MANAGEMENT OF PIGEONPEA WILT [*Fusarium udum* Butler]

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MANAGEMENT OF PIGEONPEA WILT [*Fusarium udum* Butler]

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A B S T R A C T

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important pulse (legume) crop in the Indian sub-continent. The crop has a greater significance in Indian agriculture, because of its multiple uses as rich source of protein food, feed, fodder, fuel and also sustaining agriculture productivity. More than 100 pathogens, *viz*., fungi, bacteria, viruses, phytoplasma and nematodes, are attacking the pigeonpea. Among these, the wilt of pigeonpea caused by *Fusarium udum* Butler is one of the main constraint in harvesting the economic crop yield in Gujarat. In spite of cultivation of wilt tolerant cultivars of pigeonpea (GT-100 and BDN-2), the incidence upto 30% have been reported from the major pigeonpea growing areas of Bharuch, Narmada and Vadodara districts of Gujarat. But very meagre information is available on pigeonpea wilt in Gujarat. Therefore, the present investigations were carried out as here under.

The wilt of pigeonpea is caused by *Fusarium udum* Butler, had proved by repeated tissue isolation and by positive pathogenicity test of isolated pathogen from Bharuch, Gujarat. The incidence of wilt was ranged from 8.4% to 14.3%, phytophthora blight 2.0% to 7.6% and sterility mosaic disease 0.8% to 4.0% in major pigeonpea growing areas of Bharuch, Narmada and Vadodara districts of Gujarat. The pigeonpea plant is vulnerable for attack by wilt pathogen throughout its growth development. However, wilt damage is more after rainy season, at flowering and at poding stages. The wilt in the field is characterized by gradual or sometimes sudden yellowing, withering and drying of leaves, followed by the drying of the entire plant or some of its branches. If the stem and root are broken and splited, the black streak may be traced upto a height of several feet on the centre of stem.

Among, the seven inoculation techniques, the sowing in wilt sick plot and soil inoculation are the most reliable and successful for screening large number of entries against wilt. The higher inoculum level (50 g/kg or more) in the soil was required to create high disease pressure. There was variation in virulence of the isolated pathogen, *F. udum* Butler, from Bharuch, Narmada and Vadodara districts because there is much variation observed in inducing wilt symptoms in susceptible (T-15-15) and resistant/tolerant (BDN-2) genotype.

The Czapek's Dox agar, Potato dextrose agar and Oat meal agar media are the best media for radial growth of mycelium, dry mycelial weight and sporulation of *Fusarium udum*.

The garlic bulb (63.7%) extract is significantly superior to inhibit the growth of *Fusarium udum in vitro* followed by turmeric rhizome (58.5%) and neem leaves (44.2%). The antagonist *viz*., *T. viride* had significantly inhibited the growth of *Fusarium udum* Butler and next in order is *T. harzianum* followed by *A. niger*.

All the three concentrations of carbendazim, benomyl, hexaconazole and methyl ethyl mercury chloride inhibited the cent per cent growth of *Fusarium udum* upto 10 days. The propioconazole 1000 and 1500 ppm and copper oxychloride 3000 ppm also inhibited *Fusarium udum* Butler upto seven days. The per cent inhibition was decreased significantly with increase in period from 7 to 10 days, suggesting the breakdown or degradation of fungicide. The effective fungicides namely; carbendazim, benomyl, hexaconazole and methyl ethyl mercury chloride at three concentration tested and the higher concentration of thiram persists for a long period upto ten days.

The seed treatment to pigeonpea with thiram (3 g/kg

seed) prior to sowing and two drenching of carbendazim, benomyl and hexaconazole, first after 15 days of sowing and then at 15 days of first, had significantly reduced the per cent incidence of wilt and significantly increase the grain yield (kg/ha). Two drenching as above of *T. harzianum* had also significantly reduced per cent wilt incidence and increased grain yield (kg/ha) than control, but it was significantly lesser than above three fungicides tested.

The artificial screening of pigeonpea entries revealed that BPWR-03-1, BPWR-03-2, BPWR-04-2, ICPL-87119 and SKNP-0306 were found free from wilt during both years and SKNP-0207 and BDN-2 were found free from wilt during 2006-07. These entries were rated as resistant genotypes.

The ICPL-87119 was free from sterility mosaic for both the years under natural field condition, while, ICPL-87119 x BP-9224, BDN-2 x BP-86108, T-15-15 x A-2, MS-288 x ICPL-87119 were free from sterility mosaic during 2004-05.The entries, namely; GT-101, SKNP-214, SKNP-040, SKNP-409 and BPWR-03-1 were found free against phytophthora blight in natural field condition.

The significant contributions of these investigations are the reduction in wilt incidence by drenching of carbendazim, benomyl, hexaconazole and *T. harzianum* and identification of BPWR-03-1, BPWR-03-2, BPWR-04-2, ICPL-87119 and SKNP-0306 as resistant genotypes against wilt.

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C E R T I F I C A T E

This is to certify that the thesis entitled **"MANAGEMENT OF PIGEONPEA WILT [*Fusarium udum* Butler]"** submitted by **Mr. MEHTA ASHVINKUMAR NATAVARLAL** in partial fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY** of the **NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafide research work carried out by him under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place : Navsari **Major Advisor**

Date : March, 2008 **(H. L. Chauhan)**

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT PATHOLOGY by the undersigned is the result of investigations done by me under direct guidance and supervision of Dr. H. L. Chauhan, Major Advisor and Professor of Plant Pathology, Paddy Research Laboratory, National Agricultural Research Project (N.A.R.P.), Soil and Water Management Research Unit, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

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I. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is widely grown by small farmers in the semi-arid tropics as a backyard subsistence crop. It is produced commercially in India, Myanmar, Kenya, Malawi, Uganda and a few countries of Central America. Pigeonpea commonly known as arhar or tur, is the second most important pulse crop after chickpea in India. It is one of the extensively used pulses in India as an important source of protein in human diet. Generally, it is grown all over the country, but it is cultivated extensively in Bihar, Uttar Pradesh, Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka, West Bengal and Gujarat.

Pigeonpea is a profitable and popular crop in the Middle Gujarat after cotton. It fetches good price in the market. It is a hardy plant, when intercropped with a cereals, and ensures a measure of income stability. People use the dry grain as dhal, the green pod or seed as vegetable, feed, fodder and the sticks as fuel wood. In addition, it can be cut for forage and improves poor soil through its deep strong rooting systems, leaf drop at maturity and addition of nitrogen by symbiotic activities during crop growth. Considering importance of pulses in human nutrition, Government of India is giving much emphasis on increasing production of pulses in the country.

Pigeonpea dhal i.e. tur dhal being a main source of

protein, occupies key position in Gujarati diet. In Gujarat, Pigeonpea is grown over an area of 2,96,700 ha with the production of 2,58,000 m. tonnes. (Anon, 2004.) It is mainly grown in Bharuch, Narmada, Vadodara, Sabarkantha, Kheda, Surat, Tapi, Valsad, Ahwa-Dang, Panchmahal, Mehesana, Ahmedabad and Banaskantha districts and also Saurashtra region of the state. It is grown extensively either as a rainfed or irrigated cash crop in Bharuch, Narmada and Vadodara districts of Middle Gujarat.

Pigeonpea is attacked by more than 100 pathogens (Nene *et al*., 1989). These include *viz*., fungi, bacteria, viruses, nematodes and phtoplasmas. Fortunately, only a few of them cause economic losses (Kannaiyan *et al*., 1984) and the distribution of the most important diseases is geographically restricted. At present, farmers mainly grow pigeonpea varieties have some degree of tolerance to most of the pathogens (Nene, 1988). This situation could change once the diverse varieties are replaced by a few improved tolerant cultivars.

The diseases of economic importance at present are *viz*., Fusarium wilt, Sterility Mosaic Disease (SMD), Phytophthora Blight (PB), Macrophomina root rot, Stem canker, Alternaria blight and pearly cyst nematode on the Indian sub-continent. In Gujarat, among these diseases, the wilt is one of the major constraints, followed by Phytophthora blight and Sterility

mosaic, in affecting the productivity of this crop per unit area. The wilt is caused by *Fusarium udum* Butler is one of most serious and oldest known disease (Butler, 1906) and it is known to cause heavy losses every year in India (Kannaiyan *et al.*, 1981).

Vaidya *et al.* (2003) reported that, in spite of cultivation of wilt resistant cultivar (GT-100 & BDN-2) wilt incidence up to 30 per cent reported from Pigeonpea growing region of Gujarat. The another major disease is sterility mosaic caused by *Tenuivirus* and phytophthora blight caused by *Phytophthora drechsleri* f. sp. *cajani*.

The voluminous work has been done on *F. udum* Butler in India and abroad. Very meagre information is available in Gujarat about this disease. It has caused heavy losses due to epidemic in pigeonpea growing areas of Middle Gujarat, resulting into change in the cropping system in the few pockets of Middle Gujarat in last decade of twentieth century. The pathogen is a soil and seed borne. The genus *Fusarium* have wide host range and survives for long time in the field in the absence of host plant. Therefore, chemical control is not satisfactory, adequate and economical as a long-term solution. Considering, the crop health and economic losses, the alternative to this is to explore the possibility of improving genetical disease resistance and integration of chemical and biological control, which can be

successfully adopted in modern agriculture.

Considering, the seriousness of the Pigeonpea wilt disease in the Middle Gujarat, there is an urgent need to initiate the work on management of Pigeonpea wilt. With this view to explore the possibility of identifying the wilt resistant genotypes and to identify the effective and economical chemical or antagonist, to minimize the yield losses. The present investigations on Pigeonpea wilt, (*F. udum*) and simultaneously, the information on occurrence of phytophthora blight [*Phytophthora drechsleri* Tucker f. sp. *cajani*] and sterility mosaic disease in Middle Gujarat were also carried out.

1. Survey and collection of disease sample of Pigeonpea diseases, *viz.,* wilt, phytophthora blight and sterility mosaic disease
2. Isolation and identification of causal pathogen of Pigeonpea wilt
3. Pathogenicity and symptomatology
4. Search for best rapid inoculation technique
5. Search for superior media
6. Studies on virulence pattern in wilt pathogen

7. Screening of genotypes against Pigeonpea wilt in wilt sick plot
8. Bio-efficacy of botanicals, fungicides and bioagents against *F. udum in vitro*
9. Management of Pigeonpea wilt *in vivo*

II. REVIEW OF LITERATURE

Among the several constraints responsible for low productivity of Pigeonpea, the diseases are one of them causing reduction in crop yield in terms of quality and quantity. The wilt of Pigeonpea is a major constraint in achieving yield potential. Second one is phytophthora blight [*Phytophthora drechsleri* Tucker f. sp. *cajani*] and sterility mosaic disease (*Tenuivirus*).

The much research work had carried out by various workers on different aspects of Pigeonpea wilt disease caused by *F. udum* and lot of literature is available but a few of them are included here under.

2.1 Occurrence and economic importance

The Pigeonpea wilt disease *F. udum* was first time described from India by Butler in 1906. The disease is responsible for 15–30% plant mortality in wilt prone area every year. On an average wilt incidence was 27.9 % in Maharashtra, 5.2 % in Andhra Pradesh and 1.4 % in Tamil Nadu. The grain losses was almost 100 % when wilt occurs at or prior to early pod formation stage and was around 30 % when the plants were closed to harvest (Nene *et al.,* 1979). Kannaiyan *et al.* (1980) surveyed the wilt and SMD, respectively in major state of India during 1975 to 1980. They reported average per cent incidence of wilt and SMD, respectively, in different state are 5.3 & 1.6 in

Andhra Paradesh, Bihar 18.3 & 21.4, Gujarat 5.4 & 12.2, Karnataka 1.1. & 9.8, Madhya Pradesh 5.4 & 3.7, Maharashtra 22.6 & 1.1, Rajashthan 0.1 & 5.4, Tamil Nadu 1.4 & 12.8, Uttar Pradesh 8.2 & 15.4. Kannaiyan and Nene (1981) estimated nearly 100 % grain yield losses by *F. udum* Butler when wilt occurred at pre-pod stage, 67 % at pod maturity stage and 29.5 % at preceding harvest stage. The total production losses due to wilt was around 97,000 tones per year in India (Kannaiyan *et al.,* 1984)

Kannaiyan *et al*. (1981) reported 0-45% wilt incidence in Gujarat state, with an average of 5.4% in the predominantly Pigeonpea growing regions of Vadodara, Bharuch and Godhara.

2.2 Causal organism

The fungus causing wilt in Pigeonpea was described as *Fusarium udum*. Later, Butler in 1926 reached the conclusion that, it could not be distinguished from *F. vasinfectum* which attacks cotton and sesamum. Padwick (1940) studied cultural characters of *F. udum* and found it differed from *F. vasinfectum* in that it produced abudent spores in sporodochia, and these spores were strongly hooked at the apex. He proposed the name *Fusarium udum* Butler var *cajani.* Subramaniam (1955) was of opinion that we could not even distinguished from *F. oxysporum* var. *cubense*. Snyder and Hansen (1940) named the fungus *F. oxysporum f.* sp*. udum,* a nomenclature supported by

Chattopadhyay and Sen Gupta (1967). However, the name *F. udum* Butler is commonly accepted as the macro conidia of *F. udum* Butler are distinguished by a prominent hook (Booth, 1971).

The fungus becomes systemic invading lateral roots, tap root, collar, main stem, branches, leaflets, petioles, rachies, padicel and pod hull (Nene *et al.,* 1980). Rai and Upadhyay (1979) discovered the perfect stage of *F. udum* on wilted and dead Pigeonpea plants near Varanasi in Uttar Pradesh, India and identified it as a new species of *Gibberella*. Because of the large size of the perithecia and the 2 – celled (and rarely 3 celled) ascospores, it was named as *G. indica*. The *F. udum,* like other *Fusarium* spp. shows great deal of variation in cultural characters. Butler's description (Butler, 1910) of *F. udum* was : mycelium parasitic within roots of the host plant, or saprophytic and then creeping, hyphae hyaline, slender, much branched, usually with little aerial growth, microconidia of the cephalosporium type produced successively on the ends of short simple or clustered conidiophores and remaining bound in a drop of liquid after adjunction, unicellular or with one or more septa. Elliptical, hyaline singly, salmon pink in mass, occasionally developing from the surface of minute spherical stromata and then of the Tubercularia type, 5.15 x to 2.4 µm in diameter microconidial satge in culture usually moist and bacteria like,

white to salmon pink, occasionally (on rice) orange red, never green or purple, macroconidia of the *Fusarium* type found as the microconidia but on shorter conidiophores and becoming free as soon as abjuncted, falcate 3 to 5 septate hyaline, 15- 50 x 3- 5 µm in diameter, usually late in appearing, chlamydospores, round or oval, rather thick walled, hyaline sometimes in short chains, 5 to 10 µm in diameter.

According to Booth (1977) *Fusarium udum* Butler is known to form three types of spores, i.e. microconidia, macroconidia and chlamydospores. Microconidia were 8-16 x 2-4 µm in size, cylindrical to oval, sometimes one septate, produced from long lateral phialides, laterally borne on branched conidiophores. Macroconidia were fusoid with widest point above the centre, 1–5 septate and measuring 35-55 x 4.5-6.0 µm, while chlamydospores are globose, smooth to rough walled, measuring 9-12 x 8-10 µm borne singly or in chain on short lateral branches, intercalary or terminal.

The genus *Fusarium* belongs to the family tuberculariaceae of the order moniliales. This wilt of Pigeonpea is caused by *F. udum* Butler. The synonyms of this fungus are :

F. udum Butler var. *cajani* Padwick

F. oxysporum *Schl.* Synder & Hansen *F. udum* (Butler)

F. lateritium Nees amend, Synder & Hansen f. sp.

(Padwick) Gordon

F. uncinatum Wollenw

F. laterium Nees var. *uncinatum* (Wollenw) [Singh, 1982]

2.3 Symptomatology

According to Butler (1918) and Mohanty (1946), wilt can be characterized by gradual or rather sudden withering and drying of the green parts, exactly like moisture stress inspite of having enough moisture in the soil. The infection occurs through fine lateral roots which are penetrated by infection hyphae and then blackening of tissue starts. The xylem vessels are frequently block by dumps of hypha, which are clearly visible when section of infected root or shoot is cut. A thick mass of mycelium and conidia are produced on the surface of the bark, at the collar region.

Singh (1968) observed that the attacked plants succumb completely to the disease and recovery of such plants was rare. Aerial growth of the fungus may also occur as white to pinkish cottony mass at the base of the stem near ground level. Sheldranke *et al.* (1978) observed the symptoms of Pigeonpea wilt during the reproductive phase. Examination of the main root and of the base of the stem show blackening of tissues, either uniformly or in streaks especially in the early stages. In some cases the black streaks can be traced up to the stem to a height

of several centimeters and the branches that arises from the blackened parts wither first. The streaks can be traced down to the roots and are found to arise from main or lateral roots. The disease spread in a centrifugal manner in the field up to the time of harvest.

Reddy *et al.* (1990) described the symptoms of Pigeonpea wilt; it can appear 4 to 6 weeks after sowing. The initial visible symptoms are loss of turgidity in leaves, and slight interveinal clearing. The foliage shows slight chlorosis and sometimes becomes bright yellow before wilting. Leaves are retained on wilted plants. The initial internal symptom of wilt is the browning of xylem vessels from the root system to the stems. The xylem gradually develops black streaks and brown or dark purple bands appear on the stem surface of partially wilted plants extending upwards from the base. When the bark of such bands is peeled off, browning or blackening of the wood beneath can be seen. In wilt tolerant genotypes, this bands are confined the basal part of the plant. Sometimes especially in the later stages of a crop the branches dry from the top to downwards, but symptoms are not seen on the lower portion of the main stem or branches. Similarly, small branches on the lower part of the plant also dry. When the main stem of such plant is split open, intensive blackening of the xylem can be seen. In humid weather, a pinkish mycelial growth is commonly observed on the

basal portion of the wilted plants. Partial wilting is usually associated with lateral root infection. Tap root infection results in complete wilting.

Fusarium wilt of Pigeonpea is seed as well as soil borne. Wilt symptoms usually appear when plants are at flowering and podding stage but sometimes occur earlier when plants are 1 -2 months old. Ratooning predisposes the plant to wilt. Patches of dead plants in the field at flowering or podding are the clear indication of the wilt disease. The most characteristics symptoms is a purple band extending upwards from the base of the main stem. Partial wilting of the plant is a definite indication of Fusarium wilt and distinguishes this disease from termite damage, drought and Phytophthora blight. The other characteristics symptoms of wilt is browning of the stem tissue in the region of purple band and blackening of xylem, visible when the main stem of primary branches are split open. When young plants, 1-2 months old die due to wilt, they may not show the purple band symptoms but have obivious internal browning and blackening. Plant infected by *F. udum* also exhibit a series of leaf symptoms before they die including loss of turgidity, interveinal clearing and chlorosis to bright yellow (Reddy *et al.,* 1993).

Nene *et al.* (1981) shortly described the symptoms as yellowing, drooping and drying of the leaves and presence of

black streaks under the bark, vascular tissue is discolored. Sometimes dark brown bands may be seen on the main stem. Mundkur (1991) reported that the plants are about 5 to 6 weeks old, at which time, the leaves of the attacked plants turn prematurely yellow and wither, untill finally the entire plants dry up. Death follows very soon after. As the season advances and the plant mature, the number of deaths increases, and more than 50 % of the plants in the field are known to succumb to the disease. That the death of the plants is not due to drought, it is evident from the fact that wilt is not uniformly spread in the field, and the soil will be found on examination to have enough moisture for the normal growth of the crop. If attacked plants are dug up and examined, it will be found that the vascular tissues of the roots and stem are blackened in streaks which may be thin in the early stage of attack but are thick bands in the later stages. These black streaks can be traced upon the stem to a height of several feet and the earliest branches to wither are those, which arise from such blackened parts. Wilting may sometimes be partial, as only one side gets withered, in such cases the stem is blackened on that side alone.

2.4 Identification of the Pathogen

Singh (1982) reported that the culture of *Fusarium* sp. on PDA at 25^{o}C often with deep purple discoloration of the substrate, aerial mycelium felted or almost absent and usually

with profuse development of pionnotes and sporodochia. Conidia initially produced on simple or verticillately branched conidiophores, later on found from pionnotes or normal sporodochia in pinkish or salmon coloured masses. On the host, they are usually formed on sporodochia. Microconidia small, 1 celled, hyaline, ovoid fusoid or curved, 5-15 (6-11) x 2-4 (2-3) µm, formed free on hyphal branches held together in globoid masses. Macroconidia hyaline, thin walled, 1-3 or occationally 5 septate, falcate with a distinct foot cell and an apical cell of decreasing diameter towards the tip which may be curved or hooked, 15-30 x 2-5 to 3-5 µm. Chlamydospores developed from hyphal or conidial cells, thick walled, globose, single or in short chains, terminal or intercalary in the mycelium, hyaline, 8-10 µm diameter.

Mundkur (1991) reported that, the mycelium of *F. udum* is hyaline and produces spores of three types within the tissue of the host plant, macroconidia are produced in small cushions of stromatic mycelium on the surface of the bark. They are long, curved, pointed at the ends, septate and measure 5-50 µm in length and 3-5 µm in breath. They are formed on short conidiophores and shed when mature, without being held together in a ball. Microconidia are small elliptical or curved unicellular or with one or two septa.

2.5 Pathogenicity and screening technique

Various workers have proved the pathogenicity of wilt pathogen (*Fusarium* spp.) on different host by different methods. Grewal *et al.* (1974) proved pathogenicity of *F. solani* on 90 days old gram plants grown in autoclaved soils inoculated by putting 50 g culture of *F. solani* grown on sand maize meal medium two inch deep around the plant by replacing the soil. Patel (1988) proved the pathogenicity of *F. solani* on Pigeonpea by sowing the seed equidistantly in pot soil in holes and the holes were plugged with 10 days old pure culture of *F. solani* grown on sand maize meal medium. Maheshwari *et al.* (1980) proved the pathogenicity of *F. oxysporum* and *F. solani* causing wilt and root rot complex in pea by four different methods of inoculations *viz*. Malt extract inoculum placement, sand oat inoculum placement, pin prick method and syringe method and observed that only syringe inoculation gave the best results producing typical wilt symptoms.

Nene (1979) reported pot screening and sick plot screening techniques. In his pot screening technique, he modified and described that transplanting seedling of which roots are injured and inoculated to autoclaved sand soil in pot as under.

1. Alfisol, non-autoclaved, is filled in large (35 cm) earthen pots.
2. *Fusarium udum* is multiplied on sand Pigeonpea flour (9:1)

medium (SPM) for 15 days.

3. Fungus on SPM (200 g) and autoclaved Pigeonpea stem bits (200 g) are mixed with the top 15 cm of soil in pots.
4. Susceptible cultivar ICP–6997 (approx. 50 seed) is raised in each pot. All plants wilted within 60 days are chopped and incorporated in the same pot.
5. Step 3 is repeated.
6. Step 4 is repeated.
7. Step 4 is repeated.

After step 7, he get over 90 % wilt in each pot.

He also suggested another sick plot technique well known for screening against several vascular wilt. He described procedure to developed sick plot in the proceeding of the consultant group discussion on the resistance to soil-borne diseases of legumes page 36. The planting pattern they followed for screening is one susceptible check row after every two test rows in plot that are in the process of becoming sick and one susceptible check row after every four test rows in plots that have already become sick.

Mishra and Dhar (2005) tested three inoculation methods for Pigeonpea wilt. Three methods of inoculation mainly (1) soil inoculation, (2) water culture technique and (3) Spore

suspension method in pot culture.

(1) Soil inoculation : In soil inoculation method, three isolates of *F. udum* were multiplied individually on sand Pigeonpea flour media (8:2 w/w) and mixed with soil @ 20 % inoculum (200 g/kg soil) and filled in 10 inch diameter plastic pots. The high inoculum level of 20 % w/w was used for getting maximum wilting.

(2) Water culture : In water culture technique (1) spore suspension was prepared from 10 days old culture in sterilized distilled water and adjusted to 3-4 x 10^6/ml concentration. Big size test tubes (250 x 20 mm) upto ¾ level was filled with this adjusted spore suspension. Fifteen day old seedlings were held in straight position by cotton plug.

(3) Spore suspension : In this method, fifteen day old seedlings of wilt susceptible variety Bahar grown in sterilized soil in 25 cm diameter plastic pots were used for inoculation. Spore suspension of three isolates of *F. udum* from 10 days old culture with inoculum concentration 3-4 x 10^6/ml was prepared. Seedlings were inoculated by pipetting 5 ml of spore suspension pouring around each seedling.

Sharma *et al.* (1977) suggested new stem inoculation

techniques for testing fusarium wilt of Pigeonpea. He described the stem inoculation at different growth stage of Pigeonpea. The plants were inoculated after 45, 75 and 127 days of planting by giving longitudinal cut on the stem, 10-15 cm above ground level with the help of a sharp scalpel. The inoculum consisting of mycelium and spores of the pathogen was introduced in the cut portion. Absorbent cotton swab was wrapped around the inoculated portion for the protection from desiccation and then wrapped with polythene strip.

2.6 Testing of the different types of Media

The first semi selective medium for Fusarium group appears to be that of (Snyder *et al.,* 1959), who used streptomycine, rose Bengal and sodium taurocholate in Martin's peptone dextrose agar for isolation of *F. solani f. sp. Phaseoli*. Since then, more than 15 media, both selective and semi-selective have been reported (Tsao, 1970). Media mainly based on preferential utilization of carbon sources by *Fusarium* spp. have also been reported. (Park, 1963), (Komada 1976) and (Vaartaja, 1967) used 1-2% glactose and (Bouhot and Billotte, 1964) used 0.5–2.0% insulin for selective enhancement of *Fusarium* spp.

2.7 Pathogenic variability (Wilt)

Gaur and Sharma (1989) isolated *F. udum* from the

wilted plant of Pigeonpea from various regions of India. *In vitro* studies indicated that the 11 single spore isolate differ in their cultural and morphological characteristics and showed a marked diversity in virulence towards the susceptible variety T-21.

Rajendra and Patil (1992) compared 22 isolates of the pathogen from Pigeonpea in various parts of India. They were compared with respect to morphology, colony growth, sporulation *in vitro*, mycelial dry weight, pH of culture filtrates, organic carbon. Utilization and electrical conductivity and quantitative estimation of root exudates. The differences observed indicated genetic variability in *F. udum*.

Nene (1979) reported variation in *Fusarium* spp. isolated from Hyderabad and from other locations. He observed great deal of variation in cultural characters, growth, sporulation, color and change in medium color. Many other scientists had also reported variations (Sarojini, 1951; Subramanian, 1955; Baldev and Amin, 1974).

2.8 Inoculum threshold

Various factors like, the type and quantity of inoculum, age, method of inoculation and environmental conditions influence the quantity of inoculum required to cause successful infection in any host pathogen system. Naik *et al*. (1992) studied the relationship between inoculum density and

disease incidence in wilt pathogen (*F. udum*) and found that wilt was not observed at 1:200 dilution of wilt sick soil (15 cfu/g soil) in susceptible variety ICP-2376, but occurred at 1:100 dilution (34 cfu/g soil). Bhatti and Kraft (1992) observed that wilt of chickpeas increased with the inoculum levels (10^4 or 10^5 micro and macro conidia/ml) of *F. solani* f. sp. *pisi* and *F. oxysporum* f. sp. *ciceri*. Kapoor and Kumar (1987) noticed that the percentage of wilted tomato plants varied in proportion to the quantity of inoculum of *F. oxysporum* and *F. solani* added to the soil.

2.9 Management of Pigeonpea diseases

2.9.1 Bio-efficacy of botanicals against *F. udum* Butler *in vitro*

The tests of 20 spp. of *Callistemon lanceolatus* exhibited the strongest volatile fungitoxicity inhibiting mycelial growth of *F. oxysporum f. sp. udum (F. udum)* completely. The oil was superior to some synthetic fungicides and had no damaging effect on seed germination, seedling growth and general health and morphology of Pigeonpea plants (Pandey *et al*., 1982).

Patel (1995) recorded bio-efficacy of 29 phytoextracts tested *in vitro* against *Fusarium solani* causing wilt of Pigeonpea, among these, garlic clove extract (*A. sativum*) proved strongly inhibitory to the growth of *F. solani.*

Chauhan (1997) evaluated that the garlic bulb proved

strong inhibitory effect to *F. solani* followed by neem, onion bulb and turmeric rhizome. Whereas the extract of lantana, kadvi mahendi and tulsi were found to promote growth of the pathogen. Among seven leaf extracts, *Azadirachta indica* at 100 % concentration proved highly toxic to *F. oxysporum* with complete inhibition of mycelial growth and spore germination *in vitro* (Bansal and Gupta, 2000).

Pandav (2002) recorded that the extracts of acalypha, garlic, neem, nilgiri, onion, lantana and gulmahor found much toxic to growth of *Fusarium solani.* Patel and Vala (2004) reported that bulb extract of *Allium sativum* produced maximum (4.75 mm) inhibition, while *Allium cepa, Celsia coromandeliana, Ipomea fistula, Jetropha curcas* and *Ocimum sanctum* cause slight inhibition to *F. solani*

Mamatha and Rai (2004) reported that leaf extracts of *Lantana camara* followed by *Azadirachta indica* and *Bacopa monnieri* were effective in inhibiting the growth of *F. solani in vitro.* Singh and Chand (2004) reported that leaf extract of neem completely checked the spore germination of *F. oxysporum* f. sp. *ciceri* causing wilt of chickpea.

2.9.2 Bio-efficacy of fungicides *in vitro* against *Fusarium* sp.

Sinha (1974 and 1975) reported that, benomyl and

carbendazim at all tested concentration had been found to be inhibitory to mycelial growth and spore germination of *F. udum.*

Ghosh and Sinha (1981) reported that spore germination of *F. udum* was completely inhibited by benomyl at 50 ppm while mycelial growth was inhibited by carbendazim at 25 ppm and BAS 38601 at 50 ppm.

Singh and Bhargava (1981) obtained the best control of three species of *Fusarium* causing wilt of Pigeonpea with foltaf. Sinha and Upadhyay (1990) tested eleven fungicides at different concentration *in vitro* against *F. udum* and found that MEMC and wettable sulphur at all tested concentration had completely inhibit the growth, while mancozeb and thiram were slightly less effective.

Kapoor and Kumar (1991) studied the efficacy of systemic and non-systemic fungicides against *F. solani* responsible for pre and post emergence damping off of tomato under *in vitro* condition and reported that carbendazim was most effective followed by benomyl. Sugha *et al.* (1995) evaluated 12 fungicides against *F. oxysporum* f. sp. *ciceri* causing chickpea wilt under *in vitro* condition and observed carbendazim (50 WP and 25 DS) and thiram alone and in combination were highly effective in inhibiting mycelial growth of the pathogen.

Chauhan (1997) screened fungicides by poisoned food

technique indicated that in systemic group, topsin-M (thiophanate methyl), bavistin (carbendazim) and beam (tricyclazole) were highly fungitoxic to *F. solani* followed by tilt (propiconazole) and aliette (fosetyl-AL). In non-systemic group, ziram, (cuman-L) proved best. He also found that the systemic groups of fungicides were found to be superior as compared to non-systemic group.

Mukherjee and Tripathi (2000) evaluated eight fungicides of different concentration *viz*., 2.5, 5, 10, 25, 50 and 100 g/ml *in vitro* against S. *rolfsii, R. solani* and *F. oxysporium* f. sp. *phaseoli.* Out of these fungicides, carbendazim and hexaconazole (10-20 mg/ml) inhibited cent per cent radial growth of *F. oxysporium* f. sp. *phaseoli.*

2.9.3 Bio-efficacy of bio-agents against *F. udum* Butler *in vitro*

Among 25 isolates of rhizosphere soil and healthy seed, *Trichoderma harzianum* and *Bacillus subtilis* produces a wide zone of inhibition against *Fusarium udum* and inhibited spore germination completely at 2 x 10^7 spores/ml. and 8x 10^7 cells/ml, respectively. These antagonists showed no adverse effects on Pigeonpea seed germination. Seed coated with the antagonists germinated better than untreated seed and produced longer roots, shoots when shown in either wilt infested or sterilized soil (Sumitha and Gaikwad, 1995).

Chauhan (1997) studied the effect of antagonists against *F. solani* by two methods, inhibition zone technique and culture block method, both produced consistent results by inhibiting the growth of the fungus indicating strong antagonism by *Trichoderma viride* followed by *Trichoderma longibrachiatum, Aspergillus flavus and Aspergillus niger,* whereas, *Aspergillus parasiticus, Trichoderma harzianum* (A), *Trichoderma harzianum* (J) and *Ucladium atrus* showed moderate inhibitory effect.

Ushamalini *et al*. (1997) found inhibitory effect of the antagonist *viz.*, *Trichoderma viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii, Bacillus subtilis and Pseudomonas fluorescens* against *Fusarium cxysporum* with *Bacillus subtilis* followed by *T. harzinum and Pseudomonas fluorescens.*

Ram *et al.* (2000) showed inhibition of *F. solani* and *Pythium myritylum* by the *T. harzianum, Trichoderma auroviride, T. viride* and *Gliocladium virens.*

Pandav (2002) found strong antagonism of *Aspergillus niger* and *Trichoderma* sp. against *F. solani, Bacillus subtilis, Chaetomium globosum* and *Gliocladium virens* were also appeared as potential antagonists.

Patibanda and Sen (2004) found *A. niger* Van Teigh more useful antagonist against *F. oxysporum* f. sp. *melonis* in the *in vitro*. Seven isolates of the antagonist *A. niger* Van Teigh and

three isolates of the muskmelon wilt pathogen, *F. oxysporum* f. sp. *melonis* were assessed for their *in vitro* interaction. Isolate AN 27 were found promising based on its bio control capabilities.

Jha and Jalali (2006) reported that under *in vitro* conditions, isolate of *T. viride* showed strongest antagonistic activity towards *F. solani* f.sp. *pisi* in dual culture followed by *A. niger, A. terreus, A. sydowi, A. flavus* and *Spicaria sylavatica.*

2.9.4 Screening of Pigeonpea genotypes for disease resistance

The breeding and screening for disease resistant to Pigeonpea diseases were carried out by many workers, few of them resistant entries reported are as under.

Resistant genotypes of Pigeonpea wilt

Resistant variety / genotype against wilt	**Author**
GAUT-82-9, GAUT-82-74, GAUT-82-127 and GAUT-83-23 (Gujarat)	Patel *et al*. (1988)
BP-1809, BDN-2, BP-1314, BP-2094 and BP-2061 (Gujarat)	Rajkule *et al*.(1989)
ICP-8863 was released as Maruti (Karnataka) and 71-37 was released as JA4 in Madhya Pradesh	Agrawal *et al*. (1991)
ICRISAT, ICP-10063, ICP-11289, ICP-13072-1 & BDN-1	Das and Gupta (1992)

BSMR – 175 & BDN – 2	Shinde and Zote (1993)
ICP-8863, 9174, 12745, ICPL-333, 8363, 88047 BWR-370, DPPA-85-2, 85-3, 85-8, 85-13 and 85-14 (R)	Amin *et al*. (1993)
ICP 9145 (ICRISAT in 1993)	Reddy *et al*. (1995)
ICRISAT-ICPL-87119 (R) Released as Asha (1993)	Jain *et al*. (1995)
ICRISAT-ICP-8863 and ICP-11292 resistant to both strain	Reddy *et al*. (1995)
BSMR – 736 (R)	Zote *et al*. (1995)
ICPL – 288 genetics male sterile line. Released in (1994)	Saxena *et al*. (1998)
TT – 2001 -1, JKM – 184, JKM – 186, JJ – 72-3, BSMR – 736, RA -6, DEP – 59, JA – 28-6, ICPL – 96058, 99048, 96048, 96053, 96061, 87051 and TT – 137	Anon. (2002-03)
ICPL – 96053, JKM – 186, Phule T- 25-6, WRCP – 65, KAWR -92-2 and IPA -2013	Anon. (2004-05)
AK – 222498, JKM -197, KAWR – 45, BDN – 2001-9 and ICP – 8863	Anon. (2005 –06)
IPA – 9F, IPA -12F, IPA -16F, IPA -204,JSA – 59 and WRGRE – 18	Anon. (2006-07)

Resistant genotypes to Sterility Mosaic Disease (SMD)

Resistant variety / genotype against SMD	**Author**

Pant – A – 8505 and Pant – 8508	Pal *et al*. (1989)
134B1 and 124B2	Chauhan *et al*. (1991)
ICPL-151	Saxena *et al*. (1994)
MAL -18, AL -145, AL -201, BDN -2010, ICPL –99048 and ICPL -96061	Anon. (2002-03)
ICPL – 96053, PUSA – 2002-2, IPF -16F, NDA – 98-8 and NDA – 03-7	Anon. (2004-05)
IPA -15F, IPA16F, BRG -3 and ICP -7065	Anon. (2005-06)
IPA – 8F, BDN -2029, BRG – 2-6, IPA -15F, IPA -16F and IPA -234.	Anon. (2006-07)

Resistant genotypes to Phytophthora Blight (PB)

Resistant variety / genotype against PB	**Author**
ICPW – 61 and ICPW -66	Reddy *et al.* (1996)
PANT – A- 8514, ICPL -316, PANT –A – 83-4, PANT –A – 3-1, PANT –A – 3-2, ICPL –161 and PANT- A – 1-1	Garg *et al.* (1996)
TT -2000, JSMP -08, JKM -184, JJ -28-6, BSMR –736 and JA -28-6,	Anon. (2002-03)
BDN -708, CORG -200105, JKM -198, JKE -110, JKM -186, JJ -65, PA – 232, PA -296, PA – 300, PUSA – 2004-1, PUSA – 2004-2, PUSA – 2002-1, PUSA- 2002-2, PHULE -11-39, WRG -79, WRG-81 and JKM -189.	Anon. (2004-05)

PT -01-27	Anon. (2005-06)

2.9.5 Management of Pigeonpea wilt in the field

The field trials conducted in soil naturally infested with *Fusarium udum* the best control over three years was given by captan followed by brassicol (quintozene), phenyl mercury acetate, sulpher dust, copper oxychloride (Haidar *et al*., 1979).

Gurjar *et al.* (2004) reported that *T. harzianum* and *T. viride* gave effective management of *Fusarium* sp. in okra.

Seed treatment with a mixture of benomyl and thiram completely eradicated the pathogen from the seed of susceptible varieties *in vitro* test (Haware and Kannaiyan, 1992).

Somasekhare *et al*. (1996) tested 6 isolates of *Trichoderma* spp. under green house condition against the Pigeonpea wilt pathogen, *F. udum* by adopting 2 delivery systems, seed treatment and soil application. Seed treatment with *T. viride* isolate H reduced the number of *F. udum* propagules, reduced in number from 19.4 x 10^2 to 2.5 x 10^2 c.f.u./g of soil, whereas, *T. humatam* (S) *F. udum* propagules were reduced in number from 10.9 x 102 to 4.9 x 102 cfu/g of soil and wilt incidence ranged from 7.3 to 15.5 except, with *T. harzianum* (H) and *T. Koningii* (S) after the 35th day of inoculation. *T. viride* isolate H significantly reduced the number of *F. udum* propagules and wilt incidence and it is suggested that

this isolate would be useful in the management of Pigeonpea wilt disease.

Prasad *et al*. (2002) reported the biological control of *F. udum* by using *T. harzianum* of various inoculum levels of *F. udum.* Field plots infested with pathogens at three inoculam levels (3.04, 4.98 and 5.34 colony forming unit (cfu/g of soil) were identified. *T. harzianum* was applied as a seed treatment. 10 and 20 g/kg seed and as a soil amendment (10 and 20g/9m^2). The results showed that in general, soil application of *T. harzianum* was found to be more effective than seed treatment for disease suppression. These results suggested the need to augment soil with *T. harzianum* for obtaining effective control of Pigeonpea wilt.

III. MATERIALS AND METHODS

The details of the materials used and the methods adopted in the present investigations are described below.

3.1 Pathological investigations

3.1.1 Survey for Pigeonpea wilt

The random rowing survey for Pigeonpea wilt, phytophthora blight and sterility mosaic disease was carried out in Pigeonpea growing area of Bharuch, Narmada and Vadodara district of Gujarat, during 2005-06-07 for three years during August to December. For this purpose, 10 to 15 villages from major Pigeonpea growing area of each districts were surveyed. The 5 to 7 fields were observed in each village. Two hundred plants were continuously observed in row for each field. The number of infected wilt plant, phytophthora blight plant, SMD plant and healthy plant were counted. Simultaneously, disease plant showing typical symptoms of above diseases were collected to confirm the disease. After microscopic examination of wilted plant, the standard tissue isolation was carried out from diseased plant to isolate pathogen. The isolate of different places were purified and were used for testing virulence of pathogen from different places. The data collected during survey were transformed into percentage and average percentage of

wilt, phytophthora blight and SMD at different districts were work out.

3.1.2 Collection of disease samples

The Pigeonpea plant having symptoms of wilt, phytophthora blight and sterility mosaic disease were collected in brown paper bag from various Pigeonpea growing area of South Gujarat and Middle Gujarat and brought to the laboratory for examination of pathogens. The symptoms and signs of the specimen were examined visually of each disease and they were recorded.

The visually as well as microscopic examination of the branches, stem and root of wilt infected plant were carried out for confirming the symptoms and the presence of pathogen. They were subjected to standard tissue isolation. The pathogen associated with each sample was observed microscopically and compared with those described in the literature. The samples were labelled, dried and preserved for further studies.

3.1.3 Symptoms observed in nature

3.1.3.1 Wilt of Pigeonpea

The visual and microscopic examination of Pigeonpea wilted plant were carried out in the laboratory to study the symptoms produced during natural field infection on plant and also to know the presence of pathogen, in or on the infected

tissues / plant part.

To study the symptoms expressed in the field on naturally wilted infected plants were critically observed under field condition. The examination of wilted plant, abnormalities with uprooted root and splited root and stem was carried out. The microscopic examination of abnormal part and discolored splited root and stem were carried out to know the presence of pathogen. After confirming the presence of pathogen, namely, *Fusarium* sp. the symptoms on wilted plants were recorded.

For recording the symptoms in nature, of phytophthora blight, the similar procedure was adopted as above and for SMD, abnormal leaves and twigs were observed under binocular microscope to know the presence of eriophyid mite (*Aceria cajani*).

3.1.4 Isolation of pathogen

The isolation of wilt pathogen was carried out on potato dextrose agar (PDA) medium from the diseased part/tissue of the wilted plant. The sterile PDA medium was poured in previously clean and sterilized petriplate, aseptically. After solidification of medium, Petriplate were kept inverted and same Petriplate were used for plating the disease tissue, plant part having symptoms and signs of wilt and the presence of pathogen. The standard tissue isolation from such samples were carried out by cutting the infected Pigeonpea plant or tissue into

small pieces. The infected tissues / pieces were washed with tap water and surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds, followed by subsequent three washing with sterile distilled water under aseptic conditions. The sterile pieces were then aseptically transferred to previously prepared Petriplates containing PDA. These Petriplates were incubated at a room temperature (27 $\pm$ 2^0C). The fungal hyphae developing from the infected tissue were subcultured on PDA slant. After confirming *Fusarium* sp. by microscopy of isolated pathogen, it was further purified by growing single spore. Thus, obtained culture of *Fusarium* sp. was maintained on PDA slants for further investigations.

3.2 Identification of pathogen

The identification of pathogens were made by different techniques described in literature, such as cultural characters, morphological characters of vegetative and reproductive structure and different types of spore produced. They were microscopically examined and recorded for comparison with the description in literature to identify the fungal isolate obtained.

3.3 Pathogenicity test

To prove the Koch's postulate of *F. udum* isolate from Bharuch center was used in pathogenicity test. The same isolate was multiplied on potato dextrose medium. The pathogenicity

was carried out on Pigeonpea in net house by employing well known soil inoculation method for the soil borne pathogen.

The big earthen pot of 30 cm diameter were washed with tap water, and than disinfected with 4 % formaldehyde solution and sun dried for seven days by keeping them on laboratory terrace, open to sky. These pots were filled with double sterilized soil. The isolated fungi *F. udum* Butler, was multiplied on PDA medium for 15 days and it was mixed with autoclave soil @ of 20 g / kg soil, one week before sowing, moisten with water and kept in shed for uniformed spread of inoculua. The five earthen pots of 30 cm diameter were filled with these inoculated soil, filling 4 kg soil / pot. The surface sterilized 5 seed of highly susceptible Pigeonpea cv. T-15-15 were sown by placing 4 seed equidistantly in circular fashion and one seed in the center in each pot. The proper control (without inoculum) was maintained by using double sterilized soil without adding inoculums. The sowing of sterilize healthy seed were carried out as mentioned earlier. The pots were arrange in the net house and labelled. The pots were watered as and when required and left undisturbed for disease development. The development of symptoms of infected plant were recorded till end of the experiment. The re-isolation from wilted plant part was carried out by employing standard tissue isolation technique described earlier for isolation of pathogen.

3.4 Evaluation of different inoculation techniques for the production of Pigeonpea wilt

To find out the most effective and rapid inoculation technique with high precision to produce disease in screening nursery. The various inoculation techniques were described in literature for soil borne pathogen, among them, seven different inoculation techniques were tried for Pigeonpea wilt, using highly susceptible cv. T-15–15 in pots in net house condition. The pots were sterilized with 4 % hypochloride solution and air-dried in terrace for 7 days. The seed, soil and inoculum used were according to need of inoculation techniques to serve the purpose of techniques to be employed. The pots were filled with sterilize 4 kg soil/pot for growing Pigeonpea. Five pots or replication were maintained for each treatment/technique. Each pot had five seed or seedling of cv. T-15-15 were used. The pots were place in net house after sowing. They were watered daily with sterilize water to keep the soil moist. The normal agronomical practices were adopted.

The observations on first appearance of symptoms *viz*., yellowing, loss of turgidity and withering of leaves and wilting of plant were recorded adopting recognized method regularly till end of the experiment.

Preparation of suspension : The fungus, *F. udum* Butler was multiplied in 100 ml potato dextrose broth in 250 ml conical

flask. These inoculated flasks were placed on laboratory table for incubation and shaken for four to six times daily up to ten days to increase the growth. The entire fungal content of each flask was macerated in a warring blander for one minute after adding sterile water. The concentration of suspension was adjusted to 3-4 x 10^6 spore/ml by adding sterile distilled water. It was used in different inoculation techniques mention below.

3.4.1 Soil inoculation

The well known soil inoculation technique for soil borne pathogen was employed. The technique and sowing method were described in detail in pathogenicity test.

3.4.2 Seed inoculation

The healthy surface sterilized seed were dip into spore suspension of *F. udum* Butler (3-4 x 10^6 spore / ml) for sixteen hours and then sown as usual in previously prepared sterilized pot filled with double sterile soil as described earlier.

3.4.3 Drenching of spore suspension

The double sterile soil (4 kg /pot) was filled in the previously sterilized pot. The healthy sterilized five seed Pigeonpea/pot were sown and watering was done as and when required for germination and growth. After 15 days of germination 100 ml spore suspension (3-4 x 10^6 spores/ ml) per pot were drenched around each seedling, followed by slight

watering to have sufficient moisture in the soil.

3.4.4 Wilt sick soil

The pots were filled with 4 kg sick soil/pot (sick plot having 80 % wilt mortality at Bharuch centre) for the experiment. The healthy seed of cv. T-15-15 were surface sterilized with 2% mercury chloride solution for 10 minutes and subsequently washed with distilled sterile water; then the seed were sown as described in earlier technique, watering and other practices were followed regularly as usual.

3.4.5 Root dip method

The Pigeonpea plant cv. T-15-15 were grown from healthy surface sterile seed in the pots filled with sterile soil. After 30 days of germination, these Pigeonpea seedlings were uprooted with proper care so that minimum rootlets were injured. The roots of uprooted plants were dipped into the spore hyphal suspension (3-4 x 10^6 spores/ml) of *F. udum* Butler for 10 minutes and then they were planted in the previously prepared pots filled with sterile soil.

3.4.6 Stem inoculation

The Pigeonpea plant were raised from surface sterilized healthy seed in the pots filled with sterile soil as usual as described earlier. After 75 days of germination, the plants were inoculated by giving longitudinal cut on stem of 10 to 15

cm long, above the ground level. The pathogen was introduced in the cut portion with the help of sterile scalpel then wrapped with cotton, which was again wrapped with polyethylene strip.

3.4.7 Sowing in wilt sick plot

The sowing of 25 healthy sterilized seed of cv. T-15-15 was carried out in wilt sick plot of Bharuch center, Navsari Agricultural University, Bharuch.

3.4.8 Control

The Pigeonpea seedlings were raised in the pot filled with sterile soil as described earlier. The proper control for each technique was maintained without inoculating *F. udum* Butler.

3.5 Determination of inoculum threshold level

To determine the minimum level of the inoculua required for producing the wilt disease in the soil, the inoculum of *F. udum* multiplied on PDA for 15 days and then mixed with sterile soil @ of 5, 10, 20, 30, 50, 70, 90 and 100 g/kg of soil which was used in filling the pots. After one week of soil incubation of *F. udum* Butler, different inoculua were put in 30 cm diameter sterilize earthen pots @ of 4 kg soil/pot. The surface sterilized 5 seed of Pigeonpea cv. T-15-15 were sown by placing 4 seed at equidistance in circular fashion and one in the center for each pot. The five replication were maintained in each threshold level with suitable control. The pots were labelled, watered gently, arrange in the net house and kept undisturbed.

The recognized agronomical practices were adopted as usual to grow the plants. The observations for the wilt disease development were recorded till end of the experiment using standard method.

3.6 Testing of virulence of different isolated of *F. udum* Butler

The *F. udum* Butler was isolated from different wilt samples collected from various Pigeonpea growing region during survey (Table 3.1). They were purified by single spore isolation technique. These different isolates were multiplied on PDA medium. They were tested for their virulence on susceptible genotype, cv. T-15-15 and tolerant genotype BDN-2 by employing soil inoculation technique in pot culture under net house condition. The inoculua of each isolate was mixed separately with autoclaved soil @ of 50 g/kg soil, one week before sowing, soil was gently watered with distilled sterile water and kept in shed for uniformed spread of inoculum. The 30 cm diameter earthen pots were filled with these inoculated soil keeping 4 kg soil/pot. The surface sterilized five seeds of highly susceptible Pigeonpea cv. T-15-15 and BDN-2 tolerant genotype were sown as usual. Maintaining uninoculated control and three replication

in each case. The pots were labelled and arranged in net house. The watering and another agronomical practices were adopted as usual. The appearance of wilt symptoms and their development was recorded till end of the experiment.

Table-3.1 : Testing of virulence of different isolates of *F. udum*

Sr. No.	Isolate No.	Location	District
1	Fu - 1	Vagra	Bharuch
2	Fu – 2	Jambusar	Bharuch
3	Fu – 3	Ankleshwar	Bharuch
4	Fu – 4	Dediyapada	Narmada
5	Fu – 5	Rajpipla	Narmada
6	Fu – 6	Karjan	Vadodara
7	Fu – 7	Dabhoi	Vadodara
8	Control	-	-

3.7 Search for superior media for growth and sporulation of *F. udum*

To find out the suitable medium for growth and

sporulation of *F. udum* Butler, the following synthetic and semi synthetic solid media were tested.

[A] Semi Synthetic media

(1) Potato Dextrose Agar (PDA)

Peeled potato	: 200.0 g
Dextrose ($C_6H_{12}O_6$)	: 20.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.0 ml

(2) Oat Meal Agar (OMA)

Oat (Flour)	: 100.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.00 ml

[B] Synthetic Media

(1) Richards' Agar (RA)

Potassium nitrate (KNO_3)	: 10.0 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 5.00 g
Magnesium sulphate ($MgSO_4$-$7H_2O$)	: 2.50 g
Ferric chloride ($FeCl_3$-$6H_2O$)	: 0.02 g

Sucrose ($C_6H_{22}O_{11}$) : 50.0 g

Agar agar : 20.0 g

Distilled water : 1000.0 ml

(2) Asthana and Hawker's Agar (A & HA)

Glucose ($C_6H_{12}O_6$) : 5.00 g

Potassium nitrate (KNO_3) : 3.50 g

Potassium dihydrogen orthophosphate (KH_2PO_4) : 1.75 g

Magnesium sulphate ($MgSO_4$-$7H_2O$) : 0.75 g

Agar agar : 20.0 g

Distilled water : 1000.0 ml

(3) Czapek's (Dox) Agar (CzDA)

Sucrose ($C_6H_{22}O_{11}$) : 30.0 g

Sodium nitrate ($NaNO_3$) : 2.00 g

Dipotassium hydrogen orthophosphate (K_2HPO_4) : 1.00 g

Magnesium sulphate ($MgSO_4$-$7H_2O$) : 0.50 g

Potassium chloride (KCl) : 0.50 g

Ferrous sulphate ($FeSO_4$-$7H_2O$) : 0.01 g

Agar agar : 20.0 g

Distilled water : 1000.0 ml

(4) Elliot's Agar (EA)

Sodium carbonate (Na_2CO_3) : 1.05 g

Magnesium sulphate ($MgSO_4$-$7H_2O$) : 0.60 g

Asparagin : 3.00 g

Dextrose ($C_6H_{12}O_6$) : 3.00 g

Potassium dihydrogen orthophosphate (KH_2PO_4) : 1.36 g

Agar agar : 20.00 g

Distilled water : 1000.0 ml

(5) Das Gupta Agar (DGA)

Glucose : 10.00 g

Tripotassium phosphate (K_3PO_4) : 1.25 g

Asparagin : 2.00 g

Potato starch : 10.00 g

Agar agar : 20.00 g

Distilled water : 1000.0 ml

Twenty gram of agar agar based sterile medium was prepared employing standard procedure. It was aseptically transferred to sterile petriplates. After solidification, 5 mm culture block of 10 days old pure culture of *F. udum* Butler was

cut with sterile cork borer and placed in the center of the petriplates. Colony diameter was measured at regular interval. The petriplates were incubated at 27^0 c ± temperature. Four such plates were kept in each treatment. After 10 days of incubation, the radial growth was measured and to obtain mycelial dry weight, mycelial mat was filtered through a previously weighed filter paper, washed thoroughly with warm distilled water. The filter paper with mycelial mat was dried in oven at 60^o C till constant weight was obtained. The observations of the dry mycelial weight and sporulation were recorded as under.

-- = No sporulation

\+ = 1-10 conidia / microscopic field (10 x)

++ = 11-25 conidia / microscopic field (10 x)

+++ = 26-40 conidia / microscopic field (10 x)

++++ = >40 conidia / microscopic field (10 x)

3.8 Management of Pigeonpea wilt

3.8.1 Bioefficacy of phytoextracts against *F. udum in vitro*

Effect of phytoextracts of various plant species as listed in (Table-3.2) were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of *F. udum* Butler.

Table-3.2 : Plants used for preparing phytoextracts to test the efficacy against *F. udum* Butler *in vitro*

Sr. No.	Local name	Botanical name	Plant parts used for extracts
1	Acalypha	*Acalypha juliflora* L.	Leaves
2	Ardusi	*Adhatoda vasiaca* Nees.	Leaves
3	Gando baval	*Prosopis juliflora* L.	Leaves
4	Garlic	*Allium sativum* L.	Bulbs
5	Jatropha	*Jetropha curcas* L.	Leaves
6	Kadvi mehandi	*Lawsanis inermis* L.	Leaves
7	Karanj	*Pongamia galabra* L.	Leaves
8	Kuvadio	*Cassia tora* L.	Leaves
9	Lantana	*Lantana camera* L.	Leaves
10	Neem	*Azardirachta indica* Juss.	Leaves
11	Nilgiri	*Eucalyptus citridora* Hook.	Leaves
12	Onion	*Allium cepa* L.	Bulbs
13	Tulsi	*Ocimum sanctum* L.	Leaves
14	Turmeric	*Curcuma longa* L.	Rhizomes

The healthy fresh plant parts *viz*., leaves or bulbs of

rhizomes were collected, washed thoroughly with clean fresh water and finally rinsed with sterilized distilled water. Fifty grams of plant parts were cut into small pieces and minced with the help of a grinder by adding 50 ml sterilized distilled water. The phytoextracts obtained were filtered through double-layered muslin cloth in 150 ml conical f1ask and plugged with non-absorbent cotton. These filtered extracts were autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes.

Autoclaved extracts were individually added into previously sterilized PDA @ 5 per cent (i.e. 1 ml extract + 19 ml PDA) and mixed thoroughly at the time of pouring PDA in the previously sterilized petriplates. The petriplates were inoculated aseptically after solidification by placing 5 mm diameter mycelial block at the center, the block were cut aseptically with sterile cork borer from 15 days old pure culture of *F. udum* Butler grown on PDA. Four repetition of each treatment were maintained. The plate without phytoextract served as control. The petriplates were incubated at 27 $\pm$ 2°C temperature for 10 days. The observations on colony diameter were recorded and per cent growth inhibition was worked out by using formulae suggested by Vincent (1927).

$$PGI = \frac{100\ (DC - DT)}{}$$

DC

Where,

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony of control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

3.8.2 Antagonistic effect of different microorganisms to *F. udum in vitro.*.

Eight known antagonists *viz*., *Trichoderma viride, T. harzianum, T. longibrachyatum, Aspergillus niger, A. flavus, Gliocladium virens, Chaetomium globosum,* and *Bacillus subtilis were* tested *in vitro* by dual culture method for their antagonistic effect to *F. udum* Butler. (Source of bio-agent : Department of Plant pathology, N. M. College of Agriculture, Navsari agricultural University, Navsari).

The petriplates containing 20 ml PDA medium were inoculated aseptically with the pathogen *F. udum* Butler and the test organism (antagonist) by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three repetition of each treatments were kept and the petriplates with pathogen at center served as control. All the plates were incubated at 27 $\pm$ 2°C temperature. Observations on colony diameter was recorded up to the complete coverage of plates, which was inoculated only with pathogen. The per cent growth inhibition (PGI) was

worked out by using the formulae given by Vincent (1927) as mentioned earlier.

3.8.3 Bioefficacy of fungicides against *F. udum in vitro*

Eleven fungicides belonging to different chemical groups at three different concentrations were tested for their efficacy *in vitro* against *F. udum* Butler using poisoned food technique.

The calculated quantity of each test fungicides was incorporated in a conical flask containing 100 ml molten PDA medium so as to get required concentration in parts per million (ppm). The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicide and 20 ml medium was poured in each sterilized petriplates. On solidification of the medium, the plates were inoculated in the center by placing 5 mm diameter mycelial culture block cut aseptically with the help of cork borer from 15 days old actively growing pure culture of *F. udum* Butler grown on PDA. Three repetition were kept for each concentration of respective fungicide. The inoculated plates were incubated at 27 $\pm$ 2°C temperature. The observations on linear growth of fungus were recorded at 24 hrs. interval till the entire plate in control was completely covered with mycelium. The per cent growth inhibition (PGI) of the pathogen over control was worked out by using formulae given by Vincent (1927) as mentioned earlier.

The concentration of fungicides were those of active ingredients present in commercial formulation. Each fungicide was tested at three different concentrations as sown in Table-3.3.

Table-3.3 : Details of fungicides screened against *F. udum in vitro*

Sr. No	Technical Name	Trade Name	Concentration (µg/ml)		
Systemic fungicides					
1	Carbendazim	Bavistin (50%WP)	500	1000	1500
2	Benomyl	Benlate (50% WP)	500	1000	1500
3	Hexaconazole	Contaf (5%EC)	500	1000	1500
4	Propiconazole	Tilt (25%EC)	500	1000	1500
Non-systemic fungicides					
5	Mancozeb	Dithane M-45 (75%WP)	1000	2000	3000
6	Thiram	TMTD (75%WP)	1000	2000	3000
7	Methyl ethyl mercury chloride	Emissan (6%WP)	1000	2000	3000
8	Copper oxychloride	Blitox (50%WP)	1000	2000	3000
9	Copper hydroxide	Kocide (77%WP)	1000	2000	3000
Systemic + Non-systemic fungicides (Mix formulation)					
10	Carbendazim (12%) + Mancozeb (63%)	Sixer (75%WP)	1000	1500	2000

11	Metalaxyl (64%) + Mancozeb (8%)	Master (72%WP)	1000	1500	2000
12	Control	--	--	--	--

3.8.4 Screening of Pigeonpea genotypes in sick plot

The gene therapy is safe, eco-friendly and long term solution for disease management. The screening of Pigeonpea wilt was carried out in wilt sick plot at National Agriculture Research Project, Navsari Agricultural University, Bharuch center. It is on going programme since many years. Therefore, the new breeding material and their progressing generations were procured and tested for wilt resistant. The resistant against wilt of Pigeonpea had been tested by using the technique described in Pigeonpea disease resistance screening technique in Information Bulletin No. 9 by ICRISAT, A. P., India (1981). The screening of genotypes/entries against wilt was carried out in Pigeonpea wilt sick plot at NARP, NAU, Bharuch, whereas for phytophthora blight and sterility mosaic disease, the natural incidence in different entries in the sick plot and also in other experimental plots were observed and occurrence of phytophthora blight and SMD were recorded right from germination to harvest of the crop, using 1 to 9 point scale divided into five categories for wilt, phytophthora blight and sterility mosaic as under.

The screening of 95 genotypes including the eleven tolerant/resistant entries of previous years and entries procured from research scientist (pulse) were screened in the wilt sick plot. The test entries were sown in four meter length in wilt sick plot and susceptible check was sown after two test entries. The sowing was carried out in normal *kharif* season. The periodical observations at 15 days interval for occurrence of diseases were recorded as mentioned earlier.

Rating scale for scoring Pigeonpea diseases

Rating	Wilt	Blight	Sterility mosaic	Genetical score
1	No symptoms on any plant	No symptoms on any plant	No symptoms on any plant	Resistant
3	10 % or less mortality	Symptoms on 10 % or fewer plants	Symptoms on 10 % or fewer plants	Moderately Resistant
5	11 – 20 % mortality	Symptoms on 11 – 20 % plants	Ring spot symptoms on most plants but disappearing with	Tolerant

			age; no sterility	
7	20 – 50 % mortality	Symptoms on 20 – 50 % plants	Mild mosaic symptoms on most plants causing partial sterility	Moderately Susceptible
9	51 % or more mortality	Symptoms on 51 % or more plants	Severe mosaic on most plants; almost complete sterility	Susceptible

In second year, 6 tolerant / resistant selections from previous year and the new entries procured from Research Scientist (Pulse) were sown in the wilt sick plot as previous year. The incidence of wilt was recorded adopting same procedure described earlier.

The Pigeonpea sterility mosaic and phytophthora blight are endemic/sporadic disease at N.A.R.P. farm, Navsari Agricultural University, Bharuch. Therefore, the critical observations on natural occurrence of sterility mosaic and phytophthora blight in different entries grown in different experiments were recorded during 2004-05 and 2005-06, simultaneously, to know the reaction of sterility mosaic and phytophthora blight.

3.8.5 Control of Pigeonpea wilt in sick plot at Bharuch centre

To know the effect of fungicide and antagonist to control of Pigeonpea wilt, the three fungicides and three antagonists found most effective during *in vitro* test were included in the field trial (Table-3.4). The experiment was laid down in wilt sick plot at National Agricultural Research Project, Navsari Agricultural University, Bharuch. The highly susceptible local cv. T-15-15 was used. The general recommendation of seed treatment with thiram @ of 3 g/kg seed was given to the seed of all the treatment, whereas; the seed of control plot was used untreated. The details of experiment as here under.

Table-3.4 : Fungicides and antagonists used *in vivo*

Sr. No.	*Technical name*	Trade Name	Concentration used for drenching
1	Carbendazim	Bavistin 50 % WP	0.1%
2	Benomyl	Benlet 50 % WP	0.1%
3	Hexaconazole	Contaf 5 % EC	0.1%
4	N.A.U., Navsari isolate	*Trichoderma viride*	Spore suspension 10^6 spores / ml
5	N.A.U., Navsari isolate	*Trichoderma harzianum*	Spore suspension 10^6 spores / ml
6	N.A.U., Navsari isolate	*Aspegillus niger*	Spore suspension 10^6 spores / ml
7.	--	Control (no seed treatment and no drenching)	--

The first drenching of above treatments were carried out after 15 days of germination and second drenching was carried out after 15 days of first drenching. The one litre solution was drench in one meter length of row.

1. Number of treatments : 7 (Seven)
2. Replications : 4 (Four)

3. Experimental design : Randomized block design
4. Plot size : 4.0 x 1.8 mt.
5. Date of Planting : 2nd week of July
6. Variety : T-15-15
7. Spacing : 90 x 20 cm between plant
8. Dibbling : 2 seeds/dibble
9. Fertilizer dose : NPK (20-40-00) kg/ha
10. Seed rate : 12 kg / ha

The periodical observations on wilt incidence were recorded adopting standard procedure as described earlier.

IV. RESULTS AND DISCUSSIONS

4.1 Pathological investigations

4.1.1 Survey for Pigeonpea diseases

The random survey of Pigeonpea diseases in Bharuch, Narmada and Vadodara district was carried out in Pigeonpea growing area during the year 2005, 2006 and 2007. The survey indicated that (Table-4.1) average wilt incidence was maximum in Vadodara district (14.0%) followed by Narmada (11.7 %) and Bharuch district (10.9%). Among three years, average maximum wilt (13.8 %) was recorded in 2007. The maximum wilt was recorded from Vagra (32.0%) in 2007 and 2005 (25.0%), it was followed by Tilakwada (23.0%) in 2007 whereas, minimum wilt incidence was recorded from Valia (1%) during 2006 and 2007 and from Dediyapada (1%) during 2007.

The three years survey of phytophthora blight indicated that average maximum phytophthora blight was observed in Vadodara (5.5%), followed by Bharuch (5.0%) and Narmada district (2.5%). Among the three years, average maximum phytophthora blight was found during 2007 (5.3%), followed by 2005 (3.9%) and 2006 (3.5%). The maximum phytophthora blight was recorded during 2007 at Dabhoi (9%), followed by Bharuch, Amod and Karjan (8%). The least phytophthora blight was recorded at Sagbara (1 %) during all the

Table 4.1 Per cent Pigeonpea diseases in Bharuch, Narmada and Vadodara district.

Location/Year	Wilt incidence				Phytophthora Blight				Sterility Mosaic			
	05	06	07	Ave.	05	06	07	Ave.	05	06	07	Ave.
Bharuch District												
Amod	8	5	10	7.7	3	6	8	5.7	1	0	1	0.7
Vagra	25	20	32	25.7	2	4	6	4.0	2	0	1	1.0
Jambusar	9	6	11	8.7	4	5	6	5.0	3	1	0	1.3
Ankleshwar	10	5	10	8.3	5	5	6	5.3	2	1	0	1.0
Zaghadia	3	4	5	4.0	3	2	3	2.7	0	0	1	0.3
Valia	2	1	1	1.3	5	6	6	5.7	6	3	2	3.7
Bharuch	20	18	25	21.0	6	7	8	7.0	1	2	6	3.0
Average	**11.0**	**8.4**	**13.4**	**10.9**	**4.0**	**5.0**	**6.1**	**5.0**	**2.1**	**1.0**	**1.6**	**1.6**
Narmada District												
Rajpipla	5	12	16	11.0	3	2	3	3.7	1	0	1	0.3
Sagbara	12	18	13	14.3	1	1	1	1.0	2	1	0	1.0
Selamba	12	7	15	11.3	1	1	2	1.3	3	0	1	1.3
Tilakwada	20	18	23	20.3	2	1	1	1.3	2	1	0	1.0
Dediyapada	2	2	1	1.7	5	5	5	5.0	4	2	3	3.0

	10.2	**11.4**	**13.6**	**11.7**	**2.4**	**2.0**	**2.4**	**2.5**	**2.4**	**0.8**	**1.0**	**1.3**
Average												
Vadodara district												
Dabhoi	13	13	14	13.3	8	5	9	7.0	3	2	1	2.0
Karjan	17	16	19	17.3	5	4	8	5.7	6	2	2	3.3
Shinor	11	14	10	11.7	3	2	6	3.7	3	2	6	3.7
	13.6	**14.3**	**14.3**	**14.0**	**5.3**	**3.6**	**7.6**	**5.5**	**4.0**	**2.0**	**3.0**	**3.0**
Average												
Over all average	**11.6**	**11.4**	**13.8**	**-**	**3.9**	**3.5**	**5.3**	**-**	**2.8**	**1.2**	**1.9**	**-**

three years, and at Tilakwada during 2006-2007 (1%) and at Selamba (1%) during 2005-2006.

The three years survey of SMD indicated that average maximum (3%) SMD was recorded in Vadodara district, followed by Bharuch (1.6%) and Narmada (1.3%). Among the three years, the maximum (2.8%) SMD incidence was recorded during 2005, followed by 2007 (1.9%) and 2006 (1.2%). The maximum (6 %) SMD was recorded at Valia and Karjan in 2005 and at Shinor during 2007, followed by Dediyapada (4 %) during 2005. The SMD did not appear during 2005 at Zagadia, and during 2006 at Amod, Vagra, Rajpipla and Selamba, as well as it was also not appeared in Jambusar, Ankleshwar, Sagbara and Tilakwada during 2007.

4.1.2 Collection of disease samples

The Pigeonpea plant having symptoms of wilt, phytophthora blight and sterility mosaic disease were visually observed in the field and collected from Pigeonpea growing area of Bharuch, Narmada and Vadodara districts during survey. The presence of pathogen was confirmed by critical microscopic examination of each disease part of plant. The identified sample of each diseases were labelled and preserved after proper drying for further studies.

The SMD disease samples were observed visually for morphological abnormalities on leaves, twigs and stem. They were also observed under binocular microscope to know presence of vector, namely, eriophyid mite, *Aceria cajani.*

4.1.3 Symptoms observed in nature

The symptoms of each disease were critically recorded after confirming the presence of pathogens/vectors with sample collected from fields. They are as under.

4.1.3.1 Wilt of Pigeonpea

The scattered patches of dead plants in the field during flowering or podding, is the first indication of wilt. The most characteristic symptom is a purple band extending upward from the base of the main stem. This band is more easily seen in Pigeonpea with green stem than in those with colored stem.

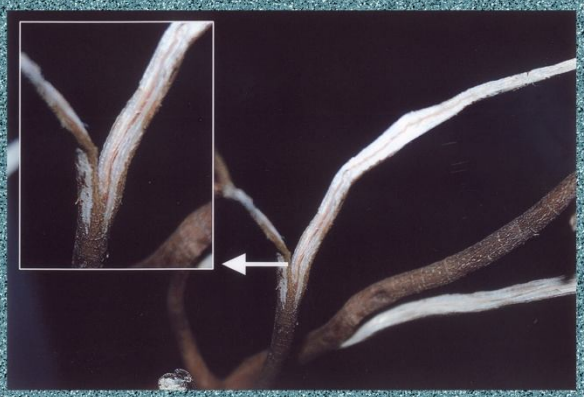
Partial wilting of the plant is definite indication of wilt and distinguishes this disease from termite damage, drought and phytophthora blight that also kill the whole plant. Partial wilting is associated with lateral root infection, while total wilt is due to tap root infection (Plate-I).

The other characteristic symptoms of wilt are browning of the stem tissue in the region of the purple band and browning or blackening of the xylem, visible when the main stem or primary branches are split open. The intensity of browning or blackening decreases from the base to the tip of the plant. Some times, branches (especially lower once) dry, even if there is no band on the main stem. These branches have die - back symptoms with a purple band extending from tip to

Plate-I : Wilt symptoms (A, B & C)

A. Gradual Wilting in wilt affected pigeonpea plant

B. Natural wilt symptoms (Black band) on pigeonpea plant



C. Wilt symptoms in splited root of pigeonpea

downward, and intensive internal blackening. When young plants (1-2 months old) die due to wilt, they may not show the purple band symptoms but have obvious internal browning and blackening. Plant infected by *F. udum* also exhibit a series of leaf symptoms before they die including loss of turgidity, interveinal clearing, and chlorosists to bright yellow. The leaves of wilted plant remain hanging on the plant.

4.1.3.2 Phytophthora blight of Pigeonpea

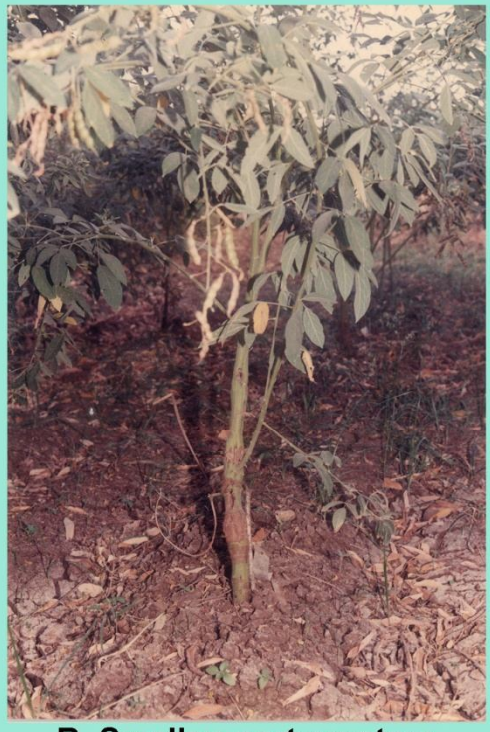
Phytophthora blight resembles to symptoms of damping off disease, in that it causes sudden death of seedlings. Infected plants have water soaked lesions on their leaves and brown to black, slightly sunken lesions on their stems and petioles. Infected leaves lose turgidity and become desiccated. Lesions girdle the affected main stems or branches which break at this point and the foliage above the lesion dries up. When conditions favour the pathogen, it is common for many plants to die. Pigeonpea plants that are infected by blight, but not killed often produce large galls on their stems especially at the edges of the lesions. In some cases, gum like materials ooze out which may remain on lesion area. The pathogen infects the foliage and stems but not the root system (Plate-II, III).

4.1.3.3 Sterility mosaic disease of Pigeonpea

In the field, the disease can be easily identified from a distance as patches of bushy, pale green plants without flowers

or pods. The leaves of these plants are small and show

A. Black spot on stem



B. Swollen part on stem

Plate-II : (A and B) Symptoms of phytophthora blight

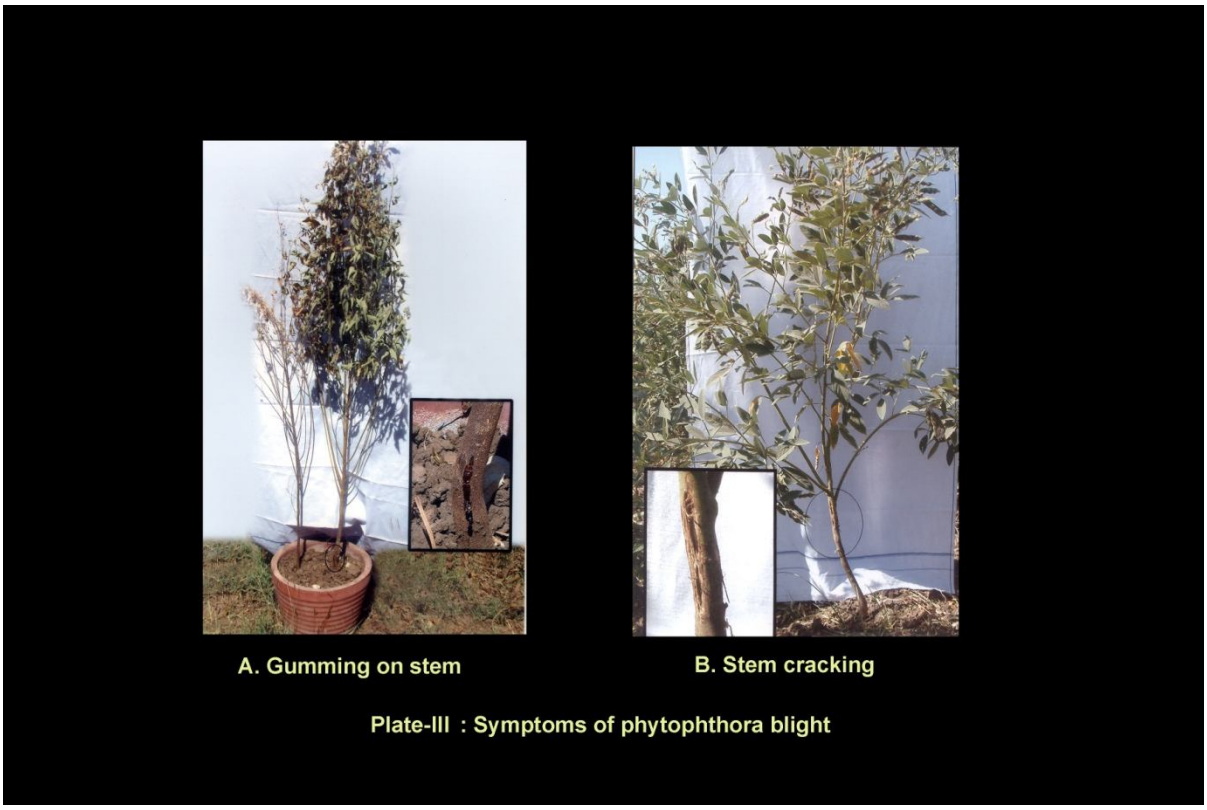


Plate-III : Symptoms of phytophthora blight

light and dark green mosaic pattern. The mosaic symptoms initially appears as vein clearing on young leaves. When infection occurs at 45 days after emergence or later only some part of the plant may show disease symptoms, while remaining parts appear normal.

Some Pigeonpea varieties exhibited ring spots symptoms on leaf, these indicated localized site of infection of the virus and such plants produced normal flowers and pods. The severe internodal shortening of the branches, clustering of leaves and sometimes, leaves become filiform (Plate-IV).

4.1.4 Isolation of pathogen

The wilt samples collected from Bharuch centre were critically observed to know the presence of pathogen in infected plant part, after confirming the presence of pathogen by microscopy. The disease tissue from infected plant parts were subjected to tissue isolation. The isolated fungus culture was purified and again subjected to microscopic examination. They revealed that the isolated fungus produce spores of *Fusarium* sp., which was evident in microscopic examination. The fungal isolates were obtained from respective wilt samples and were further purified by single spore isolation technique. These purified single spore culture of *Fusarium* sp. was maintained on PDA slant at low temperature for further investigations.

Plate-IV : Symptoms of sterility mosaic disease (SMD)

4.2 Identification of Pathogen

The fungus isolated from Pigeonpea wilt samples was purified by single spore isolation technique. The identification of fungal isolate was done by studying the cultural and morphological characters of isolate (fungus/pathogen) grown on PDA. The microscopic examination of the mycelium and their reproductive structure, different type of spores of the pathogen, was carried out. The observations on various characters were recorded as under.

4.2.1 Cultural characters

Fungal colonies grew fairly fast on PDA and attained a diameter of 35 mm in 12 days at 27 $\pm$ 2^0 C temperature. The mycelium was profuse and hyaline when young but later turned fluffy white (Plate-V).

4.2.2 Morphological Characters

The mycelium was septate, hyaline and produced three types of spores within the host tissue and in cultures, depending upon the nutrition and other environmental factors. Microconidia were small, elliptical or curved, unicellular or with one or two septa, and measure 5-15 x 2-4 µm. They developed free on hyphal branches. In cultures, many spores were held together in a ball. Macroconidia were produced in a small

cushions of stromatic mycelium on the surface of the host near ground level. The stromatic bases were tubercular in

Plate-V : Pure culture of *F. udum* Butler isolated from wilted pigeonpea plant

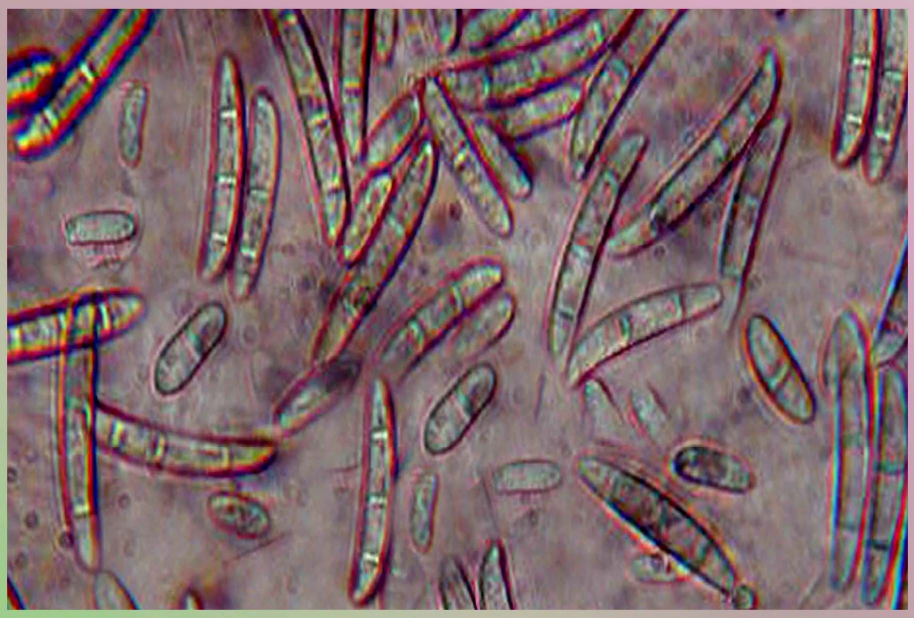


Plate-VI : Microphotograph of *F. udum* Butler (450 x) conidia (Macro and Micro)

Plate-VII : Microphotograph of *F. udum* (450x) fungal mycelium

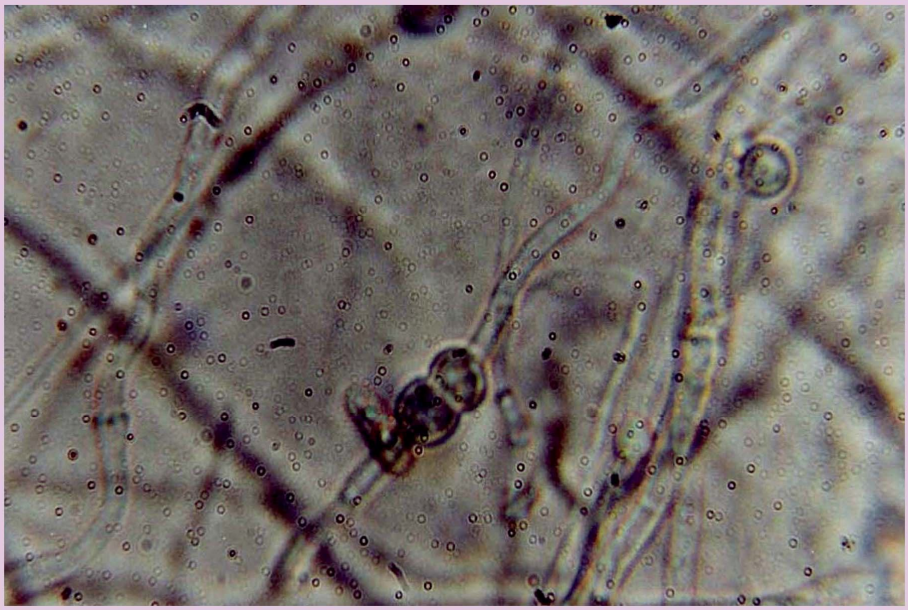


Plate-VIII : Microphotograph of *F. udum* (450x) chlamydospores

culture media. The macroconidia were long, curved, pointed at the tip, and knotched at the base, sepated (2-4 septa) and measured 15-50 x 3-5 µm. Chlamydospores are also formed on the host and in culture media when the later are old. They developed from any cell of the hypha, sometimes from cells of macroconidia, which rounded off and become thick walled. The chlamydospores were oval or spherical, single or in chains, terminal or intercalary (Plate- VI, VII, VIII).

The above description of *Fusarium* sp. isolated from the wilted Pigeonpea plant in the present study is matching with the description of *F. udum* Butler given by Booth (1977), Mehrotra (1980), Singh (1982) and Mundker (1991). Further, the fungus also produced typical wilt syndrome in the pathogenicity test. Thus, *Fusarium* sp., under study was identified and confirmed as *F. udum* Butler causing Pigeonpea wilt.

4.3 Pathogenicity test

The Pathogenicity test of isolated fungus, *F. udum* was carried out in pots by employing well known soil inoculation technique for most of the soil borne pathogen. The highly susceptible Pigeonpea cultivar, T-15-15 was used as host plant. The Bharuch isolate of *F. udum* Butler was grown on PDA medium for 15 days. It was mixed with sterile soil to fill the pot (20 g/kg soil) and allowed to establish pathogen in the soil for 7 days and then sowing of Pigeonpea seed was carried out. The results

presented in the Table 4.2 revealed that the pathogen *F. udum* Butler inoculated was capable of infecting and developing wilt in Pigeonpea plant, whereas in control, all the plant remain healthy till end of experiment.

The typical wilt symptoms were started to appear in plants after 30-35 days of sowing in the pots. Complete wilting syndrome and death of the plants took 40-45 days of sowing. The leaves of the infected plants gradually turned yellow, withered and dried, as if it suffered from moisture shortage, though adequate moisture was in the pots due to regular watering, eventually causing complete death of the entire plant. Leaves were retained on wilted plant. The visual examination of the wilted plant after uprooting, showed that roots and the base of the stem showed black streaks in the center, which became more clear and pronounced on removal of bark. The brown or black streaks were observed when the roots and the main stem was split open. In a few cases, the white mycelial growth was observed in the center of roots and stems. The infected plants did not show any symptoms of recovery upto 15 days during this time entire plant wilted and died.

The reisolation from such roots and stem of the infected wilted plant, showed the fungus *F. udum* Butler in culture. The cultural and morphological characters were studied and compared with those original one and found identical to *F.*

udum Butler. This clearly revealed that inoculated Pigeonpea plant producing wilt symptoms closely resembled to those produced under natural field conditions. Thus, isolated *F. udum* was proved pathogenic to Pigeonpea beyond doubt satisfying Koch's postulates (Plate-IX).

Table-4.2 : Number of plants wilted in pathogenicity test

Sr. No.	Treatment	Number of plants	
		Inoculated	Infected/wilted
1	Soil inoculated with fungus	25	25
2	Control (without inoculum)	25	0

4.4 Efficacy of different inoculation technique

The seven inoculation techniques were tried to find out the most efficient, effective and rapid technique to produce wilt disease, which can be adopted for screening large number of entries/genotypes in wilt screening nursery. The result presented in Table-4.3 revealed that all the inoculation technique produces more than 50% wilt in the Pigeonpea plant (cv. T-15-15) after inoculation. However, soil inoculation and sowing in wilt sick plot produced maximum wilt (92 %), followed by drenching of spore suspension and wilt sick soil (88 %) in plot culture. The minimum per cent plant were wilted in stem inoculation technique (64 %), whereas, cent per cent plants remain healthy in control treatment till end of the experiment. The various

workers had suggested different technique such as Nene (1979) suggested sick pot screening technique whereas Mishra and Dhar (2005) suggested the soil inoculation, water culture technique and spore suspension

I C

Plate-IX : Pathogenicity test

I C

I = Inoculated C = Control

Plate-X : Soil inoculation technique

method in pot culture. Sharma *et al.* (1979) suggested new stem inoculation technique. Our finding tallied with the finding of above workers. However, during our experimentation soil inoculation and sowing in wilt sick plot were found best followed by wilt sicks soil and drenching of spore suspension. This may be due to inoculum remain in the root rhizosphere right from germination, in addition to this, the environmental condition remain same during plant growth as if incase of testing in wilt sick plot and normal crop grown in the field. The test entries have to face all the environment prevails in the field during crop growth. Therefore, screening in wilt sick plot give more precision and reliability of screening results Fig- 1 and Plate- X, XI, XII.

Table- 4.3 : Per cent wilt incidence due to different inoculation techniques

Sr. No.	Inoculation techniques	Number of plants		Per cent wilted plants
		Inoculated plants	Wilted plants	
1	Soil inoculation	25	23	92
2	Sowing in wilt sick plot	25	23	92
3	Drenching of spore suspension	25	22	88
4	Wilt sick soil	25	22	88

5	Root dip method	25	20	80
6	Seed inoculation	25	16	64
7	Stem inoculation	25	16	64
8	Control	25	0	0

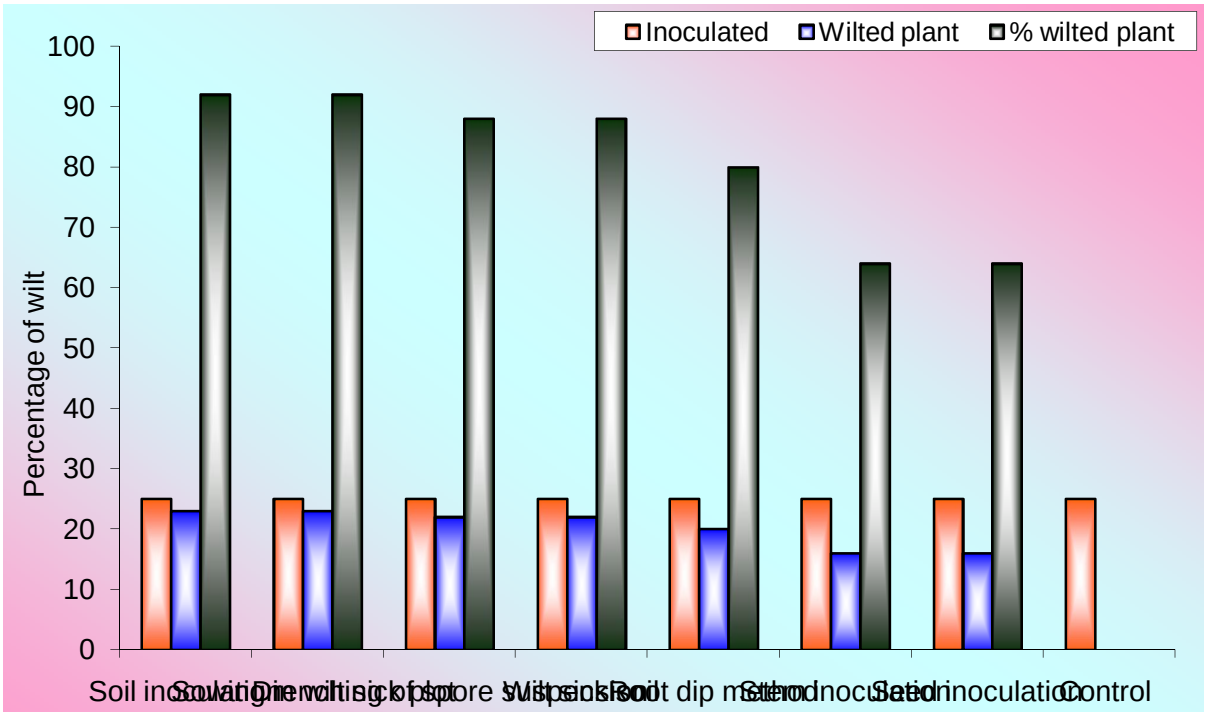
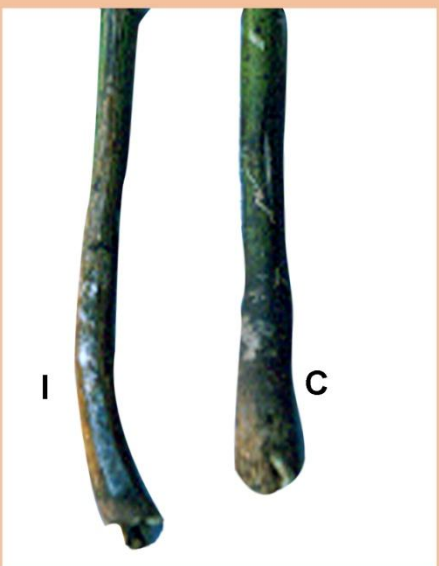


Fig-1 : Per cent wilt incidence due to different inoculation techniques

D = Drenching C = Control

Plate-XI : Drenching of spore suspension



I = Inoculated
C = Control

Plate-XII : Stem inoculation

4.5 Determination of inoculum threshold level for producing Pigeonpea wilt

The incidence and intensity of soil borne diseases such as wilt, mainly depends on quality and quantity of inoculum threshold present in the root zone of susceptible host. Therefore, it is necessary to know the minimum inoculum level i.e. inoculum threshold, required to cause the infection and to produce disease in host plant. In the present study inoculum of Bharuch isolate was prepared in the laboratory, and calculated quantity was added in the soil. Such different level or quantity of inoculum was added in the soil to grow the Pigeonpea plants of cv. T-15-15. The periodical wilt symptoms development was recorded till end of the experiment.

The result presented in Table-4.4 and Fig-2, clearly revealed that *F. udum* Butler failed to cause disease at inoculum threshold of 5 and 10 g/kg soil. But it produced more wilt when inoculum 50 g/kg soil or more was added to soil. The per cent wilt incidence was much higher at higher level of inoculua (70 g/kg) added in the soil. The first yellowing of leaves and wilting was observed in case of inoculum threshold of 90 and 100 g/kg soil treatment, after 40 days of sowing and plant completely wilted after 50 days of sowing. Incase of lower inoculum threshold 20 g/kg soil, only 5% wilt incidence was recorded after

85 days of sowing, with yellowing of lower leaves, withering and the plant wilted after 100 days of germination. With increase of threshold level above 30 g/kg soil, the percentage of wilt incidence increased.

Similar results were reported by Naik *et al.* (1992). They have not found wilt at 1:200 dilution of wilt sick soil (15 cfu/g soil) in susceptible variety ICP-2376, but found at 1:100 dilution (34 cfu/g soil). Our finding tallied with Bhatti and Kraft (1992) wherein, they reported that the increase in inoculum level, increased chickpea wilt (10^4 or 10^5 micro or macroconidia/ml). The same results were reported incase of tomato wilt, the percentage of wilted tomato plants varied in proportion to the quantity of inoculum of *F. oxysporum* and *F. solani* added to the soil.

Table-4.4 : Effect of inoculum threshold on the incidence of Pigeonpea wilt

Sr. No.	Inoculums added (g/ kg soil)	% WILT INCIDENCE 90 DAS	Yellowing and dropping of leaves at DAS	Complete wilting DAS
1	100	80	40	50
2	90	80	40	50
3	70	80	50	63

4	50	80	50	60
5	30	40	60	65
6	20	5	85	100
7	10	0	0	0
8	5	0	0	0
9	Control	0	0	0

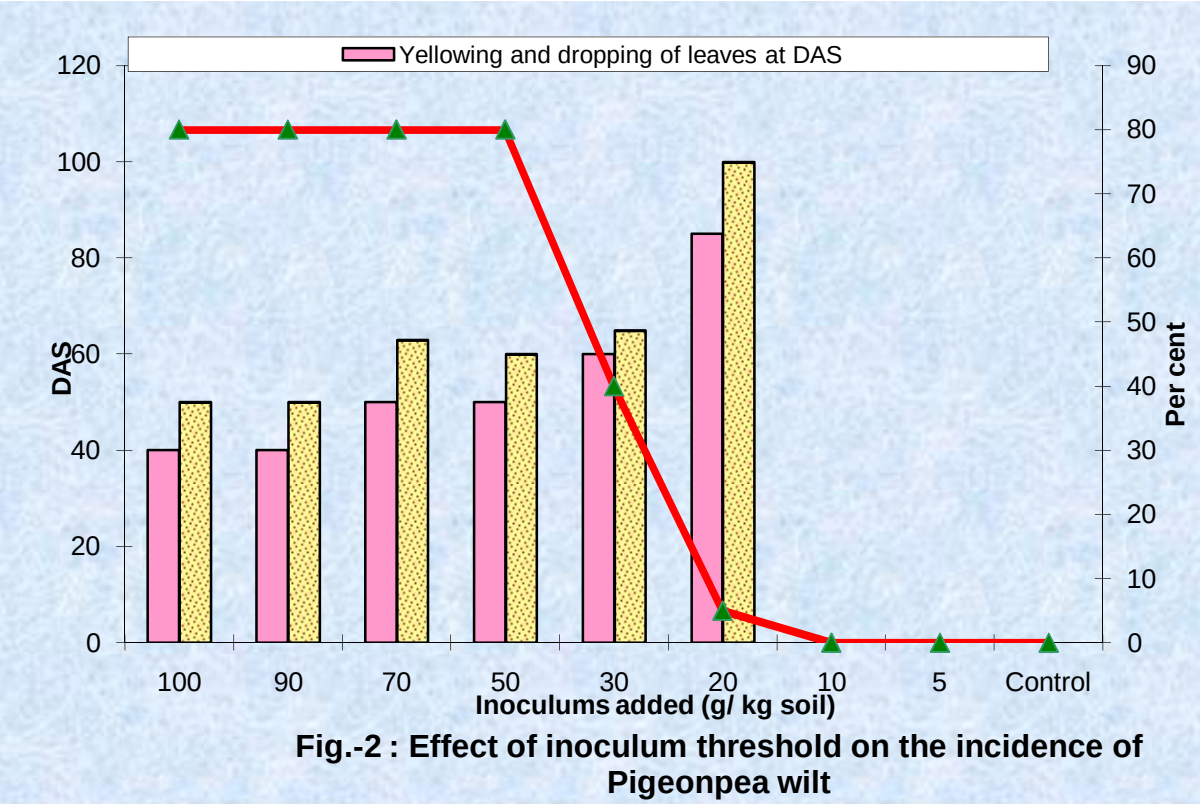


Fig.-2 : Effect of inoculum threshold on the incidence of Pigeonpea wilt

4.6 Testing virulence of different isolate of *F. udum* Butler

The seven different isolate of *F. udum* Butler, were isolated from the wilted plants collected from different Pigeonpea growing area during survey. These isolates were purified by single spore isolation technique. The virulence of different isolate was tested in pot culture by employing soil inoculation method. The results presented in Table-4.5 and Fig-3 revealed that the isolate of Rajpipla fail to infect and did not develop Pigeonpea wilt on both the cultivars. The maximum (80%) wilting were recorded incase of Vagra, Dediyapada and Dabhoi isolate. The Vagra isolate produced yellowing symptoms of wilt after 30 DAS in cv. T-15-15 and after 45 days in tolerant genotype, BDN-2. The Dabhoi genotype failed to infect tolerant genotype BDN-2, but it produces 80 % wilting in T 15 – 15. The Ankleshwar, Dediyapada and Rajpipla isolate did not infect tolerant genotype, BDN-2. It is interesting to note that virulent isolate produced wilt symptoms early i.e. within 30, 40 DAS in susceptible and tolerant genotypes. In control treatment no infection or wilting was appeared. Similar differences among the isolate was recorded by Shit and Sen Gupta (1978). They studied the reaction of different isolate to four genotypes, including susceptible to resistant genotype. Rajendra and Patil (1992) reported virulence differences by testing 22 isolate on 10

genotype. Thus, the results indicate that physiological races or variation in virulence of the pathogen exist in tested isolates of various location. The present finding tallied with the findings of Baldev and Amin (1974). They reported an existence of races in *F. udum*, similarly, Sarojini (1951) reported variation in *F. udum* on the bases of pathogenicity test.

Table-4.5 : Virulence of different isolates of *F. udum*

Sr. No.	Isolates	Per cent wilt incidence at 90 DAS		First appearance of yellowing and dropping of leaves (DAS)	
		T-15-15	BDN-2	T-15-15	BDN-2
1	Ankleshwar	10	0	50	0
2	Dabhoi	80	0	40	0
3	Dediyapada	80	0	35	44
4	Jambusar	60	10	60	75
5	Karjan	75	15	45	60
6	Rajpipla	0	0	0	0
7	Vagara	80	5	30	45
8	Control	0	0	0	0

4.7 Search for superior media for growth and sporulation of *F. udum* Butler

To find out best medium for the growth and sporulation of *F. udum* Butler, seven different media including synthetic and semi-synthetic in solid state were tested.

The results revealed Table-4.6, Fig-4 and Plate-XIII that significantly more colony growth was recorded in Czapek's Dox agar medium (88.5 mm), which was at par with PDA, and

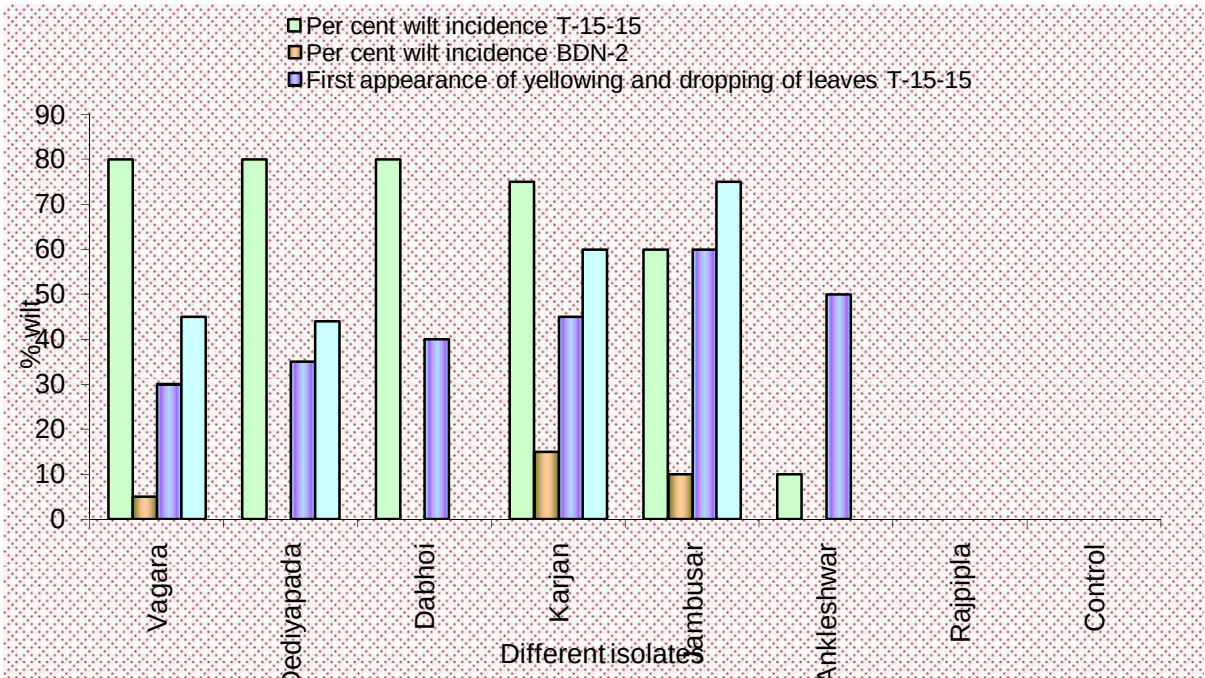


Fig-3 : Virulence of different isolates of *F. udum* on the incidence of Pigeonpea wilt at 90 DAS

Oat meal agar medium (88.0 mm). The next best was Elliot's agar medium and Richards' agar medium. The minimum growth was recorded, in case of Das Gupta medium. The maximum dry mycelial weight was recorded in PDA medium followed by oat meal agar medium and Asthana & Hawker's medium. The maximum sporulation was recorded in PDA and oat meal

agar medium, while minimum in case of Richards' agar medium.

Table-4.6 : Growth and sporulation of *F. udum* on different media *in vitro*

Sr. No.	Media	Average colony diameter (mm)	Dry mycelial weight (mg)	Sporulation
1	Czapek's Dox Agar medium	88.5	192	++++
2	Oat Meal Agar medium	88.0	290	++++
3	Potato Dextrose Agar medium	87.5	290	++++
4	Elliot's Agar medium	74.0	105	++++
5	Richards' Agar mediaum	66.5	90	++
6	Asthana & Hawker's medium	51.5	200	+++
7	Das Gupta Agar medium	45.6	102	++
	S.Em±	0.4	-	-
	C. D. at 5 %	1.3	-	-
	CV %	2.3	-	-

-- = No sporulation

+	=	1-10 conidia / microscopic field (10 x)
++	=	11-25 conidia / microscopic field (10 x)
+++	=	26-40 conidia / microscopic field (10 x)
++++	=	>40 conidia / microscopic field (10 x)

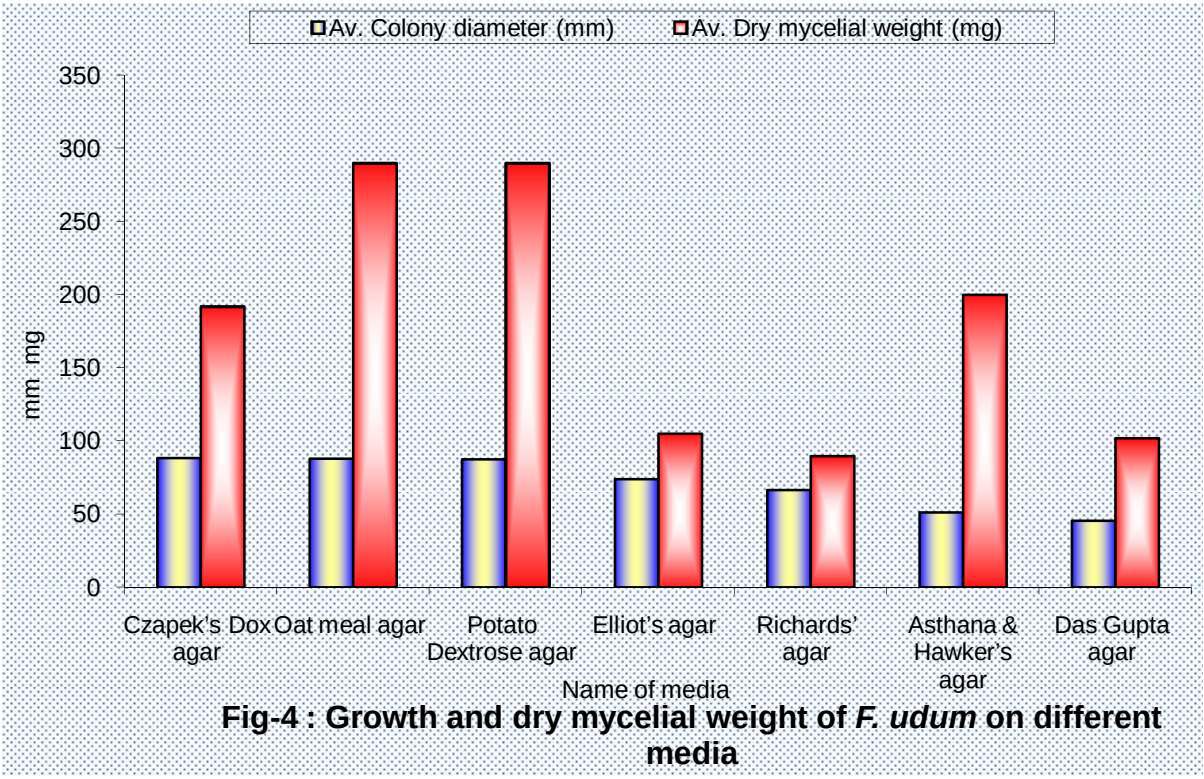
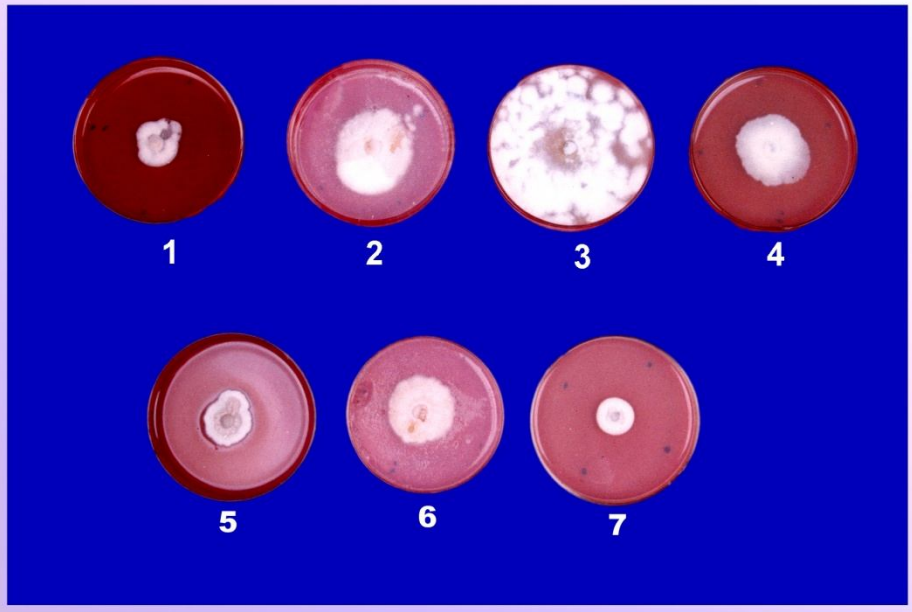


Fig-4 : Growth and dry mycelial weight of *F. udum* on different media



1. Asthana & Hawker's medium
2. Czapek's Dox Agar medium
3. Oat meal agar medium
4. Richards' agar medium
5. Elliot's agar medium
6. Potato Dextrose agar medium
7. Das Gupta agar medium

Plate-VIII : Growth of *F. udum* Butler on solid media

4.8 Management of Pigeonpea wilt

4.8.1 Bioefficacy of different phytoextracts against *F. udum in vitro*

The result presented in Table-4.7, Fig.-5 and Plate-XIV revealed that, all the phytoextracts significantly inhibited the growth of the fungus as compared to control. The extract of Garlic (*Allium sativum* L.) bulb was found significantly superior in inhibiting mycelial growth (63.70%). The next best in order of merit were Turmeric (*Curcuma longa* L.) (58.48%), Neem (*Azardiachta indica* Juss.) (44.19%) Onion *(Allium cepa* L.) (38.09%), Nilgiri *(Eucalyptus citridora* Hook.) and Acalypha *(Acalypha juliflora* L.) (32.38%) showed moderate inhibitory effect.

Kuvadio *(Cassia tora* L.), Jetropha *(Jetropha curcas* L.), Gando baval *(Prosopis juliflora* L.), Tulsi *(Ocimum sanctum* L.), Lantana *(Lantana camera* L.), Ardusi *(Adhatoda vasiaca* Nees.) and Kadvi mehndi (*Lawsonis inermis* L.) were least effective in inhibiting mycelial growth.

The inhibitory effect of Garlic bulb extract has been found better by Patel (1995), Chauhan (1997) and Patel and Vala (2004). The inhibitory effect of Turmeric rhizome extract was reported by Chauhan (1997) against *F. solani,* while inhibitory effect of Neem leaves and Onion bulb extract were reported by Chauhan (1997), Pandav (2002), Patel and Vala (2004), Mamtha

and Rai (2004) against *F. solani,* while, Bansal and Gupta (2000) and Singh and Chand (2004) against *F. oxysporum.* The present findings tallied with the studies carried out by earlier workers.

Table-4.7 : Effects of various phytoextracts on the growth of *F. udum* Butler *in vitro*

Sr. No.	Local name of phytoextract	Botanical name of phytoextracts	Av. colony diameter (mm)	Per cent growth inhibition over control
1	Garlic	*Allium sativum* L.	30.8	63.7
2	Turmeric	*Curcuma longa* L.	34.4	58.4
3	Neem	*Azardirachta indica* Juss.	46.8	44.2
4	Onion	*Allium cepa* L.	50.2	38.1
5	Nilgiri	*Eucalytus citridora* Hook.	56.5	35.4
6	Acalypha	*Acalypha juliflora* L.	59.2	32.4
7	Kuvadio	*Cassia tora* L.	70.5	17.1
8	Karanj	*Pongamia galabra* L.	72.2	15.2
9	Jatropha	*Jatropha curcas* L.	72.5	14.9
10	Gando baval	*Prosopis juliflora* L.	73.5	13.7

11	Tulsi	*Ocimum sanctum* L.	77.0	10.4
12	Lantana	*Lantana camera* L.	79.0	8.6
13	Ardusi	*Adhatoda vasiaca* Nees.	79.0	8.6
14	Kadvi mehandi	*Lawsonis inermis* L.	81.5	6.9
15	Control	--	87.5	0.0
	S.Em. ±		0.6	--
	CD at 5 %		1.9	--
	CV %		5.7	--

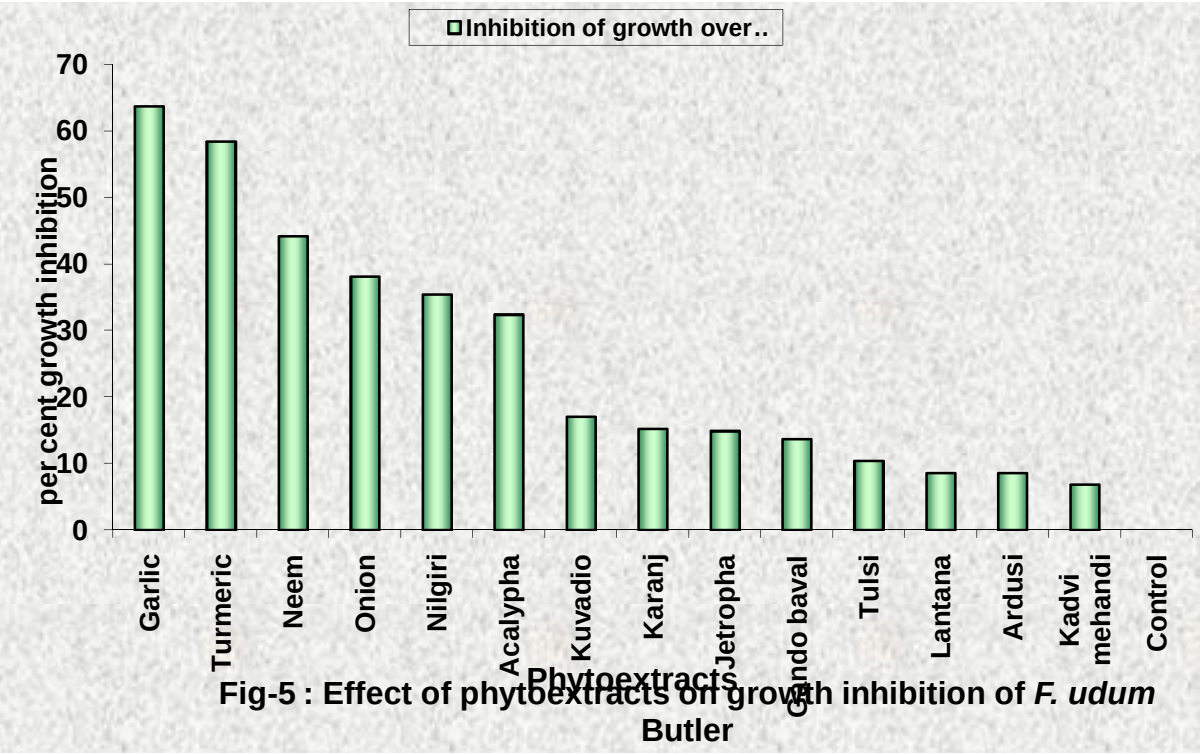


Fig-5 : Effect of phytoextracts on growth inhibition of *F. udum* Butler

1. *Trichoderma viride*
2. *Trichoderma harzianum*
3. *Trichoderma longibrachyatum*
4. *Aspergillus niger*
5. *Aspergillus flavus*
6. *Gliocladium virens*
7. *Chaetomium globosum*
8. *Bacillus subtilis*

c. *Control*

Plate-XV : Effect of antagonists on *F. udum* Butler

The effective phytoextracts *viz*; Garlic, Turmeric, Neem, Onion, Nilgiri and Acalypha provide possible alternative to over come problem of pollution in air, water and soil as well as residue with food the hazardous effect of chemicals.

4.8.2 Antagonistic effect of different microorganisms to *F. udum* Butler *in vitro*

The results presented in Table-4.8, Fig-6 and Plate-XV revealed that all the antagonists screened against *F. udum* Butler were significantly superior over control. Out of these, significantly least growth of the pathogen was recorded in *T. viride* (11.33 mm). Next best in order of merit was *T. harzianum* (13.00 mm), *A. niger* (14.33 mm), *A. flavus* (15.00 mm), *G. virens* (16.00 mm), *T. longibrachyatum* (17.00 mm), C. *globosum* (19.33 mm) and *B. subtilis* (21.66 mm).

T. viride showed maximum growth inhibition (74.04%) of the pathogen after 10 days of incubation and appeared to be the most superior over all the antagonists tested. Next best in order of merit was *T. harzianum* (70.22%) followed by *A. flavus* (65.64%), G. *virens* (63.35%), *T. longibrachyatum* (61.06%), *C. globosum* (55.72%) and *B. subtilis* (50.38%).

T. viride proved highly antagonistic followed by *T. harzianum, A. niger* and *A. flavus.* This is in harmony with the finding of earlier workers *viz.* Chauhan (1997), Ram *et al.* (2000),

Pandav (2002), Gurjar *et al.* (2004), Patibanda and Sen (2004) and Jha and Jalali (2006).

Table-4.8 : Inhibitory effect of antagonists against *F. udum in vitro* under dual culture method

Sr. No.	Test antagonists	Av. Colony diameter of pathogen (mm)	Per cent growth inhibition
1	*Trichoderma viride* (TV)	11.33	74.04
2	*Trichoderma harzianum* (TH)	13.00	70.22
3	*Aspergillus niger* (AN)	14.33	67.17
4	*Aspergillus flavus* (AF)	15.00	65.64
5	*Gliocladium virens* (GV)	16.00	63.35
6	*Trichoderma longibrachyatum* (TL)	17.00	61.06
7	*Chaetomium globosum* (CG)	19.33	55.72
8	*Bacillus subtilis* (BS)	21.66	50.38
9	Control	43.66	0.00
	S.Em. ±	0.45	--
	CD at 5%	1.36	--

CV %	4.17	--

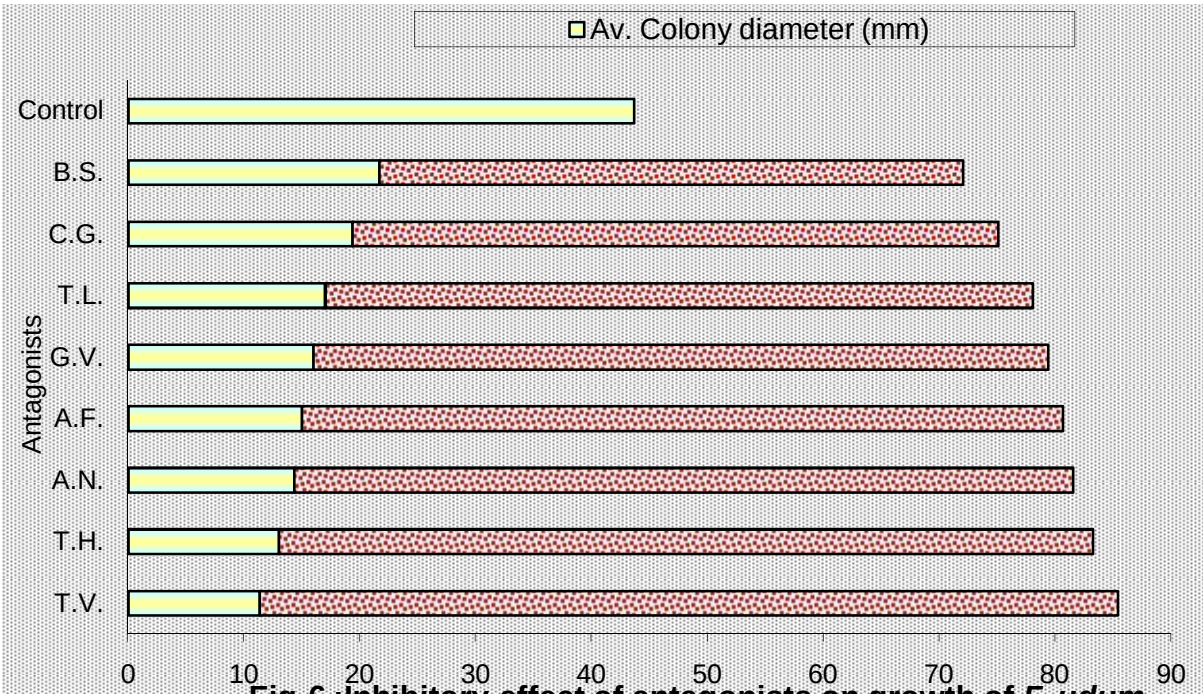


Fig-6 :Inhibitory effect of antagonists on growth of *F. udum* Butler

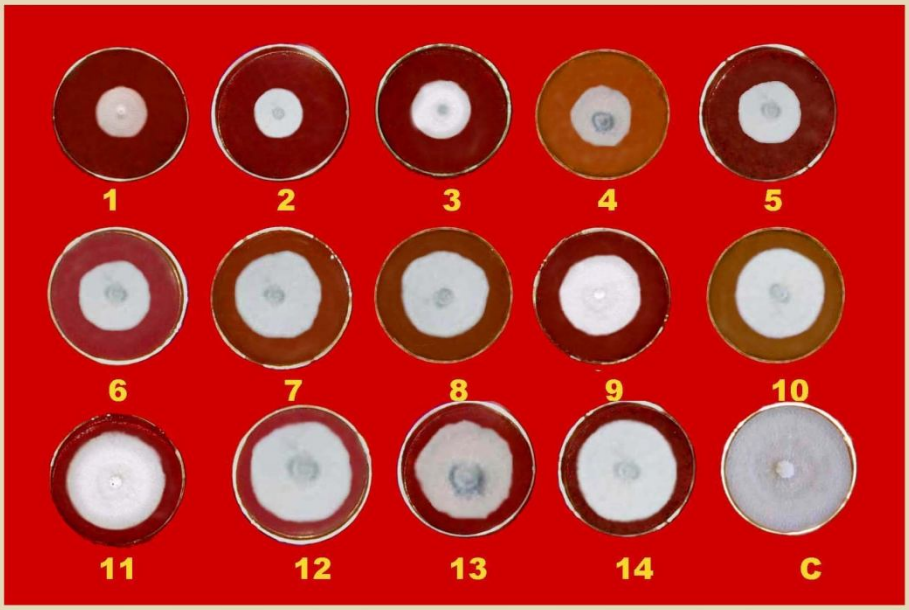


Plate-XIV : Effect of various phytoextracts on growth of *F. udum* Butler

1. Garlic
2. Turmeric
3. Neem
4. Onion
5. Nilgiri
6. Acalypha
7. Kuvadio
8. Karanj
9. Jatropha
10. Gando baval
11. Tulsi
12. Lantana
13. Ardusi
14. Kadvi mehandi

C. Control

4.8.3 Bioefficacy of fungicides against *F. udum* Butler *in vitro*

The results presented in Table-4.9 revealed that all three tested concentrations of carbendazim, benomyl, hexaconazole, MEMC and higher concentration of thiram were found significantly superior to rest of fungicides in checking the growth of *F. udum* Butler up to 10 days. This was followed by propiconazole 1000 and 1500 ppm and blitox 3000 ppm were at par up to 7 days. All these fungicides inhibited cent per cent growth of *F. udum* Butler.

Among all the concentrations, higher concentration produced maximum inhibition zone, in case of all the fungicides. The range of per cent inhibition was found from 80.7 per cent at the lowest concentration to 89.3 per cent at higher concentration, with an average of 84.9 per cent. It is evident from the results that the per cent inhibition was decreased significantly with increase in period from 7 days (87.2%) to 10 days (82.6%), suggesting the breakdown or degradation of fungicides Fig-7 and Plate-XVI.

It is also apparent form this study that interactions between fungicides and concentrations, fungicides and periods, concentrations and periods as well as interaction among fungicides, concentrations and periods were also found significant. Among the most efficient fungicides, all three

concentrations of carbendazim, benomyl, hexaconazole, MEMC and higher concentration of thiram had produced cent per cent inhibition. These fungicides persist for longer period up to 10 days. However, on an average, higher concentration produced maximum inhibition zone in all fungicides as compared to their lower concentration.

The interaction between fungicides and period was also found significant, the same trend was exhibited by carbendazim, benomyl, hexaconazole, MEMC and higher concentration of thiram produced cent per cent inhibition up to 10 days. Thus, the efficiency remained at same level up to 10 days of incubation. For the other fungicides the toxicity reduced with the passage of time. The interactions between concentrations and periods were also found highly significant for tested concentrations. However, with an increase in concentration, the percent inhibition was found significantly increased in most of the fungicides.

The study indicated that carbendazim, benomyl, hexaconazole, MEMC at all three concentrations and higher concentration of thiram (3000 ppm) were highly effective in checking the growth of fungus, followed by two lower concentration of thiram (1000 and 2000 ppm), propiconazole and blitox. These fungicides can be considered as highly effective, while, kocide and carbendazim + mancozeb were found as

moderate in their toxicity and metalaxyl + mancozeb and mancozeb were found less effective in checking the growth of *F. udum* Butler.

The similar results were obtained by Sinha (1974), Sinha (1975), Ghosh and Sinha (1981). They reported carbendazim and benomyl highly effective in growth inhibition of *F. udum*. Sugha *et al.* (1995) reported the effectiveness of carbendazim and thiram against *F. oxysporum.* Sinha and Upadhyay (1990) reported that MEMC most effective against *F. udum.*

Table-4.9 : Bio-efficacy of fungicides against *Fusarium udum* Butler *in vitro*

Sr. No.	Name of fungicides	Conc. (ppm)	Per cent Growth Inhibition at		
			7 days	10 days	Mean
1	Carbendazim (Bavistin 50% WP)	500	90.0* (100.0)**	90.0 (100.0)	90.0 (100.0)
		1000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		1500	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		Mean	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
2	Benomyl (Benlate 50% WP)	500	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		1000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		1500	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		Mean	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
3	Hexaconazole	500	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)

	(Contaf 5% EC)	1000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		1500	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		Mean	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
4	Propiconazole	500	66.6 (84.3)	64.9 (82.1)	65.8 (83.2)
	(Tilt 25% EC)	1000	90.0 (100.0)	68.2 (86.2)	79.1 (93.1)
		1500	90.0 (100.0)	70.1 (88.4)	80.1 (94.2)
		Mean	82.2 (94.8)	67.7 (85.6)	75.0 (90.2)
5	Mancozeb	1000	43.7 (47.8)	31.4 (27.2)	37.6 (37.5)
	(Dithane M-45 75%WP)	2000	46.3 (52.2)	40.3 (41.8)	47.0 (47.0)
		3000	52.5 (62.9)	49.9 (58.6)	51.2 (60.8)
		Mean	47.5 (54.3)	40.5 (42.5)	44.0(48.4)
6	Thiram	1000	72.0 (90.4)	71.5 (89.9)	71.8 (90.2)
	(TMTD 75% WP)	2000	83.8 (96.7)	73.7 (92.2)	78.8 (94.5)
		3000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		Mean	81.9 (95.7)	78.4 (94.0)	80.2 (94.9)
7	Methyl Ethyl Mercury Chloride (MEMC)	1000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		2000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
	(Emissan 6% WP)	3000	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
		Mean	90.0 (100.0)	90.0 (100.0)	90.0 (100.0)
8	Copper oxychloride	1000	68.5 (86.5)	64.9 (82.1)	66.7 (84.3)
	(Blitox	2000	71.5 (89.9)	68.5 (86.6)	70.0 (88.3)
	50% WP)	3000	90.0 (100.0)	70.1 (88.4)	80.1 (94.2)

		Mean	76.7 (92.1)	67.8 (85.7)	72.3 (88.9)
9	Copper hydroxide	1000	63.7 (80.3)	62.3 (78.4)	63.0 (79.4)
	(Kocide	2000	68.0 (85.9)	64.9 (82.1)	66.5 (84.0)
	77% WP)	3000	70.4 (88.7)	68.5 (86.6)	69.5 (87.7)
		Mean	67.4 (85.0)	65.2 (82.4)	66.3 (83.7)

Sr. No.	**Name of fungicides**	**Conc. (ppm)**	**Per cent Growth Inhibition at**		
			7 days	**10 days**	**Mean**
10	Carbendazim + Mancozeb	1000	53.8 (65.2)	50.6 (59.7)	52.2 (62.5)
	(Sixer 75% WP)	1500	57.6 (71.4)	54.6 (66.4)	56.1 (68.9)
		2000	65.3 (82.6)	60.9 (76.5)	63.1 (79.6)
		Mean	58.9 (73.0)	55.3 (67.5)	57.1 (70.3)
11	Metalaxyl + Mancozeb	1000	49.8 (58.4)	41.6 (44.0)	45.7 (51.2)
	(Master 72% WP)	1500	52.5 (62.8)	46.3 (52.2)	49.4 (57.5)
		2000	59.1 (73.6)	49.3 (57.5)	54.2 (65.6)
		Mean	53.8 (65.0)	45.7 (51.2)	49.8 (58.1)
12	Control		0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
			0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
			0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
		Mean	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)

Concentration			
1st	70.7 (82.9)	67.9 (78.5)	69.3 (80.7)
2nd	75.4 (87.2)	70.6 (82.5)	73.0 (84.9)
3rd	79.8 (91.6)	74.4 (86.9)	77.1 (89.3)
Mean	75.3 (87.2)	70.9 (82.6)	73.1 (84.9)

* Figures those out side the parenthesis are arcsine transformed values

** Figures in parenthesis are original values

Sr. No.	Source	S. Em. ±	C.D. at 5%	C.V. %
1	Fungicides (F)	0.36	1.01	
2	Concentration (C)	0.18	0.50	
3	F x C	0.62	1.76	
4	Period (P)	0.14	0.41	2.30
5	F x P	0.51	1.43	
6	C x P	0.25	0.71	
7	F x C x P	0.89	2.48	

4.8.4 Management of Pigeonpea wilt in wilt sick plot (*in vivo*)

The seed treatment with thiram before sowing and drenching of fungicide or antagonist to reduce the Pigeonpea wilt in wilt sick plot revealed that Table-4.10, Fig-8-9 and Plate-XIX all tested fungicides and antagonist significantly

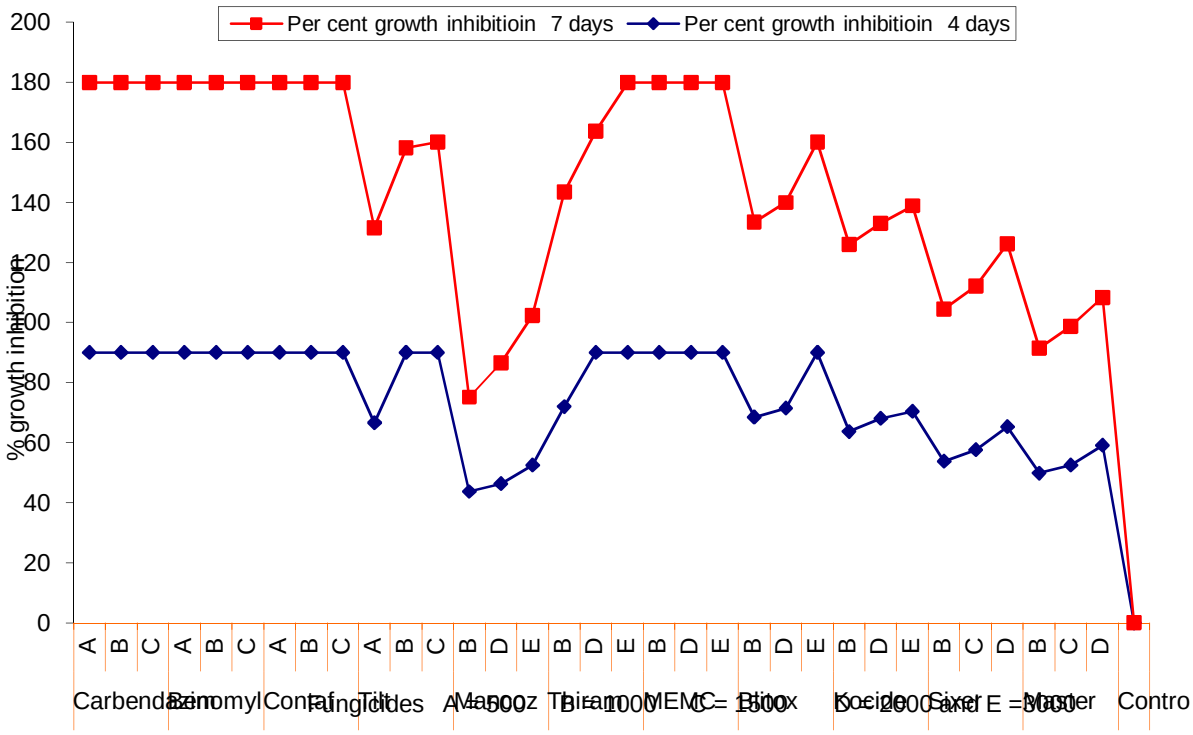
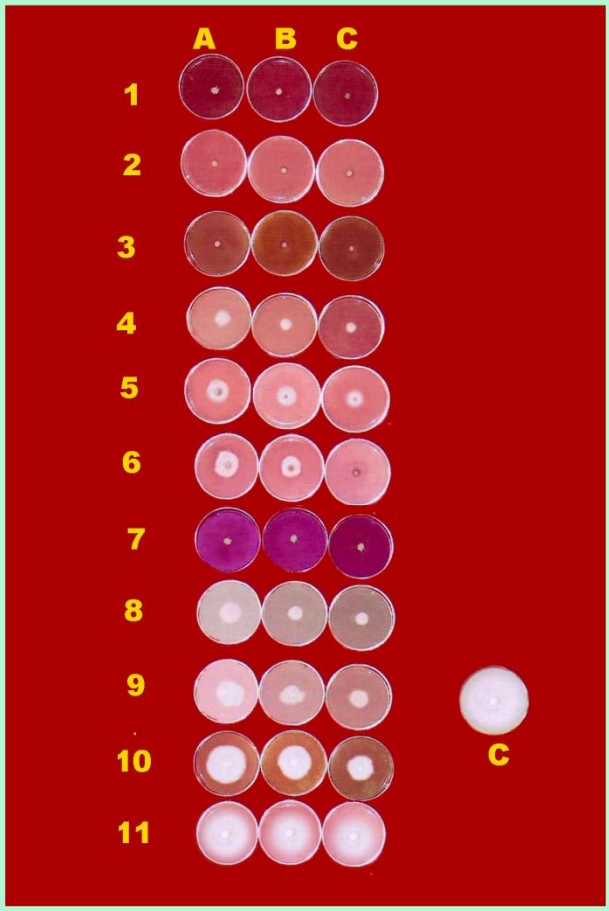


Fig-7 : Bio-efficacy of fungicides against *F. udum* Butler in vitro



Fungicides	Concentration A	B	C
1. Carbendazim(Bavistin 50% WP)	500	1000	1500
2. Benomyl(Benlate 50% WP)	500	1000	1500
3. Hexaconazole(Contaf 5% EC)	500	1000	1500
4. Propiconazole(Tilt 25% EC)	500	1000	1500
5. Mancozeb(Dithane M-45 75%WP)	1000	2000	3000
6. Thiram(TMTD 75% WP)	1000	2000	3000
7. MEMC(Emisan 6% WP)	1000	2000	3000
8. Blitox(Copper oxychloride50% WP)	1000	2000	3000
9. Kocide(Copper hydroxide77% WP)	1000	2000	3000
10. Carbendazim + Mancozeb(Sixer 75% WP)	1000	1500	2000
11. Metalaxyl + Mancozeb(Master 72% WP)	1000	1500	2000
C . Control	-	-	-

Plate-XVI : Bio-efficacy of fungicides *in vitro* against *F. udum* Butler

reduce Pigeonpea wilt than the control treatment at 40 DAS, 95 DAS and at the time of harvesting of crop. There is also significant difference between fungicides and antagonist with regards to wilt incidences, right from first observation to harvest of the crop. Among, the fungicides minimum percentage of wilt was found in drenching of carbendazim (24.9%), followed by benomyl (28.1%) and hexaconazole (30.7%). Whereas among, the antagonist minimum wilt was recorded in *T. viride* (41.1%), followed by *T. harzianum* (46%) and *A. niger* (52. 7%) at the time of harvesting of crop. The periodical observations indicated that the wilting symptoms of Pigeonpea plant started earlier, i.e. 30 DAS, and the wilt incidence was linearly increased with progress of the crop period, irrespective of treatment. This difference is more clear incase of control treatment.

The ancillary observations on phytophthora blight revealed that the drenching of fungicides and antagonists did not affect the incidence of phytophthora blight as the per cent differences of phytophthora blight was found non significant, among the treatments at 30 DAS. Whereas, after 60 DAS, there is significantly less incidence of phytophthora blight was found in drenching of fungicide than the control and drenching of antagonist.

The drenching of fungicides and antagonist did not influence the occurrence of SMD resulting into non-significant

difference, among the treatments and control even at time of harvesting.

Drenching of all the fungicides and antagonist, namely, *T. viride* and *T. harzianum* gave significantly more grain yield than the control and the drenching of *A. niger.* The yield differences among the fungicides were found significant with each other. Significantly highest grain yield was recorded in hexaconazole than the rest of treatments.

It can be concluded that the seed treatment of thiram (3 g/kg seed) and two drenching of fungicides, first at 15 days after germination and then second at 15 days after first drenching can reduced the occurrence of wilt and phytophthora blight incidence and significantly increase the grain yield than the control (no seed treatment and no drenching).

The results of Haware and Kannaiyan (1992) tallied with present findings, on seed treatment with regard to wilt. Haider *et al.* (1979) reported control of phytophthora blight by using captan, brassicol, phenylmercury acetate and copper oxychloride. The Haware and Kannaiyan (1992) suggested seed treatment with benomyl or thiram, whereas, we find seed treatment of thiram with two drenching of carbendazim, benomyl and hexaconazole to reduce the wilt incidence. Somasekaran *et al*. (1996) tested 6 isolates of *Trichoderma* spp. and observed similar finding as *T. harzianum* and *T. viride*. Our

results are also confirmed with the findings of Prasad *et al*. (2002).

Table 4.10 : Effect of fungicides and antagonists on the occurrence of Pigeonpea diseases and grain yield

Treatment	Per cent wilt incidence at			Per cent phytophthora blight		Per cent SMD at harvest	Grain yield (kg/ha)
	40 DAS	95 DAS	At harvest	30 DAS	60 DAS		
Carbendazim	*5.7	12.1	24.9	11.7	8.3	9.2	534.7
	**(0.0)	(4.50)	(18.0)	(4.3)	(11.3)	(2.7)	
Benomyl	5.7	13.8	28.1	10.2	8.8	12.2	678.8
	(0.0)	(5.80)	(21.8)	(3.3)	(1.5)	(4.7)	
Hexaconazole	5.7	16.1	30.7	10.2	8.6	8.4	709.1
	(0.0)	(7.50)	(26.2)	(3.3)	(1.5)	(2.5)	
Trichoderma viride	11.8	23.3	41.1	8.7	12.6	12	468.7
	(3.3)	(15.8)	(43.3)	(2.7)	(4.0)	(4.8)	
Trichoderma harzarinum	10.9	23. 4	46.1	9.8	10.6	10.1	456.6
	(2.8)	(16.0)	(52.0)	(3.0)	(2.8)	(3.8)	
Aspergillus niger	14.6	37.9	52.7	10.4	12.2	11.5	269.1
	(5.5)	(37.5)	(63.3)	(2.5)	(3.8)	(4.3)	

Control	25.8 (18)	46.4 (52.5)	57.1 (70.3)	11.7 (4.3)	15.6 (6.8)	15.8 (18.0	239.6
S.Em. ±	0.7	1.3	1.9	1.3	1.6	1.6	16.4
CD at 5%	2.0	3.7	5.5	NS	4.8	NS	48.7
CV %	11.9	10.2	9.3	14.1	19.1	18.2	6.8

* Figures those out side the parenthesis are arcsine transformed value

** Figures in parenthesis are original value

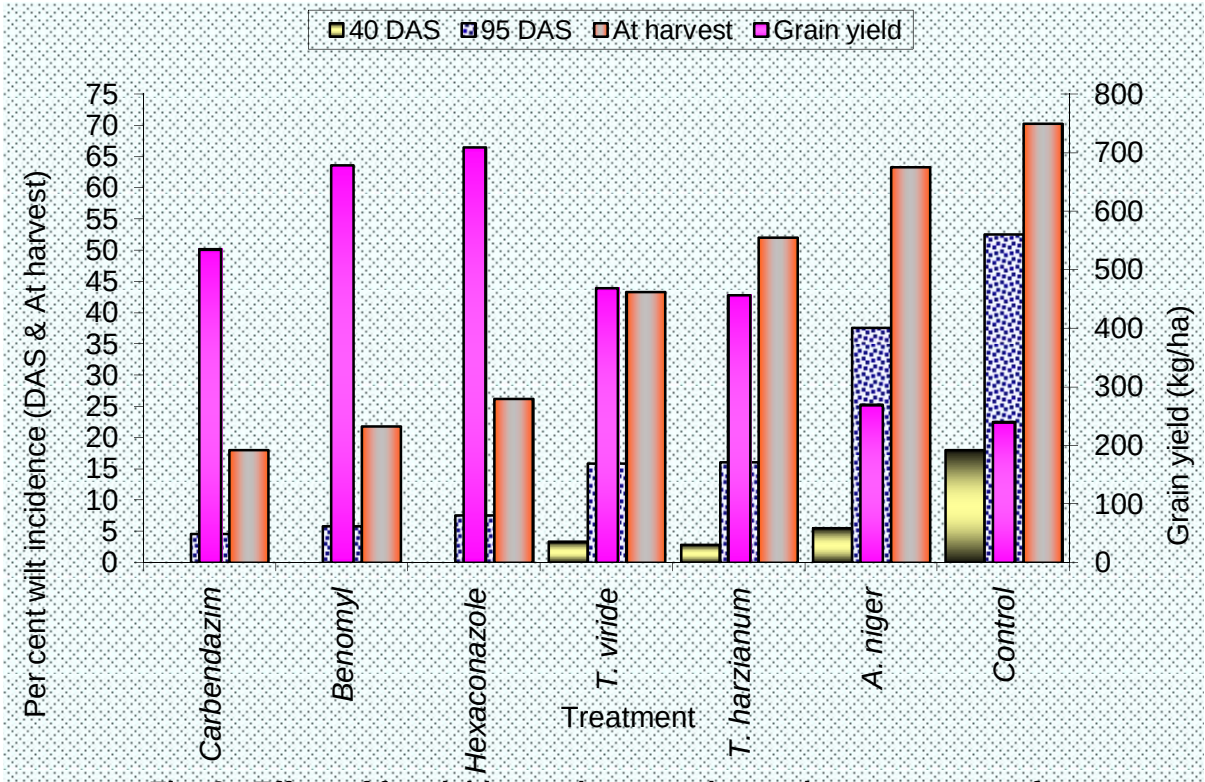


Fig.-8 : Effect of fungicides and antagonist on the occurrence of pigeonpea wilt and grain yield

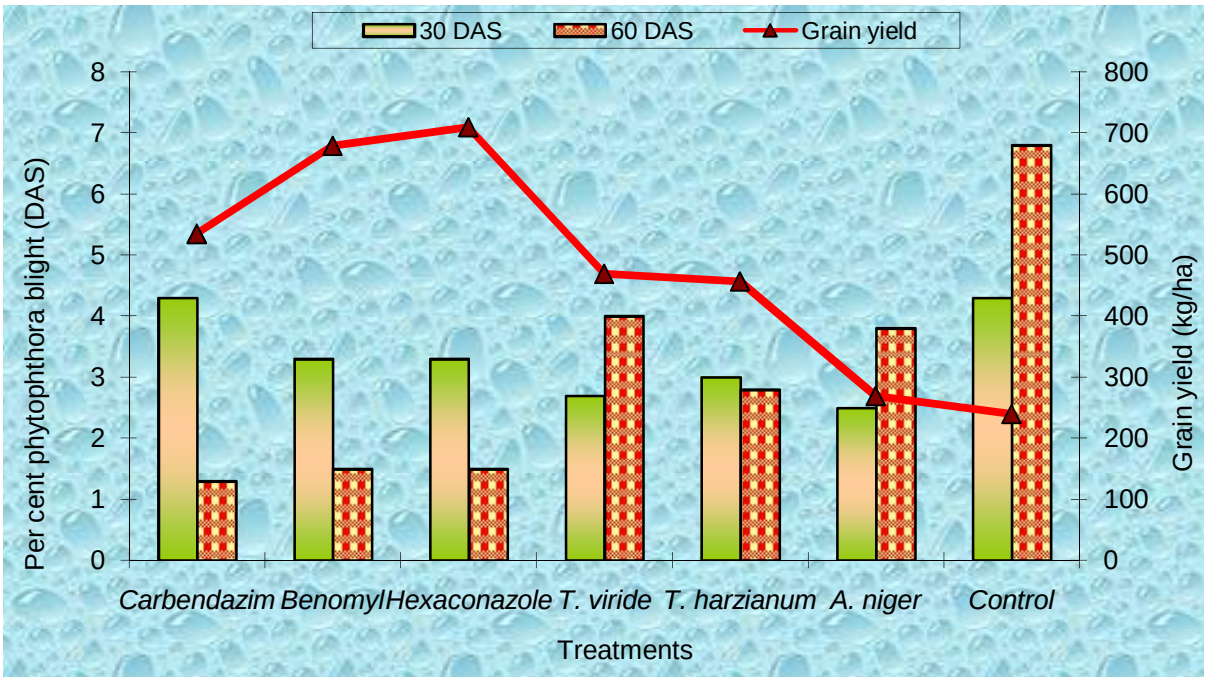
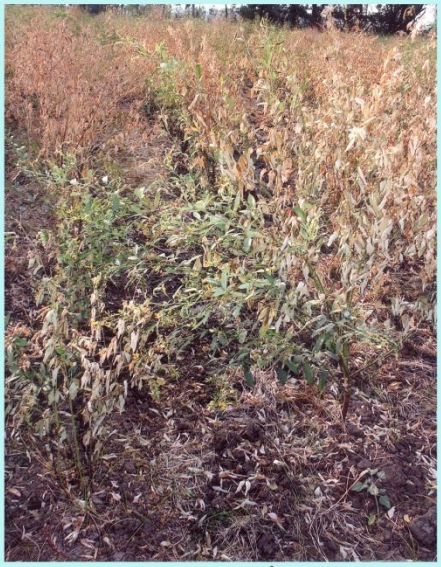


Fig.-9 : Effect of fungicides and antagonist on the occurrence of per cent phytophthora blight in pigeonpea and grain yield

A. Fungicides (Carbendazim)



B. Antagonist (*T. harzianum*)

C. Control

Plate- XIX : Effect of fungicides and antagonist on wilt incidence

4.8.5 Screening for disease resistance

4.8.5.1 Screening for wilt resistance in wilt sick plot

The screening programme of Pigeonpea wilt in wilt sick plot during 2005 and 2006 revealed that the four entries *viz*., SKNP-0306, BPWR-03-1, BPWR-03-2 and BPWR-04-2 were found resistant; the plants scoring scale-1 remain healthy till harvest. The entries SKNP-0304 and ICPL-87119 were found tolerant as they score scale-3 and rest of entries were found susceptible to wilt during 2005-06.

The wilt screening results Table-4.11 and Plate-XVII-XVIII of 2006-07 revealed that the entries of previous year *viz*., SKNP-0306, BPWR-03-1, BPWR-03-2, BPWR-04-2 and two new entries namely; SKNP-0207, BDN-2 and ICPL-87119 were found resistant, having scale-1. The seven entries namely; SKNP-0303, SKNP-0403, SKNP-0315, SKNP-0320, SKNP- 0321, SKNP-0308 and SKNP-0405 were found tolerant having scale-3. The rest of the entries were found susceptible to wilt.

The two years screening results of wilt in wilt sick plot proved that the entries BPWR-03-1, BPWR-03-2, BPWR-04-2, ICPL-87119 and SKNP-0306 were found resistance at Bharuch center. The genotype ICPL-87119 was also reported wilt resistant by Jain *et al.* (1995) from ICRISAT.

Plate-XVII : Field view of wilt sick plot of pigeonpea

Table- 4.11 : Genotype reaction to wilt in wilt sick plot

Scale	**Genotypes / Entries**		*Per cent wilt/Rating*
	2005-06	**2006-07**	
1	SKNP – 0306	BPWR – 03-1, BPWR – 03-2 BPWR – 04-2, SKNP – 0207 ICPL – 87119, BDN – 2 SKNP – 0306	0 % Mortality Resistant
3	SKNP – 0304 ICPL – 87119	SKNP – 0315, SKNP – 0320 SKNP – 0303, SKNP – 0403 SKNP – 0308, SKNP – 0321 SKNP – 0405	10 % or less Mortality Tolerant
5	SKNP – 0109 SKNP – 0307 SKNP – 0412	GT – 101, SKNP – 0206 SKNP – 0402, SKNP – O213 SKNP –0217, SKNP – 0421 SKNP – 0307, SKNP – 0309 SKNP –0318, SKNP – 0412	11 to 20 % Mortality
7	SKNP- 2006,SKNP – 2009 SKNP – 0311,SKNP -0323 SKNP – 0224,BDN – 2	SKNP- 2006, SKNP – 2009 SKNP – 0311, SKNP -0323 SKNP – 0224	21 to 50 % Mortality
9	Rest of the genotypes	Rest of the genotypes	51 % or more mortality

4.8.5.2 Natural field reaction to sterility mosaic

The natural incidence of sterility mosaic in entries of different experimental plot and in wilt sick plot was observed at NARP, NAU, Bharuch for two year. The results Table-4.12 and Plate XVII, XVIII revealed that the entries *viz*., ICPL-87119, ICPL-87119 x BP-92-24, BDN-2 x BP-86-108, T-15-15 x A-2, MS-288 x ICPL-87119 were found free from sterility mosaic during 2004-05, whereas, during 2005-06 the ICPL-87119 was only found free from sterility mosaic.

A

SKNP-0306 (R) T-15-15 (S)

B

T-15-15 (S) BPWR-03-1 (R)

Plate-XVIII : Varietal reaction in wilt sick plot (A, B, C, D & E)

Contd.. Plate-XVIII

C

T-15-15 (S) BPWR-03-1(R)

D

BPWR-04-2 (R) T-15-15 (S)

E

ICPL-87119 (R)

Table-4.12 : Genotype reaction to sterility mosaic in natural field condition

Scale	Genotypes / Entries		Per cent SMD/Rating
	2004-05	2005-06	
1	ICPL – 87119, ICPL – 87119 x BP – 9224, BDN -2 x BP – 86108, T – 15 -15x A-2, MS – 288 x ICPL – 87119	ICPL – 87119	No symptoms on any part of plant. Resistant reaction
3	GT -1 x BP – 92-24, BDN-2 x BP – 95-51, BDN -2 x BP – 86-204, ICPL -87119 x BP – 93-03, BDN – 2 x BP – 92-24, GT -1 x BP – 94 -03	ICPL -87119 x BP – 86-204, BDN -2 x BP- 94 -2, ICPL – 87119 x BP – 64 -204, ICPL – 87119 x BP -94 – 03, ICPL – 87119 x BP – 95-51, BDN -2 x ICPL – 7035, MS – 288 x BP – 87-58, MS – 3783 x ICPL – 87119, Bhadbhoot x ICPL – 1903, ICPL – 87	Symptoms found on 10 % or fewer plant. Tolerant reaction

4.8.5.3 Natural field reaction to phytophthora blight

The phytophthora blight is sporadic disease at NARP, NAU, Bharuch. Therefore the critical observation on occurence of phytophthora blight in the entries of different experimental plot and from wilt sick plot were recorded using standard method

described by Nene *et al.* (1981) for two years. These results revealed Table-4.13 that the five entries *viz.,* GT-101, SKNP-214, SKNP-040, SKNP-409 and BPWR-03-1 were found free from phytophthora blight during 2005-06. The entries *viz*., SKNP-2006, SKNP-2018, ICPL-87119, BDN-2, SKNP-0213, SKNP-403, SKNP -0412, BPWR-03-2 and BPWR-04-2 were found tolerant to phytophthora blight and the rest of the entries were found susceptible to phytophthora blight. The phytophthora blight did not appeared during 2006 -07 due to very late planting.

Table : 4.13 Genotype reaction to phytophthora blight in natural field condition

Scale	Genotypes / Entries	Symptoms
	2005-06	
1	GT -101 X SKNP -2014, SKNP 040, SKNP -0409, BPWR –031	No symptoms on any leaves/part
3	SKNP -2006, SKNP -2018, ICPL -87119, BDN -2, SKNP -2013, SKNP – 0412, BPWR -03-2, BPWR -04-2	Symptoms develop on 10 % or fewer plants.
	Diseases did not appeared during 2006-07	

V. SUMMARY AND CONCLUSION

The Pigeonpea [*Cajanus cajan* (L) Millsp.] is the most important pulse crop in Gujarat. After introduction of improve short duration, high yielding, dwarf varieties of grain and vegetable purpose, the disease spectrum has widen due to narrow genetic make up. The intensive cultivation and ratooning of Pigeonpea had predisposed to many diseases. At present, more than hundred diseases caused by fungi, bacteria viruses, phytoplasma and nematodes are reported in Pigeonpea. Among these, Pigeonpea wilt disease caused by *F. udum* Butler is the most sever problem in Pigeonpea growing area of Middle Gujarat, causing hudge production losses. The continuous occurrence of wilt epidemic had change the cropping system in the few pocket of Pigeonpea growing area of Middle Gujarat. The wilt pathogen, *F. udum* Butler had reported seed and soil borne in the nature, thus, providing primary inoculum to initiate the disease in endemic and epidemic area of Pigeonpea.

The voluminous literature on wilt of Pigeonpea (*F. udum* Butler) is available as it was reported as early as 1906 by Butler and described by him 1910 from India. However, no

systemic research work has been done on wilt of Pigeonpea caused by *F. udum* Butler in Gujarat. Hence, a few information is available on Pigeonpea wilt occurring in Gujarat. Considering this, the present investigations on various aspects are carried out on Pigeonpea to generate scientific data and to develop economical, long term and practically suitable measures to reduce the disease and to prevent the crop losses.

Therefore, the typical samples of Pigeonpea wilt caused by *F. udum* Butler was collected from National Agricultural Research Project, Navsari Agricultural University, Bharuch farm and from farmer's fields. The investigations on various aspects of Pigeonpea wilt such as, survey, pathological investigations, inoculation techniques, variation in pathogen and management of wilt disease was carried out.

The survey of Pigeonpea diseases (*F. udum* Butler), phytophthora blight (*Phytophthora drechsleri* f. sp. *cajani*) and sterility mosaic caused by *Tenuivirus* was carried out which indicated that the average percentage of wilt incidence in Bharuch district was 11.0, 8.4, 13.4 and in Vadodara district was 13.6, 14.3, 14.3 and in Narmada district, it was 10.2, 11.4, 13.6 during 2005, 2006 and 2007, respectively.

The average incidence of phytophthora blight during 2005, 2006 and 2007 was 4.0, 5.0 and 6.1 per cent in Bharuch,

2.4, 2.0 and 2.4 per cent in Narmada and in Vadodara district it was 5.3, 3.6 and 7.6 per cent during respective years.

The average incidence of sterility mosaic during 2005, 2006 and 2007 was 2.1, 1.0 and 1.6 per cent in Bharuch, it was 4.0, 2.0 and 1.6 per cent in Vadodara and 2.4, 0.8 and 1.0 per cent in Narmada district during respective years.

The pathological investigations of Pigeonpea wilt were carried out by collecting the disease samples and recording the natural symptoms and signs of a typical wilt, phytophthora blight and sterility mosaic. The microscopic examination and repeated tissue isolation from the infected wilt plant, *viz*., root and stem revealed the presence the *F. udum* Butler. The infected root and stem below the bark revealed the dark orange red discoloration in xylem tissues of the root and stem. The affected root did not exhibit any rotting. The patches of dead/wilted plants appear in the field at flowering or podding stage are the typical symptoms of wilt disease in the Pigeonpea field. Partial wilting of the plant is a definite symptom of Fusarium wilt. The spitted root and stem of wilted plant show dark, red brownish discoloration in the center of xylem. In some cases, milky white mycelial growth and pink masses of spore are present in xylem

The tissue isolation was carried out from the root and stem of wilted plant on PDA medium and it was purified by single spore isolation technique. The mycelium of isolated

fungus, *F. udum* Butler was grown on PDA medium and incubated at 27 $\pm$ 2^0C. It is septate and hyaline, often with dip purple discoloration of the substrate and fluffy white mycelium. Initially, conidia were produced on simple or verticillatelly branched conidiophores. The conidial mass may be hyaline, pinkish or salmon colored. They produce three types of spores, *viz*., macrocanidia, microconidia and chlamydospores. It is easy to distinguished *F. udum* Butler from the other *Fusarium* spp. on the basis of cultural and morphological characters of mycelium on PDA medium, their reproductive morphology and shape and size of different types of spore production. These recorded characters of isolated Fusarium with the description of *F. udum* Butler in the literature by various workers. Thus, the pathogen isolated was identified as *F. udum* Butler. This isolated fungus, *F. udum* Butler, proved Koch's postulates on Pigeonpea plant in pot culture by soil inoculation.

The pathogenicity test revealed that, the inoculated plant express symptoms of gradual chlorosis and wilting after 30-35 days of inoculation. Initially, the leaves turned yellow, loss turgidity, followed by withering, drying and wilting of the whole plant. The dried leaves of wilted plant remain hanging.

Out of seven inoculation techniques evaluated in the present study, the maximum wilting was observed incase of soil inoculation (sick plot) (92%). Thus, these two inoculation

techniques were found to be most effective for creating more wilt infection by *F. udum* Butler in Pigeonpea. However, in all the inoculation technique, the more than 60% wilting was observed. The sowing in wilt sick plot and soil inoculation in pot culture are suggested by many scientists and also by ICRISAT, as it is found most effective in creating high disease pressure artificially. These techniques can be efficiently utilized in field nursery of screening large number of genotypes against *F. udum* Butler of Pigeonpea wilt.

The calculated quantity of inoculum of, *F. udum* Butler, (for respective graded threshold level), was mixed into measure quantity of autoclave soil used for filling pots for inoculum threshold determination revealed that the lower level of inoculum 5 and 10 gm per kg soil, failed to create wilt in susceptible cv. T-15-15. The inoculum level of 50 gm/kg soil and above produced wilt in more than 50% plant. The wilting symptoms were produced earliest in highest inoculum level i.e. after 40 DAS (inoculum 100 g/kg soil). Thus, the quantity of inoculum level play an important role in wilt incidence and intensity of Pigeonpea wilt. This information is very useful for soil borne pathogen so far the efficiency of wilt sick plot depends on it. The validity of screening results are dependent on quantity of inoculum present in soil.

The seven isolates of *F. udum*, isolated from different places of Bharuch, Vadodara and Narmada district indicated a wide range in their pathogenicity i.e. virulence. The isolate of Rajpipla fail to produce wilt even in susceptible cv. T-15-15 and BDN-2, a tolerant genotype. The remaining isolate produced wilt symptoms in susceptible genotype (T-15-15), except, isolate of Ankleshwar. The isolate of Dediyapada and Dabhoi failed to produced wilt in tolerant genotype, BDN-2. The wilting symptoms among the genotypes differed with the sources of isolate used in inoculation indicated existence of variation in their virulence. Thus, the variation exists in pathogen *viz*., *F. udum*, isolated from the various Pigeonpea growing areas of Gujarat state.

The present investigations indicated that Czapek's Dox agar was best medium followed by Oat meal agar and PDA medium for the radial mycelial growth of *F. udum* Butler, however, all the tested media were found statistically at par. The maximum sporulation was observed in Czapek's Dox agar medium. Whereas, maximum dry mycelial weight was found incase of PDA and oat meal agar medium. Considering, the dry mycelial weight, sporulation and radial mycelial growth, the PDA medium is the best medium for the study and growing the *F. udum* Butler *in vitro*.

Among, the different phytoextracts tested for the inhibition of the mycelial growth, (*F. udum* Butler) revealed that the extract of garlic bulb (63.7%) was found significantly superior in growth inhibition, followed by turmeric (58.5%) and neem extract (44.2%). It is necessary to find out the active chemical involved in inhibition of fungal growth.

The maximum mycelial growth inhibition zone of *F. udum* was observed incase of *T. viride* (74%), followed by *T. harzianum* and *Aspergillus niger* (67%). This finding is important because they are eco-friendly, safe, non-polluting bioagent in water and environment as well as effective against soil borne pathogen.

The bioefficacy of fungicides against *Fusarium udum in vitro* indicated that all the three concentrations of carbendazim, benomyl, hexaconazole and MEMC were significantly superior upto 10 days of incubation. Thus, the lower concentrations of the above three fungicides require further testing below 500 ppm, so that the cost of fungicides can be reduce if lower dose is effective. Similarly, the higher concentration of thiram (3000 ppm) was also effective *in vitro* condition. Higher concentration produced higher inhibition zone in all fungicides. The range of per cent inhibition was found between 81 at lower concentration and 89% at higher concentration. The per cent inhibition was decreased significantly with increase in period from 7 days (87%)

to 10 days (83%) suggesting the breakdown or degradation of fungicides.

The seed treatment with thiram and two drenching, first after 15 days of sowing and then after 15 days of first drenching either with carbendazim or benomyl or hexaconazole significantly reduced the wilt and phytophthora blight incidence than control and antagonist. Similarly, the seed treatment with thiram and two drenching the antagonist as above also reduce wilt incidence significantly than the control (no seed treatment and no drenching) but disease incidence was significantly more than the above fungicides. Similarly, grain yield (kg/ha) was significantly higher in all the treatments except, *A. niger* than the control. The significantly highest grain yield was harvested in drenching of hexaconazole followed by benomyl than the rest of treatments and control.

The ancillary observations of above study indicated that effect of seed treatment with thiram and drenching of fungicides or antagonists did not influence the occurrence of SMD, as the per cent incidence of SMD was found non-significant among the treatments and control.

There was significant difference in percentage of phytophthora blight at 60 DAS by drenching of all the fungicides and *T. harzianum* than the control. The present finding indicated that the seed treatment with thiram and two drenching, either

of, carbendazim, or hexaconazole or benomyl or *T. harzianum* had reduced the incidence of wilt and phytophthora blight and increase the grain yield.

The genotypes/entries reaction to wilt in wilt sick plot clearly indicated that the entries BPWR-03-1, BPWR-03-2, BPWR-04-2 and SKNP-0306 were found free from wilt infection for both the years, whereas, ICPL-87119, BDN-2 and SKNP-0207 during second year, emerged as new genotype free from wilt, i.e. resistant reaction against wilt of Pigeonpea.

The entries of SKNP-0304, SKNP-315, SKNP-0303, SKNP-0403, SKNP-0308, SKNP-0321 and SKNP-0405 were found tolerant to wilt, having less than 10% mortality and rest of the entries were rated susceptible to wilt. The entries found resistant or tolerant to wilt and have good yield potential will be incorporated in yield evaluation programme and also as resistant donor in breeding programme for wilt resistance.

The natural field reaction of different entries/segregating generation of Pigeonpea against phytophthora blight and SMD at NARP, NAU, Bharuch indicated that ICPL-87119, ICPL-87119 x BP-9224, BDN-2 x BP-86108, T-15-15 x A-2, MS-288 x ICPL-87119 are found free from SMD infection, but the screening by artificial inoculation is necessary to know genetical resistance. Similarly, the natural field reaction against phytophthora blight revealed that the entries *viz*., GT-

101 x SKNP-2014, SKNP-040, SKNP-0409 and BPWR-03-1 were found free from natural field infection to phytophthora blight where less than 10% plant exhibited symptoms of phytophthora blight in natural field infection. Incase of entries SKNP-2006, SKNP-2018, ICPL-87119, BDN-2, SKNP-2013, SKNP-0412, BPWR-03-2, BPWR-04-2 the phytophthora blight did not appear during 2006-07 at Bharuch center, due to late planting.

The multiple resistance of ICPL-87119, BDN-2, BPWR-03-2 and BPWR-04-2 to wilt and phytophthora blight is out standing contribution and will provide multiple disease resistant donor as one of the parent in the breeding programme.

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***Original not seen**

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