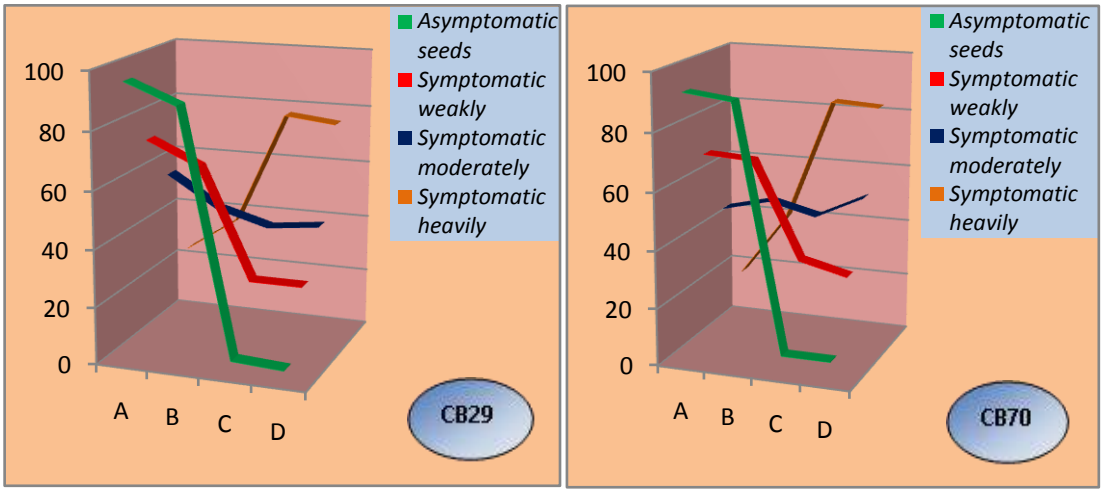
D

A=Germination **B=Surviving seedling with Symptoms**

C= Seedling Mortality **D=Total loss**

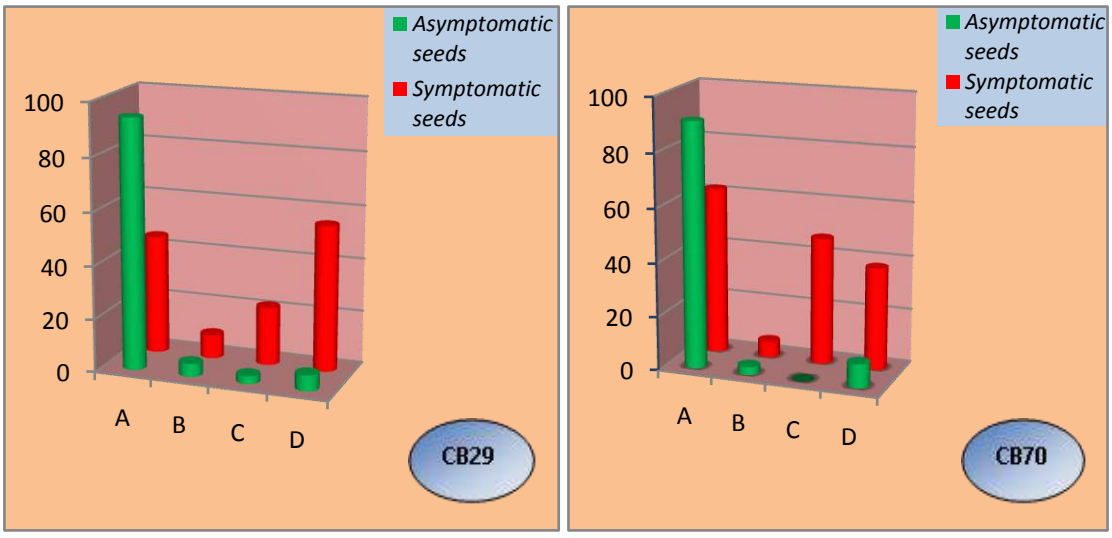
Fig. 14

A-B: PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* AFTER 30 DAY OF SOWING IN POT EXPERIMENT (5 SEEDS/CATGORY/POT)



A B

C-D: PHYTOPATHOLOGICAL EFFECTS OF NATURAL INFECTION OF *Fusarium solani* IN FIELD EXPERIMENT



C D

A=Germination **B=Surviving seedling with Symptoms**

C= Seedling Mortality **D=Total loss**

Fig. 15

Fig. 16 (A-D): Phytopathological effects of *Fusarium solani* during transmission studies in Standard blotter method

A. Petriplate showing normal seedling (left) and ungerminated seeds covered with fungal mycelium (right)

B. Poor germination without fungal growth

C. Petriplate showing failure of germination with presence of white fungal growth

D. Asymptomatic and Symptomatic seedlings showing various degrees of infection

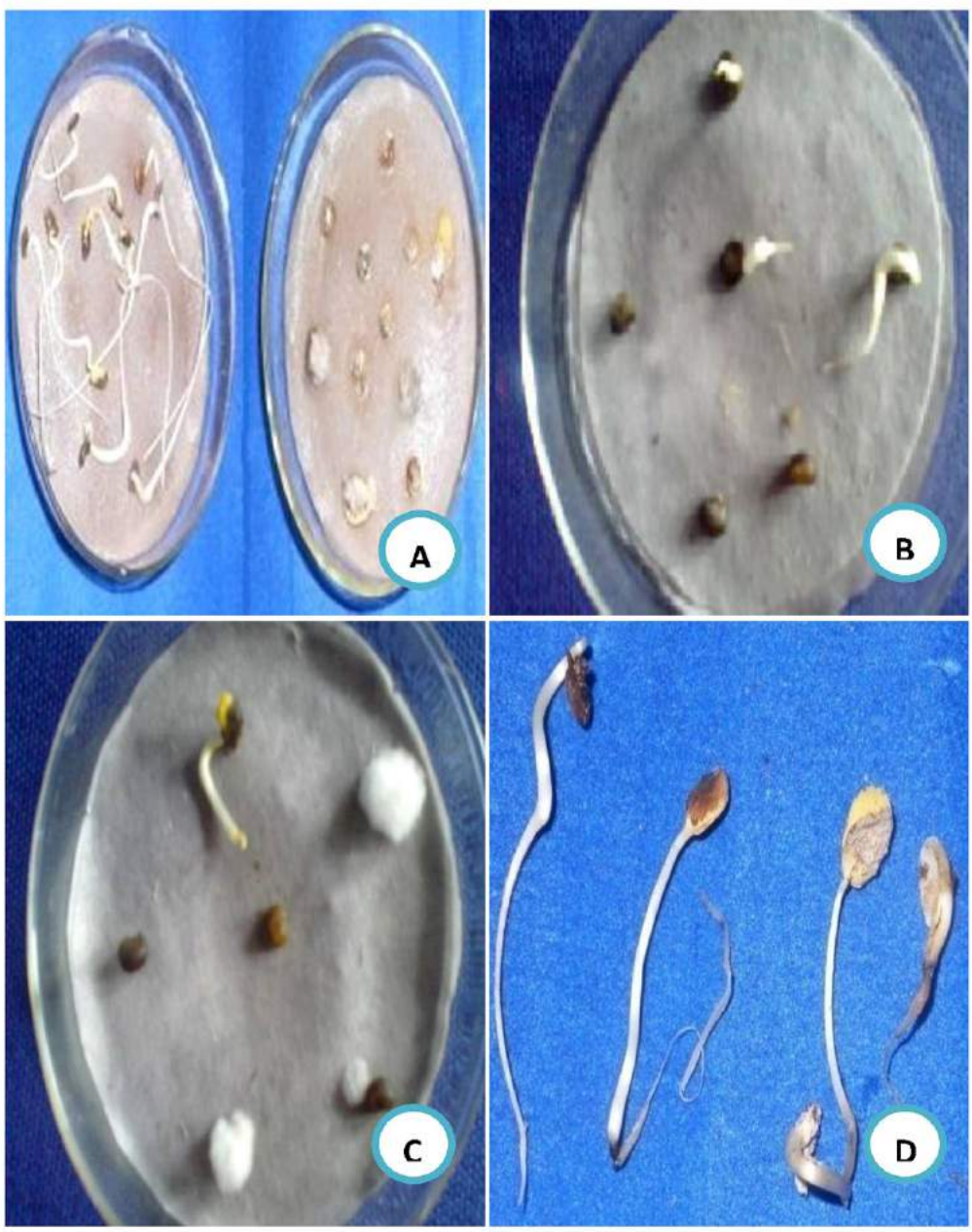


Fig. 16

Fig. 17 (A-C): Phytopathological effects of *Fusarium solani* during transmission studies of seeds in water agar seedling symptom test

A. Healthy seedling and heavily infected ungerminated seed

B. Test tube showing healthy, weakly, moderately and heavily infected seedlings (right) on 15^{th} day of sowing

C. Asymptomatic and infected symptomatic seedlings harvested from water agar method showing various degrees of infection (control, weakly, moderately and heavily)

Fig. 17

Fig. 18 (A-H): Phytopathological effects of *Fusarium solani* during transmission studies in pot experiment

A. Pot showing healthy seedling

B. Infected seedling

C. Yellow brown patches on leaves

D. Collar region of the stem showing black spots or streaks

E. Nodal region of the stem showing white mycelial patches

F. Infected pods on the plants

G. Cortical region of the split half of the stem showing fungal infection

H. White fungal growth on seeds in split half of the pods

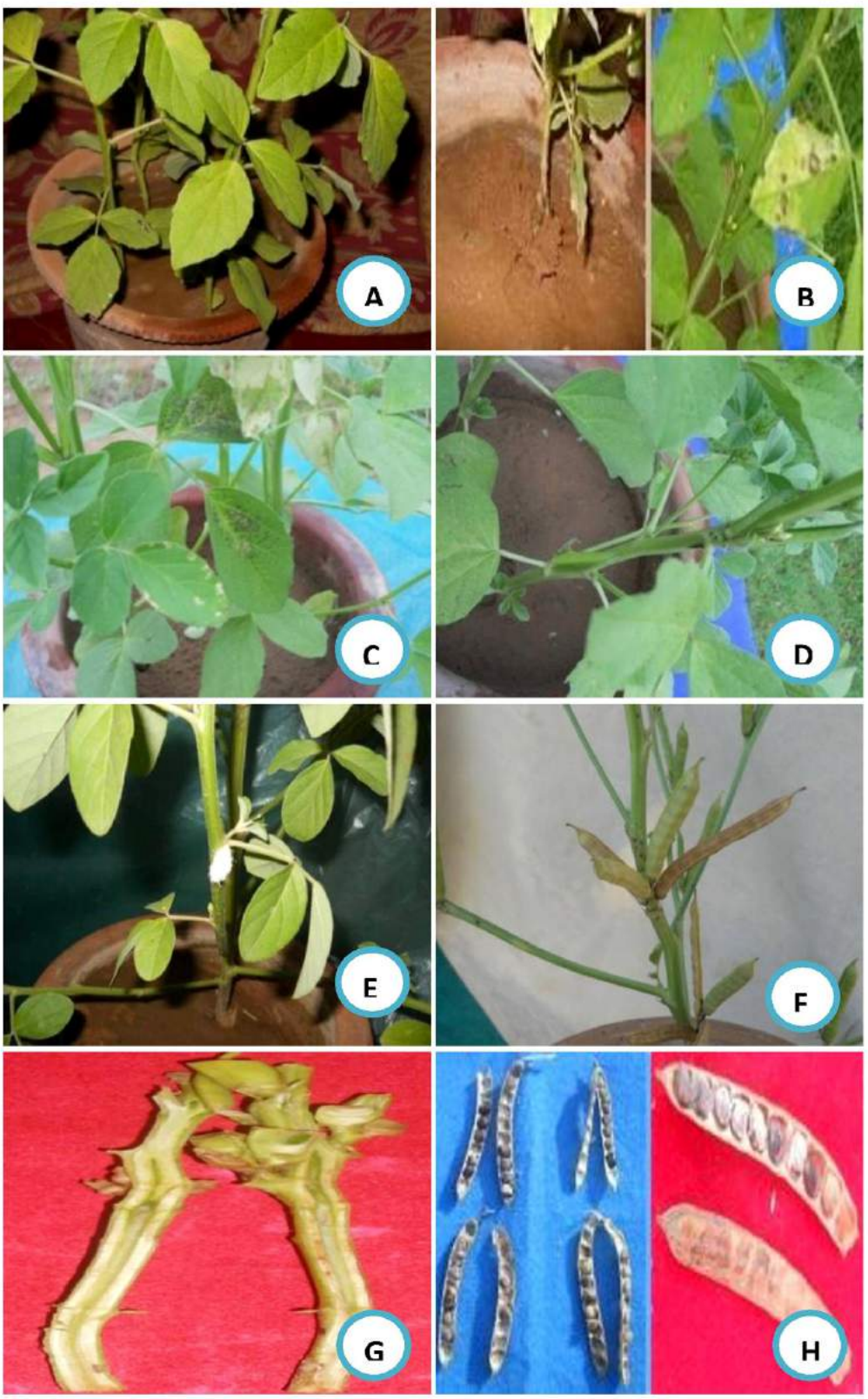


Fig. 18

Fig. 19 (A-H): Plant parts collected from fields after 30 days of sowing

A. Plants showing various degree of infection

B. Healthy to infected stem

C. Various degree of infection in leaves

D. White brown patches on root region

E. Plant showing infected pods

F. Normal and infected pods harvested from fields

G. Healthy looking seeds from harvested pods

H. Infected shrivelled seeds from infected pods

Fig. 19

Fig. 20 (A-H): Harvested pods and seeds from the fields

A. Pods showing fungal infection

B. Enlarged view of infected pods

C. Split half of pods with seeds showing fungal infection

D. White fungal growth of seeds in split half of the pods

E. Harvested seeds showing infection

F-G. Mature seeds showing white fungal growth

H. Harvested seed lot from the experimental fields

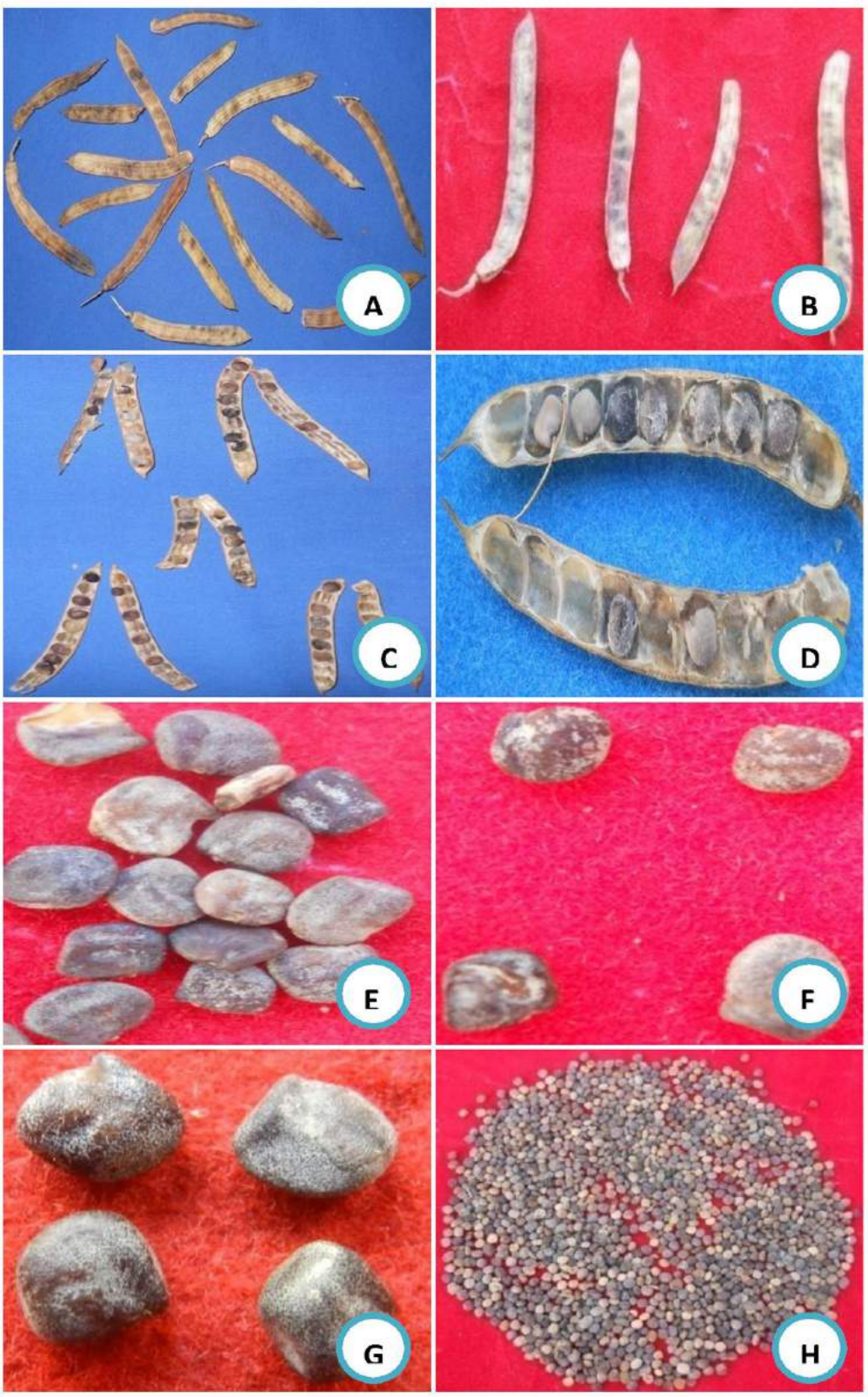


Fig. 20

Fig. 21 (A-H): Hand cut sections of asymptomatic and infected plant parts collected from fields

A. T.S. of normal leaf X 150

B. T.S. of infected leaf X 150

C. T.S. of normal stem X 150

D. T.S. of infected stem X 150

E. T.S. of normal root X 150

F. T.S. of infected root X 150

G. T.S. of normal pod X 150

H. T.S. of infected pod X 150

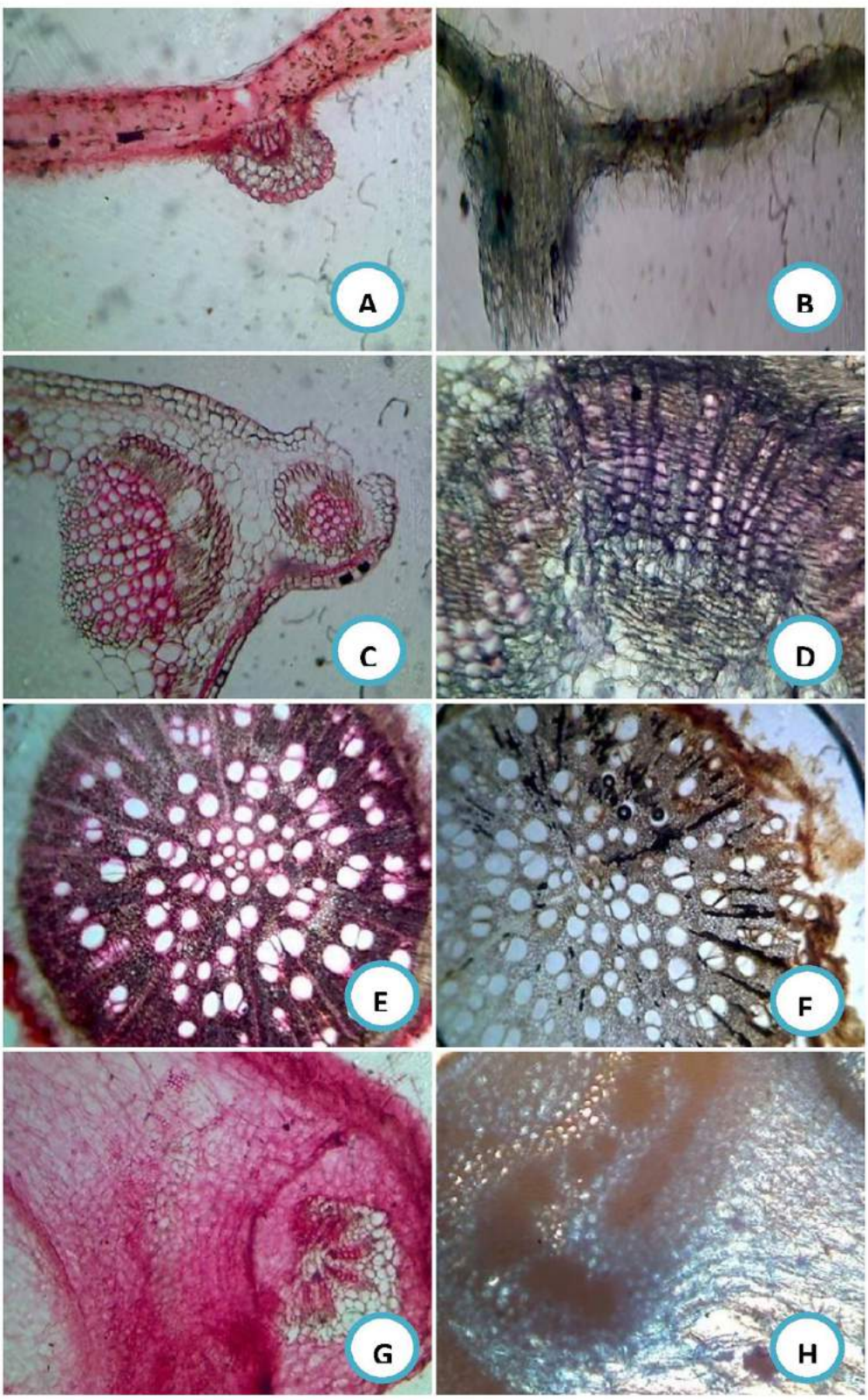


Fig. 21

BIOCHEMICAL ANALYSIS

Cluster bean seed sample (CB-70) infected with *Fusarium solani* were grown under aseptic conditions to estimate changes in important constituents like total protein content (mg/gm) and protease enzyme estimation; total starch content(mg/gm) and α-amylase enzyme estimation and total phenol content and phenol peroxidase enzyme estimation using standard quantitative techniques of biochemical analysis. Changes in different parameters were comparatively analyzed in infected seeds with healthy seeds of cluster bean from 4 hrs of soaking till 8th day of germination. (Fig. 22 and Fig. 23)

Total Protein Contents

Protein in infected leaf was lower than the healthy leaves of cluster bean, in sample used and studied. It was recorded 0.296 mg/gm in healthy leaves and 0.172mg/gm in the infected leaves. It was lower in infected stem than the healthy stem in sample used and studied. It was recorded 0.236 mg/gm in healthy stem and 0.132 mg/gm in the infected stem sample of cluster bean.

Protein in infected seeds was lower than the healthy seeds. It was recorded 0.290 mg/gm in healthy seeds and 0.206 mg/gm in the infected seed sample (Fig. 22A; Fig. 23A and Table 13A).

Protease enzyme

Protease enzyme in infected leaves was higher than the healthy leaves, in the samples used and studied. It was recorded 0.008 units/ sec/mg of fresh weight of tissue in healthy leaves and 0.017 units/ sec/mg of fresh weight of tissue in the infected leaves sample.

Protease enzyme in infected stem was higher than the healthy stem. It was recorded 0.005 units/ sec/mg of fresh weight of tissue in healthy stem and 0.007 units/ sec/mg of fresh weight of tissue in the infected stem sample.

Protease enzyme in infected seeds was higher than the healthy seeds. It was recorded 0.006 units/ sec/mg of fresh weight of tissue in healthy seeds and 0.008 units/ sec/mg of fresh weight of tissue in the infected sample (Fig. 22B; Fig. 23B and Table 13A).

Starch Contents

Starch in infected leaves was lower than the healthy leaves. It was recorded 0.054 mg/gm in healthy leaves and 0.036 mg/gm in the infected leaves sample.

Starch in infected stem was lower than the healthy stem. It was recorded 0.315 mg/gm in healthy stem and 0.108 mg/gm in the infected stem.

Starch in infected seeds was lower than the healthy seeds. It was recorded 0.063 mg/gm in healthy seeds and 0.054 mg/gm in the infected seed sample (Fig. 22C; Fig. 23A and Table 13B).

***α- Amylase*:-**

α- amylase enzyme in infected leaves was higher than the healthy leaves in the samples used and studied. It was recorded 0.008 units/ sec/mg of fresh weight of tissue in healthy leaves and 0.012 units/ sec/mg of fresh weight of tissue in the infected leaves.

α- amylase enzyme in infected stem was higher than the healthy stem. It was recorded 0.003 units/ sec/mg of fresh weight of tissue in healthy stem and 0.009 mg/gm in the infected stem.

α- amylase enzyme in infected seeds was higher than the healthy seeds. It was recorded 0.008 units/ sec/mg of fresh weight of tissue in healthy seeds and 0.016 units/ sec/mg of fresh weight of tissue in the infected seeds (Fig. 22D; Fig. 23B and Table 13B).

Phenolics Contents

Slight increase in total phenols was observed in infected leaf. It was 0.06 mg/gm in healthy leaves whereas 0.15 mg/gm in infected leaves samples.

Very little increase in total phenols was observed in infected stem. It was 0.07 mg/gm in healthy stem whereas 0.16 mg/gm in infected stem samples.

Very little increase in total phenols was observed in infected seeds samples. It was 0.49 mg/gm in healthy seeds whereas 0.53mg/gm in infected seeds samples (Fig. 22E; Fig. 23A and Table 13C).

Poly Phenol Oxidase

Very little increase in Polyphenol oxidase activity was observed in uninfected leaves or healthy samples. It was 0.021 units/ sec/mg of fresh weight of tissue in healthy leaves whereas 0.012 units/ sec/mg of fresh weight of tissue in infected leaves.

Very little increase in Polyphenol oxidase was observed in healthy stem samples. It was 0.03 units/ sec/mg of fresh weight of tissue in healthy stem whereas 0.008 units/ sec/mg of fresh weight of tissue in infected stem samples.

Very little increase in Polyphenol oxidase was observed in healthy seed samples. It was 0.026 units/ sec/mg of fresh weight of tissue in healthy seeds whereas 0.018 units/ sec/mg of fresh weight of tissue in infected seeds (Fig. 22F; Fig. 23B and Table 13C).

TABLE -13 A : QUANTIFICATION OF TOTAL PROTEIN AND PROTEASE ENZYME

Concentration	NORMAL SEEDS	INFECTED SEEDS	NORMAL STEM	INFECTED STEM	NORMAL LEAVES	INFECTED LEAVES
Protein (mg/g)	0.290	0.206	0.236	0.132	0.296	0.172
Protease activity (units/sec/mg. weight of fresh tissue)	0.006	0.008	0.005	0.007	0.008	0.017

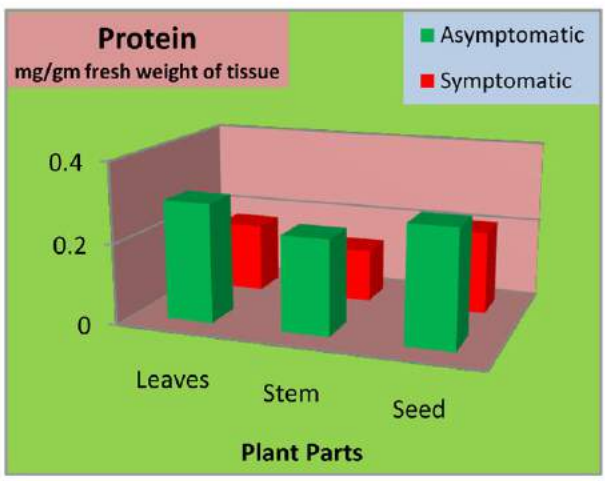
B : QUANTIFICATION OF TOTAL STARCH AND α- AMYLASE ENZYME ACTIVITY

Concentration	NORMAL SEEDS	INFECTED SEEDS	NORMAL STEM	INFECTED STEM	NORMAL LEAVES	INFECTED LEAVES
Starch(mg/g)	0.063	0.054	0.315	0.108	0.054	0.036
α- Amylase activity (units/sec/mg. weight of fresh tissue)	0.008	0.016	0.003	0.009	0.008	0.012

C : QUANTIFICATION OF TOTAL PHENOL AND POLYPHENOL OXIDASE ENZYME ACTIVITY

Concentration	NORMAL SEEDS	INFECTED SEEDS	NORMAL STEM	INFECTED STEM	NORMAL LEAVES	INFECTED LEAVES
Phenol (mg/g)	0.49	0.53	0.07	0.16	0.06	0.15
Polyphenol Oxidase activity (units/sec/mg. weight of fresh tissue)	0.026	0.018	0.03	0.008	0.021	0.012

A-F: QUANTIFICATION OF PROTEIN, STARCH AND RELATED ENZYMES



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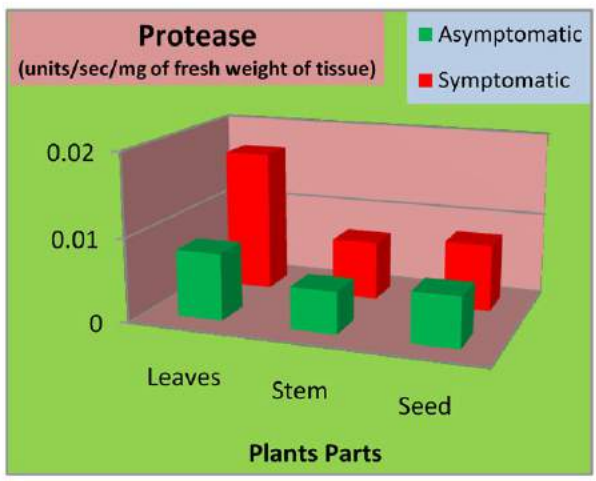

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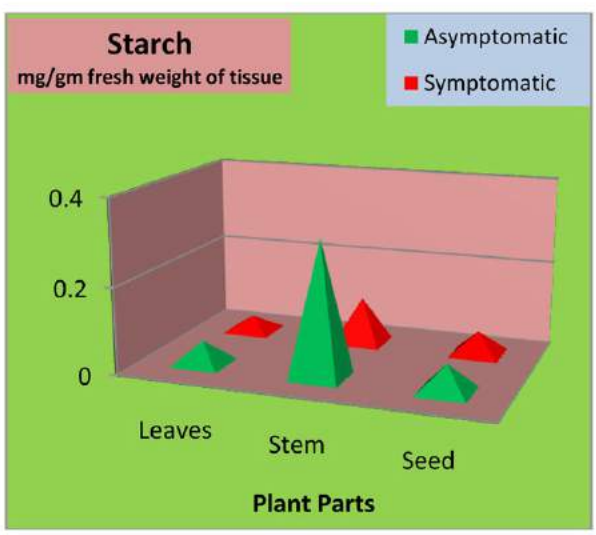

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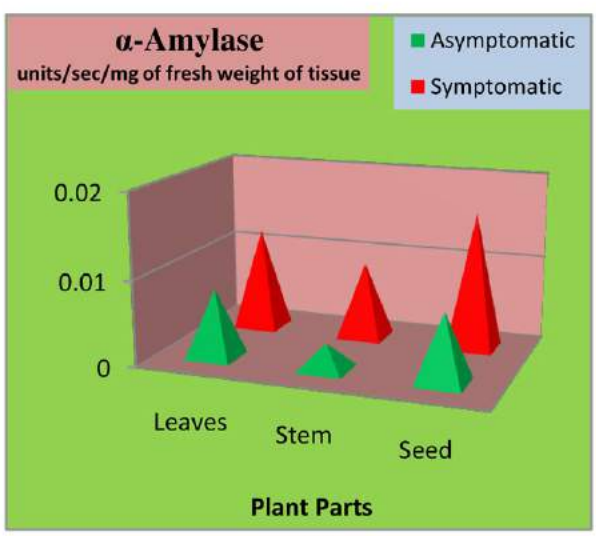

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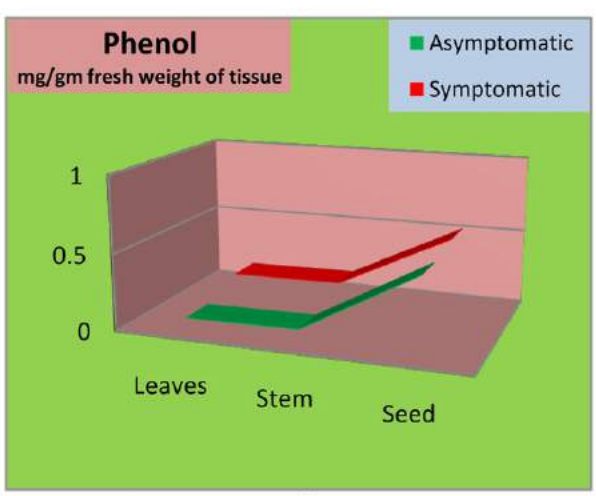

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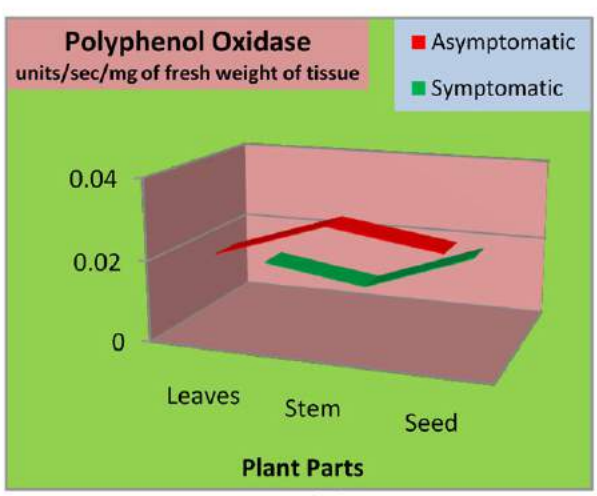
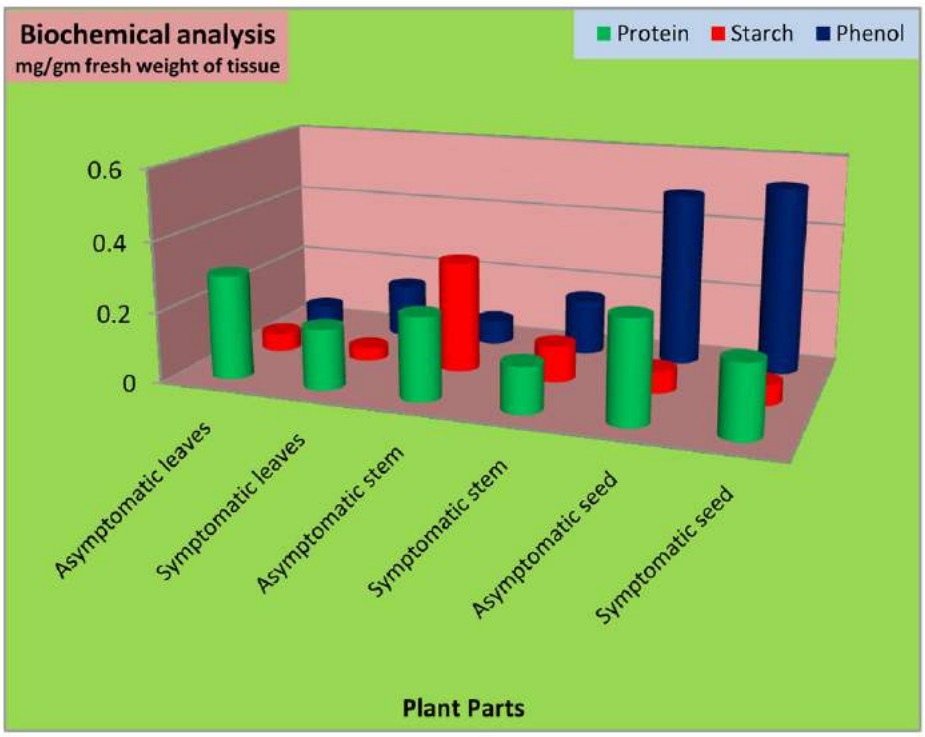


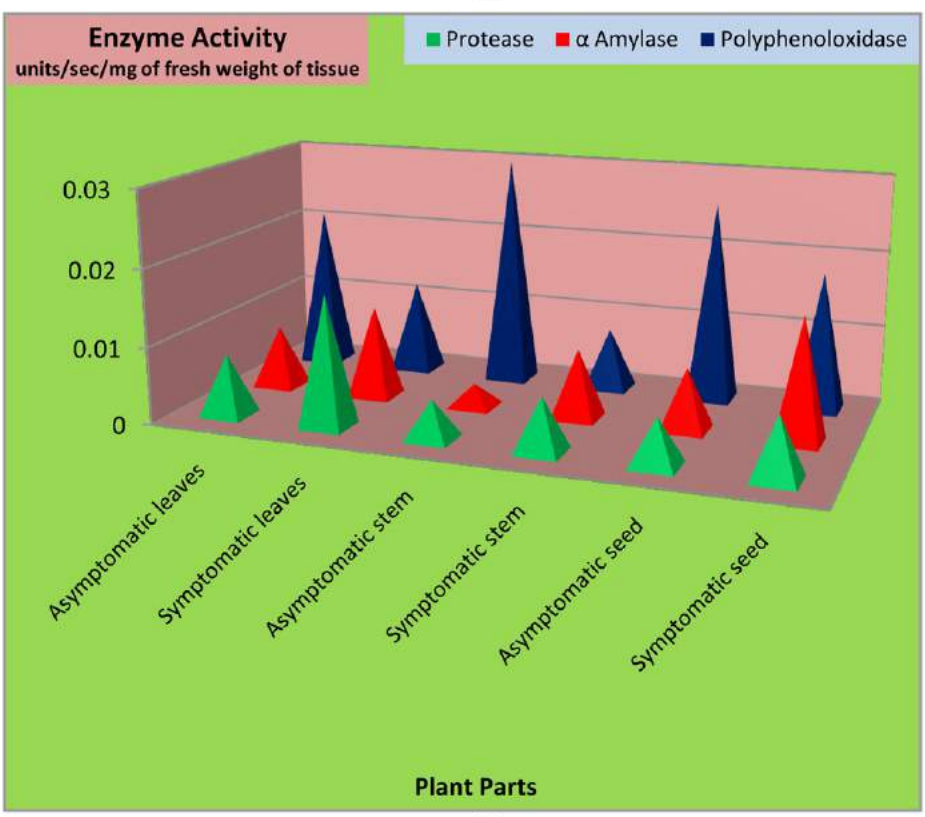
F

Fig. 22

A-B: COMPARATIVE BIOCHEMICAL AND ENZYME ACTIVITY ANALYSIS



A



B

Fig. 23

CONTROL OF SEED -BORNE INFECTION

PHYSICAL CONTROL

Oil Thermotherapy

Seed samples ac nos CB29 (Jaipur) and CB70 (Sikar) carrying natural infection were used for study.

Oil treatment at 50° C **(Fig. 24A-H)**

The seed germination was showed maximum in sesame oil (96%) followed by mustard oil (86%), linseed oil (82%), neem oil (76%), castor oil (72%), mahua oil (66%) and coconut and groundnut oil (62%) in 5 min treatment. While in 10 minute treatment, maximum germination was observed in seed treated with heated linseed oil (92%) followed by mustard oil (84%), sesame oil (68%). Seed treatment with coconut oil, groundnut oil, mahua oil and neem oil showed 58% germination. Minimum percent germination was observed in castor oil treatment (56%) in 10 min treatment.

The effective control of pathogen incidence was observed in sesame oil (94.73%) in 5 min treatment followed by linseed and mustard (93.42%), neem oil (71.05%), castor oil (65.78%), mahua oil (57.89%), coconut oil (50%) and groundnut oil (44.73%). While in 10 min treatment maximum pathogen control was observed in sesame oil (92.10%) followed by mustard oil (88.15%), linseed oil (77.63%), neem oil (71.05%), castor oil (63.15%), mahua oil (56.57%), coconut oil (42.11%) and groundnut oil (39.47%).

For the control of seedling infection, 5 min treatment of sesame oil was most effective (88.09%) followed by mustard and linseed oil (83.33%), neem oil (66.66%), castor oil (61.90%), coconut, groundnut and mahua oil (57.14%).

While In 10 min treatment the best effective control of seedling infection was observed in sesame oil (78.51%) followed by mustard oil (78.57%), linseed oil(64.28%), castor oil (61.90%), coconut, groundnut, mahua and neem oil (57.14%). (Fig. 28A and Table 14)

***Oil treatment at 70° C* (Table 15)**

The maximum percent seed germination (78%) observed in mustard oil in 5 min treatment followed by sesame oil and linseed oil (74%), castor oil (68%), neem oil (66%), mahua (62%) and groundnut oil (60%). Minimum seed germination was observed in coconut oil (58%) in 5 min treatment.

While percent germination was observed maximum in linseed oil (72%) in 10 min treatment followed by mustard oil (68%), sesame oil (62%), castor oil (58%), coconut and neem oil (56%) and groundnut and mahua (54%).

The best control of pathogen incidence was observed in sesame oil (85.52%) followed by mustard (77.63%), linseed oil (77.63%), castor oil (65.78%), neem oil (65.78 %), mahua (57.89%), coconut oil (44.73%) and groundnut oil (39.47%) in 5 min treatment. While in 10 min treatment sesame oil (82.89%) showed maximum pathogen control followed by mustard and linseed oil (82.89%), neem oil (71.05%), castor oil (63.15%), mahua (56.57%), groundnut and coconut oil (39.47%).

Highest seedling control was observed in sesame oil (76.19%) followed by linseed oil (73.80%), mustard oil (73.80%), neem oil (66.66%), castor oil (57.14%), mahua oil (47.61%), groundnut and coconut oil (38.09%) in 5 min treatment.

While in 10 min treatment it was highest in mustard (64.28%) followed by sesame oil (61.90%), linseed oil (59.52%), castor oil and neem oil (57.14%), mahua (47.61%), coconut (42.85%) and groundnut (38.09%). (Fig. 28B and Table 14)

CHEMICAL CONTROL

Systemic Fungicides

Four systemic fungicides Bavistin, Kitazin- P, Systhane and Topas were used at four different concentrations viz. 2000ppm, 1000ppm, 500ppm and 250ppm for overcome seed borne infection of *Fusarium solani* in cluster bean seed samples. The common name of these systemic fungicides are Carbendazim; Iprobenphos(Ibp); Myclobutanil and Penconazole subsequently (Fig. 25A-D and Table 15).

Maximum germination was observed in Bavistin (90%) at 2000ppm concentration followed by Kitazin-P (85%), Systhane (82%) and Topas (70%) at the same concentration.

The percent germination by using Bavistin at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 79%, 81%, 83% and 90% respectively. The percent germination by using Kitazin- P at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 70%, 75%, 80% and 85% respectively. The percent germination by using Topas at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 60%, 64%, 67% and 70% respectively. The percent germination by using Systhane at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 68%, 73%, 79% and 82% respectively.

Maximum or highest percent control of seedling infection was expressed by Bavistin (98.38%) at 2000ppm followed by Kitazin-P (95.16%); Systhane (90.32%) and Topas (85.48%) at same concentration.

The percent control of seedling infection by using Bavistin 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 61.29%, 70.96%, 75.80% and 98.38% respectively. The percent control of seedling infection by using Kitazin-P at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 58.06%, 67.74%, 74.19% and 95.16% respectively. The percent control of seedling infection by using Topas at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 54.83%, 61.29%, 64.51% and 85.48% respectively. The percent control of seedling infection by using Systhane at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 58.06%, 64.51%, 70.96% and 90.32% respectively.

Maximum or highest percent control of pathogen incidence was expressed by Bavistin (98.75%) at 2000ppm conc. followed by Topas (91.25%), Kitazin-P (90%) and Systhane (86.25%) at same conc.

The percent control of pathogen incidence by using Bavistin at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 80%, 85%, 91.25% and 98.75% respectively. The % control of pathogen incidence by using Kitazin-P at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 81.25%, 86.25%, 88.75% and 90% respectively. The percent control of pathogen incidence by using Topas at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 71.25%, 73.75%, 76.25% and 91.25% respectively. The percent control of pathogen incidence by using Systhane at 250ppm, 500ppm, 1000ppm and 2000ppm conc. were recorded 80%, 81.25%, 83.75% and 86.25% respectively (Fig. 29A).

BIOLOGICAL CONTROL

Plant Extracts

Seed borne infection of *Fusarium solani* (CB29 and CB70) of cluster bean seed was controlled by using leaf extracts of plants viz. *Azadirachta indica, Dalbergia sisso, Eucalyptus rudis, Lantana camera, Parthenium officinalis, Ricinus communis and Saracca indica.* (Fig. 26A-C and Table 16)

Extracts of bulbs of *Allium cepa* and *Allium sativam* were also used for overcome infection of *Fusarium solani* in cluster bean seeds.

The latex of *Calotropis procera, Datura innoxia* and *Ficus religiosa* were also utilized to controlling infection of pathogen in cluster bean seeds.

Disease infected seeds were observed at random by using SBM.

Leaf extracts

Out of seven leaf extracts *Dalbergia sisso* expressed maximum germination (83%) followed by *Azadirachta indica* (76%), *Ricinus communis* (73%), *Parthenium officinalis* (62%), *Eucalyptus rudis* (61%), *Lantana camera* (59%) and *Saracca indica* (58%).

Out of seven leaf extracts the maximum percentage of seedling infection control of infected cluster bean seeds was observed by leaf extracts of *Dalbergia sisso* (89.55%) followed by *Azadirachta indica* (82.08%), *Ricinus communis* (80.59%), *Lantana camera* (74.62%), *Saracca indica* (73.13%), *Parthenium officinalis* (71.64%) and *Eucalyptus rudis* (68.65%).

Out of seven leaf extracts the maximum percentage control of pathogen incidence of infected cluster bean seeds was observed by leaf extracts of *Dalbergia sisso* (87.14%) followed by *Azadirachta indica* (84.28%), *Ricinus communis* (82.85%), *Saracca indica* (77.14%), *Lantana camera* (75.71%), *Parthenium officinalis* (72.85%) and *Eucalyptus rudis* (70%). (Fig. 26A and Table 16)

Bulb extracts

Out of two bulb extracts *Allium sativam* bulb extracts (77%) show better germination than *Allium cepa*(55%).

Allium sativam bulb extracts (83.58%) expressed better percentage control of seedling infection than *Allium cepa* bulb extracts (64.17%).

Allium sativam bulb extracts (81.42%) expressed better percentage control of pathogen than *Allium cepa* bulb extracts (67.14%). (Fig. 26C and Table 16)

Latex extract

Latex of *Calotropis procera* (81%) showed better percent germination than *Datura innoxia* latex (61%) and *Ficus religiosa* latex (60%). (Fig. 26B and Table 16)

Latex *of Calotropis procera* expressed maximum percentage control of seedling infection (91.04%) followed by *Ficus religiosa* (76.11%) and *Datura innoxia* (70.14%).

Latex *of Calotropis procera* expressed maximum percentage control of pathogen incidence (91.42%) followed by *Ficus religiosa* (74.28%) and *Datura innoxia* (71.42%).

Maximum percent germination expressed by seed treatment of *Fusarium solani* infected seeds with leaf extract of *Dalbergia sisso* (83%) while percent control of seedling infection and percent control of pathogen incidence were expressed maximum by seed treatment with *Calotropis procera* latex (91.04% and 91.42%) respectively and minimum percent germination; minimum percentage control of seedling infection and minimum percentage control of pathogen incidence expressed by only *Allium cepa* bulb extracts (55%; 64.17% and 67.14%) respectively in *Fusarium solani* infected cluster bean seeds. (Fig. 29B and Table 16)

Fungal Antagonists

Pure suspension culture of *Trichoderma viride* and *T. harzianum* and 4 dilutions i.e. 1:1, 1:2 and 1:4 and 1:8 (v/v) were used for infected seed treatment of cluster bean seeds. These biological antagonists overcome growth of *Fusarium solani* in cluster bean seeds (Fig. 27 and Table 17).

Trichoderma viride

The results of treatment of *Fusarium solani* disease infected seeds of cluster bean expressed that use of these bioagent like *trichoderma viride* overcome pathogen incidence in all four kinds of dilution as compared to control disease infected cluster bean seeds.

Maximum or highest percentage of germination expressed by 1:1 was 77% followed by 1:2 was 70%, 1:4 dilution was 64% and 1:8 dilution was 60%.

Maximum or highest percentage of control of seedling infection of *Fusarium solani* on cluster bean seeds was expressed by 86% by using 1:1 dilution of *Trichoderma viride* followed by 80% by using 1:2 dilution, 70% by using 1:4 dilution and 64% by using 1:8 dilution of *T.viride.*

Maximum or highest percentage of control of pathogen incidence of *Fusarium solani* on cluster bean seeds was expressed by 84.28% by using 1:1 dilution of *Trichoderma viride* followed by 81.42% by using 1:2 dilution, 78.57% by using 1:4 dilution and 71.42% by using 1:8 dilution of *T.viride.*

The maximum nonhazardous control expressed by using 1:1 dilution and minimum overcome of disease expressed by using 1:8 dilution of *T. viride* (Fig. 27A; Fig. 29C and Table 17).

Trichoderma harzianum

All four kinds of dilutions of *T. harzianum* were significant to overcome the disease infection caused by *Fusarium solani* in cluster bean seeds, also helpful to increase percentage germination; increase in percent seedling infection control and increase in pecent pathogen incidence control.

Use of *T. harzianum* bio-agent as overcome *Fusarium solani* disease infection on cluster bean seeds is more useful than the use of *T. viride* on cluster bean seeds.

Maximum or highest percent of germination expressed by 1:1 dilution of *T. harzianum* were 84% followed by 1:2 was 76%, 1:4 dilution was 73% and 1:8 dilutions was 70%.

Maximum or highest percentage of control of seedling infection of *Fusarium solani* on cluster bean seeds was expressed by 90% by using 1:1 dilution of *Trichoderma harzianum* followed by 82% by using 1:2 dilution, 76% by using 1:4 dilution and 72% by using 1:8 dilution of *T.harzianum.*

Maximum or highest percentage of control of pathogen incidence of *Fusarium solani* on cluster bean seeds was expressed by 90% by using 1:1

dilution of *Trichoderma harzianum* followed by 84.28% by using 1:2 dilution, 81.42% by using 1:4 dilution and 80% by using 1:8 dilution of *T. harzianum* (Fig. 27B and Table 17).

The maximum nonhazardous control expressed by using 1:1 dilution and minimum overcome of disease expressed by using 1:8 dilution of *T. harzianum* as in *T.viride* (Fig. 29C).

TABLE -14 : CONTROL OF SEED BORNE INFECTION OF *Fusarium solani* IN CLUSTER BEAN SEEDS BY HEATED OIL TREATMENT AT 50° C AND 70° C

Oil	Temp.	Germination (%)		Seedling Infection Control (%)		Pathogen Control (%)	
		5 min	10 min	5 min	10 min	5 min	10 min
Castor oil`	50° C	72 (7.2)	56(5.6)	61.90(1.6)	61.90 (1.6)	65.78(2.6)	63.15 (2.8)
	70° C	68(6.8)	58(5.8)	57.14 (1.8)	57.14 (1.8)	65.78(2.6)	63.15 (2.8)
Coconut oil	50° C	62 (6.2)	58 (5.8)	57.14 (1.8)	57.14 (1.8)	50 (3.8)	42.11 (4.4)
	70° C	58 (5.8)	56 (5.6)	38.09(2.6)	42.85 (2.4)	44.73(4.2)	39.47 (4.6)
Groundnut oil	50° C	62 (6.2)	58 (5.8)	57.14(1.8)	57.14 (1.8)	44.73(4.2)	39.47 (4.6)
	70° C	60 (6.0)	54 (5.4)	38.09(2.6)	38.09 (2.6)	39.47 (4.6)	39.47 (4.6)
Linseed oil	50° C	82 (8.2)	92 (9.2)	83.33 (0.7)	64.28 (1.5)	93.42 (0.5)	77.63 (1.7)
	70° C	74 (7.4)	72 (7.2)	73.80(1.1)	59.52 (1.7)	77.63(1.7)	82.89 (1.3)
Mahua oil	50° C	66 (6.6)	58 (5.8)	57.14(1.8)	57.14 (1.8)	57.89(3.2)	56.57 (3.3)
	70° C	62 (6.2)	54 (5.4)	47.61(2.2)	47.61 (2.2)	57.89(3.2)	56.57 (3.3)
Mustard oil	50° C	86 (8.6)	84 (8.4)	83.33(0.7)	78.57 (0.9)	93.42(0.5)	88.15 (0.9)
	70° C	78 (7.8)	68 (6.8)	73.80(1.1)	64.28 (1.5)	77.63(1.7)	82.89 (1.3)
Neem oil	50° C	76 (7.6)	58 (5.8)	66.66(1.4)	57.14 (1.8)	71.05(2.2)	71.05 (2.2)
	70° C	66 (6.6)	56 (5.6)	66.66(1.4)	57.14 (1.8)	65.78(2.6)	71.05 (2.2)
Sesame oil	50° C	96 (9.6)	68 (6.8)	88.09(0.5)	78.51 (0.9)	94.73 (0.4)	92.10 (0.6)
	70° C	74 (7.4)	62 (6.2)	76.19 (1.0)	61.90 (1.6)	85.52 (1.1)	82.89(1.3)
Control	60 (6.0)			(4.2)		(7.6)	

Figures in Parenthesis are mean value of 5 replicates.

TABLE -15 : CONTROL OF SEED BORNE INFECTION OF *Fusarium solani* IN CLUSTER BEAN SEEDS BY SYSTEMIC FUNGICIDES

Common name	Trade name	Concentration In ppm	% germination	% control of seedling infection	% control of pathogen incidence
Carbendazim	**Bavistin**	**250ppm**	79(7.9)	61.29(2.4)	80(1.6)
		500 ppm	81(8.1)	70.96 (1.8)	85(1.2)
		1000 ppm	83(8.3)	75.80(1.5)	91.25 (0.7)
		2000 ppm	90 (9.0)	98.38 (0.1)	98.75 (0.1)
Iprobenphos(IBP)	**Kitazin-P**	**250 ppm**	70(7.0)	58.06(2.6)	81.25(1.5)
		500 ppm	75(7.5)	67.74 (2.0)	86.25(1.1)
		1000 ppm	80(8.0)	74.19 (1.6)	88.75(0.9)
		2000 ppm	85(8.5)	95.16 (0.3)	90(0.8)
Penconazole	**Topas**	**250 ppm**	60(6.0)	54.83 (2.8)	71.25(2.3)
		500 ppm	64(6.4)	61.29 (2.4)	73.75(2.1)
		1000 ppm	67(6.7)	64.51(2.2)	76.25(1.9)
		2000 ppm	70(7.0)	85.48(0.9)	91.25(0.7)
Myclobutanil	**Systhane**	**250 ppm**	68(6.8)	58.06 (2.6)	80(1.6)
		500ppm	73(7.3)	64.51(2.2)	81.25(1.5)
		1000ppm	79(7.9)	70.96 (1.8)	83.75 (1.3)
		2000ppm	82(8.2)	90.32(0.6)	86.25(1.1)
Control			**60(6.0)**	**6.2**	**8.0**

Figure in parenthesis are mean value of 5 replicates.

TABLE -16 : CONTROL OF SEED BORNE INFECTION OF *Fusarium solani* IN CLUSTER BEAN SEEDS BY LEAF EXTRACTS, BULB EXTRACTS AND LATEX YIELDING PLANTS

Source	Name of plants	% Germination	% Control of seedling infection	% Control of pathogen incidence
Leaf Extracts	***Azadirachta indica***	76(7.6)	82.08(1.2)	84.28(1.1)
	Dalbergia sisso	83(8.3)	89.55(0.7)	87.14(0.9)
	Eucalyptus rudis	61(6.1)	68.65(2.1)	70(2.1)
	Lantana camera	59(5.9)	74.62(1.7)	75.71(1.7)
	Parthenium officinalis	62(6.2)	71.64(1.9)	72.85(1.9)
	Ricinus communis	73(7.3)	80.59(1.3)	82.85(1.2)
	Saracca indica	58(5.8)	73.13(1.8)	77.14(1.6)
Bulb extracts	***Allium cepa***	55(5.5)	64.17(2.4)	67.14(2.3)
	Allium sativam	77(7.7)	83.58(1.1)	81.42(1.3)
Latex yielding plant extracts	***Calotropis procera***	81(8.1)	91.04(0.6)	91.42(0.6)
	Datura innoxia	61(6.1)	70.14(2.0)	71.42(2.0)
	Ficus religiosa	60(6.0)	76.11(1.6)	74.28(1.8)
Control		**63(6.3)**	**6.7**	**7.0**

Figure in parenthesis are mean value of 5 replicates.

TABLE -17 : BIOLOGICAL CONTROL OF *Fusarium solani* IN CLUSTER BEAN SEEDS BY FUNGAL ANTAGONISTS IN THEIR CULTURE SUSPENSION

Dilutions	% Germination		% Control of seedling infection		%Control of pathogen incidence	
	T.v.	***T.h.***	***T.v.***	***T.h.***	***T.v.***	***T.h.***
1:8	60(6.0)	70(7.0)	64 (1.8)	72(1.4)	71.42(2.0)	80(1.4)
1:4	64(6.4)	73(7.3)	70(1.5)	76(1.2)	78.57(1.5)	81.42(1.3)
1:2	70(7.0)	76(7.6)	80(1.0)	82(0.9)	81.42(1.3)	84.28(1.1)
1:1	77(7.7)	84(8.4)	86(0.7)	90(0.5)	84.28(1.1)	90(0.7)
Control	**65 (6.5)**		**5.0**		**7.0**	

Figure in parenthesis are mean value of 5 replicates of cluster bean seeds.

T.h. = Trichoderma harzianum

T.v. = Trichoderma viride

Fig. 24 (A-H): Physical control of seed borne infection of *Fusarium solani* by seed treatment with heated oil at 50° C

Treated seeds with heated oil showing better germination, less symptomatic seedlings and low incidence of pathogen in comparison to their control

A. Sesame oil

B. Mustard oil

C. Linseed oil

D. Neem oil

E. Castor oil

F. Mahua oil

G. Groundnut oil

H. Coconut oil

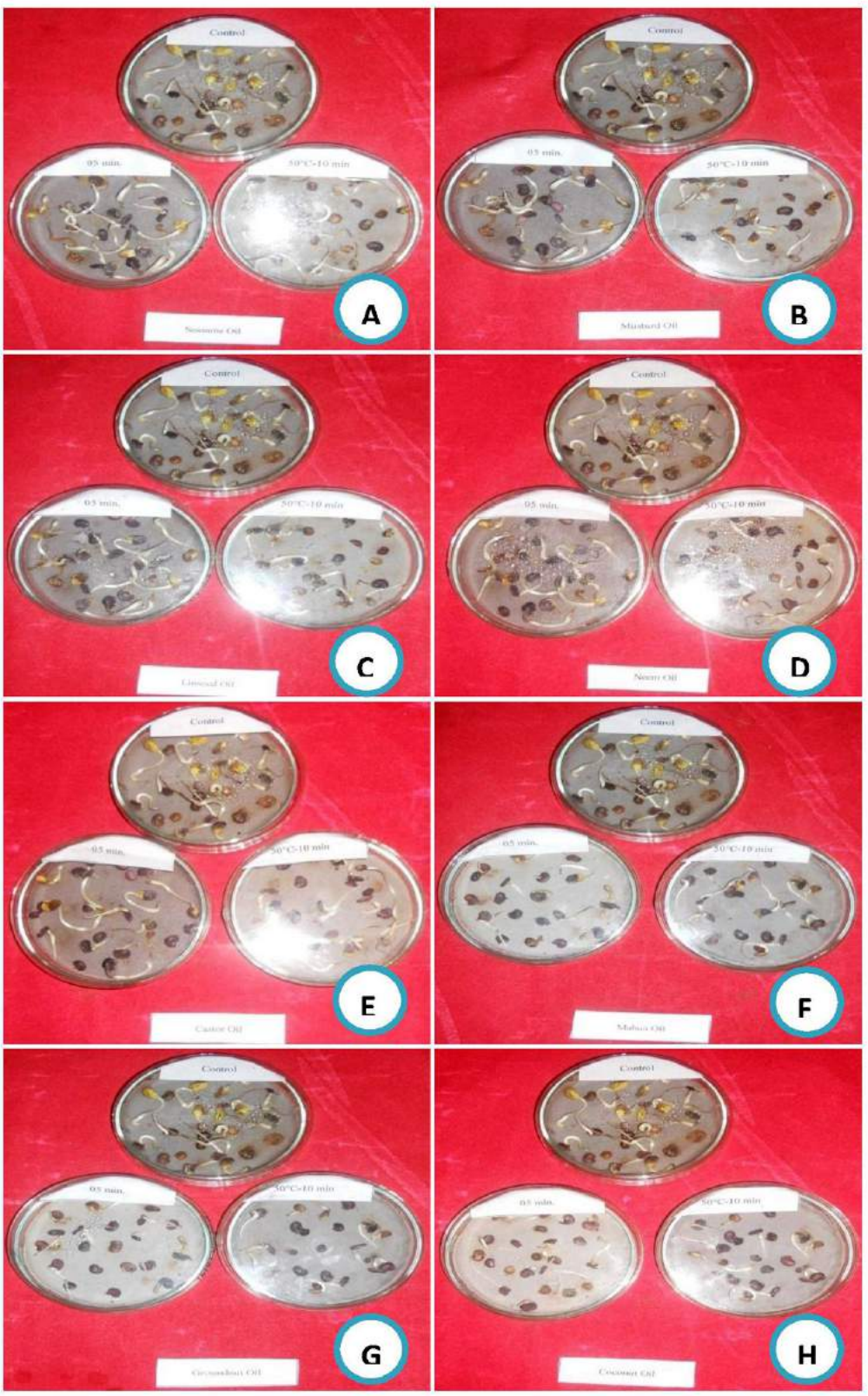


Fig. 24

Fig. 25 (A-D): **Chemical control of seed borne infection of *Fusarium solani* by seed treatment with systemic fungicides at 2000 ppm, 1000 ppm, 500 ppm and 250 ppm concentrations respectively**

A. Bavistin

B. Kitazin-P

C. Systhane

D. Topas

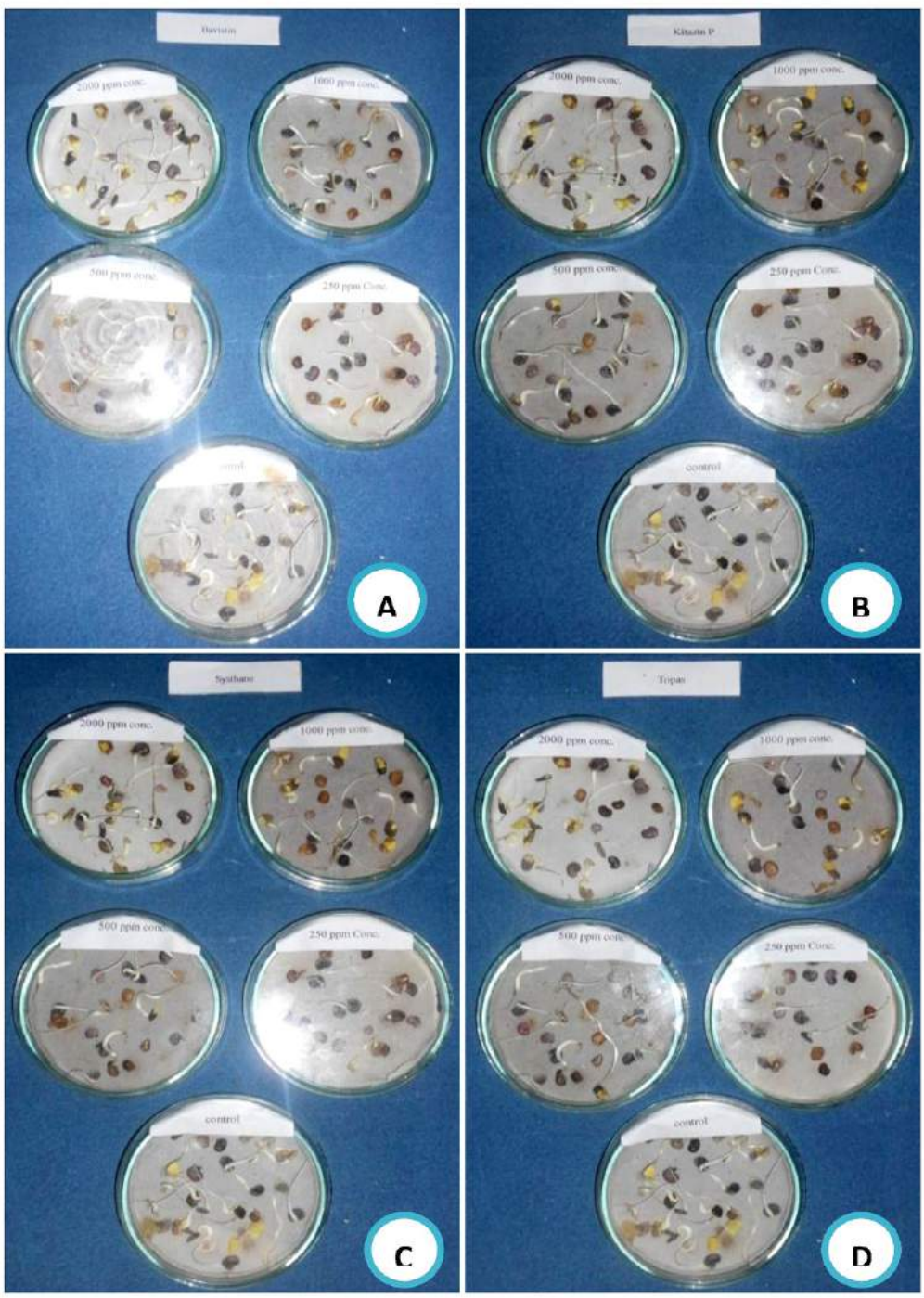

Bavistin
2000 ppm conc.
1000 ppm conc.
500 ppm conc.
250 ppm Conc.
control
A
Kitazin P
2000 ppm conc.
1000 ppm conc.
500 ppm conc.
250 ppm Conc.
control
B
Systhane
2000 ppm conc.
1000 ppm conc.
500 ppm conc.
250 ppm Conc.
control
C
Topaz
2000 ppm conc.
1000 ppm conc.
500 ppm conc.
250 ppm Conc.
control
D

Fig. 25

Fig. 26 (A-C): Biological control of seed borne infection of *Fusarium solani* by seed treatment with leaf extracts, latex yielding plant extracts and bulb extracts of different plants

A. Leaf Extracts- *Dalbergia sisso, Azadiracta indica, Ricinus communis, Parthenium officinalis, Eucalyptus rudis, Saraca indica, Lantana camera*.

B. Latex yielding Plant Extracts- *Calotropis procera, Datura innoxia, Ficus religosa*.

C. Bulb Extracts- *Allium sativum* and *Allium cepa*.


Dalbergia sissoo
Azadirachta indica
Ricinus communis
Parthenium officinalis
Eucalyptus rudis
Control
A
Calotropis procera
Datura inoxia
Ficus religosa
Control
B
Latex Yielding Plant Extracts
Onion (Allium cepa)
Control
Bulb Extracts
C

Fig. 26

Fig. 27 (A-B): Biological control of seed borne infection of *Fusarium solani* by seed treatment with fungal antagonists at 1:1, 1:2, 1:4 and 1:8 dilutions

A. Petriplate showing effect of fungal antagonist *Trichoderma viride* at 1:1, 1:2, 1:4 and 1:8 dilutions

B. Petriplate showing effect of fungal antagonist *Trichoderma harzianum* at 1:1, 1:2, 1:4 and 1:8 dilutions

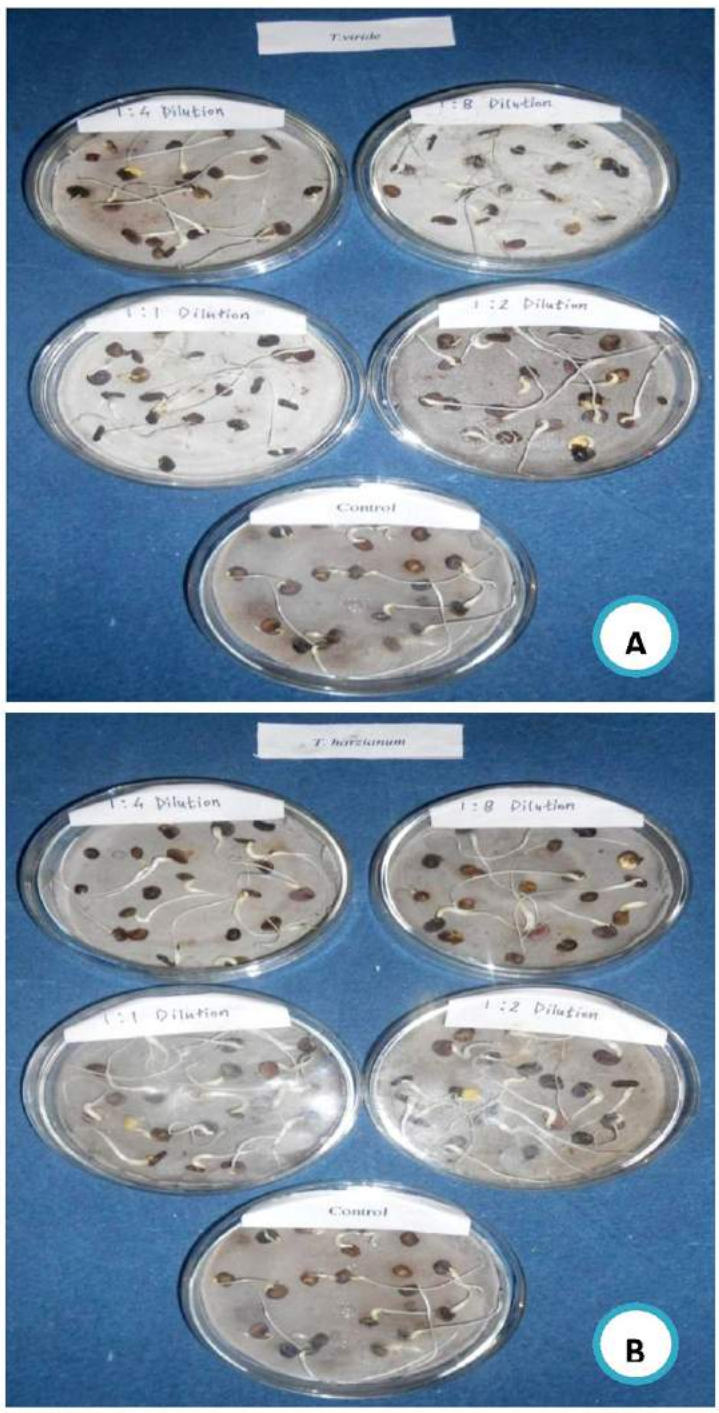
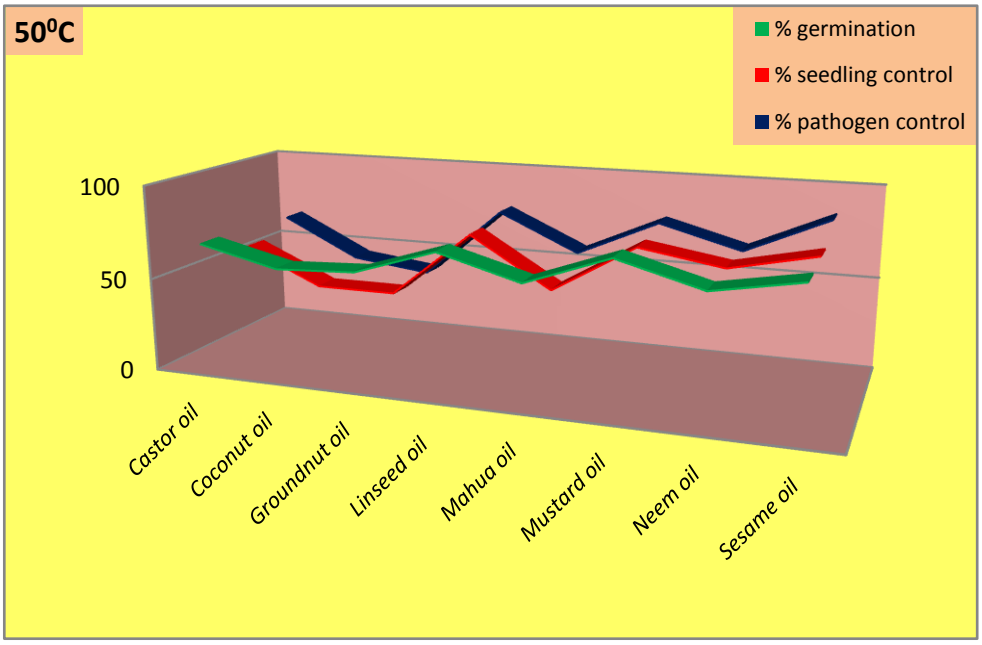
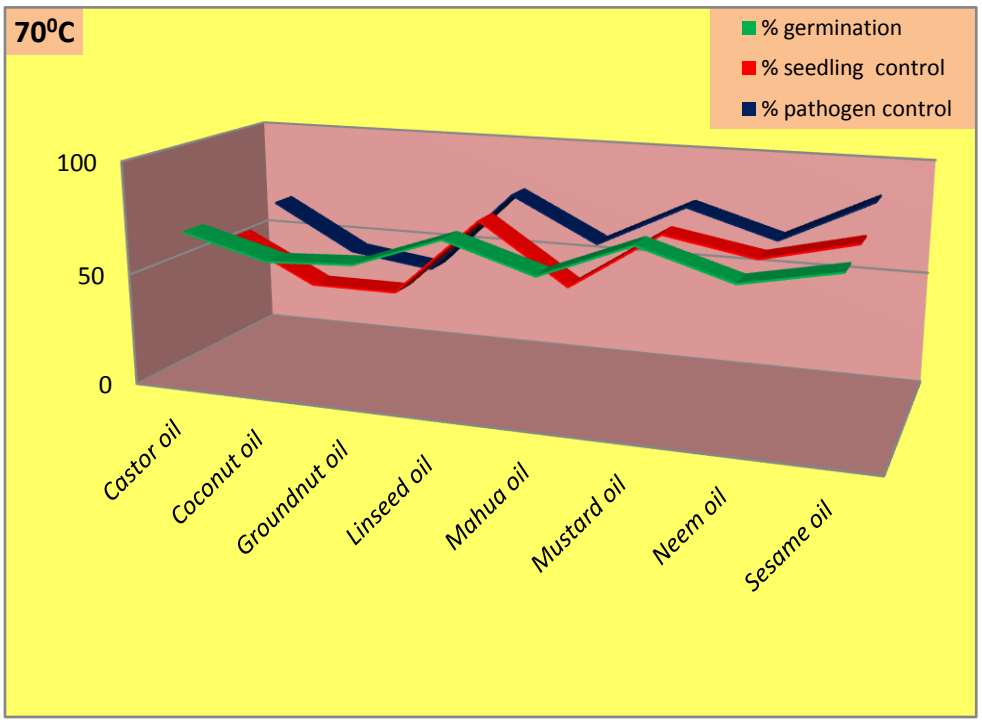

T.viride
1:4 Dilution
1:8 Dilution
1:1 Dilution
1:2 Dilution
Control
A
T.harzianum
1:4 Dilution
1:8 Dilution
1:1 Dilution
1:2 Dilution
Control
B

Fig. 27

A-B: CONTROL OF SEED BORNE INFECTION OF *Fusarium solani* BY HEATED OIL TREATMENT FOR 5 MIN



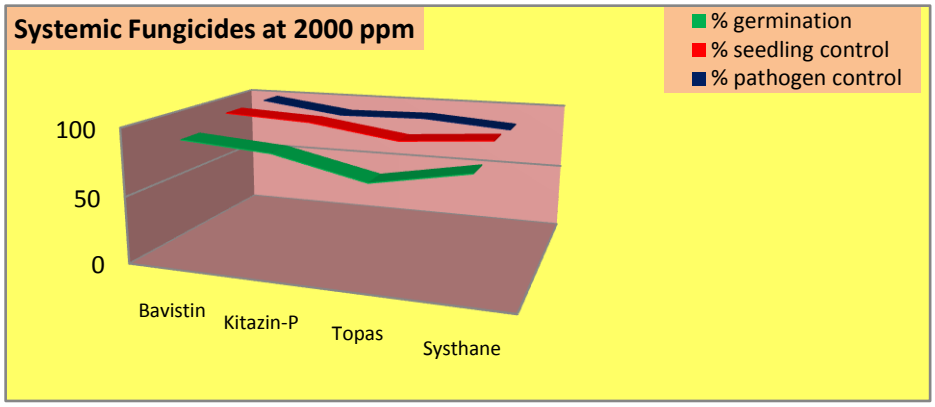
A



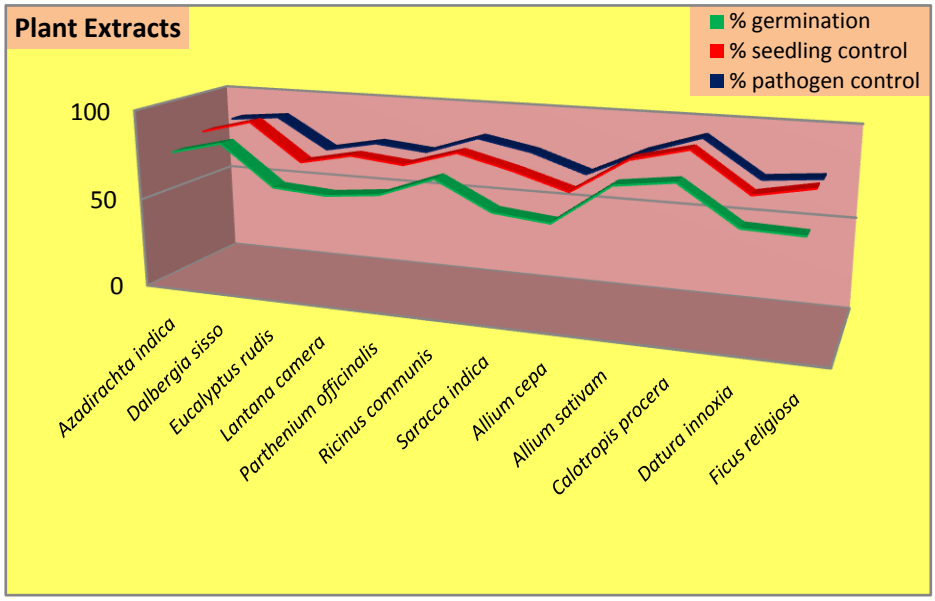
B

Fig. 28

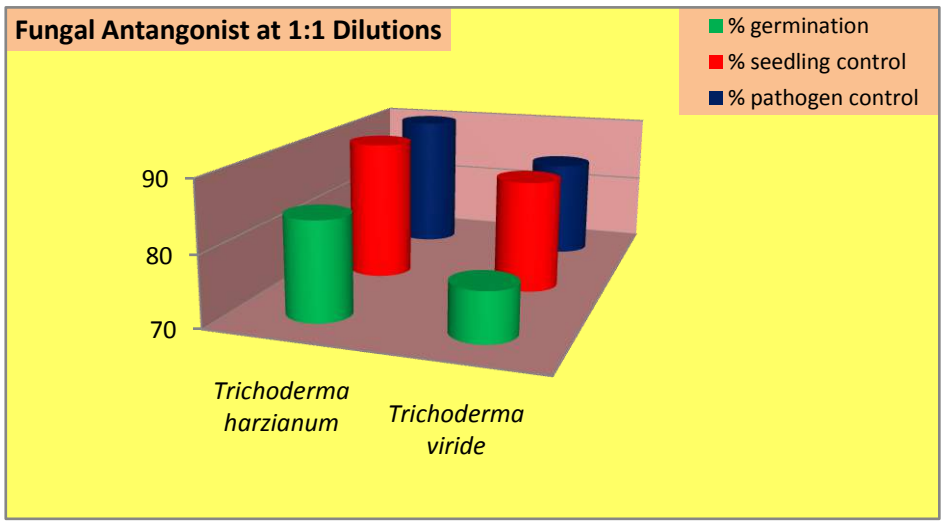
A-C: CONTROL OF SEED BORNE INFECTION OF *Fusarium solani* IN CLUSTER BEAN SEEDS BY CHEMICAL & BIOLOGICAL METHODS



A



B



C

Fig. 29

CULTURAL PRACTICE

DRIP IRRIGATION

Introduction

Drip irrigation is of recent origin and in India is being used on a limited scale in Tamil Nadu, Karnataka, Kerala and Maharashtra states mainly for coconut, coffee, grape and vegetable production. Drip irrigation systems (DIS) are extremely effective in arid and drought prone areas where water is scarce and have been used experimentally in India for over 15 years. In the states of Tamil Nadu, Karnataka, Maharashtra, and Andhra Pradesh progressive farmers started using this method of irrigation in the late-1970s without the benefit of any subsidies or support from central or state governments. However, as a result of subsequent sustained efforts by the state and central governments, agricultural universities and private sector manufacturers; use of drip irrigation systems spread through the drought prone areas of southern and western India. The use of Drip irrigation system is primarily to irrigate high value horticultural crops. Sometimes DIS are used for irrigation of vegetable and other commercial crops in Maharashtra, Karnataka, and Tamil Nadu. The sharp rise in the area under DIS irrigation between 1988 and 1989 is due to the significant increase in the use of these systems in the Maharashtra State.

Drip irrigation also known as “trickle irrigation” or “micro irrigation” or “localized irrigation”. It is an irrigation method that saves water and fertilizer by allowing water to drip slowly to the roots of plants either onto the soil surface or directly onto the root zone through a network of valves, pipes, tubing and emitters. It is done through narrow tubes that deliver water directly to the base of the plant (Fig. 30; Fig. 31A-C).

Drip irrigation is gaining popularity for production of some vegetable crops. It can be used to save water and enhance crop yields.

In India, the irrigated area consists of about 36 per cent of the net sown area. Presently, the agricultural sector accounts for about 83 per cent of all water uses rest 5, 3, 6 and 3 percent uses by domestic, industrial, energy sectors and other consumers respectively. Increasing competition with the other water users in the future would limit the water availability for expanding irrigated area. In traditional surface irrigation methods, the losses in water conveyance and application are large. These losses can be considerably reduced by adopting drip and sprinkler irrigation methods. Among all the irrigation methods, the drip irrigation is the most efficient and it can be practiced in a large variety of crops especially in vegetables, orchard crops, flowers and plantation crops. In drip irrigation, water is applied near the plant root through emitters or drippers, on or below the soil surface, at a low rate varying from 2 - 20 lit res per hour. The soil moisture is kept at an optimum level with frequent irrigations. Drip irrigation results in a very high water application efficiency of about 90-95 per cent.

History of drip irrigation

Head irrigation has been used since ancient times when buried clay pots were filled with water which would gradually seep into the grass. Perforated pipe was introduced in Germany in the 1920s.

The usage of plastic to hold and distribute water in drip irrigation was developed in Australia by Hannis Thill after second world war. Usage of a plastic emitter in drip irrigation was developed in Israel by Simcha Blass and his son Yeshayahu. Instead of releasing water through tiny holes blocked easily by tiny particles, water was released through larger and longer passage ways by using velocity to slow water inside a plastic emitter. The first experimental system of this type was established in 1959 by Blass who partnered later in 1964 with

Hatzerim to create an irrigation company called Netafim. Together they developed and patented the first practical surface drip irrigation emitter.

In the United States, the first drip tape called Dew Hose was developed by Richard Chapin of Chapin watermatics in the early 1960s.

Modern drip irrigation has arguably become the world's most valued innovation in agriculture since the invention of the impact sprinkler in the 1930s, which offered the first practical alternative to surface irrigation. Drip irrigation may also use devices called micro-spray heads which spray water in a small area instead of dripping emitters. These are generally used on tree and vine crops with wider root zones. Subsurface drip irrigation (SDI) uses permanently or temporarily buried dripper line or drip tape located at or below the plant roots. It is becoming popular for row crop irrigation especially in areas where water supplies are limited or recycled water is used for irrigation. Careful study of all the relevant factors like land topography, soil, water, crop and agro-climatic conditions are needed to determine the most suitable drip irrigation system and components to be used in a specific installation.

The use of drip irrigation can be traced back to the ancient custom in certain parts of India of irrigating vegetable crops and some other plants also. During the summer months, the plant was irrigated by a hanging pitcher containing water and a minute hole at its bottom to allow the trickling of water on to the plant. The tribal farmers of Arunachal Pradesh practiced a primitive form of drip irrigation system using a slender bamboo as the conduit for water flow. The modern drip irrigation began its development in Afghanistan in 1866 when researchers began experimenting with irrigation using clay pipe to create combination irrigation and drainage system. The use of drippers in sub-surface irrigation network was first experimented with in Germany in 1869. The drip system was developed for field crops in Israel in the early 1960s and in Australia and North America in the late 1960s. In India, there has been a tremendous growth in the area under drip irrigation during last 15 years. At present around

3.51akh ha area is under drip irrigation with the efforts of the Government of India. Many crops are irrigated by the drip method in India with the tree crops occupying the maximum percentage of the total area under drip irrigation followed by vine crops, vegetables, field crops, flowers and other crops.

Principle of drip irrigation

Drip irrigation or micro irrigation or trickle irrigation is the slow, precise application of water and nutrients directly to the plant root zones in a predetermined pattern. A drip irrigation design can be customized to meet specific needs.

At the present time as long as water is inexpensive; irrigation inefficiency and over-watering may be overlooked. Once water supply is low due to a drought and water restrictions are applied; the inefficiencies of a poorly designed and installed irrigation system quickly become apparent. For an irrigation system to be successful, it must include proper design, correct installation, the right component selection, the proper layout and appropriate maintenance. Through appropriate management of drip irrigation the root zone moisture content can be maintained near the field capacity throughout the season providing a level of water and air balance closed to optimum for plant growth and drip irrigation have very significant effect on quality and quantity of yield, pest control harvest timing of a crop in humid and arid areas during the dry season.

Drip irrigation system delivers water to the crop using a network of mainlines, sub-mains and lateral lines with emission points spaced along their lengths. Each dripper/emitter, orifice supplies a measured, precisely controlled uniform application of water, nutrients and other required growth substances directly into the root zone of the plant.

Water and nutrients enter the soil from the emitters then moving into the root zone of the plants through the combined forces of gravity and capillary. The

plant's withdrawal of moisture and nutrients are replenished almost immediately by drip emitters to ensuring that the plant never suffers from water stress. This process is useful to enhancing quality; achieve optimum growth and high yield.

The principle operation and maintenance requirements associated with the implementation of this technology include the need for regular cleaning of the system and careful monitoring of the quality of the source water, as the drip irrigation systems are very sensitive to the clogging of the drippers. The systems also require a relatively high degree of skill to design, install and operate.

Materials and methods

Cyamopsis tetragonoloba and drip irrigation system

Cyamopsis tetragonoloba is a drought tolerant plant. So the Drip irrigation system was not usually applied in cluster bean crop due to its drought tolerant capacity. *Cyamopsis tetragonoloba* crop can be easily grown at only 250 mm of annual rainfall. The drip irrigation system normally used in vegetable crops but rarely it also applied in cluster bean field for obtaining higher quality and yield of crop. It also applied in cluster bean field for experimental purpose by various agriculture research institutes. It is also used to obtain disease free or infection free crop of cluster bean crop by various farmers and research institutes.

Need of Drip Irrigation

Water deficits during the establishment of cluster bean delay maturity and may cause gaps in uneven stands. Water stress in early vegetative stages results in reduced leaf area and reduced yield. The most serious yield reduction result from water stress during flowering and fruit development. Water stress at this time may also result in small and / or misshapen fruit (Anthony and Kerry, 2009). Arid and semi arid areas of Rajasthan are depending upon the amount and quality of water

available for agriculture. So the drip Irrigation system is most suitable process to increase yield of cluster bean with low water availability (Fig. 32A-D).

Results

Research Highlights

The experiments were conducted at farm situated in tapiplya village near the Reengus station of Sikar districts of Rajasthan to compare the performance and evaluation of drip irrigation system with sprinkler/ surface irrigation method. The general observation indicated that there was beneficial effect on yield and water saving due to irrigation applied through drip in comparison to surface method of irrigation. The drip system could save 35-40% water and gave higher yields of vegetables as compared to surface method**.** By using drip irrigation method in cluster bean crop the labour cost, rate of infection and energy / fertilizer and pesticide cost can be reduced.

Observation

The comparative observation between surface or sprinkler irrigation and drip irrigation revealed that the %yield, % water saving and % size and quality of crop were higher in drip irrigation than the surface irrigation. The % yield were observed 85%, 110%; % water saving were 65% and 90% and % size and quality of crop were 85%, 105% in surface irrigation and drip irrigation respectively. The % range of infection, % energy/ fertililizer / pesticide cost and % labour cost were observed lower in drip irrigation than the surface irrigation. The % range of infection were 90%, 45%; % energy/ fertilizer / pesticide cost 85%, 60% and % labour cost were 95%, 57% in surface irrigation and drip irrigation respectively. These results showed that by using drip irrigation in field the % yield, % water saving and % size and quality of crop increased while % range of infection, % energy/ fertilizer/ pesticide cost and % labour cost decreased or reduced than the use of surface or sprinkler irrigation (Fig. 33 and Table 18).

Advantages of Drip irrigation systems

- Fertilizer and nutrient loss is minimized due to localized application and reduced leaching.
- Water application efficiency is high.
- Field leveling is not necessary.
- Fields with irregular shapes are easily accommodated.
- Recycled non-potable water can be safely used.
- Moisture within the root zone can be maintained at field capacity.
- Soil type plays less important role in frequency of irrigation.
- Soil erosion is minimized.
- Weed growth is minimized.
- Water distribution is highly uniform, controlled by output of each nozzle.
- Labour cost is less than other irrigation methods.
- Variation in supply can be regulated by regulating the valves and drippers.
- Fertigation can easily be included with minimal waste of fertilizers.
- Foliage remains dry, reducing the risk of disease.
- Usually operated at lower pressure than other types of pressurized irrigation, reducing energy costs also.
- The advantages of drip irrigation systems include a high efficiency of water use and greater crop yields compared to other irrigation methods. In addition, crops irrigated using drip irrigation systems generally require less tillage and are of better quality. Drip

irrigation system also contribute to improved plant protection and reduced occurrences of plant diseases and greater efficiencies in the use of fertilizers because water containing the agrochemicals is applied directly to the plant roots in the quantities necessary for optimal plant production. For a similar reason, Drip irrigation system can also make use of lower quality water and results in no return flows, tail water losses or increased soil erosion. Because of water is applied in optimal quantities, plants generally have a shorter growing season and produce fruit earlier with less weed growth and pest damage than conventionally irrigated crops. The lower labour requirements result in relatively low operational costs with savings in labour of up to 90% of the costs associated with conventional systems because mechanical operations can be carried out simultaneously with the application of irrigation water. The use of drip irrigation system in hilly terrain and on lands with problem soils also improved infiltration in soils with low conductivity. Drip irrigation systems are low pressure systems which can be adapted for use in greenhouses and with automated control systems.

TABLE -18: COMPARISION BETWEEN SURFACE IRRIGATION AND DRIP IRRIGATION SYSTEM

	Surface Irrigation System	**Drip Irrigation System**
Yield(quarter/hectare) (in %)	85	110
Water Saving (in %)	65	90
Rate of Infection (in %)	90	45
Size and Quality of Crop (in %)	8 5	105
Energy/Fertililizer/Pesticide Cost (in %)	85	60
Labour cost (in %)	95	57

Fig. 30:

Diagrammatic representation of drip irrigation layout

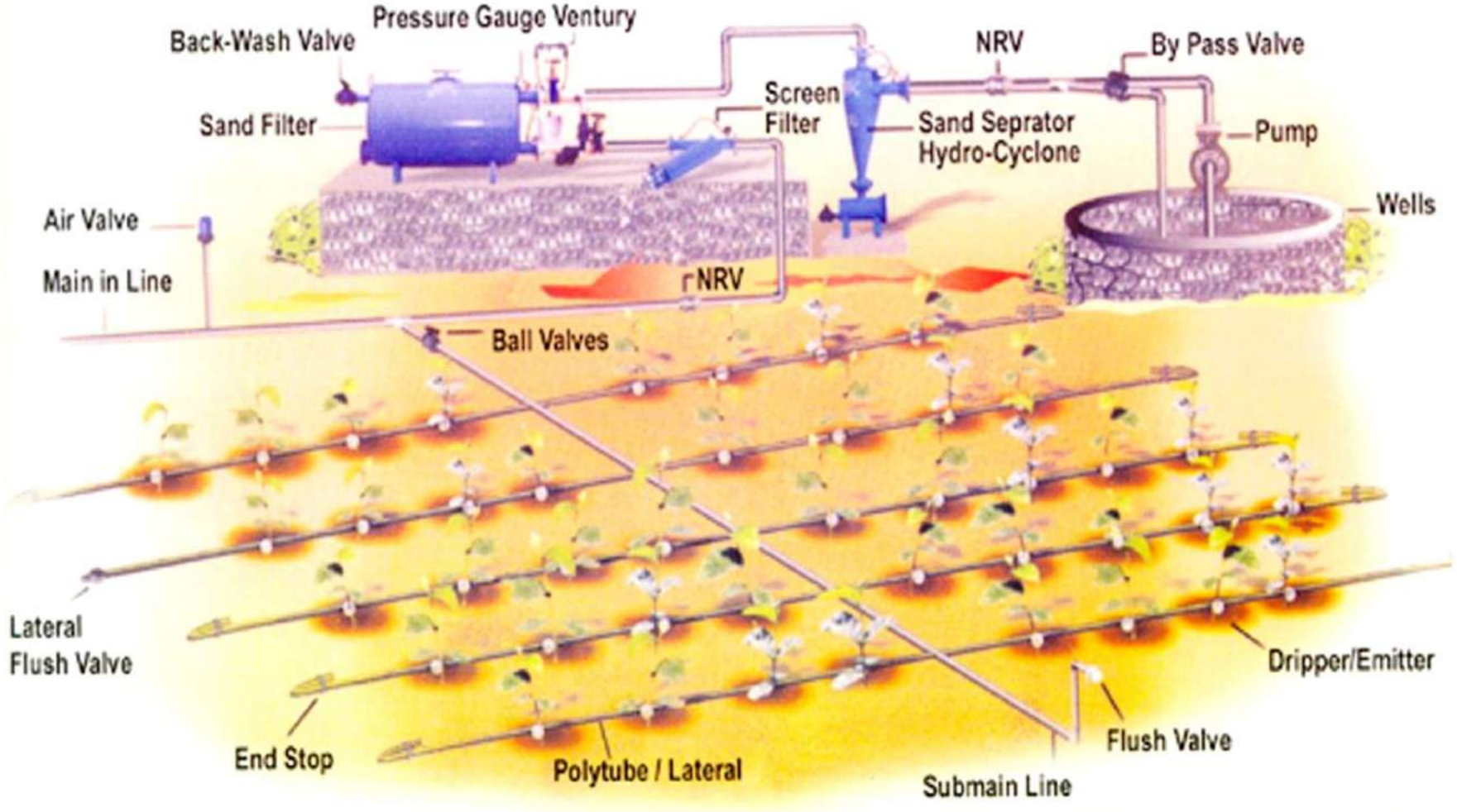

Back-Wash Valve
Pressure Gauge Ventury
NRV
By Pass Valve
Screen Filter
Sand Filter
Sand Seprator Hydro-Cyclone
Pump
Air Valve
Wells
Main in Line
NRV
Ball Valves
Lateral Flush Valve
Dripper/Emitter
End Stop
Polytube / Lateral
Flush Valve
Submain Line

Fig. 30

Fig. 31 (A-C): Control of *Fusarium solani* in cluster bean by adopting drip irrigation in fields as a cultural practice

A. Drip Irrigation Pump set up.

B. Drip irrigation pump set up being manually operated

C. Emitters and pipes used for drip irrigation

Fig. 31

Fig. 32(A-D): Control of *Fusarium solani* in cluster bean by adopting drip irrigation in fields as a cultural practice

A. Young seedling of cluster bean with the network of drip pipes in root system in field

B. Mature stage of cluster bean field

C. Lush green field of cluster bean crop by adopting drip irrigation

D. Enlarged view of cluster bean field showing emitters and pipe

Fig. 32

COMPARATIVE GRAPHICAL REPRESENTATION BETWEEN SURFACE IRRIGATION SYSTEM AND DRIP IRRIGATION SYSTEM (VALUES IN %)

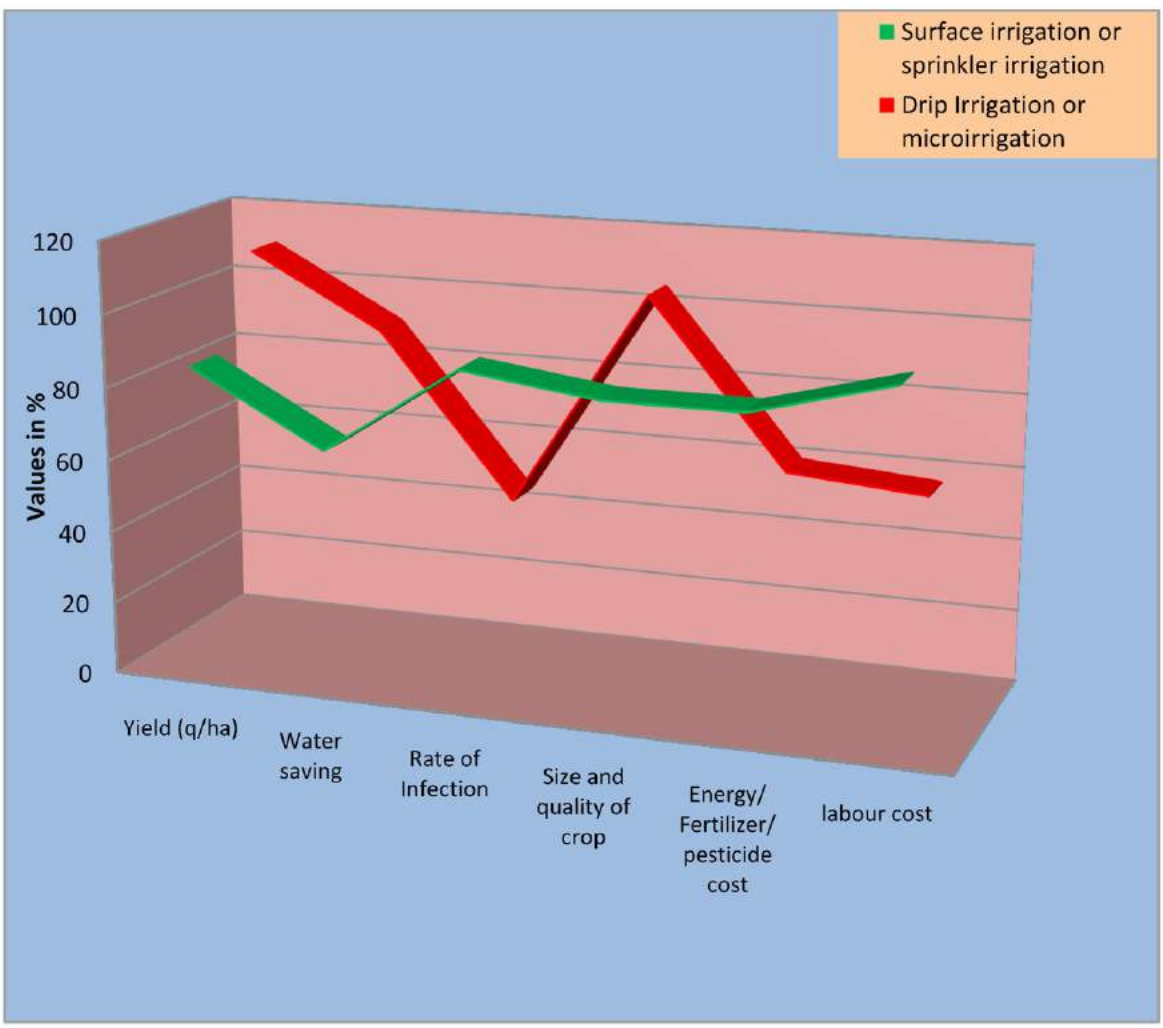


Fig. 33

Chapter – 5

Discussion

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] is an important legume crop extensively grown in arid and semi arid regions of India. It is widely cultivated in all 33 districts of Rajasthan. It is grown for green manuring, green fodder, vegetables, production of seeds, pharmaceuticals, cosmetics and extraction of guar gum purposes in India. Cluster bean also has wide importance as industrial and commercial purpose due to presence of glactomannon gum found in spherical-shaped endosperm. It suffers a number of diseases but heavy losses are obtained due to infection of *Fusarium solani*.

SURVEY AND DETECTION OF SEED BORNE MYCOFLORA

A number of methods have been followed to determine seed borne mycoflora of *Cyamopsis tetragonoloba*. Konde et al. (1980) used blotter paper and agar plate method for isolation of seed borne fungal species in pearl millet. In present study dry seed examination, standard blotter method (SBM) and Potato Dextrose Agar (PDA) test were used, which are the standard methods recommended for seed health testing (ISTA, 1996; Anonymous, 1985). The reports on seed borne fungi of cluster bean are by Chand and Gandhi (1978), Shivanna and Shetty (1988a), Dwivedi and Dube (1992), Bhatia and Singh (1995), Nagerabi and Elshafie (2001) and Singh, Chandil and Tripathi (2005). These observations are based on limited number of samples from different localities and thus provide little opportunity about the fungi on cluster bean seeds, and do not provide detailed information about seed borne mycoflora. Some of these reports are elementary.

During the present study 120 seed samples of cluster bean seeds collected from 11 districts of Rajasthan were studied to obtain comprehensive information regarding the seed borne fungi of cluster bean seeds in Rajasthan state.

DRY SEED EXAMINATION

Dry seed examination of seed samples posses various degrees of deformities and discolourations. Neergaard (1977) has described three basic

categories of seed discolouration viz. superficial necrotic lesions, fungal coating and pigmentation on seed surface.

In Present study, seed samples contained besides the normal or healthy looking seeds; symptomatic seeds with various types of deformities like seeds with black streaks; grey coloured seeds with white mycelial growth; shrivelled seeds; broken and insect damaged seeds; debris and inert matter.

On incubation, various type of discolouration associated with various type of fungi. Seeds with black streaks revealed mainly the infection of *Colletotrichum dematium, Curvularia lunata, Rhizoctonia bataticola, R.solani, Macrophomina phaseolina and Phoma betae;* grey colour seeds with white mycelial growth yielded *Fusarium moniliforme, Fusarium oxysporum* and *Fusarium solani;* shrivelled seeds yielded a group of fungi and bacteria viz. *Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Chaetomium sps., Drechslera tetramera, Fusarium moniliforme, F. oxysporum, Penicillium sps., Rhizoctonia solani, Myrothecium roridum* and Bacterial ooze; Broken and insect damaged seeds carried infection of *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Drechslera tetramera, Rhizoctonia bataticola, Chaetomium spp., Rhizopus sps. and Trichothecium roseum* and Debris and inert matter carried infection of *Aspergillus fumigatus, A. niger, Curvularia pallescens, Drechslera rostrata, F. monaliforme, F.oxysporum, F. solani, Mucor sps., Phoma betae, Rhizopus nigricans* and *Trichothecium roseum.*

Ramnath, Neergaard and Mathur (1970) observed seed necrosis due to the infection of *Fusarium equiseti, F. semitectum* and *Macrophomina phaseolina* in mungbean. Agarwal and Shrivastava (1981) developed a seed soak method to detect kernel smut pathogen (*Tilletia barclayana*) in rice. Shivanna and Shetty (1988b) have reported that *Colletotrichum dematium* caused discoloured, Shrivelled and light weight seeds of guar.

Gupta, Sindhu and Naaz (1989) observed darker patches on the surface of okra seeds at micropylar end found to be associated by *Alternaria alternata, Drechslera sp., Curvularia lunata* and *Aspergillus flavus*.

Varma, Singh and Singh (1989) revealed brown to black discolouration with few to numerous microsclerotia in *Rhizoctonia solani* infected seeds of moth bean.

Dwivedi, Dubey and Dwivedi (1991) isolated *Fusarium solani* from stored guar seeds showing pale- brown discolourations. Seeds with black spots invariably yielded *Colletotrichum dematium* but this symptom (black spots) became prominent only after soaking the seeds in distilled water for 30-35 min.

Varma, Singh and Singh (1992a) reported black discolouration with and without pale areas in *Colletotrichum dematium* infected *Vigna aconitifolia* seeds. Bhatia and Singh (1998) and Kumar (2000) revealed brown discoloured and shriveled seeds of guar yielded predominant growth of *Aspergillus flavus.*

Varma and Shrivastava (2002) revealed seed borne mycoflora of soybean in Hadoti region of Rajasthan. They reported various deformities viz. purple, reddish brown, dark reddish brown, dark brown black, purple black discolorations, brown microsclerotial seeds, pycnidial seeds, pycnidial seeds with whitish growth, shriveled and broken seeds. On incubation reddish brown discolouration were shown by *Fusarium oxysporum* infected soybean seeds.

Sadda and Varma (2010) reported various discolourations in dry seed examination of smooth gourd seeds viz. brown, black, white, small discoloured seeds, shriveled seeds, insect damaged seeds and debris and inert matter. On incubation white discoloured seeds of smooth gourd revealed the presence of *Fusarium oxysporum*.

Gupta, Dubey and Singh (2011) revealed either dark brown discolouration or seeds covered with white mycelial crust showed the infection of *Fusarium semitectum* in seeds and causing wilt disease in *Dalbergia sisso*.

Zaidi and Pathak (2013) reported various seed deformities due to fungal infection in chick pea seeds besides normal looking viz. wrinkled big seeds, wrinkled small seeds and damaged seeds.

Fusarium oxysporum is a highly pathogenic fungus and its different species have been reported to cause various diseases viz. root rot, seedling blight, wilt and damping off in various leguminous crops. It is found to cause various types of discolourations in many crops of Rajasthan, such as brown discolouration with or without mycelial growth in cowpea and moth bean (Varma, Singh and Singh, 1989), redish brown discolouration in soybean (Mathur, 1992), white crust on guar (Bhatia and Singh, 1998), seeds with white mycelial growth in pigeon pea (Sharma, 1996), *Vigna mungo* (Singh, 1997), mung bean (Sharma, 1999), white crust on okra seed (Agrawal, 2000) and dark brown discolouration or mycelial crust in *Dalbergia sisso* (Gupta, Dubey and Singh, 2011).

INCUBATION TESTS

In present studies, 53 fungal species belonging to 28 genera in SBM were found in 120 seeds samples and 40 fungal species belonging to 23 genera in PDA in 35 seed samples in incubation test of cluster bean collected from 11 districts of Rajasthan. This is the largest number of fungi recorded for the first time in the study on seed borne mycoflora of cluster bean and could be attributed to the large number of seed samples of cluster bean. Fungi viz. *Alternaria brassicola, A. tenuissima, Curvularia pallescens, Drechslera halodes, D. maydis, Fusarium moniliforme, Graphium sp., Memnoniella levispora, Mucor sp., Myrothecium verrucaria, Penicillium spp., Thielavia terricola* and *Trichurus spiralis* recorded in SBM, could not be detected in PDA test.

In Rajasthan, Jain and Patel (1969) isolated 8 Fungi in guar seeds viz. *Alternaria sp., Aspergillus sp., Curvularia sp., Helminthosporium sp., Fusarium sp., Penicillium sp., Rhizopus sp.* and *Cephalosporium sps.* While Singh and Solanki (1974) recorded 5 fungi viz. *Aspergillus sp., Fusarium sp., Penicillium sp., Rhizopus sp*. and *Rhizoctonia sp.* Singh and Chouhan (1973) and Karwasara and Singh (1982) isolated 9 fungi from Panjab and Haryana during study of seed borne mycoflora. 22 fungi on guar seeds belonging to different cultivars from Mysore (Shivanna and Shetty, 1988a). Dwivedi and Dubey (1992) recorded 19 and 17 fungi from unsterilized and sterilized stored seeds of guar at Varansi.

Among these fungi *Aspergillus flavus*, *A. fumigatus* and *A. niger* were dominant. Dwivedi and Dwivedi (1994) recorded 25 fungal species on guar seeds using agar plate and blotter method. *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Trichoderma viride* and *Aspergillus terreus* were dominant.

Sharma et al. (1996) reported five isolates of *F.solani* causing root rot in mulberry using PDA. Elwakil and El- Metwally (2001) isolated total 27 fungal species belonging to 17 genera from peanut seeds in Egypt. Among them *Cephalosporium sp., Fusaium moniliforme, F. oxysporum, F. solani, Rhizoctonia solani, Sclerotium bataticola* and *Verticillium sp.* were dominant.

Nagerabi and Elshafie (2001) isolated 59 species and 8 varieties of fungi of 24 genra of cluster bean were found. *Aspergillus*, *Rhizopus*, *Curvularia*, *Alternaria* and *Fusarium* genus were dominant.

Varma and Shrivastava (2002) yielded 52 fungal species belonging to 33 genera in soybean seeds by using SBM. Among them *Alternaria alternata, Cephaliophora irregularis, Colletotrichum dematium, Curvularia lunata, Drechslera tetramera, Fusarium oxysporium, Macrophomina phaseolina, Papulospora coprophila, Rhizoctonia bataticola* and *Trichothecium roseum* were dominant.

Singh, Chandil and Tripathi (2005) reported association of 8 fungal species in guar seeds viz. *Alternaria cyamopsidis, Aspergillus sp., Chaetomium sp., Curvularia lunata, Memnoniella echinata, Penicillium vinaceum, Rhizopus oryzae* and *Strachybotrys sp*. Among them *Alternaria cyamopsidis* and *Aspergillus sp.* were found to be dominant. They also reported grayish to dark brown circular patches on leaves by *Alternaria cyamopsidis*.

Begum et al. (2007) isolated 17 fungal species belonging to 11 genera by using blotter and PDA method in naturally infected soybean seeds. Among them *Colletotrichum truncatum, Diaporthe phaseolorum* and *Fusarium oxysporum* were found in higher frequencies (Nasir, 2003; Agarwal et al., 2006).

Habib et al. (2007) yielded various fungi including *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum* and *F. solani* from 25 seed samples of different varieties of egg plant.

Sultana and Ghaffar (2007) isolated 29 fungal species of 15 genera in bitter gourd. Out of which *Aspergillus spp., Chaetomium spp., Cladosporium spp., Fusarium semitectum, Fusarium solani and Rhizopus sp.* were dominant pathogens.

Farrag and Moharam (2012) isolated 6 fungal species viz. *Alternaria alternata, Helminthosporium oryzae, Fusarium oxysporum, F. solani, Penicillium italicum* and *Rhizoctonia solani* from cucumber seeds. Among them *Fusarium oxysporum, F. solani* and *Rhizoctonia solani* were dominant.

Ratod et al. (2012) used SBM and agar plate method for detection of seed borne mycoflora of *Arachis hypogea* (Ground nut) and isolated 16 fungi out of which *Alternaria tenuis, Aspergillus flavus, A. niger* and *Fusarium oxysporum* were dominant.

Embaby et al. (2013) isolated eight fungal species viz. *Aspergillus flavus, A. niger, A. paarsiticus, Fusarium moniliforme, F. oxysporum, Fusarium spp., Penicillium spp.* and *Sclerotinia sclerotiorum* from thirty seed samples of *Phaseolus vulguris, Pisum sativum* and *Glycine max.* Out of these *Aspergillus flavus* was most prominent in *Phaseolus vulguris* and *Pisum sativum* seeds whereas *Aspergillus niger* in *Glycine max* seeds. Earlier five fungal genera i.e. *Alternaria, Aspergillus, Epicoccum, Fusarium and Trichoderma* were isolated from some legume seeds of beans, cowpea, and lupine (Embaby and Mona, 2006).

Ghangaokar and Kshirsagar (2013) recorded 40 fungal species from seven samples of legume seeds viz. *Pisum sativum, Macrotyloma uniflorum, Lens culinaris, Phaseolus vulgaris, Vigna unguiculata, Cajanus cajan* and *Cicer arietinum*. Out of which *Aspergillus flavus, A. niger, Alternaria alternata, Fusarium moniliforme, Rhizopus nigricans* and *Trichoderma viride* were common seed borne fungi in all legume seeds.

Sharma et al. (2013) isolated 32 fungal species and 3 bacterial species on okra seeds from Jaipur (Rajasthan). *Actinomycetes, Arthrobotrys supberba, Aspergillus fumigates, Cladosporium oxysporum, Drechslera sp., Fusarium moniliforme, Stachyobotrys spp., Verticillium alboatrum* were dominant.

Zaidi and Pathak (2013) isolated 13 fungal species during incubation test from chickpea seeds. Among them *Alternaria alternata, Aspergillus flavus, A. niger, Chaetomium spp., Fusarium oxysporum, Penicillium citrinum* and *Rhizopus nigricans* were dominant.

Ashwini and Giri (2014) reported association of 10 fungi belonging to 8 genera viz. *Acremonium strictum, Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium oxysporum, Fusarium semitectum, Fusarium solani, Macrophomina phaseolina, Phoma medicaginis, Penicillium* sp. and *Rhizopus* sp. by using blotter paper method in green gram (Ali et al., 2010). Similar seed borne fungi were observed in mung bean by Barua et al. (2007).

Varma and Sadda (2015) reported association of seed borne fungi with the discoloured seeds of *Luffa aegyptica*. They isolated 29 fungal species belonging to 18 genera in incubation test. *Among them Alternaria alternata, Chaetomium globosum, C. spinosum, Colletotrichum orbiculare, C. dematium, Curvularia lunata, Drechslera halodes, D. bicolor, Rhizoctonia bataticola, R. solani, Rhizopus nigricans* and *R stolinifer* were dominant.

Of the total fungi recorded in the present study, *Alternaria alternata; Aspergillus flavus; A. niger; Cladosporium oxysporum; Curvularia lunata; Fusarium oxysporum; F. solani; Rhizoctonia solani* and *Rhizopus nigricans* in untreated and pretreated seeds in SBM and PDA were important and showed high percentage and occurrence in cluster bean seed samples collected from various districts of Rajasthan. They are also known to cause various diseases in cluster bean.

High incidence of *Alternaria alternata* was found in the samples of Jaipur, Jhunjhunu, Jodhpur and Sikar; *Aspergillus flavus* in Jaipur, Churu, Nagaur and

Sikar; *A. niger* in Jaipur, Sikar and Nagaur; *Cladosporium oxysporum* in Jaipur, Sikar and Jhunjhunu; *Curvularia lunata* in Jaipur, Nagaur and Sikar; *Fusarium oxysporum* in Bikaner, Churu, Jaipur, Jhunjhunu, Nagaur and Sikar; *F. solani* in Jaipur, Sikar, Shriganganagar and Jhunjhunu; *Rhizoctonia solani* in Churu, Jaipur, Jhunjhunu and Sikar; *Rhizopus nigricans* in Churu, Jaipur, Jhunjhunu and Sikar. The seed samples of Jaipur and Sikar carried high infection percentage of all these fungus. In general, the samples of Jaipur and Sikar carried heavy infection of fungi in comparison with seeds collected from other districts of Rajasthan. High humidity and temperature during the rainy seasons might favour the development of disease leading to heavy infection of these districts. However according to Mihail and Alcorn (1986) concurrent heat and water stress favoured the development of the charcoal rot disease of guar caused by *Macrophomina Phaseolina*.

Fusarium solani is the major pathogen of cluster bean crop in the Rajasthan state during present study. Total 57 samples were infected with *F. solani* showing 0.25-62% incidence in SBM. Out of these 13 and 18 seed samples were infected with *Fusarium solani* collected from Jaipur and Sikar districts respectively and showing 0.5-58% and 0.25-62% incidence respectively. The heavy infection of *F. solani* suggested that the pathogen have wide occurrence in Rajasthan state.

EFFECT OF SODIUM HYPOCHLORITE PRETREATMENT ON SEED-BORNE FUNGI IN BLOTTER METHOD

The pretreatment of seeds with 0.5% available chlorine in blotter test was useful in reducing the incidence of superficial and fast growing as well as common seed borne fungi. The growth of fungi was rendered sparse hence permitted easy recording of the pathogens. All the fungi recorded in untreated were commonly recovered in pretreated seeds. Total elimination or decrease in incidence of parasitic fungi as suggested by Limonard (1968) was not recorded in the present study. Saxena, Kumari and Karan (1982) revealed that untreated seeds showed highest percentage of fungi than the pretreated seeds in blotter test. Teggi and Hiremath (1990) reported reduction in seed mycoflora due to surface

sterilization. Varma (2003) reported the seed borne inoculum of *Rhizoctonia bataticola* in moth bean seeds and also suggested that the chlorine pretreated standard blotter test was good for evaluating the seed borne inoculum of pathogen. Similar results were also observed by Sultana and Ghaffar (2007).

Niaz and Dawar (2009) observed that surface disinfection of seed with 1% $Na(OCl)_2$ reduced the incidence of *Aspergillus* spp., *Chaetomium* spp., *Rhizopus spp.* and *Cephalosporium* spp. Surface sterilization also has the advantage of minimizing competition among fungi on the rice seeds (Kaur, 2010).

In present study incidence of *F. solani* fungus were recorded 0.25-62% in untreated seeds and 0.25-35% in pretreated seeds (SBM) and 0.25-30% in PDA tests.

PHYTOPATHOLOGICAL EFFECTS OF SEED BORNE FUNGI

Seed borne infection of various fungi caused an adverse effect on the seed germination of the cluster bean seeds and resulted in symptomatic seedling. The main fungi hindering seed germination in cluster bean were *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium oxysporum, Curvularia lunata, Fusarium oxysporum, F. solani, Rhizoctonia solani* and *Rhizopus nigricans.*

Fusarium solani infection caused radical browning and black discontinuous streaks on hypocotyls. Seeds showed white mycelial growth. Yellow-brown to black discolouration appears on stem affected with wilt disease. Symptomatic seedling showed characteristic yellowing and drying. Azaz, Azam and Ansari (2001) reported similar symptom on cucurbits due to *Fusarium oxysporum*.

Sinha and Prasad (1981) reported adverse effects on seed germination of Mung due to *Alternaria alternata, Bortyodiplodia theobrome, Curvularia lunata, Fusarium moniliforme* and *Macrophomina phaseolina*. Inhibition of seed germination and yellowing of seedling by seed borne inoculum of fungi including *Alternaria alternata, Aspergillus niger, Fusarium oxysporum, F. moniliforme, Rhizopus stolinifer* (Chandiram and Maheshwari, 1992). The infection of

Aspergillus flavus and *Alternaria alternata* caused browning of radicle, brown to black streaks on hypocotyls and finally rotting of seed and seedling. It also reduces the percent germination or sprouting of cucurbits (Pandey and Gupta, 1986). Shanker and Rao (1995) studied effects of soaking of *Vigna radiata* L. seeds for six hours in culture filtrates of *Aspergillus niger* and found that it caused reduction in seed germination. *Rhizoctonia solani* and *R. bataticola* infection caused brown black streaks on transition zones, radicle and hypocotyls, browning and finally rotting of basal part of hypocotyls. Similar symptoms were observed on black gram due to *Rhizoctonia solani* by Singh (1997). Howlett (2006) reported toxins of the seed-borne fungi responsible for inhibition of normal growth of seedlings in different crops. Anjorin et al. (2009) revealed poor germination and seedling caused by *Rhizopus nigricans* in watermelon.

HISTOPATHOLOGY OF NATURALLY INFECTED SEEDS

Fusarium solani (Mart.) Sacc. was first described by Martius (1842) from rotted tubers of potato (*Solanum tuberosum*). The species was transferred to the genus *Fusarium* by the Italian mycologist Saccardo (1881). *F. solani* was emended by Snyder and Hansen (1941) to comprise a complex group of species that are widely distributed in soils and cause tuber, root, and stem rots of plants worldwide. **(**Luginbuhl, 2010; Desjardins, 2006).

Present study deals with the location of dominant pathogen viz. *Fusarium solani* carried out in cluster bean seeds is a detailed and critical attempt which determines the exact penetration in various seed tissues. In order to appreciate the comparison, the following features deserve attention. (i) An extensive survey preceded the studies on location. (ii) Selected seed samples carrying various degrees of infection were used. (iii) Seeds were categorised as symptomatic (weakly, moderately and heavily) and healthy looking asymptomatic. (iv) The techniques of component plating, cleared wholemount preparation (Singh, 1983) and microtome sectioning (Johanson, 1940) were followed.

The study has proved the accurate determination of incidence and spread of inoculum in seed. Component plating and incubation were used for incidence.

Cleared wholemount preparation are good for preliminary detection of mycelium but the detailed information on location and distribution of mycelium in seed tissue can be obtained only from microtome sectioning.

Fusarium solani caused a severe wilt, root rot and damping off and resulted a significant loss of cluster bean crop (Satyaprasad and Ramarao, 1981). The seed-borne nature of *F. solani* was reported on guar crop by Dwivedi, Dubey and Dwivedi (1991). They also reported brownish discolouration of *F. solani* infected cluster bean seeds. *Fusarium solani* is widely found most important soil borne as well as seed borne pathogen causes various diseases such as root rot, wilt, damping off in vegetables, legumes, oilseeds and ornamental crops and causes severe losses to economic, medicinal and industrial value of the crop (Sultana and Ghaffar, 2007; Abu-Taleb et al., 2011; Khair et al., 2011; Bahar and Shahab, 2012; Karima and Nadia, 2012; Hafizi et al., 2013).

In present study, asymptomatic seeds revealed the infection of pathogen, either restricted to seed coat or found with very low incidence in hilar region while symptomatic (weakly, moderately and heavily infected) seeds carried inoculum in all components of seed and its distribution was directly correlated to the degree of infection. The mycelium in weakly infected seeds was localized in different layers of seed coat and hilar region. The cells of cotyledons revealed some hyphal bits. Hyphae and chlamydospores were also observed in hilar region. Embryonal axis also showed cell division as a result of fungal invasion. Similar observation was also reported *Fusarium oxysporum f.sp. udam* in pigeon pea by Kumar et al. (2001), in watermelon seeds by Boughalleb and El- Mahjoub (2006), in *Dalbergia sisso* by Gupta, Dubey and Singh (2011) and in cucumber seeds by Farrag and Moharam (2012).

In moderately and heavily infected seeds carried both inter as well as intra cellular mycelium in all the parts including embryo (seed coat, cotyledons, hilar region, endosperm and hypocotyl shoot root axis). Dense growth and mycelial bits was also observed in hourglass layer and parenchyma. Disintegration, lysis and vacuolation of cells and tissues were observed in epidermal and hypodermal cells of heavily infected seeds during present study. This is because of the activity of

pectolytic and cellulolytic enzymes produced by the pathogen as reported by Goel and Mahrotra (1974). It may be recorded in present study that seeds of moderately and heavily infected categories usually failed to germinate.

The presence of abundant mycelium in hilar region, stellate parenchyma and cotyledon region is suggestive that the pathogen penetrates the seed through the hilar region and from there spread rapidly. Its entry through the creaks in seed coat is not ruled out. Singh and Sinclair (1985) observed that the penetration of *Cercospora sojina* in soybean seeds takes place through seed pores and hilar trachids. Carlson (1973) reported that heavy aggregation of fungal mycelium in the hilar region may be accounted to the high moisture content of these tissues and high level of gaseous exchange takes place during seed development. Nemec (1978) observed *Fusarium solani* infection on rough lemon and revealed the presence of chamydospores in xylem and cortex. Hyphae penetrated the cortex intracellulary as well as intercellulary.

Bottle gourd seed yielded *Fusarium sp.* mostly from micropylar end, which is the region of placental attachment (Palodhi and Sen, 1983). Hyphal strands were also produced in abundance from the setures. They also reported seed infection was confined to testa and tegmen in asymptomatic seeds but heavily infected seed had inoculum in embryo also. These results showed that pathogenic *Fusarium sp.* was not borne in embryo but presence of mycelium at the point of seed attachment is very significant for germinating seedling.

Varma, Singh and Singh (1989) reported infection of *Rhizoctonia solani* localized in seed coat and hilar region in symptomless seeds of moth bean while it was observe deep seated and showed microsclerotia and inter and intracellular mycelium in all components of symptomatic seeds viz. seed coat, cotyledon and hypocotyl radicle axis. Weakening and depletion of cell contents were also observed in severe infection of pathogen. Similar observations were reported in rubber seeds infected with *Botryodiplodia theobromae* by Varma, Singh and Singh (1990). Thin hyaline, branched septate mycelium and conidia in *Trichothecium roseum* infected cowpea seeds in cleared whole mount preparation. In microtome sectioning both inter and intracellular mycelium and hyphal bits

were observed in all seed components of symptomatic seeds (Varma and Singh, 1991).

In *Vigna aconitifolia* seeds hyaline, pale brown to dark brown and septate mycelium of *Colletotrichum dematium* were seen in all seed components (Varma, Singh and Singh, 1992a). Whereas in cowpea seeds few to many pinhead like black microsclerotia and mycelium on surface infected with *Rhizoctonia bataticola*. Presence of profuse, thick, knotty, hyaline, dark brown, branched, septate mycelium and microsclerotia were seen in cleared wholemount preparation of all seed parts infected with *Rhizoctonia bataticola*. In microtome sectioning, hyphal mat and immature and mature microsclerotia were seen below the palisade layer in seed coat of weakly infected seeds. In moderately to heavily infected seeds the dense mycelial growth interlocked the seed coat with cotyledons. The fungus colonization was observed in hypocotyl shoot root axis. Disintegration of cells was seen in all severely infected seed parts. Similar observations were reported for moth bean seeds infected with *Rhizoctonia bataticola* (Varma, Singh and Singh, 1992b,c). Kumar et al. (2001) studied seed infection of *Fusarium oxysporum f.sp. udam* in pigeon pea. They reported that in asymptomatic seeds the mycelium remained confined to seed coat and hilar region. Heavily infected symptomatic seed carried inter as well as intracellular mycelium in all seed components. Mycelial mat, chlamydospores, necrotic cells and lytic cavities were also reported in heavily infected seeds.

Varma (2002) reported deep seated infection of *Rhizoctonia bataticola* in *Vigna aconiitifolia* seeds and showed microsclerotia and inter and intracellular mycelium in different layers of seed coat, hilar region, aleurone layer, cotyledons and hypocotyls shoot root axis. She also reported direct penetration of fungal hyphae through the seed coat whereas Sultana et al. (2009) reported the infection of *Macrophomina phaseolina* in all the seed components of cucumber seeds. In *Dalbergia sisso* infection of *Fusarium semitectum* restricted to layers of seed coat in asymptomatic seeds and it invades endosperm and embryonal axis in symptomatic seeds (Gupta, Dubey and Singh, 2011). Similar effects were also observed in seeds of moth bean (Varma, Singh and Singh, 1989); soybean

(Sharma, 1992); Guar (Bhatia, Singh and Singh, 1998); *Abelmoschus esculantus* (Agrawal, 2000);; wheat (Kaur, Singh and Chahal, 2007); in smooth and ridge gourd (Sadda, 2012) and in lentil (Singh 2013).

Farrag and Moharam (2012) revealed both internal and external heavy infection of *Fusarium oxysporum* in seed coat and cotyledons and minimum infection was observed in embryo of cucumber seeds. Similar results were reported in watermelon seeds by Boughalleb and El- Mahjoub (2006).

Thus the present study clearly indicates that inoculum of *F. solani* is occurs in asymptomatic seeds as well as in symptomatic seeds. In the asymptomatic seeds the incidence remains low and infection confined to seed coat. In symptomatic seeds the infection is intra-embryonal and deep seated and distributed in all the seed components. The deep infection is difficult to inactive using normal conventional methods of control.

PHYTOPATHOLOGICAL EFFECTS AND DISEASE TRANSMISSION

In guar seeds phytopathological effects and disease transmission have not been studied in detail. Singh (1951) reported *Fusarium caeruleum* causing wilt in guar while Prasad and Desai (1951) observed blight due to *F. moniliformae*. Root rot by *F. solani* by Satyaprasad and Ramarao (1981) and Mathur and Sekhawat (1988). Ramarao (1983) tested 30 varieties of guar for disease transmission of *Fusarium solani*. Dwivedi, Dubey and Dwivedi (1991) observed damping off caused by *F. solani* as a seed borne inoculum of guar.

The present study on the basis of data from blotter, water agar, pot and field experiment has clearly shown that seeds of cluster bean infected with *Fusarium solani* gave poor germination and seedling underwent pre and post emergence mortality. The pre- emergence mortality was higher than post emergence mortality due to the infection of wilt disease caused by *Fusarium solani*. It is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. The margins of the cotyledonary leaves curl downward and

inwards. Entire seedling appeared dried and collapsed. The stem near the soil line may be slightly thickened and brittle. Brown to black discolouration appears on stem affected with wilt disease. Seeds covered with white mycelial growth. The failure of seed germination and incidence of seedling mortality are correlated with degree and nature (superficial to deep) of infection. The total loss was very high in heavily infected seeds than the asymptomatic seeds.

Initial symptoms appear as white mycelial growth on seed coat. Dark brown patches on root shoot transition zone which progressed up towards hypocotyls and downwards to radicle. The fungal infection resulted in brown radicle, discontinuous black streaks on hypocotyls and necrotic spots on cotyledons. The association of infected seed coat with cotyledons also resulted in spread of infection to cotyledons and hypocotyl. Similar effects were also observed in guar seeds (Bhatia, 1995), in maize seeds (Basak and Woong, 2002), in *Dalbergia sisso* (Gupta, Dubey and Singh, 2011), in cucumber seeds (Farrag and Moharam, 2012) and in lentil (Singh, 2013). Kumari and Karan (1981) observed lesions on cowpea seedlings infected with *Fusarium oxysporum* (Varma, 1990). Shivanna and Shetty (1988a) reported blight and damping off symptoms resulting into pre and post emergence mortality in cluster bean seedlings infected by *C. dematium*.

In pot experiment, mortality rate was increased gradually and none of the heavily infected seedling could survive after five days of emergence. The symptomatic seedling developed characteristic symptoms and mycelium on plumule and radicle region. Initial symptoms appeared as yellow brown patches on cotyledonary leaves. The seedling from asymptomatic seeds show low intensity of symptoms but the seedlings raised from symptomatic seeds showed wilting progressed from above ground part of stem to lower part of seedling. The wilt symptoms appeared in pots after 30-40 days of sowing. The infected plant leaves showed yellowish brown patches on their surface followed by their shrivelling and drooping. The infected root showed browning at transition zone. Basal part of the stem near the collar region showed brown black patches, black streaks on pods and entire plant seedling produced by symptomatic seeds

appeared dried and collapsed. Similar observation was reported in chickpea seeds by Haware et. al. (1978). Pande, Rao and Sharma (2007) observed wilt symptoms caused by *Fusarium oxysporum f. sp. Ciceris* in all pots after 41 days of sowing in chickpea seeds.

In field experiment, seed germination was observed maximum in asymptomatic seeds than symptomatic seeds. Seedling produce from symptomatic seed showed yellow brownish necrotic patches on the leaves followed by their shriveling, dryness and drooping ultimately resulting in the death of such plants. This was observed as initial stage of wilting. Black or brown discoloration was observed on infected root and Brown blackened patches appear on basal part of infected stem near the collar region. The pod harvested from the infected plants had black patches and produced infected seeds. As the disease progresses the patches become larger and eventually the cotyledons, stem and leaves dry up and die. These symptoms develop without notice still in the field and continue to progress after harvest, resulting in infected pods in storage. Pale brown patches were also observed in split half of infected samples. Similar findings were reported in moth bean infected with *Rhizoctonia bataticola* (Varma, 2003), sponge gourd and ridge gourd infected with *Colletotrichum orbiculare* (Sadda, 2012), in lentil infected with *Fusarium oxysporum* (Singh, 2013) and in tomato plants infected with *Fusarium oxysporum* (Askar et al., 2014).

Coskuntuna and Ozer (2004) were found *Aspergillus niger* and *Fusarium equiseti* at high rates in all Hungarian vetch (*Vicia pannonica* Crantz) plant parts during field experiment. Seed-borne fungi are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. The infected seeds may fail to germinate, or transmit disease from seed to seedling and/or from seedling to growing plant (Islam and Borthakur, 2012). In barley crop during field experiment, Ramadan and Zrary (2014) reported significant increase in the effect of six fungal pathogens viz. *Aspergillus candidus*, *A. niger*, *A. sulphurous*, *Cladosporium herbarium*, *Curvularia lunata*, *Drechslera tetramera* and *Penicillium sp*. on pre-emergence damping-off seedlings with soil infestation.

Singh and Chouhan (1973) reported formation of stubby roots without root hair and browning of stem due to *Aspergillus flavus* infection in cluster bean seedlings whereas *Colletotrichum dematium* produced irregular black streaks on hypocotyls shoot root region and transition zone. Satyaprasad and Ramarao (1981) and Mathur and Shekhawat (1988) were reported yellowing and drooping of leaves and black lesions at soil surface of root of guar in *F. solani* infected plant. Nene et al. (1991) reported dark brown discolouration of internal xylem of roots when split open vertically in chickpea. Till than roots of wilting plants do not show any external rotting whereas Varma (2003) reported pre and post emergence losses in symptomatic seeds infected with *Rhizoctonia bataticola* was high than the asymptomatic seeds of moth bean. Initial symptoms appeared on transition zone as brown black patches on $3^{rd} – 4^{th}$ day in symptomatic seeds. It progressed to hypocotyls and root causing brown streaks, and circular or oval concentric spots on cotyledons. Sclerotia were observed on dry necrotic tissues of leaves. Pods harvested from infected plant carried sclerotia on outer and inner surface of pericarp. Sultana, Azeem and Ghaffar (2009) revealed the transmission of *Macrophomina phaseolina* from seed to seedling and cause pre and post emergence loss in cucumber seeds. Seeds which failed to germinate were found to be covered with sclerotia of pathogen. Partial emerged seedlings were rotted and blackened.

Rajput et al. (2010) reported the colonization percent of *Fusarium solani* was highest as compared to other fungi in shisham tree and maximum reduction in seed germination was also reported. In smooth gourd Sadda and Varma (2010) reported anthracnose caused by *Colletotrichum orbiculare* showed the small, water soaked or yellowish patches on older leaves and the leaves at the centre of plant often attacked first than leaving stem and runners bare.

High pre and post emergence losses in *Dalbergia sisso* infected with *Fusarium semitectum* causing wilt disease like necrotic spots on cotyledonary leaves and browning of radicle. Later these spots were turned into wilting of seedling/ plant and succumb to death (Gupta, Dubey and Singh, 2011).

Farrag and Moharam (2012) reported transmission of pathogenic fungi of cucumber seeds viz. *Fusarium oxysporum, F. solani* and *Rhizoctonia solani* from the germinated seeds to seedling causing pre and post emergence death. The transmission rate of tested fungi causing seed rot or pre emergence death was higher than the seedling mortality. Symptoms were observed after 10-15 days of inoculation with *Fusarium oxysporum* as linear cortical lesions on died seedlings or vascular wilt on the plants and ultimately caused seedling death and in maize seeds by Basak and Woong (2002). Pale yellow discolouration on hypocotyl was observed in lentil seeds by Singh (2013). This discolouration spreads rapidly on shoot than the primary root region. Similar observations observed in bottle gourd by Kuniyasu and Kishi (1977). Sharma and Sharma (2014) revealed that the seed borne inoculum of *Ralstonia solanacearum* caused pre- and post-emergence losses in brinjal seeds and the symptoms appears as browning of radicle, plumule later turned into necrotic spots with bacterial oozing. Similar symptoms on seeds were reported earlier in tomato (Sharma and Agarwal, 2010) and in cluster bean (Jain and Agarwal, 2011).

In the present study the cleared wholemount preparation of radicle and hypocotyl also showed intercellular mycelium in cortical and vascular regions. In leaf the hyphae was mostly localized in mesophyll cells. The fungus was also detected from cortical region of the radicle. Transverse hand cut sections of root, stem, leaves and pods revealed inter as well as intracellular mycelium in epidermis, cortex, xylem, vessels and in pith tissues. Seed setting is very less in the infected pods with small, shriveled and infected seeds. The harvested seeds were showed white mycelial growth. Split half of the stem and root showed the presence of fungal hyphae. Satyaprasad and Ramarao (1981) also observed an internal brown discolouration in split half of root of cluster bean.

Enough evidences of phytopathological effects of seed borne nature of *Fusarium solani* and its transmission from seed to seedling/plant was observed in present study. Weak infection transmitted to plants with wilt symptoms and drooping of leaves while heavy infection causes failure of seed germination or low germination. These observations revealed that seed borne inoculum play an

important role in disease transmission. Pod and seed harvested from weakly infected plant were found free of infection but moderately to heavily infected plants were produce infected and abortive pods and seeds.

BIOCHEMICAL ANALYSIS

Biochemical study revealed alteration in metabolism of the host tissues at various stages of disease development caused by the pathogen. The pathogen alters the metabolism of host cell by releasing its secretion which consist enzymes and toxins with the disease development. Metabolism equilibrium is established between host and parasite during the localized phase of infection. In present study biochemical estimation of *Fusarium solani* infected plant parts and healthy plant parts of cluster bean were comparatively analyzed regarding changes in different parameters. These tests were applied to estimate changes in important constituents of seeds like total protein, total starch content and total phenol content and their related enzyme.

The deterioration of its food reserves and other constituents caused by fungi associated with seed and their effect on mycoflora on ground nut seeds (Gupta and Chauhan, 1970; Kamble and Gangawane, 1987), in *Cajanus* seeds by Sinha, Singh and Prasad (1981) and in wheat by Agarwal, Thakur and Awasthi (1982), in soybean seeds by Varma (2003), in sorghum by Dicko et al. (2005), in sugarcane by De Armas et al. (2007), in chilli by Anand et al. (2009), in tomato by Baker et al. (2010), in lentil by Singh and Varma (2010) and in *Luffa* seeds by Sadda and Varma (2011) in chickpea by Sharma et al. (2011) .

Total Protein Contents and Protease activity

In present study total soluble protein in *F. solani* infected leaf, stem and seed were lower than the healthy leaves, stem and seeds of cluster bean respectively while protease enzyme activity were higher in *F. solani* infected leaves, stem and seeds than healthy counter parts.

Shukla, Dube and Tripathi (1988) found that the protein content of arhar seeds with a mycelial mat was lower than the asymptomatic seeds. Agarwal (1989) reported reduction in protein contents after inoculation with *Phytophthora drechsleri f. sp. cajani* in the resistant cultivar of pigeon pea, in sesame (Singh et al., 1972); in taramira and safflower (Singh and Sinha, 1979); in sunflower seeds (Pustovoit and Borodin, 1983); in pea (Singh et al., 2005) and in lentil (Singh, 2013). Dubey (1984) studied on protease activity in diseased tissue. Sujathamma and Reddy (1985) reported high protein content in groundnut hypocotyl due to infection of *Rhizoctonia solani*. Increase in protein contents at early stage of infection was reported by Agarwal et al. (1982), Srivastava and Dhawan (1985). Alteration in protein metabolism of plant tissue during pathogenesis has been reported by Bhatnagar (1992), Debnath (2000) and Yadav (2003). Yadav et al. (1996) studied the changes in protein content of mustard plant parts infected with *Albugo candida*. The fungi deteriorated biochemical qualities of seed due to change of nutritional profiles such as protein content and decrease the value for sowing, as food or feed. Varma (2003) reported that storage fungi cause deterioration of the soybean seeds by affecting planting and edible value. Both asymptomatic seeds and symptomatic seeds of soybean infected with *Rhizoctonia bataticola* showed initial enhancement of protease activity. Later it declined continuously in symptomatic seeds. Adenkunle et al. (2008) reported insignificant increase in protein content of unhealthy seeds of melon than the healthy seeds. Begum et al. (2008) reported that the fungi has strong ability to infect all components of seeds and therefore as reflected by reduction of seed quality parameters. Sadda and Varma (2011) reported higher protein contents in *Colletotrichum orbiculare* infected *Luffa cylindrica* plant parts than the healthy counterparts whereas protease enzyme activity was higher in case of healthy plant parts than the anthracnose infected counterparts. Akhtaruzzaman et al. (2012) were isolate and characterize the protease from 7 leguminous seeds viz. soyabean, lentil, black gram, bengal gram, groundnut and pea. Highest concentration was observed in groundnut and lowest concentration was observed in lentil.

Starch Contents and α -Amylase enzyme activity

In present study the starch content in infected leaves, stem and seeds were also lower than healthy leaves, stem and seeds respectively and the alpha amylase enzyme activity were recorded higher in infected leaves, stem and seeds than healthy leaves, stem and seeds respectively. Similar changes in carbohydrate reported in *Mangifera indica* by Karnawat and Kant (1990), in *Braassica juncea* by Debnath (2000), in tree species by Mathur (2002), in cauliflower by Singh and Sexena (2003), in soybean by Varma (2003), in lentil by Singh and Varma (2010) and in *Luffa cylindrica* by Sadda and Varma (2011).

According to Bateman and Millar (1966) sugars play an important role in the inhibition of pectinolytic and cellulolytic enzymes which are essential for pathogen.

Purohit et al. (1980) and Rao (1989) reported significant increase in hydrolyzing enzyme activity in MLO induced phyllody in sesame. Similar increase in α -amylase activity in leaf spot disease of turmeric caused by *Taphrina maculans* were also observed by Agarwal et al. (1982).

Singh et al. (1990) observed more amount of total sugar in susceptible varieties of various pulses due to infection of *Aspergillus flavus*. Starch, the primary storage material in most seeds has been studied for its deterioration by mycoflora in many crops like rice (Kondo and Okamura, 1934), *Cajanus* seeds (Sinha, Singh and Prasad, 1981), wheat (Agarwal, Thakur and Awasthi, 1982) and Groundnut (Kamble and Gangawane, 1987). Decrease in starch content also observed in pigeon pea seeds due to *Aspergillus* infection (Sinha and Prasad, 1977; Sinha, Singh and Prasad, 1981). Sharma (1992) reported that α- amylase activity was high in infected seed than the healthy seeds of soybean. Jain and Yadav (2003) reported positive significant correlation of leaf infection with total and reducing sugars in finger millet. Singh and Saxena (2003) also reported similar type of disruption in cauliflower plant cells. They suggested that accumulation of total soluble sugar in infected tissue may involve their translocation from the adjacent healthy tissue. Similar findings were also reported

by Marmit and Sharma (2008) for induced leaf galls tissues of *Mangifera indica*. Varma (2003) revealed that the amount of starch was low in the *Fusarium oxysporum* infected soybean seeds and seedlings as compared to their healthy counterparts. She also reported initial increase in starch content during the initiation of seed germination. Varma (2003) reported high α- amylase activity in *Rhizoctonia bataticola* infected soybean seeds at initial stage later it becomes lower than the healthy ones. Yadav (2003) and Sharma (2004) reported that carbohydrate may accumulate in "physiological sink" (galls) by depletion of starch due to α- amylase and other enzymes activity.

Singh (2004) reported increase in α- amylase activity in leaf spot disease of *brassica*. High level of α- amylase might be attributed to the involvement of fungus in enzyme activity. Thus, correlation established between amino acid content and pod damage was positive and significant. High level of α- amylase might be attributed to the involvement of fungus in enzyme activity. Thus, correlation established between amino acid content and pod damage was positive and significant. Such a relation was established in general either the infection by fungal diseases (Kushwaha and Narain, 2005).

Marmit and Sharma (2008) observed higher total soluble sugar, starch and α- amylase activity in insect induced leaf galls tissue than the normal tissue of *Mangifera indica*. Parashar and Lodha (2008) revealed lower starch content and higher α- amylase activity in *Ramularia foeniculi* infected fennel (*Foeniculum vulgare*) plant parts than the healthy counterparts. Afiukwa et al. (2009) reported about determination of amylase activity of crude extracts from germinated mango seeds. Sawant and Gawai (2011) reported that the fungi influenced stored substance or nutrient by absorbing them or by converting some of the substance complex into simple one. Singh et al. (2011) reported increased activity of α-amylase in *Albugo candida* infected tissues of *Brassica* plant parts. The increase activity of these enzymes might be due to their enhanced synthesis by the host to meet the catabolic reactions in the enhanced state of host metabolism after infection. Nwaukwu et al. (2012) reported that the pathogenic fungi affect the

nutritional composition of the edible fruit of *Dialium guineense*, a fruit mostly eaten in Africa. The reduction in nutrient components is due to disease pressure.

Shrivastav and Kumar (2013) revealed losses in reducing sugars and α-amylase activity after infection of *Botrytis* in onion and *Colletotrichum* in capsicum.

Phenolics Contents and Poly Phenol Oxidase activity

Several workers (Cruickshank and Perrin, 1964; Bhatia, Uppal and Bajaj, 1972; Chopra, Jhooty and Bajaj, 1974; Nemec, 1978) have emphasized a definite correlation between degree of resistance and level of phenol while Singh, Nagra and Mehrotra (1982) observed that phenol have no relation with resistance.

In the present study total phenol content were observed little higher in infected leaves, stem and seeds than the healthy leaves, stem and seeds while the poly phenol oxidase enzyme activity were recorded higher in healthy leaves, stem and seeds than infected leaves, stem and seeds respectively. Total phenolic compounds were also increased with disease severity as compared to control. The deterioration of its food reserves and other constituents were clearly obtained.

Kamble and Gangwane (1987) reported increase in total phenol content in ground nut seeds due to infection of *Curvularia lunata*, *Aspergillus flavus*, *Penicillium fumiculosum*, *P. varians* and *Fusarium oxysporum*. Singh and Shrivastav (1988) have showed increase in total phenol content in seedlings of moth bean infected with *Macrophomina phaseolina*. The increase in quantity of total phenols might be attributed with defense mechanism (Jain and Yadav, 2003). Agarwal (1989) observed that total phenol content in pigeon pea increased after infection of *Phytophthora drechsleri* in resistant variety whereas it was reduced in leaves of susceptible variety. Rao and Panwar (2001) revealed decrease poly phenol activities in *Sesamum indicum* infected with *Curvularia phaseoli*.

Saharan et al. (2001) observed phenolic compound and oxidative enzymes in cluster bean leaves infected with *Alternaria* blight. They were reported that the amount of poly phenol oxidase (PPO) increased with the increase in intensity of

Alternaria blight up to 50% in highly susceptible varieties as compared to their respective healthy leaves. Similar observations were reported in cluster bean by Joshi, Gupta and Singh (2003) during study on root and leaves infected with *Macrophomina phaseolina* and by Joshi, Gupta and Kumar (2004) during study on *Alternaria* blight.

Jain and Yadav (2003) reported the increase in quantity of total phenols quantity of total phenol in infected finger millet plant parts might be attributed to defense mechanism (Parashar and Lodha, 2007).

Varma (2003) reported that both asymptomatic seeds and symptomatic soybean seeds infected with *Rhizoctonia bataticola* showed initial increase in phenol content but the amount was always low in symptomatic seeds than the asymptomatic during germination whereas peroxidase activity was high in asymptomatic soybean seeds than the symptomatic seeds infected with *Rhizoctonia bataticola*. Singh (2004) reported increase in the phenolics in relation to resistance in *Brassica* (Ghosal et al., 2004).

Accumulation of total phenol caused the hyperphenolicity in infected resistant host tisuue in pearl millet infected with *Sclerospora graminicola* whereas polyphenol oxidase activity increased (Arun, Mali and Manga, 2010).

Singh and Varma (2010) estimated total phenol and related polyphenol oxidase enzyme activity in *Lens culinaris Medic* infected with *Fusarium oxysporum* and reported higher phenol contents in infected plant parts than the healthy plant parts whereas poly phenol oxidase activity was higher in normal plant parts viz. leaf, stem, fruit and seeds compared to infected counterparts.

Anjum, Fatima and Amjad (2012) determined the dynamics of total phenolic content and polyphenol oxidase activities in wheat infected with *Ustilago tritici*. Sharma, Joshi and Sharma (2012) studied defense mechanism in cluster bean.

CONTROL OF SEED-BORNE INFECTION

Different methods (physical, chemical and biological) of seed treatment were employed for eradication of contamination and seed borne infection of pathogens. Earlier worker have also employed physical method by using heated oil (Dubey, 2000), chemical by fungicides (Sharma et al., 2002), biological methods by plant leaf extracts (Owolade, Amusa, Osikanlu, 2000), by bulb extracts (Upadhyaya and Gupta, 1990), fungal antagonist (Michereff, Menezes and Mariano, 1993) and drip irrigation (Breazea, Neufeld, Mayer and Davision, 2000; Sadda, 2012).

In the present study physical (oil thermotherapy), chemical (systemic fungicides), biological (plant extract and fungal antagonist) and culture practice (drip irrigation) have been tried on naturally infected seed samples CB-29(Jaipur) and CB-70 (Sikar) of *Fusarium solani* for the control experiments whereas naturally infected seed samples of CB 70 (Sikar) were used for cultural practice (Drip irrigation).

PHYSICAL CONTROL

Oil Thermotherapy

Oil thermotherapy probably first employed by Chaturvedi and Tripathi (1989). They carried out screening of 21 essential oil against the 17 fungal species isolated from *Cicer arietinum* and *Cajanus cajan* seeds stored for twelve month. They reported that best fungitoxicity expressed by seed treatment with *Seseli indicum* oil.

In oil thermotherapy, by treating the seeds at 50 °C and 70 ° C temperature for 5 and 10 minutes in eight oils viz. castor, coconut, ground nut, linseed, mahua, mustard, neem and sesame oils were applied during present study. Although all treatments were effective and reduced incidence of pathogen and improved percent seed germination but best results were obtained from heated oil of sesame, mustard and linseed at 50°C for 5 min. Maximum inhibition in incidence of *F. solani* was observed by seed treatment with sesame at 50°C for 5 min. Similar

observations have also been reported for control of *F. oxysporum* in pigeon pea at 60° - 70°C (Kumar, 2000).

Dwivedi, Pandey and Dubey (1991) tested essential oils from leaves and seeds of angiosperm against *Aspergillus flavus*. They reported that volatile oil from seeds of *Doccus carota* L. at 2000 ppm inhibits the fungal growth in *Cyamopsis tetragonoloba* (L.) Taub. Inhibition of *A. flavus* by treating guar seeds with *Cuminum cyminum* oil at 1000 ppm (Dwivedi et al., 1992).

Neem oil at 2-10% completely controlled the infection of *Alternaria alternata, Aspergillus niger* and *Fusarium oxysporum* (Locke, 1995). Niaz and Kazmi (2005) reported effective control of *Aspergillus sps.* in maize grain at 0.025% concentration of neem oil.

Antifungal activity of dill seed oil against *Aspergillus flavus, A. niger, Alternaria alternata, Helminthosporium sp.* and *Fusarium solani* in *Arachis hypogea* was reported by Rizki et al. (1997). Agarwal (2000) observed significant reduction in infection of *Rhizoctonia bataticola* by treating okra seeds with heated oil of mustard, sesame, castor and ground nut.

Dubey (2000) revealed that treatment of heated neem oil at 80ºC against *F. moniliforme* was most effective in sesame. All treatments at 80ºC or 90ºC for 5 min significantly reduced the recovery of fungi. Singh (2013) was reported maximum control of *Fusarium oxysporum* pathogen in infected seedlings was observed by 2 min heated oil treatment at 60ºC by mustard and coconut oil followed by sesame oil and the maximum percent germination was observed with the treatment of mustard and neem oil followed by sesame and groundnut oil in lentil seeds.

In present study, radicle emergence and percent seed germination in cluster bean was generally decrease with an increase in temperature since they have hard seed coat. Seed treatment at 50°C temperature showed higher germination than 70°C in all kind of oils used for treatment. These observations clearly indicate that 50°C and 5 min is appropriate temperature and time for

control the *F. solani* infection in cluster bean seeds. Oil thermotherapy may not be practical for large scale but is of practical use for small farmers, primarily in developing countries, who save seeds from year to year, for the control of seed borne pathogen.

CHEMICAL CONTROL

Systemic Fungicides

Four systemic fungicides viz. Bavistin, Kitazin-P, Systhane and Topas were used in present study for chemical control. Although all systemic fungicides is found to be effective against *F. solani* but maximum germination were obtained after seed treatment with Bavistin at 2000 ppm concentration followed by Kitazin –P, Systhane and Topas at same concentration. The percentage germination, percentage seedling infection control, percentage pathogen incidence control were increase with an increase in concentration of systemic fungicides.

Various fungicides have been suggested for the control of *F.solani* which cause root rot, damping off and wilt respectively in cluster bean. Among them Benomyl, Vitavax, Carbendazim, Mancozeb, Benlate, Thiram and Captan were important. (Satyavir and Grewal,1972; Shivanna and Shetty, 1992; Mohamed et al., 2006). 22 fungicides were used against wilt pathogen (*F. caeruleum*) and found most effective control of wilt pathogen by ethyl mercury chloride at 25 ppm by Satyavir and Grewal (1972). The role of conventional fungicides in the treatment of vegetable disease is mainly for the protection than cure (Maude, 1977).

Yadav (1979) reported that surface sterilized seeds with 0.1% mercuric chloride planted in inoculated soil showed 25% pre emergence damping off. They also reported Captan, Benomyl, Vitavax and Mancozeb reduced incidence of *Rhizoctonia solani* in *Luffa aegyptiaca*. Benlate has been reported to be most effective for checking the mycelial growth of *F. solani* (Borboru, 1984; Ahmad et al., 1996). Complete inhibition of the growth of *F. solani* was reported by treatment with Benlate at 50.0 ppm (Baird et al., 1994; Mathre & Johston, 1995;

Nawar, 2007). Seed treatment with Bavistin obtained most effective control of *Fusarium solani* when used singly (Shivanna and Shetty, 1992). Kumar (2000) also observed similar result by seed treatment with aqueous Foltaf, Vitavax and Mancozeb for control of *F. oxysporum* in pigeon pea seeds. There was no mycelial growth of *Fusarium oxysporum f. sp. lini* causing linseed wilt at 500, 1000, and 1500 ppm concentration of Bavistin and Benomyl (Sharma et al., 2002).

The efficacy of Mancozeb, Thiram, Carboxyin, Dithane M-45, Sulfur dust, Carbendazim, Ziram, Streptocycline, Thiophanate-methyl and Blue copper at 2500 ppm in controlling *S. rolfsii* [*Corticium rolfsii*] causing collar rot in lentil was determined in vitro. Mancozeb, Thiram, Carboxin and Dithane M-45 recorded 100% control of the pathogen (Singh et al., 2005). Best control of *Fusarium oxysporum* obtained from seed treatment of guar seeds with Topsin M fungicides at 4000ppm (Mohamed et al., 2006) and in cashew Carbendazim (0.1%) was most effective (Karande et al., 2007). Maheshwari et al. (2008) were tested seven fungitoxicants against *Fusarium oxysporum f. sp. lentis*. Among them Carbendazim proved most effective fungitoxicant for checking the fungal growth followed by Captan and Hexaconazole and Diniconazole.

Maude et al. (2008) found that seed soaked in thiram, eradicated 16 seed borne fungal pathogens of vegetable, cereal and flower seeds but it did not completely eradicate *Alternaria brassicola* from brassica seeds.

Carbendazim and Carbendazim + Mancozeb gave 100 % inhibition of mycelial growth of *F. solani* at 0.2 and 0.3% concentrations (Chavan et al., 2009). Jaiman et al. (2009) reported Carbendazim 50 WP, Thiophenate 70WP, Thiram 70WP and Captan 50 SP as effective fungicides against root rot caused by *Macrophomina phaseolina* in cluster bean. Masum et al. (2009) revealed that Vitavax -200 expressed the best control of seed borne infection of six pathogenic fungi viz. *Agrostis tenuis, Bipolaris sorghicola, Botrytis cinerea, Crinum graminicola, Curvularia lunata* and *Fusarium moniliforme.* Best germination in *Fusarium solani* infected seeds of bottle gourd treated with Aliette (92%) followed by Benlate (90%), Carbendazim, Ridomil, Mancozeb and Vitavax significantly

increased germination by 84-88% as compared to control (78%) (Sultana and Ghaffar, 2010).

Kumar, Tapwal and Borah (2012) utilized 4 fungicides viz. Bavistin 50%WP, Dithane M-45, Dithane Z-78, and Fytolan 50%WP against *Verticillium* wilt of *Parkia roxburghii*. They reported Bavistin 50% WP was most effective at 75 ppm and minimum inhibition was recorded in treatment with Fytolan 50%.

BIOLOGICAL CONTROL

Plant Extracts

In present study, control by leaf extracts of seven plants viz. *Azadirachta indica, Dalbergia sisoo, Eucalyptus rudis, Lantana camera, Parthenium officinalis, Ricinus communis* and *Saracca indica;* Bulb extracts of two plants viz. *Allium cepa* and *Allium sativum* and Latex yielding plant extracts of three plants viz. *Calotropis procera, Datura innoxia* and *Ficus religiosa* were used for their antifungal properties against the pathogen.

Although all the plant extracts proved better germination but the best control of *F.solani* obtained by leaf extracts of *Dalbergia sisso* followed by latex yielding plant extract of *Calotropis procera,* bulb extracts of *Allium sativum* and other plant extracts used for treatment. Minimum percent germination was obtained by treatment with bulb extract of *Allium cepa.* Maximum percentage control of seedling infection and percentage control of pathogen incidence was observed with *Calotropis procera* latex and minimum was observed with *Allium cepa* bulb extracts.

Earlier workers have also tried plant extracts for control of pathogen. According to Narayanan and Ayer (1967) *Azadirachta indica* gave the moderate effect against the pathogen and its fungitoxic activity may be due to the presence of azadiractin containing desactylimbin.

Meena and Mariappan (1994) has been reported use of neem leaf powder to be effective in controlling storage fungi of sorghum. Srivastava and Lal (1997)

reported moderate fungicidal response of *Lantana camara* against the *Alternaria alternata* isolated from fruits of pomegranate due to presence of thymol.

Agrawal (2000) observed that *F. oxysporum* was best controlled by leaf extract of *Eucalypltus rudis, Lantana and cammara* and *Azadirachta indica*. Extract of *Eucalyptus rudis, Catharanthus roseus* were found best to control *F. oxysporum* in pigeon pea (Kumar, 2000). The fungicidal property of *Allium cepa* may be due to the presence of sulpher compounds and allicin (Dutta et al., 2004).

Akinbode and Ikotun (2008) reported that *Moringa oleifera* leaves extract treated seeds showed highest control of *Collectotrichum destructivum* in cow pea (*Vigna uniguculata*).

Menna, Godara and Gangopadhyaya (2010) reported leaf extracts of *Calotropis* and *Azadirachta* were effective against blight disease in cluster bean.

Khair and Nadia (2011) studied the effect of aqueous extracts of Chilli, Lantana, Lemon grass and Onion seeds against *F. solani* and *R. solani.* They observed high reduction in mycelial growth of *F. solani* and *R. solani* in *Phaseolus vulgaris* plants.

Kiran et al. (2012) reported maximum control of *Fusarium solani, F. oxysporum* and some other fungus by using extract of *Millingtonia hortensis* at 50% concentration in maize.

Tongbram and Chhetry (2012) was observed maximum control of *Fusarium udam* causing wilt in pigeonpea by *Azadirachta indica* and minimum control by *Eryngium foetidum*. Highest inhibition of spore germination was observed in bulb extracts of *Allium sativum* extract and under field condition maximum control of disease obtained with bulb extracts of *Allium sativum.*

Fungal Antagonists

Various fungal and bacterial antagonists have been tried for control of *Fusarium* wilt in legumes. The most commonly used antagonist are *Bacillus subtilis, Glicladium virens, Rhizobium leguminosorum, Trichoderma hamatum, T.*

harzianum, T. koningii, T. longiforum , T. *viride* and *Streptomyces gourgereti* (Mehrotra and Cladius, 1972; Singh and Mukhopadhyay, 2002; Essalmani and Lahlou, 2003; Deore, Sawant and Ilhe, 2004; Muhammad and Dawar, 2010).

In present study two fungal antagonists *Trichoderma harzianum* and *T. viride* were used for seed treatment against *F. solani*. Both fungal antagonist were observed highly antagonistic and restrict the mycelial growth of pathogen but *Trichoderma harzianum* gave the better control for *Fusarium solani* than *Trichoderma viride*. Maximum percentage germination obtained by *Trichoderma viride* and *T. harzianum* at 1:1 dilution. 1:8 dilutions of both antagonists showed minimum percentage germination.

Tricholderma viride and *T. harzianum* were used as biocontrol agents by various workers (Deore, Sawant and Ilhe, 2004; Jatav and Mathur, 2005; Muhammad and Dawar, 2010; Sadda, 2012; Singh, 2013).

According to Bandhopadhyaya et al. (2002) *Trichoderma spp.* were highly antagonistic against *R. bataticola* and *F. oxysporum*. Bhat et al. (2003) reported great reduction in chickpea wilt caused by *F. oxysporum* by *Trichoderma viride* and *T. harzianum* seed treatment.

Deore, Sawant and Ilhe (2004) used *Trichoderma spp.* viz. *T. viride, T. harzianum, T.hamatum, T. longiforum* and *T. koningii* for management of Powdery mildew of cluster bean and they recorded that combination of *T. viride, T. harzianum* and *T. hamatum* was most effective in control of Powdery mildew of cluster bean.

Jatav and Mathur (2005) used 8 biocontrol agent including *Trichoderma spp* (5 isolates) as seed treatment of *Rhizoctonia solani* and *Fusarium solani* infected cluster bean seeds. They reported highest germination with *T. harzianum* followed by *Bacillus subtilis.*

Shrivastava and Mishra (2008) used antagonistic fungi in seed dressing for management of chickpea and pigeonpea wilt respectively.

Khair, Khalifa and Haggag (2010) tested the antagonist effect of four *Trichoderma album, T. hamatum, T. harzianum* and *T. viride* against *Fusarium solani* and *Rhizoctonia solani* in vitro.

Muhammad and Dawar (2010) recorded maximum reduction in charcoal rot of sunflower by *Trichoderma reesei* followed by *T. harzianum*.

Biological control of plant pathogens by Microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Agarwal, Malhotra and Trivedi, 2011).

Sadda (2012) reported better control of *Rhizoctonia solani* and *Colletotrichum orbiculare* by *Trichoderma harzianum* than *T. viride* in smooth gourd and rough guard. Singh (2013) reported better control of *Fusarium oxysporum* and *Rhizoctonia solani* by seed treatment with *Trichoderma viride* than the *T. harzianum* in lentil.

CULTURAL PRACTICE

DRIP IRRIGATION

Studies on drip Irrigation have been done by various workers in different crops mostly in vegetables due to popularity and effectiveness of this method in world viz. in alfalfa by Breazea, Neufeld, Mayer and Davision (2000); in fruit crops by Malik and Luhach (2002); in hybrid tomato by Hebbar et al. (2004); in sugarcane by Goel et al. (2005); in chilli by Kumar (2008); in cotton by Narayanamoorthy (2008); in cowpea by Rao and Shahid (2011); in *Luffa* by Sadda (2012) and in okra crop by Soomro et al. (2012).

The present study revealed that the drip irrigation system is not only useful for increasing yield and water saving but also it is effective methods to increase size and quality of the crop by controlling the fungal disease. This method also reduced the labour cost, rate of infection and energy / fertilizer and pesticide cost. These results are due to directly reach of water, fertilizer and pesticides in to the root zones of plants and water were falling down only in the form of drip. The

present study revealed that drip irrigation or micro irrigation is more effective method than the surface irrigation or sprinkler irrigation in terms of high and quality yield of cluster bean crop and water saving. According to Singh (1992) water requirement by drip irrigation is only 30-40% of surface irrigation. Similar results were reported by Kumar (1999).

Al-Jamal et al. (2000) conducted experiment on comparison of sprinkler trickle and furrow irrigation efficiencies for production. They found that maximum irrigation water use efficiency 0.084 t/ha mm-1 of water applied was obtained using the sprinkler system followed by drip 0.059 t/ha mm-1 and furrow 0.046 t/ ha mm -1 of onion.

Breazeale, Neufeld, Myer and Davison (2000) reported increase in yield and reduction in water use through drip irrigation in Alfalfa crop. Hebbar et al. (2004) reported fruit yield of tomato was 19.9% higher in drip irrigation over furrow irrigation. Goel et al (2005) also reported similar result of increase in yield of sugarcane by drip irrigation method.

Punam et al. (2003) conducted research on okra crop under drip irrigation system and compared it to the conventional irrigation method. They concluded that drip irrigation increased okra yield by 30.34%, and brought about water saving of 41.23% compared to the conventional method.

According to Maisiri et al. (2005) and Polak and Yodder (2006) drip irrigation reduced the total amount of water required to grow a crop than conventional flood and sprinkler system.

Erdem (2006) stated that potato yield and water use efficiency were higher under drip method of irrigation as compared to furrow irrigation system. Similar observation was reported on sunflower by Asgari et al. (2007).

Ibragimov et al. (2007) conducted an experiment on water use efficiency of irrigated cotton in Uzbekistan under drip and furrow irrigation on a deep silt loam soil (Calcic xerosol). Seed-lint cotton yield was increased 10-19% relative to

that for furrow-irrigated cotton. Kumar et al. (2008) reported general perception about micro irrigation adoption leads to increase in yield and water saving.

Kumar (2008) suggested use of drip irrigation for vegetable crops viz. chilli, tomato and brinjal while they suggested sprinkler system for potato, cluster bean and ground nut.

Narayanamoorthy (2008) reported 114% increase in yield and 45% reduction in applied water by using drip irrigation in cotton. Sadda (2012) was also found drip irrigation system better than surface irrigation system in terms of high yielding and water saving in rough gourd and smooth gourd.

According to Kumar and Jos (2013) micro irrigation and drip irrigation is very useful method in semi arid and arid climate.

Increase in yield and water saving were obtained through drip irrigation in okra field (Soomro et al., 2012). Similar finding were reported on maize earlier by Gomez et al. (2006).

Chapter – 6

Summary and Conclusions

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] vernacularly known as guar is an important Fabaceae crop grown during Kharif season in arid and semi arid regions of India. It is drought tolerant crop suitable for cultivation in Rajasthan. Guar is also cultivated under rainfed conditions of kharif season in India. It is grown from April to October and July- November. India is a largest producer of cluster bean with 80% of total production of world. Rajasthan is leading producer of guar with 75% of total production of India. In Rajasthan guar is grown in all 33 districts but Alwar, Barmer, Bikaner, Churu, Hanumangarh, Jaipur, Jaisalmer, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Pali, Shri-Ganganagar and Sikar account for 80% of the total production in Rajasthan. Cluster bean is grown for green manuring, green fodder, vegetables, production of seeds, pharmaceuticals, cosmetics and extraction of guar gum purposes in India. Cluster bean also has wide importance as industrial purpose due to presence of glactomannon gum found in spherical-shaped endosperm.

During the year 2011-12 total area under its cultivation in Rajasthan was 3444 thousand hectares with a total production of 2218 thousand tonnes and the yield was 644 kg/ hectares; in the year 2012-2013 the average area of Rajasthan under their cultivation was 5152 thousand hectares with a total production of 2461 thousand tones and the yield was 478 kg/ hectares and in the year 2013-14 total area under its cultivation was 5070 thousand hectares with a total production of 2862 thousand metric tonnes and the yield was 564 kg/ hectares.

The present study has been carried out on seed-borne diseases of the cluster bean crop and their control by non hazardous methods on following aspects-

1. Survey and Detection of seed borne mycoflora associated with the seeds of *Cyamopsis tetragonoloba* in Rajasthan.

2. Find out the incidence and occurrence of various types of seed symptoms and fungi associated with them.

3. Histopathology of naturally infected guar seeds to determine invasion and spread of *Fusarium solani*.
4. Study of Phytopathological effects and disease transmission of seed borne inoculum of *Fusarium solani* from seed to seedling/ plant using seeds carrying natural infection.
5. Biochemical analysis (protein, starch and phenol and their related enzymes viz. protease, α- amylase and polyphenoloxidase) of seed, stem and leaves of *Fusarium solani* infected cluster bean plants.
6. Control of seed borne inoculam of *F. solani* by using physical (Heated oil), Chemical (Systemic fungicides), non-hazardous methods including Biological (leaf extracts) and by using Fungal antagonists (*Trichoderma harzianum and Trichoderma viride*).
7. Control of seed and soil borne inoculum by drip irrigation method as a culture practice in cluster bean field.

SURVEY AND DETECTION OF SEED BORNE MYCOFLORA

Six field surveys of several cluster bean fields were carried out during harvest seasons of 2011-2014 and 120 seed samples of cluster bean were collected from 11 districts of Rajashtan namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar. Survey of the districts showed that cluster bean was highly susceptible to *Fusarium solani* which appear in rainy season from July to September when cloudy weather and high humidity conditions prevail.

30-40 days old seedlings showed the wilt symptoms in all above ground parts. Initially the wilt causes a yellowing and wilting of lower leaves on infected plants and margin of cotyledonary leaves rolled inwards and fall off. The wilting progress upward to the plant as the fungus spreads. Wilted leaves often dry up and drop prematurely. Eventually the entire plant wilts and dies. In some plants pods and stem showed fungal growth and dryness. Pods either killed or become malformed. Seed setting is very less in the infected pods. Disease is most severe

in rainy season when the humidity and optimal temperature of the air favor rapid development and spread of disease.

DRY SEED EXAMINATION

One hundred twenty seeds samples of cluster bean were collected from 11 districts of Rajashtan namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shri Ganganagar and Sikar were studied. Seed samples revealed besides normal healthy looking (asymptomatic) seeds, symptomatic seeds with different deformities and discolourations. Dry seed examination of seed samples of cluster bean revealed the occurrence of variously discoloured seeds and seeds with fungal bodies, such as seeds with black streaks 34 (0.25-12.5%), grey colour seeds with white mycelial growth 43(0.25-40.5%), shrivelled seeds 80(0.25-25%), broken and insect damaged seeds 75(0.25-50%) and debris and inert matter 34 (0.25-20%) respectively. On incubation grey colour seeds with white mycelial growth yielded *F. solani*.

INCUBATION TESTS

One hundred twenty seed samples of cluster bean from eleven districts of Rajasthan were screened using untreated and chlorine pretreated seeds in standard blotter method. Thirty five seed samples of cluster bean crop were also incubated using potato dextrose agar plate method. 53 fungal species of 28 genera, saprophytic as well as parasitic, were recorded on cluster bean seeds in SBM and 40 fungal species of 23 genera in PDA were recorded. The fungi common to both untreated as well as pretreated seeds were *Alternaria alternata* , *A. brassicola*, *A. tenuissima, Aspergillus candidus* , *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. sulphureus*, *A. sydowi*, *Botryodiplodia theobromae*, *Cephaliophora tropica*, *Chaetomium globosum*, *C. indicum*, *C. megalocarpum*, *C. murorum*, *C. spinosum*, *Choanophora cucurbitarum*, *Cladosporium oxysporum*, *Colletotrichum dematium*, *Curvularia clavata*, *C. lunata*, *C. pallescens*, *Drechslera halodes*, *D. maydis*, *D. rostrata*, *D. tetramera*, *Eurotium amstelodami*, *Fusarium moniliforme*, *F. oxysporum*, *F. pallidoroseum*, *F. solani*, *Graphium sp*. , *Macrophomina phaseolina*, *Melanospora zamiae*, *Memnoniella echinata*, *M. levispora*, *Mucor*

sp., Myrothecium roridum, M. verrucaria, Neocosmospora vasinfecta, Paecilomyces varioti, Penicillium spp., Phoma betae, Rhizoctonia bataticola, R.solani , Rhizopus nigricans, R. stolonifer, Stachybotrys atra, S. chartarum, Thielavia terricola, Trichothecium roseum, Trichurus spiralis. 13 fungi viz., *Alternaria brassicola, A. tenuissima, Curvularia pallescens, Drechslera halodes, D. maydis, Fusarium moniliforme, Graphium sp., Memnoniella levispora, Mucor sp., Myrothecium verrucaria, Penicillium spp., Thielavia terricola* and *Trichurus spiralis* were not recorded in PDA method.

Among these, the important and dominant fungal species encountered were *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium oxysporum, Curvularia lunata, Fusarium oxysporum, F. solani, Rhizoctonia solani* and *Rhizopus nigricans*.

The pretreatment with 0.5% available chlorine reduced percent incidence of both parasitic and saprophytic fungi. Number of fungal species observed in PDA test (40 fungal species of 23 genra) were less in comparison to the SBM (53 fungal species of 28 genra).

INFECTION OF *Fusarium solani*

Fusarium solani is the major pathogen found in the eleven districts of the Rajasthan. In present study 57, 53 and 15 samples were infected with *Fusarium solani* showing 0.25-62%, 0.25-35% and 0.25-30% incidence in untreated, pretreated (SBM) and PDA respectively.

HISTOPATHOLOGY OF NATURALLY INFECTED SEEDS

Component plating, cleared whole mount preparation and microtome sectioning were used to determine the location and extent of penetration of seed borne pathogen.

Seed samples ac. no. CB29 (Jaipur) and CB70 (Sikar) carrying natural infection of *F. solani,* categorized as asymptomatic (healthy seeds) and symptomatic (weakly, moderately and heavily infected) seeds were used for

histopathology of cluster bean seeds. The seed components viz. seed coat, endosperm, cotyledon, hilar region and hypocotyl shoot root axis of the heavily infected seeds were separated with difficulty in comparison to weakly infected seeds.

In component plating, peach- white fluffy growth of fungus was observed only on seed coat (5, 7%) of asymptomatic seeds of cluster bean collected from Jaipur (CB29) and Sikar (CB70) respectively. Infection of this fungus was not observed in other components of asymptomatic seeds.

In weakly infected seeds, pathogen were recorded 57, 50% in seed coat; 26, 20% in endosperm; 15, 35% in cotyledons; 14, 24% in hypocotyl shoot root axis and 12, 17% in hilar region of both the seed samples respectively. In moderately infected seeds, the infection was found 84, 72% in seed coat; 77, 62% in endosperm; 72, 64% in cotyledons; 67, 60% in hypocotyl shoot root axis and 60, 58% in hilar region of both the seed samples respectively. Whereas in heavily infected seeds, it was 99, 97% in seed coat; 99, 97% in endosperm; 98, 95% in cotyledons; 98, 94% in hypocotyl shoot root axis and 95, 94% in hilar region of both the seed samples respectively.

The cleared wholemount preparation of seed coat, cotyledons, endosperm, hilar region and hypocotyl shoot root axis revealed the presence of thin, hyaline, branched septate, inter-and intracelluar mycelium of *F. solani* in their cells of both CB29 (Jaipur) and CB70 (Sikar) seed samples. The mycelial net was dense and spore were also found in all the components of heavily infected seeds of both the seed samples.

In microtome sectioning, asymptomatic seeds showed the mycelium only in soft tissues of seed coat (hourglass and parenchyma layers) and hilar region. Infection was not observed in endosperm, cotyledons and hypocotyl shoot root axis.

Weakly symptomatic seeds revealed thin, hyaline, branched and septate mycelial bits in seed coat. Hyphae were also observed in hilar tracheids and

stellate parenchyma. Hyphal bits were observed in storage region and between the cotyledons and endosperm. However infection does not reaches up to the inner cotyledonary tissues. Thus the amount of reserve food material remains unaffected.

In moderately infected seeds, mycelial bits were observed in hourglass and parenchyma layer of seed coat. Mycelium colonized to all the parts namely seed coat, hilar region, storage region of the cotyledons, endosperm, embryonal axis and cotyledonary space. Cell content of cotyledon was depleted due to the presence of mycelium.

In heavily infected seeds, heavy aggregation of mycelium and conidia in tissue of all components of the seed was observed. Aggregation of mycelium and chlamydospores was found all around the seed surface. Mycelium aggregates and penetrates the epidermal cells and the conidiophores produce conidia which accumulate in a slimy white mass. The palisade and hourglass cells were highly disintegrated and withering of loose palisade layer. Mycelium also fills in the space between two cotyledons which leads to disintegration and depletion of cell contents. A thick mycelial mat was formed in the parenchyma layer of seed coat. The infection was deep seated in the cells of cotyledons, these cells appeared under stress, vacuolated with poor cell content. The epidermis of cotyledons show intracellular mycelium. The maximum infection was localized towards proximal and distal ends of cotyledon. Inter and intracellular mycelium observed in epidermal and peripheral layers of hypocotyl shoot root axis.

PHYTOPATHOLOGICAL EFFECTS AND DISEASE TRANSMISSION

Seed samples ac. no. CB29 (Jaipur) and CB70 (Sikar) carrying natural infection were used to study phytopathological effects and disease transmission by using blotter method, test tube seedling symptom test, pot experiment and field experiment.

Standard blotter method, the germination was 94, 97% on 8th day in asymptomatic, where as in symptomatic weakly seeds the germination was 27, 35%; 18, 30% in moderately and 7, 22% in heavily infected seeds of both the seed samples respectively. Initial symptoms appeared as pale to brown patches on root-shoot transition zone which progressed upwards and radicular region. These symptoms spreads to cotyledonary leaves. Due to heavy infection these leaves fall down and plumule dries up. On incubation these seedlings showed the presence of pathogen.

Water agar seedling symptom test, the germination was 89, 87% on 8th day in asymptomatic whereas in symptomatic weakly seeds it was 34, 37%; 26, 35% in moderately and 9, 22% in heavily infected seeds of both the seed samples respectively. The seeds which showed failure of germination were fully covered with mycelium and spores. Symptomatic seedlings are small in size and show brown-black patches on plumule-radicle transition zone. Heavily infected seeds donot show plumule emergence.

Pot experiment, germination were observed 96, 93% in asymptomatic seeds whereas in weakly infected seeds 73, 69%; 57, 46% in moderately and 25, 18% in heavily infected categories of both the seed samples respectively. Heavily infected seeds were failed to germinate or not able to grow normally as other seeds. Leaves of infected plant showed yellowish brown necrotic patches on their surface followed by their shriveling, dryness and drooping. On incubation these parts yielded the growth of fungal mycelium. Basal part of the stem near the collar region showed yellow black patches. Split half of the infected stem show the presence of mycelium in cortical region. These patches progressed upward and plant show wilting. The infected roots showed brown or black discolouration and are very small in size with only main root system. Pods were very small with black discolouration and less number of immature seeds are found, some seeds are covered with white crust of mycelium. Seedling produced by symptomatic seeds appeared dried and collapsed after a short period of emergence.

In field experiment, seed germination was maximum after 8th day of sowing, it was 94, 91% in asymptomatic seeds and 45, 62% in symptomatic seeds

of both the seed samples. Infected seedlings or mature plants show various types of symptoms.

Dark brown to black patches appears after two weeks of sowing, on the basal part of the stem near the transition zone. Roots from these plants showed the presence of pale-brown discolourations. Leaves of infected plants showed yellow brown necrotic patches. Pod setting per plant is very less than the normal plants. These pods have small seeds with white crust and brown black patches are seen on outside and inside the pod. These symptoms develop without notice still in the field and continue to progress after harvest.

Cleared wholemount preparations of infected parts revealed thin, hyaline, inter-and intracellular mycelium in cortical and vascular region of the stem, root, leaves and pods. The mycelium was mostly intercellular in the cortical cells of radicle and hypocotyl. In cotyledonary cells mycelium was sparse but dark, necrotic cells with depleted cell contents were common. Hand cut sections of root, stem, leaves and pod revealed inter as well as intracellular mycelium in epidermis, cortex and in pith region.

BIOCHEMICAL ANALYSIS

According to Mitter et al. (1987) for understanding the host- pathogen interaction the identification of differences in biochemical events between diseased and healthy tissue is prerequisite. It may be hypothesized that after causing disturbances in normal host metabolism the fungus obtains some metabolites from the host tissue. The pathogen utilizes these transformed metabolites and leads to changes in chemical constituents of plant (Shah and Daniel, 1998).

Biochemical estimations of healthy and infected cluster bean (Ac. no. CB70) by *Fusarium solani* at various stages of disease development were carried out. The seed infection caused deterioration of food reserves. These estimations were carried out by using standard quantitative techniques to estimate alteration in

the host metabolism due to the fungal infection. The revealed alterations are summarized below.

Total Protein Contents and Protease activity

Total protein content in *F. solani* infected leaf, stem and seeds were lower than the healthy leaves, stem and seeds of cluster bean while protease enzyme activity were higher in infected leaves, stem and seeds than healthy leaves, stem and seeds.

Starch Contents and α -Amylase enzyme activity

Total starch content was higher in healthy leaves, stem and seeds than infected counterparts whereas α- amylase activity was found higher in *F. solani* infected leaves, stem and seeds than healthy counterparts.

Phenolic Contents and Poly Phenol Oxidase activity

Total phenol content was higher in infected plant parts as compared to healthy plant parts while PPO (Polyphenol oxidase enzyme) activity was higher in healthy parts than infected leaves, stem and seeds.

CONTROL OF SEED-BORNE INFECTION

CB29 (Jaipur) and CB70 (Sikar) seed samples were used for physical, chemical and biological control of *Fusarium solani* infection. The experiments to control pathogen included oil thermotherapy or hot oil treatment (physical), systemic fungicides (chemical), leaf extracts, biological antagonists (biological control) and drip irrigation (cultural practice). Data on the effect on seed germination, control of infected seedling and incidence of pathogen after various treatments were recorded.

PHYSICAL CONTROL

Oil Thermotherapy

In oil thermotherapy, treatment of infected seeds at 50° C and 70° C temperature for 5 and 10 minutes in castor, coconut, groundnut, lineseed, mahua, mustard, neem and sesame oils were done. All the test oils were useful in reducing the fungal infection and disease intensity. Among these, maximum percent germination was recorded 96% in sesame oil in 5 min and 92% in linseed oil in 10 minute treatment in comparison to 60% in control. The effective control of pathogen was observed in sesame oil (94.73%) in 5 min followed by mustard oil and linseed oil (93.42%). Radicle emergence and percent seed germination in cluster bean was generally decrease with an increase in temperature since they have hard seed coat. Treatment at 50°C temperature showed higher germination than 70°C in all kind of oils used for treatment. The treatment of infected seeds with different oils at 50°C for 5 min. shows more control of infection of pathogen than treatment for 10 min.

CHEMICAL CONTROL

Systemic Fungicides

In systemic fungicides treatment, Bavistin, Kitazin-P, Systhane and Topas were used at 4 different conc. 2000, 1000, 500 and 250ppm. All the test fungicides were effective in reducing the disease intensity. Among these, maximum germination was observed in Bavistin (90%) at 2000ppm followed by Kitazin-p (85%), Systhane (82%) and Topas (70%) at same concentration. Percent germination, seedling infection control, pathogen incidence control were increase with an increase in concentration of systemic fungicides.

BIOLOGICAL CONTROL

Plant Extracts

Leaf extracts of 7 plants viz. leaf extracts of plants *Azadirachta indica, Dalbergia sisso, Eucalyptus rudis, Lantana camera, Parthenium officinalis,*

Ricinus communis and *Saracca indica;* extracts of bulbs of *Allium cepa* and *Allium sativam* and latex yielding plant extracts of *Calotropis procera, Datura innoxia* and *Ficus religiosa* were used for treatment. Most of the plant extracts were found to be effective and significantly reduced the disease in comparison with the control. Among these, maximum percent germination was observed in plant leaf extracts of *Dalbergia sisso* (83%) and Minimum percent germination were recorded in bulb extracts of *Allium cepa* (55%). Maximum percent germination was expressed by treatment with *Dalbergia sisso* while percent control of seedling infection and maximum percent control of pathogen incidence were expressed by treatment with latex of *Calotropis procera* and minimum were observed by *Allium cepa*.

Fungal Antagonists

For biological control with biological antagonists pure culture suspension of *Trichoderma viride* and *T. harzianum* at 4 dilutions i.e. 1:1, 1:2 and 1:4 and 1:8 (v/v) were used for infected seed treatment of cluster bean seeds. Maximum percent germination was expressed by *Trichoderma viride* and *Trichoderma harzianum* at 1:1 dilution. 1:8 dilutions of both antagonists showed minimum percent germination. Although both antagonists found to be effective as they significantly restricted the growth of *Fusarium solani* but *Trichoderma harzianum* expressed better control than *Trichoderma viride*.

CULTURAL PRACTICE

DRIP IRRIGATION

For progress of disease incidence in relation to the prevailing environmental conditions and irrigation method was used. A field trial was conducted with local cultivar in experimental plot in tapiplya village near the Reengus station of Sikar district of Rajasthan during growing season from 2012-2014 to compare the performance and evaluation of drip irrigation system with surface irrigation method. The drip lines were spread in field from May to July till the arrival of monsoon. The field trial revealed that the drip irrigation system is

useful for increasing yield, water saving, size and quality of the crop. This method also reduced the labour cost, rate of infection and Energy / fertilizer and pesticide cost. Increase in yield of crop and water saving were obtained due to direct reach of water, fertilizer and pesticides in to the root zones of plants and water were falling down only in the form of drip. The drip irrigation systems are extremely effective in arid and drought prone areas where water is scarce. The progress of disease was recorded which were influenced due to the variation in varying weather conditions. It was found that disease intensity with drip irrigation facility was comparatively less than other simple irrigation methods viz. surface irrigation or sprinkler irrigation in cluster bean.

CONCLUSIONS

1. One hundred twenty seed samples of cluster bean collected from 11 districts of Rajasthan carried seeds with black streaks (*Colletotrichum dematium, Curvularia lunata, Rhizoctonia bataticola, R.solani, Macrophomina phaseolina* and *Phoma betae*); grey colour seeds with white mycelial growth(*Fusarium moniliforme, Fusarium oxysporum* and *Fusarium solani*); shriveled seed (*Alternaria alternata, Aspergillus flavus, A. niger, Curvularia lunata, Chaetomium sps., Drechslera tetramera, Fusarium moniliforme, F. oxysporum, Penicillium sps., Rhizoctonia solani, Myrothecium roridum* and Bacterial ooze); broken and insect damaged seed (*Alternaria alternata, Aspergillus flavus, Curvularia clavata, Curvularia lunata, Drechslera tetramera, Rhizoctonia bataticola, Chaetomium spp., Rhizopus sps.* and *Trichothecium roseum*) and debris and inert matter seeds (*Aspergillus fumigatus, A. niger, Curvularia pallescens, Drechslera rostrata, F. monaliforme, F.oxysporum, F. solani, Mucor sps., Phoma betae, Rhizopus nigricans* and *Trichothecium roseum*).
2. *Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium oxysporum, Curvularia lunata, Fusarium oxysporum, F. solani,*

Rhizoctonia solani and *Rhizopus nigricans* were dominent fungi. Both pathogenic and saprophytic fungi recorded during incubation test.

3. 53 fungal species of 28 genera and 40 fungal species of 23 genera were recorded in SBM and PDA respectively by incubation test.
4. The important pathogenic fungi hampering seed germination and causing seedling symptoms were *Fusarium solani*.
5. Seed samples collected from Jaipur (CB29) and Sikar (CB70) carried higher percentage incidence of fungi in comparison with seed samples collected from other districts of Rajasthan.
6. The percentage range of *F. solani* in dry seed examination and incubation test was recorded highest in samples collected from Sikar (CB70) 12 (0.25-40.50%), 18 (0.25-62%), 15 (0.5-35%), 5(0.25-30%) followed by Jaipur (CB29) 10 (0.5-35.5%), 13 (0.5-58%), 12 (0.5-34%), 3(0.5-29%) in DSI, untreated, pretreated (SBM) and PDA respectively.
7. Standard blotter method using pretreated seeds has been recommended for the detection of seed borne infection of *F. solani*.
8. Histopathology of naturally infected seeds with *F. solani* confirmed that *Fusarium solani* in cluster bean were internally seed-borne and the infected seeds could be asymptomatic or symptomatic (weakly, moderately and heavily infected). Infection in asymptomatic seeds was localized to seed coat only and in symptomatic seeds, the infection was deep seated, found in all components, extra-or intra embryonal infection causing damage to seed tissue.
9. Seed-borne infection of *F. solani* caused pre-and post emergence losses, which were low in asymptomatic and high in symptomatic seeds.
10. Phytopathological effects and disease transmission experiments confirmed the pathogencity and disease transmission of pathogen in cluster bean.
11. The natural infection of *F. solani* caused decrease in total protein and starch and increase in total phenol.
12. The seed infection of *F. solani* caused deterioration of food reserves and showing increase in their enzyme activity (Protease, α- amylase) while in case of PPO (poly phenol oxidase) decrease observed in infected parts.

13. Hot oil treatment of *F. solani* infected seeds with sesame, mustard, linseed and neem oil at 50° C were most effective in reducing mycoflora, seedling abnormality and disease symptoms during storage.
14. Among all systemic fungicides used for treatment of *Fusarium solani* infected seeds, Bavistin and Kitazin-P at 2000 ppm were found most effective.
15. Leaf extracts of *Dalbergia sisso, Calotropis procera, Allium sativam* and *Azadirachta indica* were most effective against the infection of *F. solani.*
16. For control of seed borne infection the most effective biological antagonist were *Trichoderma harzianum*.
17. The use of drip irrigation in cluster bean field was observed effective to increase yield, water saving; reduce labour cost and rate of infection. This method is also effective to control infection and produce healthy and quality crop.

FUTURE PERSPECTIVES

DRIP IRRIGATION

Drip irrigation have world wide acceptance with horticultural and commercial crops because of their economic consideration. The use of drip irrigation primarily to irrigate high value horticultural crops. India has a wide diversity in climatic condition than other countries. In India , irrigated area consist of approximately 36% of net sown area. Increasing competition of agricultural sector with other water uses sectors (Domestic, industrial and energy sector) causing water scarcity. To promote production of horticultural and commercial crop, aggressive efforts are required to bring more area under cultivation through area expansion schemes. At primarily the dry land or arid and semiarid crops should get attention with technological interventions and utilizing drip irrigation method to enhance quality and productivity. At present around 3.51 lakh hectare area is under drip irrigation with special efforts of government of India. It will provide a better economy. The most appropriate advantage of drip irrigation method is water saving up to 35-40% than other irrigation methods. Drip

irrigation method is also useful to enhance the productivity and quality of crop and reduce the labour cost, rate of energy and infection and fertilizer and pesticide cost. Hence drip irrigation method will play a key role in future by fulfilling the requirement of intensive, water saving and energy saving agricultural production.

CLUSTER BEAN

- Cluster bean has world wide recognition due to glactomannan gum derived from endosperm of guar which makes it commercial product.
- A high concentration of flavanoids and other phenolics compound like kaempherol in guar seeds may expand its neutraceutical and pharmaceutical uses.
- **Antioxidant:** Results showed that consumption of cluster bean pod can supply antioxidant constituents to the organism due to the presence of hydrophobic antioxidant constituents.
- **Anti-diabetic:** An aqueous extract of pods of the plant at a dose of 250 mg/kg of body weight significantly reduces blood glucose level due to marginal antihyperglycemic effect expressed by *Cyamopsis tetragonoloba* on blood glucose level in normal fasted rats. This effect can be attributed due to presence of flavanoids and other phenolics of the plant.
- **Anti-ulcer:** An ethanol extract of the pods was showed anti ulcer activity. The intensity of gastric lesions induced by hypothermic restraint stress and indomethacin was reduced significantly by guar extract.
- **Anti bacterial:** Study showed that methanolic extract of *Cyamopsis tetragonoloba* expressed mild antibacterial effect against *Escherichia coli*, *Staphylococcus aureus, lactobacillus* & *Salmonella typhimurium*.
- **Anticoagulant:** Study of sulphate derivatives of galactomannan from seeds showed anticoagulant activity
- **Antimicrobial activity:** Methanolic extract of pods showed antibacterial and antifungal activity.
- **Anti-asthmatic:** Alcoholic and aqueous extract of the leaves of this plant showed antiasthmatic activity. Anti-asthmatic activity may be attributed to its anti-histaminic, anti-allergic and adaptogenic effect.

- **Anti-inflammatory activity:** Alcoholic and aqueous extract of seeds of guar in acute, subacute and neurogenic inflammation against various phelogestic agents. Ethenolic extract showed significant inhibitory effect on inflammation caused by them. Anti-inflammatory activity of seeds may be attributed to its rich flavonoidal and saponin contents.
- **Hemolytic activity:** Saponin rich extract prepared from guar meal showed hemolytic activity. This hemolytic activity may thought to be due to effect on cell membrane permeability by forming pores in membrane, altering the sodium-potassium and calcium-magnesium ATPase activities or insertion of the hydrophobic saponin nucleus into the lipid bilayer.
- In cosmetics, guar gum derived from endosperm of seeds and pods used for its antifungal, antibacterial and anti inflammatory properties. The seeds and pods have laxative properties. The pods provide essential, valuable and useful minerals needed for good body development.
- Different parts of this plant are traditionally claimed to be used for the treatment of asthma, arthritis, cure night blindness and inflammation. As per ayurveda the plant is used to reduce fire and also useful in constipation, dyspepsia, anorexia, agalatia, hyetalopia and vitated condition of kapha and pitta. The medicinal properties of exhibited by plant may be attributed to the presence of flavonoids in plant parts.
- Pods and seeds of *Cyamopsis tetragonoloba* are a very useful local source of fibers. The wide application of *Cyamopsis tetragonoloba* in traditional medicine, pharmaceutical, cosmetics, paper industries, textile, bakery and oil field expressed the commercial values and future perspectives of this research work to increase the profitability and the production.

Chapter – 7

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Chapter – 8

Appendices

S. No. 38

Certificate

National Conference on

PLANT BIORESOURCE MANAGEMENT AND BIOTECHNOLOGY (PBMB)

January 29-31, 2014

Organized by Department of Botany, University of Rajasthan, Jaipur

This is to certify that Prof./Dr./Mr./Ms. ....VIKAS PAREEK.....
.....GOVT. COLLEGE, KOTA.....has participated in National Conference on Plant Bioresource Management and Biotechnology from January 29 - 31, 2014 in the Department of Botany, University of Rajasthan, Jaipur - 302004.

He / she also ~~presented paper~~ / ~~poster~~ / ~~delivered plenary lecture~~ / ~~chaired a session~~ on ..... x ..... x ..... x ..... x ..... January 29/30/31, 2014.

(Prof. Meenakshi Sharma)
Organizing Secretary

(Dr. R.D. Agrawal)
Organizing Secretary

(Prof. K.P. Sharma)
Chairperson

P-S3(84)

FUSARIUM OXYSPORUM IN CLUSTER BEAN SEEDS OF RAJASTHAN

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One hundred two seed samples of the harvest year 2009-2011 collected from 11 districts of Rajasthan. 37 seed samples contained gray coloured seeds with white mycelial growth. The symptoms ranged from 0.25-50.25%. In inoculation tests, the pathogen was recorded in 45, 43 and 17 samples in untreated, pretreated and PDA respectively with 1-24.5% (Untreated), 1-27.5% (Pretreated) and 2.5-38% incidence in standard blotter and PDA tests respectively. High infection percentage and relative percent recurrence (RPO) were recorded in samples from Sikar, Jaipur, Bikaner, Churu, Jalore and Jhunjhunu etc. Histopathology confirmed its presence in different seed parts. Infection was localized in seed coat and hilar region in symptomless and weakly symptom seed but in moderately to heavily symptomatic seeds, mycelium were recorded in seed coat, cotyledons and hypocotyl shoot root axis. The amount of colonization by pathogens and the damage caused to host tissue varied with the degree of seed infection.

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SEED-BORNE NATURE OF *FUSARIUM SOLANI* (MART.) SACC. CAUSING WILT DISEASE IN CLUSTER BEAN GROWN IN RAJASTHAN

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ABSTRACT : 120 seed samples of cluster bean collected from eleven districts of Rajasthan were studied. In dry seed examination occurrence of variously discloured seed, seeds with black streaks and grey coloured with white fungal growth. 10 Seed samples from Jaipur (35.5%) and Sikar 12 (40.5%) carried higher frequency of *Fusarium solani* causing wilt disease in guar component planting whole-mount preparations and microtome sections confirmed its presence in different parts of the seed. Infection was localized in seed coat region of symptom less seeds. Whereas in symptomatic seeds infection was deep-seated and showed fungal hyphal bits in different layers of seed coat, endosperm, cotyledons, hypocotyl shoot root axis and hilar region. Amount of colonization by pathogen varied with severity of seed infections. Heavily infected seeds did not germinate. Control of *Fusarium* wilt begins by planting certified seeds and avoiding overhead irrigation and watering through drip lines.

Key words : *Cyamopsis tetragonolola, Fusarium solani, Wilt seeds.*

INTRODUCTION

Fusarium solani (Mart.) Sacc. a well known wilt pathogen has been reported seed-borne in many leguminous crops. It causes destructive wilt disease in guar during warm, wet growing season. But there is no much information on its being seed-borne in the crop. During the survey the fungus was found to the invariably associated with guar seeds of Jaipur and Sikar district of Rajasthan and the study was concentrated on its incidence, penetration and host parasite relationship in seeds and above ground plant parts.

MATERIAL AND METHODS

Survey was done in various areas of Rajasthan district namely Alwar, Bikaner, Churu, Jaipur, Jalore, Jhunjhunu, Jodhpur, Kota, Nagaur, Shiganganagar and Sikar to find out the areas of production of guar. 120 seed samples and plant parts were collected and screened using ISTA methods. Dry seed examination of the seed samples was done and they were categorize das symptomless and symptomatic carrying infection of grey coloured seed with white mycelial growth. Samples of infected plant material were also collected and subjected to dry seed examination, SBM and PDA. In standard blotter test 25 seeds per plate were spaced blotters in sterilized petri plate containing three well moistened flatter and incubated at 26±2°C under 12 h of alternating cycles of artificial light and darkness for seven days. Percent incidence of seed mycoflora, germination abnormalities in seedlings was recorded on eight day of incubation. Sterilized seeds and plant parts were aseptically plated on petri plates (10 seeds/petriplate) containing 15-20 ml of PDA media and incubated for 7 days. Percent incidence of mycoflora, seed germination and abnormality of seedlings were examined by naked eyes as well as under stereo binocular microscope on eight day of incubation.

For component plating seeds were soaked for 3 hrs in sterilized water individually, and seed components were inculcated at 28±°C. For whole mount preparations seeds were boiled in 10% KOH for 30 minutes, washed thoroughly and seed coat endosperm, cotyledons embryonal axis and hilar region were separated. Each component was failed separately in 10 ml of lacto phenol containing 2 ml of trypan blue and mounted in polyvinyl alcohol (Singh *et al.*,1977). For microtomy usual microtome techniques were followed (Johanson,1940). The wax embedded seeds were chopped from one side and immersed in 1% aqueous solution of sodium lauryl sulphate for 24 hours washed thoroughly in water and immersed in glycerol and glacial acetic acid (1:1) for 7 days (Singh and Singh,1978). Serial microtome sections were cut at 10-20 thickness stained with safranin and light green combination and mounted in DPX. For further studies two samples were selected one form the Jaipur (CB-29) and other from Sikar (CB-70) of the Rajasthan.

RESULTS AND DISCUSSION

102 seed samples were collected from various guar growing areas of Rajasthan. Dry seed examinations of seed samples were done. Besides normal seeds of Jaipur (CB-29) and Sikar (CB-70) showed variously discolored seeds with black streaks (0.25-12.5%), grey coloured seeds with white mycelial growth (0.25-40.5%), shriveled seeds (0.25-25%), Broken and insect damaged seeds (0.25-50%) and debris and inert matter (0.25-20%) (Fig.1).

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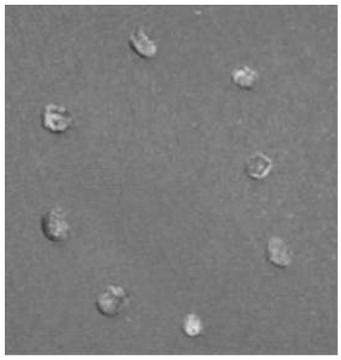
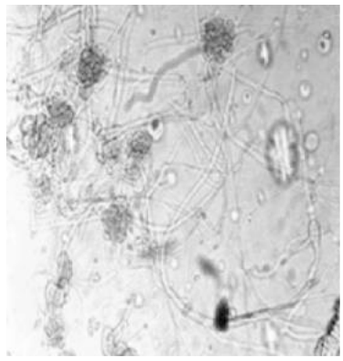


Fig. 1


Fig. 2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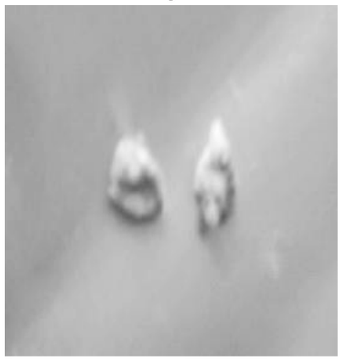
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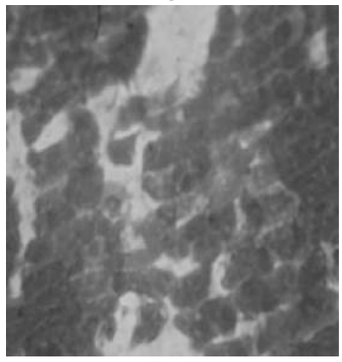
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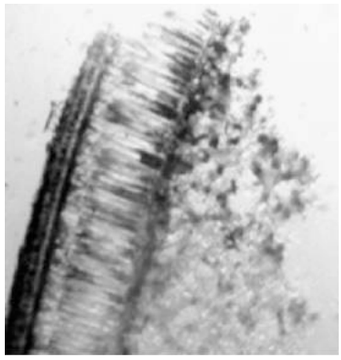
Fig. 6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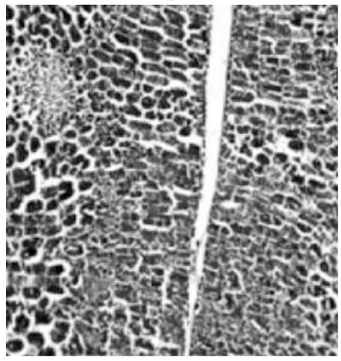
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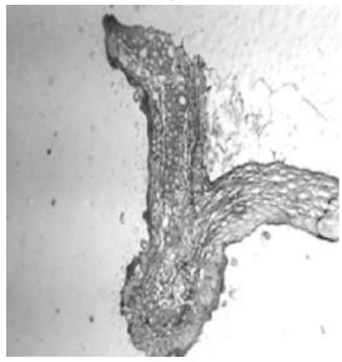
Fig. 9

Fig.1 Infected guar seeds with *Fusarium solani* x5, **Fig.2** Heavy infection of *F.solani* in SBT x5, **Fig.3** Fungal growth of *F.solani* x10 **Fig.4** Component plating of seed coat and cotyledon x20, **Fig.5** Cleredwhole mount preparation of seedcoat showing Hyphal bits x20 **Fig.6** Whole mount preparation of hypocotyle shoot root axis x20, **Fig.7-9** T.S. of infected seed, **Fig.7** Inter and intra cellular mycelium in parenchyma layer of seed coat x20, **Fig.8** Weathered cotyledonary cells with hyphal bits x 20 **Fig.9** Mycelium was observed in hypocotyls shoot root axis x 20

Incubation tests showed that these discolourations were caused by various fungi such as seeds with black streaks- *Macrophomina phaseolina, Curvularia lunata, Colletotrichum dematium* and *Rhizoctonia bataticala*; Grey coloured seeds with white mycelial growth- *Fusarium solani* and *F.oxysporum*; shriveled seeds *Aspergillus flavus, A.niger* and bacterial ooze; broken and insect damaged seeds. *Aspergillus flavus* and Rhizopussps; Debris and inert matter *Fusarium oxysporum, F.solani, F.monaliforme, Aspergillus niger* and *A.fumigates* (Table.1).

Fusarium solani infected symptomatic grey coloured seeds show white mycelial growth (Figs.2&3). The pathogen was recovered in 0.5-35.5%; 0.25-40.5% seeds in dry seed examination of both the seeds samples of Jaipur and Sikar where as in untreated, pretreated standard blotter test and potato dextrose agar it was 58%, 62%; 34%, 35% and 29%, 30 in both the samples respectively. Observations of the collected infected samples lead to the following conclusions.

In dry seed inspection Jaipur and Sikar disease occurrence was found to be maximum 35.5% and 40.5% in comparison to other areas. If was found to be minimum in Jodhpur (0.5-2.5%) and in Alwar 0.25-3%. It was found to be minimum in PDA methods. The guar is commonly susceptible to *Fusarium solani* causing wilt diseases during summer to rainy season when the conditions are favorable for the growth of fungi (Kamal and Khan,1967). *Fusarium solani* was recorded on incubated components of asymptomatic as well as symptomatic seeds (Fig.4). In asymptomatic seeds 5, 7% seed coat developed white mycelial growth and other components are free from infection in both the seed samples. Whereas in symptomatic weakly moderately and heavily infected seed samples the growth of the fungus was observed in seed coat (57-50%, 84-72%, 99-97%), endosperm (26-20%, 77-62%, 99-97%), cotyledons,15-35%, 72-64%, 98-95%), Hypocotyl shoot root axis (14-24%, 67-60%, 98-94%) sand in hilar region it was 12-17%, 60-58%, 95-94%) in both the seed samples respectively (Table.2).

The seed coat endosperm and embryo could be easily separated in normal seeds. But it was difficult to do so in moderate to heavily infected seeds. In cleared whole mount preparations thin, hyaline, septate, branched inter and intra cellular and clumps of the mycelium was observed in all the components of symptomatic seeds. Mycelium net work was dense in all the components of heavily infected seeds of both the seed samples (Figs.5&6). Occasionally chlamydospores were also observed in the seed coat. Anatomically guar seed consists of seed coat, endosperm hypocotyl-shoot-root axis and hilar region. Which is composed of two large flashy cotyledons, plumule, hypocotyl, radicle axis and hilar region. The seed coat shows characteristic leguminous features *i.e.* the epidermis of palisade cell, hourglass cells and parenchyma. The endosperm is represented by the aleuronic and compressed parenchyma cell with negligent cell contents.

In weakly infected symptomatic seeds showed presence of thin, hyaline, branched, septate mycelial fragments in the seed coat. The lumen of palisade and hourglass cells showed thin hyaline and branched mycelium in the layers of seed coat,

Table. 1 Dry seed examination of guar seed showed the association of fungal bodies.

Type of seed disorder	Percent range	Fungi associated with seeds	Seedling symptoms
Seeds with black streaks	0.25-12.5(34)	*Macrophomina phaseolina* *Curvularia lunata* *Colletotrichum dematium* *Rhizoctonia bataticola*	Pale-yellow coloured seeding. Blackening at the base of stem. Necrosis on the root region. Brown spots on seedlings.
Grey coloured seed mycelial growth	0.5-40.5% (43)	*Fusarium solani* *F.oxysporum*	Yellowing of stem bases, drooping of leaves and with wilting of seedling. Premature leaf fall. Blighting of leaves.
Shrivelled seed	0.25-25% (80)	*Aspergillus flavus* *A.niger, Bacterialooze*	Browning of leaves and basal part of shoot. Crinkling of leaves. Browning and rotting of seedling.
Broken and insect damaged seed	0.25-50% (75)	*Aspergillus flavus* *Rhizopus* sps.	Rotting of seedlings.
Debris and inert matter	0.25-20% (34)	*F.oxysporum, F.solani*, *F.monaliforme*, *A.niger* *A.fumigatus*	Necrotic streaks and spots on radicle and basal part of shoot.

Table. 2 Percent infection of *Fusarium solani* in cluster bean seeds in component plating and cleared whole mount preparation.

Component plating

AC. No.	Asymptomatic seeds					Symptomatic Seeds														
						Weakly					Moderately					Heavily				
	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR
CB-29 JAIPUR	5	0	0	0	0	57	26	15	14	12	84	77	72	67	60	99	99	98	98	95
CB-70 SIKAR	7	0	0	0	0	50	20	35	24	17	72	62	64	60	58	97	97	95	94	94

Cleared whole mount Preparation

AC. No.	Asymptomatic seeds					Symptomatic Seeds														
						Weakly					Moderately					Heavily				
	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR	SC	Endo	Cot	HSRA	HR
CB-29 JAIPUR	10	0	0	0	2	59	57	13	12	16	75	73	61	57	59	99	99	98	98	95
CB-70 SIKAR	8	0	0	0	2	46	46	34	30	14	71	71	67	63	60	97	97	97	96	92

SC = Seed Coat, ENDO = Endosperm COT = Cotyledon, HSRA = Hypocotyle-shoot-root axis, HR = Hilar region.

hyphal bits were also seen in the hilar tracheid and stellate parenchyma, cotyledons showed discontinuous but distinct patches of uninfected and infected cell divisions. The infected regions show in an increase the number of cells which were smaller, shapeless vacuolated with very little cell content. The cell division mostly occurred in the marginal cells of cotyledons which progressed in deeper tissues. In moderately to heavily infected seeds the heavy aggregation of mycelium was observed in all the seed components. The cells of the palisade and hourglass layers was loosely arranged and withered (Fig.7).

A thick mycelia mat was found in the parenchyma layer. The cells were indistinguishable. Profuse mycelium was seen in all the parts of the seed-coat, cotyledons and hypocotyl-shoot-root-axis (Figs.8&9). *Fusarium solani* has been separated to be seed-borne in seeds of *Cyamopsis tetragonoloba*. The infected seeds may be asymptomatic or with symptoms. The symptomless infected seeds show infection in inculcation tests and produce profuse growth of white mycelium. Guar seed samples showed various seed discolouration and disorders which follow the classes of discolouration given by Neergaard (1977). Dwivedi and Dubey (1992) isolated *F.solani* from stored seeds of guar showing brown discolouration. Shivanna and Shetty (1988b) have reported that *C.dematium* caused discolouration, shriveled and light weight seeds of guar. Pande *et al.* (2007) observed *F.oxysporum* on chick pea seeds. Neeraj and Singh (2009) reported Macrophomina root-rot losses. In present study grey coloured seeds with white mycelial growth show the presence of *F.solani* and seeds with black streaks show the presence of *Rhizoctonia* sps.

In India reports on seed-borne fungi are by Singh & Chauhan (1973), Manoharachary *et al.* (1978), Karwasara & Singh (1982), Shivamma & Shetty (1988a) and Dwivedi *et al.* (1991). In present study once the infection reaches the seed at appropriate stage and time of development the infection spreads quickly. This may account for lack of distinction between moderate and heavily infected seeds. Seed borne nature of *F.oxysporum* was observed in moth bean seeds Varma (1990) and in guar seeds Bhatia (1995). The present study clearly indicates that *F.solani* is not only seed born but deep seated. The infection causes distortion and depletion of host cell. Control of *Fusarium* wilt begins by planting certified seeds, no seed saving from infected plants and use of disease free seeds of variety resistant to wilt disease. Other measures include planting in well drained soil free from surface runoff water, practicing crop rotation avoiding overhead irrigation and watering through drip lines or tapes or haoses (Singh and Singh,1978). In Luffa seeds Sadda (2013) used the dripirrigation system for the irridication *Colletotrichum orbicular.* Removal and destroying of infected plant parts at the end of season is also recommended.

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Research Article

PHYTOPATHOLOGICAL EFFECTS AND DISEASE TRANSMISSION IN CLUSTER BEAN SEEDS GROWN IN RAJASTHAN

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ABSTRACT

Guar (*Cyamopsis tetragonoloba*) is known to suffer many diseases, which are responsible for its quality and low yield resulting in severe economic losses. In petriplate and water agar seedling symptom test incidence of pathogen was high in symptomatic seeds as compared to the asymptomatic seeds of both the seed samples. It was 85%, 79% (SBM) and 83%, 73% on 8th day of sowing in both the samples respectively. Where as in pot experiment germination started after 3rd day of sowing. The infection of *Fusarium solani* affected the emergence and growth of seedling. It was 89%, 91% in control and 65% - 40% in all the categories of symptomatic seeds of both the seed samples respectively. Infected seedlings show brown to black streaks on collar region of the stem and brown to black necrotic patches on leaves, Root tip portion showed the rotting and small in size. In pot experiment seedlings obtained from symptomatic seeds, pods and seed setting are very few. On incubation these infected parts showed the presence of pathogen. Control by drip irrigation is very effective.

Keywords*: Cyamopsis tetragonoloba, Disease Transmission, Fusarium solani, Phyto-Pathological Effect, Seeds*

INTRODUCTION

Cluster bean drought hardy crop of the arid and semi-arid zones and cultivated under rain fed conditions of kharif season.

It is used mainly as feed, fodder and vegetable purposes. It is a rich source of protein and galactomannose gum which is stored in endosperm and utilized in wide range of industrial processes.

Guar is known to suffer many diseases which are responsible for its quality and low yield resulting in severe economic losses to the country as it is an important cash crop with a great potential for foreign exchange (Chand and Gandhi, 1978). The major disease causing low planting value of the crop includes fungal, bacterial and viral diseases. Among the different pathogens attacking the crop *F. solani* is the most common fungus causing considerable yield losses. The pathogen caused wilt of seedlings. At later stages of plant growth, the infected plants exhibit rot near the soil which results in wilting of host plant. In present study sequential transmission studies were made using blotter method, water agar seedling symptom test and pot experiment.

MATERIALS AND METHODS

For the phytopathological effects and disease transmission was carried out by using naturally infected seeds of Sikar (CB-70) and Jaipur (CB-29). Asymptomatic seeds of the same sample were used as control. The study was carried out by employing following methods.

1. Petriplate method
2. Water agar seedling symptom test
3. Pot experiment

In petriplate method two replicates of 100 seeds per category per sample pretreated with aqueous solution of sodium hypochlorite with 0.5% available chlorine were spaced on moistened blotters and incubated at 20± ºc under 12 h of alternating cycles of light and darkness. Data were recorded at 24 hr intervals up to 8 days.

In water agar seedling symptom test 100 seeds per category per sample were sown on 1% sterilized water agar medium in test tube (1 seed/test tube) under aseptic conditions and incubated at 26± 2º c. the observations were taken daily up to 15 days and data were recorded.
In pot experiment 100 seeds per category per sample (5 seeds/ pot) were sown in 12" size earthen pots containing sterilized soil in end of the month July 2013, 2014. The pots were watered on every alternate day and data recorded weekly to 15 day intervals up to the maturity. For isolation and presence of the pathogen in different parts of seedling / plant, both symptomatic and healthy looking seedlings/ plants were uprooted at regular intervals, washed in running water and each split longitudinally into two halves. One half surface sterilized with 0.5% available chlorine was sown on moistened blotters and incubated for 7 days, while other half was cleared by boiling for 5-10 min. in 10% aqueous solution of KOH washed with distilled water stained with cotton blue and mounted in PVA. Hand cut sections of root and stem were also made and stained with cotton blue.

RESULTS AND OBSERVATIONS

The performance of seed samples of guar carrying natural infection of *Fusariumsolani* was studied in the sample ac.no.CB-29 (Jaipur) and CB-70 (Sikar) (Figure 2.A). Effect of pathogen on the seed germination, seedling survival, mortality and disease transmission was studied in petriplate (Figure 1.A-B), water agar (Figure 1.C-D) and pot experiments (Figure 1.E-F). Sequential observations were made on the percent germination, seed rot, seedling/ plant survival and incidence of the pathogen.
In petriplate and water agar seedling symptom test the germination started after 24 hr of sowing and maximum germination was 94%, 97% and 89%, 87% on 8^{th} day in control seeds, where as in symptomatic seeds it was 27%,18%, 7%; 35%, 30%, 22% and 34%, 26%,9%; 37%, 35%, 22% in both the samples respectively. The ungerminated seeds of both the samples were covered with fungal growth and showed seed rot (Figure 2.B-C). Initial disease symptoms appeared as pale- yellow discolouration on the hypocotyl region on 3^{rd} to 5^{th} day (SBM and Water agar test). Irregular necrotic spots were on the cotyledons and browning of radicle. Symptoms were later turn yellow and seedlings fell prematurely (Figure 2.D). Finally whole shoot turned yellow to brown, pulpy and show wilting. These seedlings finally rotted between the 8^{th} to 10^{th}day of sowing in both the seed samples. On incubation infected seedling show microconidia and profuse growth of fungal mycelium on hypocotyl and necrotic spots on cotyledons along with the browning of radicle. Some seedlings obtained from asymptomatic seeds or weakly infected seeds showed descrete black streaks.
In pot experiment germination started after 3^{rd} day of sowing. On 8^{th} day it was maximum. The infection of *F. solani* affected the emergence and growth of seedlings it was 89%, 91% in control and 65%, 68% ; 46%, 50%; 37%, 40% in catagorised seeds of both the seed samples, where as in heavily infected seeds very few seeds are germinate. Seedlings show symptoms after 20day of sowing seeds in pot (Figure 2.E). Total losses was 4%, 7% in control and 27%, 31%; 43%, 54%; 75%, 82% in categorized symptomatic seeds. Some seedlings obtained from asymptomatic seeds or weakly infected seeds showed descrete black streaks on hypocotyls and necrotic spots on cotyledons along with the browning of radicle. Seedlings from weakly infected seeds showed wilting (after 20 days), drying and drooping off of leaves and ultimately died (Figure 2.F). Very few plants attained maturity showing black streaks on collar region of the stem and brown to black necrotic patches on leaves, stem and pods (Figure 2.G). Symptomatic leaves are small in size. Such plants produced symptomless or symptomatic pods, whereas seedlings obtained from moderately to heavily infected seeds, flowering and seed setting in pods are very few or infected (Figure 2.H). Presence of the pathogen is on the stems (Figure 2.I) and also in the pith region of the stem (Figure 2.J). On incubation these stem and leaves showed the presence of pathogen, while cleared and microtome preparation revealed the presence of inter and intra cellular mycelium in the cortical cells, pith and vascular region. Necrotic cells were quite evident in cleared preparations of leaves whereas in stems and roots brown discolouration with fungal growth was observed. Presence of conidia and mycelium were also seen in split half of the stem and roots. Control by drip irrigation is very useful at the time of flowering and fruit setting. Use of certified seeds is very useful to get high yield of seeds.

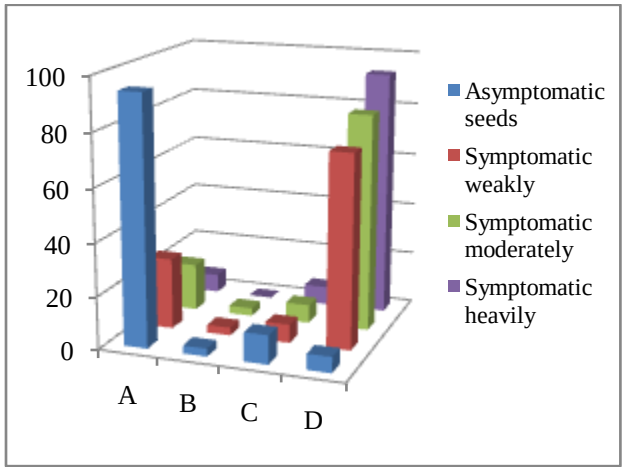


FIGURE : (A) CB29

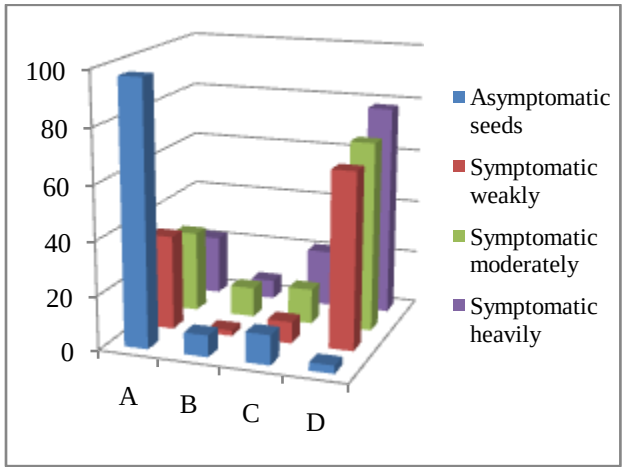
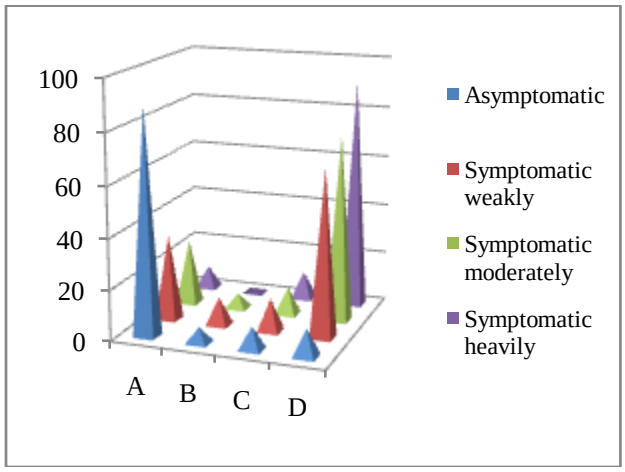


FIGURE : (B) CB70



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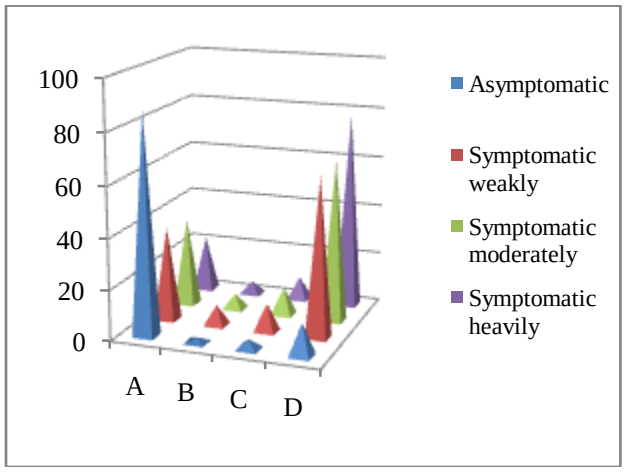

FIGURE : (D) CB70

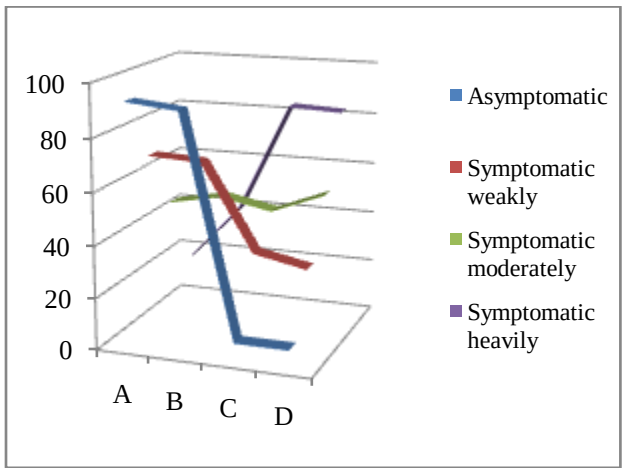

FIGURE : (E) CB 29

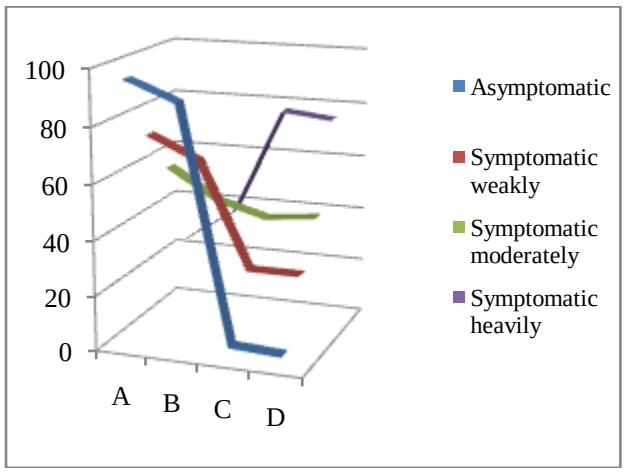

FIGURE : (F) CB70

A-Germination **B-Surviving seedling with Symptoms**
C-Seedling Mortality **D-Total loss**

Figure 1 (A- F): Phytopathological effect of *Fusarium solani* on 8th day in SBM (A-B) and 15th day in water AGAR (C-D) and after 60 days in pot experiment (E-F)

A **B** **C**

D **E** **F**

G **H** **I** **J**

Figure 2A-J: Phytopathological Effects of *Fusarium solani* in SBM, Water agar Test and Pot Experiment

A. White mycelial growth in dry seed inspection
B. Heavily infected seeds covered with pathogen in standard blotter method
C. Test tube showing healthy (Right), symptomatic seedlings (Left) on 14th day in water agar test tube seedling symptom test
D. Symptomatic seedlings
E. Symptomatic plant in Pot experiment
F. White brown Necrotic Patches on leaves
G. Symptomatic pods
H. Pods showing brown black patches
I. Stem infected with *F. solani*
J. Split half of the stem showing infection of *F. solani*

DISCUSSION

Prasad and Desai (1951), observed blight due to *F. moniliforme***.** Satyaprasad and Rama Roa (1981) and Mathur and Sekhawat (1988) recorded root rot by *Fusariumsolani*. The seed borne inoculum of *F. solani* caused damping off in guar seeds (Dwivedi *et al.*, 1991) and wilt caused by *F. oxysporum* in lentil seeds (Singh 2012).

During the present study seed borne infection of *F. solani* resulted in pre and post emergence of losses. Pre emergence mortality was higher than post emergence mortality. The failure of seed germination and incidence of seedling mortality are correlated with degree and nature of infection. Heavily infected seeds failed to germinate. In pot experiment plants under went wilting shows the brown patches on leaves, leads to the shredding and roting. Collar region of the stem shows the brown- black streaks. Such plants failed to produce flowers and pods. Satyaprasad and Rama Rao (1981) observed similar symptoms caused by *F. solani* in guar seeds. Rot symptoms are induced by *M. phaseolina* in guar seeds was observed by Lodha (1998), Lodha *et al.,* (2002) and also pre and post emergence of mortality in comparision to control in guar seeds (Jaiman and Jain 2004). However wilt caused by *Fusariumoxysporum* in ground nut seeds showed similar symptoms (Awurum and Uwajimgba, 2013), Bhatia (1995) in guar seeds and in lentil seeds by Singh (2012). Control by drip irrigation was studied in *Luffa* seeds (Sadda, 2012).

The present study provides enough evidences of effects of seed borne inoculum of *F. solani*. Heavy to moderate infection causes failure of seed germination and wilting of surviving seedlings/ plants. Weak infections results in plants which reach maturity and have infection confirmed to basal parts only. Seeds harvested from such plants were found free of infection.

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CARBOHYDRATES AND ITS RELATED ENZYMES WAS ESTIMATED IN GUAR SEEDS INFECTED BY *FUSARIUM* WILT IN RAJASTHAN

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ABSTRACT

Quantitative estimation of carbohydrates and their related enzymes was carried out in seeds and seedlings of *Cyamopsis tetragonoloba* infected with Fusarium solani causing wilt disease. Infection of *Fusarium solani* was symptomatic and it was characterized by sudden yellowing and drooping followed by drying of leaves and entire seedling. Estimation of carbohydrates and its related enzymes was done in healthy and wilted counterparts. These plant parts showed variation in their carbohydrate contents. In healthy plant parts total soluble sugar and starch were found higher than the wilt infected plant parts. It was observed 0.06, 0.054; 0.12, 0.108; 0.04, 0.036 mg/g in infected seed, stem and leaves respectively while in its healthy counterparts it was 0.07, 0.063; 0.35, 0.315; 0.06, 0.054 mg/ g respectively. ?- amylase activity was higher in case of infected plant parts 0.016, 0.009, 0.012 units/ sec/ mg weight of fresh tissues of seed, stem and leaves respectively than the healthy counterparts 0.008, 0.003, 0.008 units/ sec/ mg weight of fresh tissues respectively.

Key words : Cyamopsis tetragonoloba, Fusarium solani, Carbohydrate contents., Guar Seeds.

INTRODUCTION

Fusarium Solani (Mart.) Sacc. is well known phytopathogenic fungus and is an important causal agent of several crop diseases in legumes viz. wilt, root rot and damping off. *Fusarium Solani* was emended by Synder and Hansen (1941). The fungus is chiefly soil borne as well as seed borne (Satyaprasad and Ramaroa, 1985; Dwivedi, Dubey and Dwivedi, 1991). *Cyamopsis tetragonoloba* is an important legume crop grown in warm and rainy season for green manuring, green fodder and vegetable crop for human consumption since ancient times in Rajasthan (India). It also has wide and special importance due to presence of glactomannan gum content. The green pods serve as a nutritious vegetable contains 82.5% water, 3.7% protein, 9.9% carbohydrate, 0.2% fat, 2.3 % fiber and 1.4% other minerals. It is a rich source of protein and carbohydrate in comparison to other legumes. *Cyamopsis tetragonoloba* seeds are prime source of carbohydrates such as starch and glactomannan. So it has wide industrial uses viz. food, textile and paper industries. Various disease such as wilt, blight, root rot and damping off cause reduction in yield of *cyamopsis tetragonoloba crop*. Out of which *Fusarium* wilt caused by *Fusarium solani* (Mart.) Sacc. is important and characterized by sudden yellowing and drooping followed by drying of leaves and the entire seedling.

Study is done to understand the physiological change of diseased plant parts. Comparative quantification of carbohydrates and their related enzymes were carried out in healthy and Fusarium solani infected plant parts of Cyamopsis tetragonoloba (L.) Taub.

MATERIALS AND METHODS

Normal and fungal infected cluster bean plant parts grown in the fields of Sikar (ac.no.CB-70) districts of Rajasthan were collected and their morphological studies were undertaken. Quantification of total starch content and related enzyme viz. ?- amylase extract of infected and healthy counterparts viz. leaf, stem and seeds of cluster bean plants was carried out by using standard methods. (Loomis and Shull, 1937).

Estimation of carbohydrates

A. Total soluble sugar.

500 mg of fresh healthy and disease infected plant parts of cluster bean viz. stem leaves and seeds were homogenized with 10.0 ml of 80% ethanol than grind in pestle and mortar. The samples were centrifuged at 2000 rpm for 20 minutes. After centrifugation the supernatant was discarded and residue was collected; concentrated on water bath. The volume of these aqueous solution were raised to 50 ml with distill water (Ext. A) and processed further by following the method of Loomis and Shull (1937) for soluble sugars. However the residual pallet obtained by centrifugation was used for estimation of starch.

B. Starch content

Residue was used for the estimation of starch, suspended in 5.0 ml of water and subsequently 6.5 ml of 52% perchloric acid was added to the residue. Stir the mixture and the contents were centrifuged at 2000 rpm for 20 min. The supernatant was decantant and collected and the whole procedure was repeated thrice. Supernatants of each step were then pooled together and the total volume was made up to 100 ml with distilled water (Ext. B). The mixture was then filtered through whatmann filter paper (No. 42); 1 ml of aliquot of this filtrate was taken for estimating starch content (Mc Cready *et al.*, 1950).

Quantification of carbohydrates

Aliquot (1 ml) of each of the test samples from Ext. A and Ext. B were used for the quantification of total level of carbohydrates using phenol sulphuric acid method (Dubois et al., 1951) A regression curve for standard sugar (glucose) was also prepared.

A stock solution of glucose was made in distill water, 0.1 to 0.9 ml was transferred to test tube and the volume was raised to 1 ml with distill water readily 1 ml of 5% aqueous phenol was also added; kept cool in an ice chest and shaken gently. 5 ml of conc. H2SO4 was added by agitating gently during the addition of the acid. Subsequently the test tube was kept on the water bath (26-30°C) for 20 min. Optical density of yellow orange colour was measured using UV spectrophotometer (Systronics UV - VIS-118) at 490 nm after setting for 100% transmission against the blank. Three replicates of each samples were run and there mean values were calculated. A regression was computed between its known concentration and their respective ODs. The concentration of total soluble sugar was directly work out from the regression curve of the standard glucose. Three replicates of each experimental sample were taken and their mean values were calculated. The sugar content in term of glucose equivalent and the use of conversion factor (0.9 to convert the values of glucose to starch) was made in each case.

Extraction of α - amylase

1 gm fresh weight of normal looking and disease infected cluster bean sample each was crushed with 1.0 ml of 1 M phosphate buffer pH=7.0. The homogenate was centrifuged at 3000 rpm for 10-15 min and supernatant was collected which served as enzyme extract.

Estimation of α - amylase activity

1 ml of enzyme extract which mixed with 1.0 ml of substrate (Prepared by dissolving 10 mg soluble starch in 100 ml of 0.02 M phosphate buffer PH=6.9 containing 0.0065 M NaCl) forms the reaction mixture. Incubate the reaction mixture at 30° C for 15-20 min and end the reaction by adding 2.0 ml of dinitrosalicylic acid reagent, and solution of was kept in water bath for 5 min then 1.0 ml of potassium sodium tartrate was added, after this cool the test tubes solution in running tap water and made up the volume to 10 ml by adding 6 ml of distilled water. Read the optical density of the yellow coloured solution developed at 560 nm against blank. The activity was expressed in terms of mg starch hydrolysed/sec/mg fresh weight of tissue.

RESULTS AND DISCUSSIONS

The starch content significantly decreased in *Fusarium solani* infected cluster bean plant parts viz. seed, stem and leaves (Fig. 1A and Table 1). Stem of cluster bean showed higher amount of starch content than other counter parts viz. leaves and seeds in both infected and healthy conditions. Total soluble sugar also expressed similar decrease in infected plant parts (Fig 1B) while infected plant parts expressed higher amount of α - amylase activity than the healthy counterparts (Fig. 1C; Table 1). However, in case of ?- amylase activity, comparative increase was found to be somewhat more in seeds than the stem in both infected and healthy conditions (Fig. 1D). Comparative analysis of these three components expressed that the total soluble sugar and starch content were higher in stem than the other counterparts viz. leaves and seeds while α - amylase activity were recorded higher in seed than other counterparts in both infected and healthy condition. Similar observations were reported in lentil by Singh and Varma (2010) and in *Luffa cylindrica* by Sadda and Varma (2011).

Starch, the primary storage material in most seeds has been studied for its deterioration by fungal infection in many crops like *Cajanus seeds* (Sinha, Singh and Prasad, 1981), wheat (Agarwal, Thakur and Awasthi, 1982) and Groundnut (Kamble and Gangawane, 1987). Decrease in starch content also observed in pigeon pea seeds due to *Aspergillus* infection by Sinha and Prasad (1977). Bateman and Millar (1966) reported sugars play an important role in the inhibition of pectinolytic and cellulolytic enzymes which are essential for pathogen. Jain and Yadav (2003) reported positive significant correlation with leaf wilt infection with total and reducing sugars. Yadav (2003) and Sharma (2004) reported that carbohydrate may accumulate in "physiological sink" (galls) by depletion of starch due to ?- amylase and other enzymes activity. Kushwaha and Narain (2005)

suggested that increase in total soluble sugars might be because of their accumulation as a result of disruption of normal phloem transport on pathogenesis. Earlier Singh and Sexena (2003) also reported similar type of disruption in plant cells. They suggested that accumulation of total soluble sugar in infected tissue may involve their translocation from the adjacent healthy tissue. Similar findings were also reported by Marmit and Sharma (2008) for induced leaf galls tissues of *Mangifera indica*.

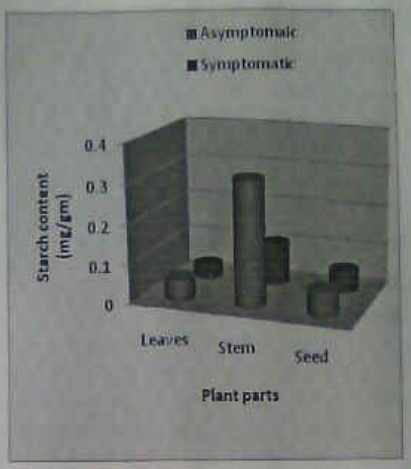
Singh (2004) reported increase in ?- amylase activity in leaf spot disease of brassica. High level of ?- amylase might be attributed to the involvement of fungus in enzyme activity. Thus, correlation established between amino acid content and pod damage was positive and significant. Parashar and Lodha (2008) revealed lower starch content and higher ?- amylase activity in *Ramularia foeniculi* infected fennel (*Foeniculum vulgare*) plant parts than the healthy counterparts. Marmit and Sharma (2008) observed higher total soluble sugar, starch and ?- amylase activity in insect induced leaf galls tissue than the normal tissue of *Mangifera indica*.

Afiukwa *et al.* (2009) reported about determination of amylase activity of crude extracts from germinated mango seeds. Singh *et al.* (2011) reported increased activity of α - amylase in *Albugo candida* infected tissues of *Brassica* plant parts. The increase activity of these enzymes might be due to their enhanced synthesis by the host to meet the catabolic reactions in the enhanced state of host metabolism after infection. Sawant and Gawai (2011) reported that the fungi influenced stored substance or nutrient by absorbing them or by converting some of the substance complex into simple one.

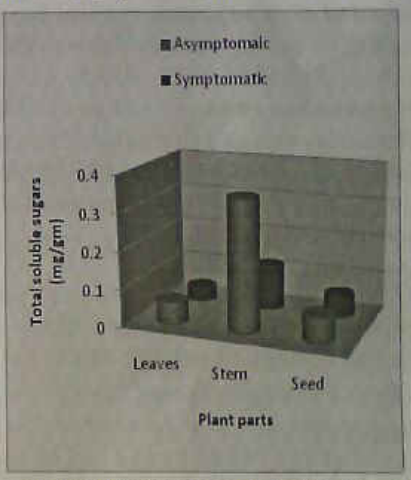
TABLE 1: Quantification of Carbohydrates (Starch and total soluble sugar) and related enzyme (?- amylase) activity in Cyamopsis tetragonoloba (L.) Taub.

Concentration (mg/g)	NSd	ISd	NS	IS	NL	IL
Total soluble sugars (mg/g)	0.07 ±	0.06 ±	0.35 ±	0.12 ±	0.06 ±	0.04 ±
Starch (mg/g)	0.063 ±	0.054 ±	0.315 ±	0.108 ±	0.054 ±	0.036 ±
α - Amylase activity (units/sec/mg. weight of fresh tissue)	0.008 ±	0.016 ±	0.003 ±	0.009 ±	0.008 ±	0.012 ±

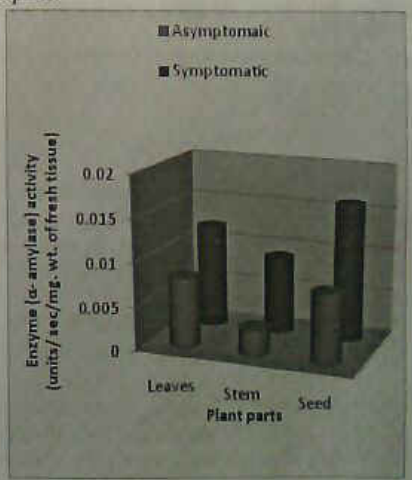
NSd = Normal seeds ISd = Infected seeds,
NS = Normal stem IS = Infected stem
NL = Normal leaves IL = Infected leaves



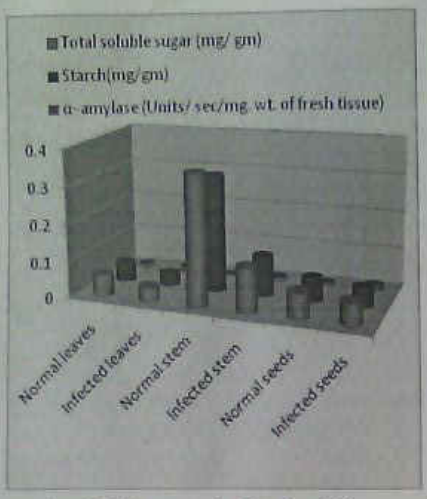
A. Comparison of starch in healthy (Asymptomatic) and *Fusarium solani* infected (Symptomatic) cluster bean counterparts.



B. Comparison of total soluble sugars in healthy (Asymptomatic) and *Fusarium solani* infected cluster bean counterparts.



C. Comparison of enzyme (?-amylase) activity in healthy (Asymptomatic) and *Fusarium solani* infected (Symptomatic) cluster bean counterparts.



D. Comparative 3D histogram for Total soluble sugar, Starch and α - amylase activity.

Nwaukwu *et al.* (2012) reported that the pathogenic fungi affect the nutritional composition of the edible fruit of *Dialium guineense*, a fruit mostly eaten in Africa. The reduction in nutrient components is due to disease pressure. Shrivastav and Kumar (2013) revealed losses in reducing sugars and ?- amylase activity after infection of Botrytis in onion (*Allium cepa*) and colletotrycum in capsicum (*Capsicum annuum*).

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Fusarium solani a dominant seed borne pathogen in seeds of cluster bean grown in Rajasthan

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ABSTRACT

The present study has been carried out to illustrate the seed borne nature of the pathogen and its transmission from seed to seedling/plant. For those purpose one hundred twenty seed samples of *Cyamopsis tetragonoloba* collected from 11 districts of Rajasthan were examined by SBM and PDA tests. *Fusarium solani* is a serious pathogen in guar seeds causes wilt disease which is responsible to reduce the quality and yield of the crop it causes severe economic losses. The pathogen is seed borne both extra and intra embryonal. Guar seeds naturally infected with *Fusarium solani* showed white discolouration with irregular shape or covered with white mycelial crust 43(0.25-40.5%) causing wilt disease. These seeds on incubation yielded pure growth of the pathogen. On the basis of severity of infection seeds were characterized as asymptomatic and symptomatic (weakly, moderately and heavily). The transmission of seed borne inoculum of *Fusarium solani* from seed to seedling caused high pre and post emergence losses in symptomatic seeds than the asymptomatic seeds. The pathogen is transmitted from seed to seedling and causes heavy losses. From heavily infected plants, leaves showed necrotic patches where as stem and roots showed presence of yellow black streaks. On inoculation fungal mycelium and spores were observed in the split half of the stem and root cortical region. Thus the present study highlights the brief outlines about the seed borne nature and transmission of pathogen from seed to seedling/ plant and also provides enough detail about the severity of infection of wilt disease caused by *Fusarium solani* in cluster bean crop which is useful to obtain disease free seeds and high commercial, industrial and medicinal value of the crop express the significance of present research work to increase the profitability and the quality production.

KEY WORDS: ASYMPTOMATIC SEEDS, *CYAMOPSIS TETRAGONOLOLA*, *FUSARIUM SOLANI*, INFECTION, INOCULUM, TRANSMISSION.

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INTRODUCTION

Fusarium solani is widely found most important soil borne as well as seed borne pathogen causes various diseases such as root rot, wilt, damping off in vegetables, legumes, oilseeds and ornamental crops and causes severe losses to economic, medicinal and industrial value of the crop (Richardson, 1990; Sultana and Ghaffar, 2007; Abu-Taleb *et al*., 2011; Khair *et al*., 2011; Bahar and Shahab, 2012; Karima and Nadia, 2012; Hafizi *et al*., 2013).

Cluster bean is a highly commercial, industrial and medicinal important leguminous crop due to presence of glactomannan gum in endosperm of seeds. It is highly adapted to arid and semi arid regions of the world requiring low inputs and care. It is cultivated mainly in rainy season as a rainfed crop in arid zones of India and various other parts of the world (Pathak *et al*., 2010; Pachundkar *et al*., 2013). Pods and seeds of *Cyamopsis tetragonoloba* are a very useful local source of fibers. Guar gum extracted from seeds is used as an appetite depressant and also used as a bulking agent in laxative, in gastric ulcer and asthama treatment. It also reduced blood cholesterol and glucose levels significantly by using an aqueous extract of pods of the plant at a dose of 250mg/kg of body weight (Sharma *et al*., 2011).

Fusarium solani is serious pathogen which causes wilt, root rot and damping off in cluster bean (Satyaprasad and Ramaroa, 1981; Dwivedi, Dubey and Dwivedi, 1991). The pathogen is seed borne and transmitted in all plant parts of cluster bean (Mathur and Shekhawat, 1988; Dwivedi, Dubey and Dwivedi, 1991). The heavy infection of pathogen causes severe losses in quality and yield of crop which reduce the medicinal and industrial value of the crop.

There is very little information on the seed borne nature of *Fusarium solani* in guar seeds. Therefore, the objectives of this study were to (i) isolate and identify the seed borne nature of *Fusarium solani* in cluster bean by using incubation tests (ii) to determine the exact location and extent of penetration of seed borne pathogen (iii) to illustrate the disease transmission of the pathogen from seed to seedling/ plant parts. Thus, the present study has significant role to understand the seed borne nature and severity of infection of the pathogen in cluster bean crop which is very useful in obtaining disease free seeds and the wide application of cluster bean seeds in traditional medicine, pharmaceutical, cosmetics, paper industries, textile, bakery and oil field expressed the high commercial values and future perspectives of present research work to increase the profitability and the quality production.

MATERIALS AND METHODS

One hundred twenty seeds samples of cluster bean collected from 11 districts of Rajasthan in the year 2011-2014 were subjected for dry seed examination and incubation tests by Standard blotter method and Potato Dextrose Agar method as recommended by ISTA (Anonymous, 1985). In dry seed examination test besides normal looking asymptomatic seeds of various discolourations viz. seeds with black streaks; grey colour seeds with white mycelial growth; shriveled seeds; broken and insect damaged seeds and debris and inert matter were observed. Seeds were incubated on moistened blotters both untreated and 0.5% chlorine pretreated for 2 min in PDA test. Pretreated seeds were spaced (20 seeds/ plate) on petriplate containing PDA medium. Out of these two samples CB29 (Jaipur) and CB70 (Sikar) were used for seed health testing and transmission studies. Disease transmission from seed to seedling/ plant was studied by using asymptomatic and categorized symptomatic seeds (weakly, moderately and heavily). 100 seeds per category per sample were sown in petriplate (20 seeds/ plate) and 50 seeds per category per samples were sown in water agar seedling symptom test (1 seed / test tube) (Khare *et al*., 1977) and in earthen pots (5 seeds/ pot).

RESULTS AND DISCUSSION

In dry seed examination seeds infected with *Fusarium solani* showed white mycelial growth on seeds in 43 (0.25-40.5%) seed samples (Fig. 2-A). On incubation the fungus was isolated from 57 (0.25-62%), 53 (0.25-35%) and 15 (0.25-30%) in untreated, pretreated in SBM and PDA respectively. The incidence was high in untreated seeds in SBM. Histogram gives the comparative account of percentage incidence of pathogen on 8th day of incubation in both CB29 (Jaipur) and CB70 (Sikar) seed samples (Fig. 1). Highest infection of *Fusarium solani* in both the samples was obtained in untreated seeds in SBM. Seed germination as well as incidence of pathogen was very low in both pretreated seeds and PDA. In standard blotter test germination varied from 94% to 97% in asymptomatic seeds of cluster bean whereas in weakly moderately and heavily infected seeds it was 25-35%, 18-30% and 7-22%. In water agar test the percentage germination was almost similar except asymptomatic seeds. In asymptomatic seeds the pathogen suppresses the germination after radicle emergence and expressed yellow brown to black discoloration (Fig 2-B). Konde *et al*. (1980) used blotter paper and agar plate method for isolation of fungal species. Bhatia (1995) have reported reduction in germination by seed borne inoculums of

Fusarium oxysporum. Shakir & Mirza (1992) have also reported *F. solani* caused significant reduction in seed germination in bottle gourd and sponge gourd (Shakir *et al*., 1995). In Pots the germination varied from 93-96% in asymptomatic seeds and 69-73%, 46-57% and 18-25% in categorized symptomatic seeds (weakly, moderately and heavily) of cluster bean. Survival and vigour of seedling was observed lower in symptomatic seed than the asymptomatic seeds. The pre and post emergence loss in symptomatic seeds was observed very higher than the asymptomatic seeds.

Symptomatic seeds showed initial symptoms on transition zone in the form of pale to brown patches on 3rd to 5th day but in asymptomatic seeds delayed symptoms were observed. Later the symptoms increased rapidly and spread to the cotyledons in the form of irregular necrotic brown colored spots. The severe infection of fungus caused seedling mortality after 15th days. The surviving plants which grew to their full height showed black patches in the form of streaks on basal part of stem near the collar region (Fig 2-C). 30 to 40 days old seedling showed complete yellowing and wilting. Split half of the infected stem showed brown to black discolouration and on incubation these halves expressed fungal mycelial bits in cortex region (Fig 2-E). The infected plant leaves showed yellowish brown necrotic patches on their surface followed by their shrivelling and drooping (Fig. 2-D). On incubation heavily infected leaves showed the presence of fungal spores. The infected pods having less number of seeds than healthy ones. Split half of infected pods showed brown-black patches on outer and inner surface and white colored fungal growth also appeared on harvested seeds (Fig 2-F). Satyaprasad and Ramarao, (1981) reported yellowing and drooping of leaves and black lesions at soil surface of root of guar in *F. solani* infected plant. The pathogen was isolated from cotyledonary leaves of seedling and plant parts. Cleared and hand cut sections of these seedling and plant parts expressed the fungal mycelium in epidermis, cortex and vascular regions. In present study hilar region of the seed showed aggregation of fungal mycelium. This study suggests that the hilar region is the entry point of fungal mycelium. Gupta, Dubey and Singh, (2011) reported high pre and post emergence losses in *Dalbergia sisso* infected with *Fusarium semitectum* causing wilt disease. They also reported symptoms of wilt disease as necrotic spots on cotyledonary leaves and browning of radicle. Later these spots were turned into wilting of seedling/ plant and succumb to death. They also revealed transmission of pathogen from seed to seedling/ plant.

The guar is commonly susceptible to *Fusarium solani* causing wilt diseases during summer to rainy season when the conditions are favorable for the growth of fungi (Kamal and Khan, 1967). Dwivedi *et al*., (1991) reported that *Fusarium solani* infected seeds may be symptomatic or asymptomatic. In case of asymptomatic seeds or weakly infected seeds pathogen colonized the seed coat while in case of moderately and heavily infected seeds pathogen colonized to endosperm and embryo of seeds. The seed borne inoculum of *Fusarium solani* (Mart.) Sacc. deeply present in embryo later spreads rapidly and cause failure of seed germination while in asymptomatic and weakly infected seeds it is transmitted to developing plants. Varma *et al*., (1989) revealed the colonization of *Rhizoctonia bataticola* pathogen in all components of moth bean seeds.

Varma *et al*., (1992) reported seed borne nature of *Colletotrichum damatium* in *Vigna aconitifolia*. Varma, (2002) reported *Rhizoctonia baticola* as a serious pathogen in the seeds of *Vigna aconitifolia* crop. The initial symptoms on transition zone as brown black patches on 3rd to 4th day in symptomatic moth bean seeds infected with *Rhizoctonia bataticola* (Varma, 2003).

Rajput *et al*., (2010) revealed that maximum infection of frequency exhibited by *Fusarium solani* colonizing stem tissue of Shisham trees followed by bark tissues from seeds and roots. The colonization percent of *Fusarium solani* was highest as compared to other isolated fungi. Maximum reduction in seed germination was also reported by *Fusarium solani*. Sadda and Varma, (2010) reported small water soaked or yellowish spots on above ground parts viz. leaves and stem of smooth gourd infected with *Colletotrichum orbiculare* caused Anthracnose disease.

Farrag and Moharam, (2012) reported transmission of pathogenic fungi of cucumber seeds viz. *Fusarium oxysporum, F. solani* and *Rhizoctonia solani* from the germinated seeds to seedling causing pre and post emergence death. The transmission rate of tested fungi causing seed rot or pre emergence death was higher than the seedling mortality. Similar observations were reported in tomato seeds (Askar *et al*., 2014) and in chickpea seeds (Zaidi and Pathak, 2013). Seed-borne fungi are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. The infected seeds may fail to germinate, or transmit disease from seed to seedling and/or from seedling to growing plant (Islam and Borthakur, 2012).

Singh, (2013) reported seed borne nature of *Fusarium oxysporum* caused wilt disease in lentil and reported pale yellow discolouration on hypocotyl. Later this discolouration spreads rapidly on shoot than the primary root region. Ramadan and Zrary, (2014) reported significant increase in the effect of six fungal pathogens viz. *Aspergillus candidus, A. niger, A. sulphurous, Cladosporium herbarium, Curvularia lunata, Drechslera tetramera* and *Penicillium sp*. on pre-emergence damping-off seedlings with soil infestation. Sharma and Sharma

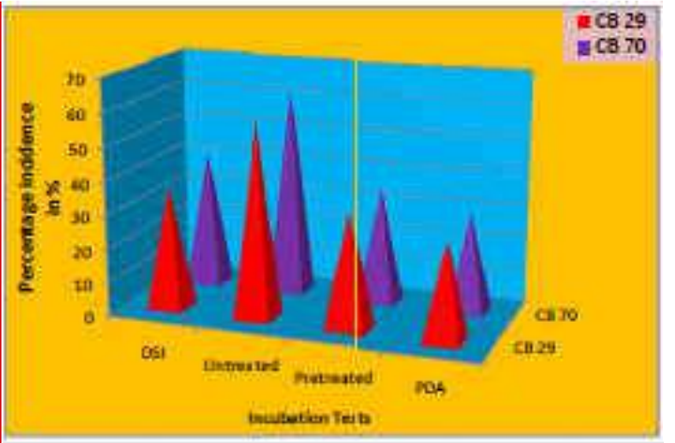

FIGURE 1: Histogram showing comparative percent incidence of *Fusarium solani* in two seed samples in DSI (Dry seed inspection), SBM (untreated and pretreated) and PDA. Note that untreated seed samples showing maximum count of pathogen in all the samples tested.

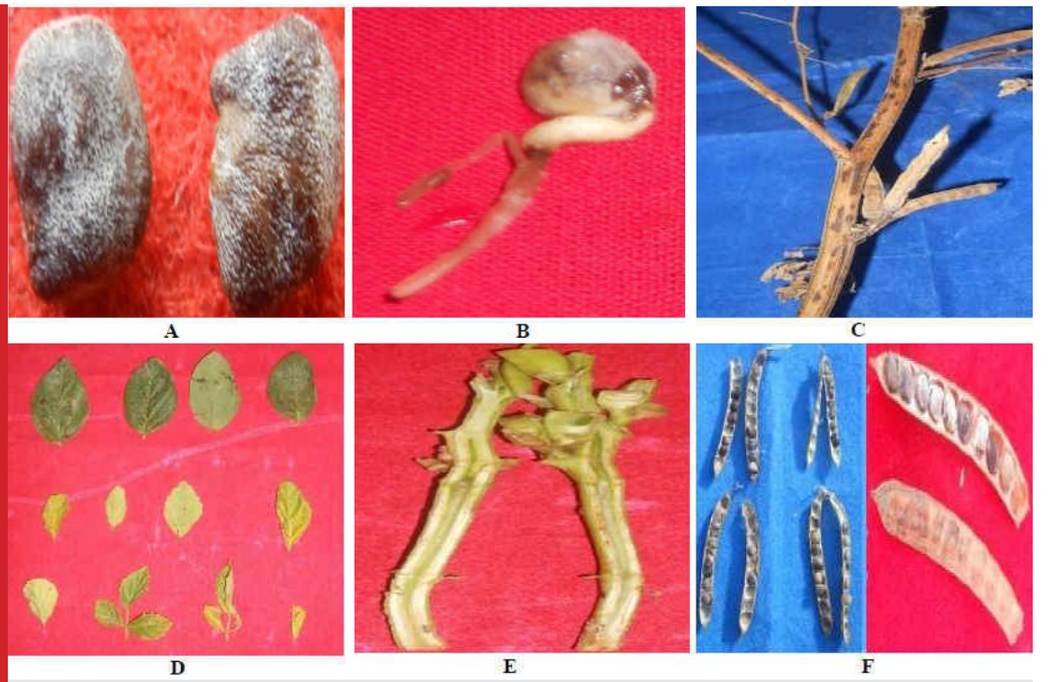

FIGURE 2: (A) White fungal mycelial growth on seed surface in DSI X-22, (B) Brown to black discoloration on radicle region X-12, (C) Brown black patches on Infected stem X-12, (D) Various degree of infection in leaves X-12 , (E) Cortical region of split half of the stem showing brown discoloration and white mycelial growth X-22, (F) Split half of infected pod showing black discoloration on outer surface and seeds with white fungal growth X- 12.

(2014) revealed that the seed borne inoculum of *Ralstonia solanacearum* caused pre- and post-emergence losses in brinjal seeds and the symptoms appears as browning of radicle, plumule later turned into necrotic spots with bacterial oozing. Similar symptoms on seeds were reported earlier in tomato (Sharma and Agarwal, 2010) and in cluster bean (Jain and Agarwal, 2011).

CONCLUSIONS

The results suggest that *Fusarium solani* is most dominant pathogen of cluster bean which caused wilt disease and revealed highest infection in untreated seeds in standard Blotter Method. The pre and post emergence loss in symptomatic seeds was observed higher than the asymptomatic seeds. The present study clearly indicates the seed borne nature of *Fusarium solani* and its transmission and pathogenicity from seed to seedling/plant parts. It also suggests that the pathogen affect all the plant parts and cause severe losses to quality and yield of cluster bean crop. Seed borne inoculum of *F. solani* was occurs in both asymptomatic and symptomatic seeds of both the samples. In the asymptomatic seeds the incidence remains low and infection confined to seed coat whereas in symptomatic seeds the infection is intra-embryonal, deep seated and distributed in all the seed components and transmitted to all plant parts viz. root, stem, leaves and pods. The deep infection is difficult to inactive using normal conventional methods of control. Thus, the present study revealed the severity of infection of wilt disease caused by *Fusarium solani* in cluster bean crop which is useful to obtain disease free seeds of important cash crop and cluster bean seeds have larger future perspectives in terms of higher economic, industrial and medicinal value of the crop than the other legume crops due to presence of glactomannan gum in endosperm of their seeds.

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