BIOLOGICAL CONTROL OF PIGEONPEA WILT CAUSED BY *Fusarium udum* Butler

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DECEMBER, 1997

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"BIOLOGICAL CONTROL OF PIGEONPEA WILT CAUSED BY *Fusarium udum* Butler"

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled "BIOLOGICAL CONTROL OF PIGEONPEA WILT CAUSED BY *Fusarium udum* Butler" *submitted by* **Mr. S. B. GOWDAR** *for the degree of* **MASTER OF SCIENCE (AGRICULTURE)** *in* **PLANT PATHOLOGY** *of the University of Agricultural Sciences, Dharwad, is a record of research work carried out by him during the period of his study in this university, under my guidance and supervision and the thesis has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles.*

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Affectionately Dedicated

to

my beloved

Parents

&

Mrunalini

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And so comes to time to look back on the path traversed during this endeavour and to remember the faces and spirits behind the action with a sense of gratitude.

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DHARWAD
December, 1997

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INTRODUCTION

I. INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the major grain legume crops of tropics and subtropics having several unique characteristics. It finds an important place in the farming systems adopted by small holding farmers in a large number of developing countries. In India, it is commonly known as redgram, arhar or tur. It is mainly used as dhal and also tender vegetable. Pigeonpea is of dietary importance with a seed protein content of approximately 21 per cent that compares well with that of other important grain legumes (Nene and Sheila, 1990). In India, it is predominantly grown during *kharif* both as sole crop and an intercrop. It is especially useful in areas of low and uncertain rainfall because of its deep rooting and drought tolerant characters. It is also grown on mountain slopes to reduce soil erosion.

Redgram accounts for 45 per cent of the output of all pulses. India accounts for 90 per cent of world output with an area of 3.73 m.ha and production of 2.19 m.tonnes of grains (Singal , 1995). In India, the major pigeonpea growing states are Andhra Pradesh, Madhya Pradsh, Gujarat and Karnataka. In Karnataka, redgram ranks second among important pulse crops next to bengalgram.

In Karnataka, it is grown on an area of 3.61 lakh ha with an annual production of 1.55 lakh tonnes. It is largely grown in the northern parts of the state especially in Gulbarga, which is called as "Redgram bowl of Karnataka". In this district, it occupies an area of 1,66,954 ha with a production of 56,940 tonnes but the productivity is 359 kg/ha, which is very low compared to the state average productivity of 453 kg/ha, (Anon., 1995a and 1995b). It is also grown in Bidar, Raichur, Bijapur, Dharwad, Bellary and Belgaum districts.

Among the various factors that affect the yield in pigeonpea, incidence of diseases is considered to be one of the main factors. Pigeonpea is known to be affected by more than hundred pathogens (Nene *et al.*, 1989). Among these Fusarium wilt (*Fusarium udum* Butler.) was found to be very severe followed by pigeonpea sterility mosaic and Phytophthora blight (*Phytophthora drechsleri* Tucker). Local cropping patterns and monoculture of the susceptible cultivars continuously for longer periods and also due to intensive cultivation of high yielding varieties with diversified genetic makeup, wilt causing pathogen has taken upper hand and is a limiting factor in higher grain production. Wilt caused by *F. udum*, is serious in India. It's incidence in dryland situation of Karnataka was reported as high as 67 per cent.

Fusarium wilt is one of the most important soil borne disease of pigeonpea and was reported for the first time by

Butler in 1906 from Bihar state. Further, he isolated, identified and established the wilt causing pathogen as *Fusarium udum* (Butler, 1910). The disease appears on young seedlings but the highest mortality occurs at flowering and podding stages. Although the disease first appears in patches in a field, it can extend to the entire field if pigeonpea is cultivated repeatedly in the same field.

The loss in yield of pigeonpea depends on the stages at which the plants wilt. It can approach 100, 67 and 30 per cent, when wilt occurs at pre-pod and pre-harvest stages respectively (Kannaiyan and Nene, 1981).

Most of the commercial cultivars grown in Karnataka have become highly susceptible to wilt and hence its management is needed.

Chemical control though necessary to control many diseases at present, but undesirable and even inadequate as a long term solution to the crop health. Most of the workers in India concentrated their efforts in identification of resistant genotypes and strain variation of the pathogen. During past few years, some notable success of the disease control were achieved through introduction of antagonistic microorganisms. No single measure would be sufficient and durable in disease control. Investigations were undertaken to search for effective antagonists, association of rhizosphere organisms,

useful organic amendments and compatibility of biocontrol agents with chemicals for management of fusarium wilt of pigeonpea. The investigations were undertaken with the following objectives.

i. Isolation and proving pathogenecity of the *Fusarium udum*,

ii. Isolation of antagonists from the rhizosphere of pigeonpea field,

iii. Survival of the pathogen in the presence of organic amendments, and

iv. Evaluation of antagonists against pathogen and compatibility of chemicals with antagonists.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the important grain legumes in India. Of the two serious diseases on this crop *viz.*, sterility mosaic and wilt, the loss in crop yield due to the latter to the tune of 37 crores of rupees annually (Kannaiyan *et al.*, 1984).

The pathogen *Fusarium udum* responsible for the disease is of a soil borne one is difficult to control. Chemical control is not only less effective but also not economical and also hazardous to health of human beings and cattle. In the absence of these, investigations have been done by various workers on the ecology of the pathogen particularly with a view of developing suitable disease management practices. Hence, the present review pertaining to soil amendments, effect of antagonistic microorganisms, compatibility of chemicals with antagonists have a direct or indirect bearing on the present investigations.

2.1 Symptoms

Butler (1918) had described the symptoms as gradual or rather sudden withering and drying up of the green plants, exactly as if they were suffering from drought even though there may be plenty of moisture in the soil. The wilt symptoms

appear in the early stages of plant growth i.e. when the plants are about eighteen days old (Satyanarayana and Kalyanasundarum, 1952). The symptoms appeared when the plants were about four to six week old (Chaube, 1968).

Wilt symptoms can appear 4 to 6 weeks after sowing. The initial visible symptoms are loss of turgidity in leaves and slight chlorosis which sometimes becomes bright yellow before wilting. Leaves remain on the wilted plants. The initial characteristic internal symptoms in the stems are the xylem tissue gradually develops black streaks and brown or dark purple bands appear on the stem 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 (Subramanian, 1963).

However, maximum severity of the disease occurs during flowering and pod formation (Nene *et al.*, 1980). Sheldrake *et al.* (1978) stated that the symptoms of pigeonpea wilt appear during the reproductive stage particularly during pod filling stage.

The pathogen becomes systemic invading tap root, lateral roots, collar, mainstem, branches, leaflets, petioles, rachis, pedicel and pod hull (Nene *et al.*, 1980). Khune (1990) studied the transmission of *F. udum* Butler in pigeonpea and isolated *F. udum* from tap root, lateral roots, collar region, mainstem, lateral branches and pods hull of diseased pigeonpea.

Partial wilting is usually associated with lateral root infection. Tap root infection results in complete wilting. Chari *et al.* (1984) using an electric current, could predict infection before the appearance of wilt symptoms with 94 per cent validity. An isolate from completely wilted plants caused wilt in 60 per cent of inoculated plants and partial wilt in ten per cent whereas the one isolate from partially wilted plant caused complete wilt in 40 per cent plants and no partial wilt (Kotasthane *et al.*, 1987).

Rajendra and Patil (1993) collected 22 isolates of *F. udum* from wilted pigeonpea plants and pathogenecity was studied using 10 pigeonpea cultivars and a pathogenic variation in the isolates was observed.

2.2 Microorganisms associated with rhizosphere

Upadhyay and Rai (1982) revealed that, fungi in the root region of healthy and diseased pigeonpea plants varied qualitatively and quantitatively. *F. udum* was recorded on the rhizosphere of wilted plants and comprised 90 per cent of the total fungal population of the rhizosphere.

Aspergillus spp., predominated in the rhizosphere of uninoculated pigeonpea plants (Khan and Prakash, 1987). Shaik and Nusrath (1987) reported soil saprophytes and antagonists such as *Trichoderma viride* Pers., and *Aspergillus niger* Var. Tiegh., among the microflora of the wilt resistant cultivar.

The microflora of the susceptible cultivar showed a predominance of *F. udum* and other *Fusarium* spp.

Pseudomonas fluorescens Migula., isolated from rhizosphere of rape seedlings inhibited *Fusarium roseum* (Link) Snyder and Hansen and *Pythium ultimum* (Dahiya *et al.*, 1988).

Gaur and Sharma (1991) said that *Trichoderma viride* present in the rhizosphere soil of pigeonpea was most effective in controlling the disease.

Patil (1993) isolated fungi like *Aspergillus candidus* Lk. ex Fr., *A. flavus* Link., *A. niger* Van Teigh., *A. terreus* Thom., and *Trichoderma viride* Pers. ex. Fr., and were found to be antagonistic to the *Sclerotium rolfsii*, *Macrophomina phaseolina* (Tassi) Goid and *Fusarium oxysporum* Schlecht.

Ramanamma *et al.* (1994) reported that an antagonistic bacterium from rice rhizosphere showed a large inhibition zone and inhibited the rice pathogens such as *Rhizoctonia solani* Kunh and also *Curvularia* spp.

A rhizosphere isolate of *Aspergillus niger* Van Tiegh (A-27) was found to check *F. oxysporum* f sp. *melonis* both *in vitro* and *in vivo* (Sen *et al.*, 1995). Vidhyasekaran and Muthamilan (1995) obtained *Pseudomonas fluorescens* strains from the rhizosphere of different crops and observed that, the strains inhibited the chickpea wilt pathogen.

2.3 Effect of organic amendments on soil borne plant pathogens

The use of organic and inorganic manures have been suggested by several workers to control soil borne pathogens. Millard (1921) suggested the use of green manuring with grass cuttings to control potato scab.

Organic and inorganic amendments have shown profound effects on the soil microbes (Alexander, 1957). Various amendments have been employed in different forms by the earlier workers (Adams *et al.*, 1968 ; Huber and Watson, 1970 ; Khanna and Singh, 1974 ; Goyal and Mehrotra, 1979 ; Chattopadhyaya and Mustafee, 1980 ; Sudhirchandra *et al.*, 1981 ; Singh and Singh, 1982 and 1985 ; Lakshmanan and Nair, 1984 ; and Gupta, 1986) to findout the quantitative and qualitative changes in mycoflora of amended and unamended soils besides evolving a suitable control method of soil borne diseases.

Srivastava (1961) recorded a significant reduction in soil population of *Fusarium oxysporum* as well as wilt incidence in case of coriander by amending soil with linseed, groundnut or sesamum oilcakes. Linseed was most effective followed by groundnut and sesamum oil cakes.

Chauhan (1963) reported significant reduction in the incidence of *Fusarium orthocerans* var. *cicer* in soil amended with cakes of groundnut, sesamum and mustard.

Mahmood (1964) also reported, reduction of pigeonpea wilt incidence in soil supplemented with groundnut cake, molasses and sweet clover after inoculation with *Bacillus subtilis* which produces bulbiformin. Peanut oilcake suppressed the *Fusarium* population in soil, when amended at more than one per cent (W/W). At while lower dosages stimulated the population (Bhalla, 1966).

Assay of autoclaved or unautoclaved field soil, reinfested with *F. udum* and amended with 0.5 to 5.0 per cent of oil cakes of margosa, groundnut or mustard 15 days after amendment, revealed that these oil cakes either stimulate or inhibit vegetative growth or sporulation or both (Singh and Singh, 1970).

Srivastava and Sinha (1971) showed that, oilcakes were more effective in reducing the disease in case of coriander wilt caused by *F. oxysporum* f. sp., *corianderii* Kul.

Zakaria and Lockwood (1980) stated that, linseed and soybean meal reduced the soil population of *Fusarium oxysporum* and *Fusarium solani* (Mart) Apple et Wr., from 10^5/g to 10^2/g in 4 to 5 weeks.

Singh and Singh (1982 and 1985) noticed that neem cake inhibited radial growth of *F. udum*, when exposed to 1 or 2 week after amendment. Amendment with chitin, cellulose and starch at 0.1 per cent reduced the population of *Fusarium* spp., in sugarcane soils (Gupta, 1986).

DasGupta and Gupta (1989) reported, the effect of different soil amendments on wilt of pigeonpea. In an experiment with potted soil inoculated with *F. udum* and amendments of green manure (*Sesbania aculenta*), oil cakes (mustard and *Azardirachta indica*) and urea reduced both the pathogen population and the number of wilted plants.

Patel (1991) reported that, soil application of *Trichoderma harzianum* + FYM was better than individual application and recorded highest chickpea seed yield. He hypothesised that soil amendments with FYM may have improved soil properties which might have further helped in better solubilization of minerals and checked the disease.

Kulkarni (1992) reported that, safflower oilcake was more effective in reducing the mortality of groundnut seedlings followed by sunhemp, sesbania, neemcake, glyricidia and wheat straw.

Patil (1993) observed maximum per cent seedling survival of cowpea in neem cake (92.50) followed by safflower oil cake (82.50), groundnut oil cake (82.50) and farm yard manure (71.6) and this was significantly more than in untreated control (63.33).

Voland and Epstein (1994) reported that, manure and compost were more effective than urea alone in inducing suppression of the damping off of radish caused by *R. solani*.

2.4 Effect of antagonistic organisms on plant pathogens

Garrett (1956) has defined the biological control of plant diseases as "Any condition under which, or practice whereby, survival and activity of a pathogen is reduced through the agency of any other living organisms (except man himself) with the result that, there is a reduction in the incidence of the disease caused by the pathogen".

The biological control is defined as use of organisms, genes or gene products to regulate a pathogen (Baker and Cook, 1979).

Millard (1921) demonstrated the first successful control of root disease by biological means. He reported the control of scab in potato caused by *Streptomyces scabies* (Thaxter) Waksman by green manuring.

2.4.1 Effect of antagonists on *Fusarium*

Several microorganisms have been tested for their antagonistic effects against species of *Fusarium*. The growing realization of importance of biological control of plant pathogens has been discussed in many reviews (Garett, 1956 ; Baker, 1968 ; Garett, 1970 ; Papavizas, 1973 ; Cook, 1977; Lumsden, 1980 ; Mukhopadhyay *et al.*, 1989).

Vasudeva and Roy (1950) observed low incidence of pigeonpea wilt in unsterilized soil due to the inhibitory effect of *Aspergillus niger*, *A. terrus* Thom., and *Bacillus subtilis* Cohn. Emend. Praz. These organisms also secreted inhibitory substances in PDA broth restricting *F. udum*. *Bacillus subtilis* appeared to be the principle inhibitory agent.

Vasudeva *et al.* (1962) reported that *Bacillus subtilis* posses strong antagonistic property against *F. udum* through production of antibiotic bulbiformin. A reduction in the wilt incidence was observed when *B. subtilis* added to the soils.

Isarlischvili *et al.* (1968) reported that, introduction of *Trichoderma* spp., into rhizosphere reduced infection of basil (*Ocimum* sp.) by *Fusarim oxysporum*. They said the antibiotic substances released by *Trichoderma* spp. in nutrient media killed *F. oxysporum*.

Kaiser and Gupta (1977) recorded 77.50 to 81.25 per cent control of pigeonpea wilt by prior inoculation of the host with non pathogenic *F. oxysporium* f. sp. *ciceri* and *F. vasinfectum*. However, these species produced initial wilt symptoms but the seedlings later on recovered as the vascular tissues were partially affected.

Kotasthane and Agarwal (1978) reported promising results obtained by the use of *Trichoderma harzianum* Rifai as biocontrol agent against chickpea wilt. They observed 91.0 and 31.7 per cent seedling mortality (pre and post emergence) in *T. harzianum* inoculated soil as against 61.8 and 51.9 per cent in untreated soil.

Singh and Singh (1980) reported inhibition of *Fusarum oxysporum* f. sp. *udum* by soil bacteria. *Bacillus subtilis* (B4, B6, B18 and B19) and *B. cereus* (B12). They were found to inhibit growth and spore germination and caused lysis of the mycelium and germ tubes of the test fungus. They also reported that *Pseudomonas fluorescens* neither inhibited the growth of *F. udum* nor brought about significant lysis of the growing mycelium. They had an inhibitory effect on spore germination.

Mahalinga (1982) reported that among the four antagonistic organisms *Bacillus subtilis* was most effective and produced inhibition zone of three mm against *Fusarium oxysporum*. *Trichoderma* spp., *Curvularia lunata* (Wakker) Boedijn., and *Streptomyces* sp. over grew the species of *Fusarium*.

There is evidence that Pseudomonads have a role in the suppressiveness of certain soils to fusarial wilt of flax, radish and cucumber (Scher and Baker, 1982). Simeoni *et al.* (1987) also reported significant suppression of chlamydospore germination of *F. oxysporum* f. sp. *cucumerinum*.

Podile and Dube (1985) suggested that, amendment of wilt sick soils with a ten-fold concentration of cell-free culture filtrate of the AF1 strain of *Bacillus subtilis* might provide biological control of fungal wilt disease and this isolate of *B. subtilis* inhibited the growth of *Verticillum albo-atrum, V. dahliae, F. udum, F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *vasinfectum* more than 10 per cent on PDA.

Shaik and Nusrath (1987) observed the mycoflora of wilt resistant pigeonpea cultivar 8858 and reported that antagonists such as *Trichoderma viride* and *Aspergillus niger* dominated. The mycoflora of wilt susceptible cultivar 8518 however showed a predominance of *F. udum* during all the stages of plant growth.

Several strains of siderophore producing *Pseudomonas fluorescens* inhibited *Fusarium oxysporum* f. sp. *vasinfectum* (Sakthivel *et al.*, 1986). Dahiya *et al.* (1988) reported that, *P. fluorescens* isolated from rhizosphere of rape seedlings inhibited *F. roseum* and *Pythium ultimum*. Sivan *et al.* (1987) also reported 26.2 per cent increase in yield of tomatoes in *Trichoderma harzianum* treated plots over *Fusarium* infested plots.

Trichoderma harzianum has shown potential to control diseases caused by *Fusarium* spp. Thus, strain T-35 of

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T. harzianum controlled fusarium wilt of cotton and melons caused by *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* respectively. It also controlled fusarium seedling blight in wheat caused by *F. culmorum* all under natural soil conditions (Kempf and Wolf, 1989 ; Sivan and Chet, 1986).

Upadhyay and Rai (1988) reported that antagonists *viz., Pencillium citrinum, T. harzianum* and *T. viride* contributed to suppressivenss of soils against *F. udum*.

Mukhopadhyay *et al.* (1989) studied the biocontrol potentiality of *Trichoderma harzianum* against chickpea collar rot and wilt *in vitro* as well as under field conditions. They found that application of wheat bran-saw-dust (WBSD) preparation of *T. harzianum* gave excellent control of the disease. Hartman and Fletcher (1991) reported *T. harzianum* to be efficient against fusarium root rot of tomato reducing disease to 21 per cent over control.

Gaur and Sharma (1991) studied microorganisms antagonists to *F. udum*. They isolated microorganisms from the rhizosphere soil of pigeonpea and tested for their antagonistic action against *F. udum*. *Trichoderma* was the most effective in controlling the disease followed by *Aspergillus niger, Streptomyces* sp., *Pencillium* sp., and *Bacillus* sp.

Patel (1991) found that *Trichoderma harzianum* initially showed two mm inhibition zone to *Fusarium* spp. and later it overgrew the colony of pathogen.

Deshmukh and Raut (1992) reported that in *in vitro* test *Trichoderma harzianum* Rifai and *Trichoderma viride* Pers: Fr. overgrew colonies of *F. moniliforme, F. oxysporum* and *M. phaseolina* with *T. harzianum* more aggressive than *T. viride*.

Patil (1993) reported that, forty-five day old culture filtrates of *T. viride* showed complete inhibition of germination of sclerotia of *S. rolfsii, M. phaseolina* and conidia of *F. oxysporum*.

Vastrad (1994) isolated *Trichoderma viride, T. harzianum, Penicillum* spp. and *Aspergillus* spp. from the rhizosphere soil of pigeonpea and were found to be highly antagonistic to *F. udum*.

2.5 Compatibility of chemicals and antagonistics

Integration of chemical and a biotic agent (*Trichodema* spp.) has been the subject of research during recent years. Integration of biological and chemical control seems to be a very promising way of controlling pathogens with a minimal interference with biological equilibrium (Papavizas, 1973).

Curl *et al.* (1976) observed that ineffective amounts (1 to 2 ug/g soil) of Pentachloronitrobenzene, applied together with *T. harzianum* controlled *Rhizoctonia solani* more effectively than *T. harzianum* alone in cotton seedling disease in the green house. Similarly, Henis *et al.* (1978) obtained control of *R. solani* damping off of radish by integration of PCNB (4 ug/g soil) and *Trichoderma harzianum* in green house experiments.

Langerak (1977) reported that after treatments of bulbs of narseins with aretan, pimaricin and thiram, the newly developing roots were more densely colonized by *T. viride* during the entire growing season. These fungi were less sensitive to these fungicides than the *Fusarium* spp. and thus more or less selectively favoured by the treatment.

Bollen (1979) critically analyzed and presented possible effects on antagonistic relationships. The indirect effect of toxicants on disease incidence may be positive or negative. Pesticides sometimes selectively enhance the antagonistic microbes or antagonism which may lead to indirect control or integrated control.

Chandra (1984) reported that integration of both chemical and biological control measures has a synergistic effect on the control of damping off in sugarbeet. Seed treatment with metalaxyl alone at 0.01 per cent was not very

effective, but when used with 10.5 g of *T. harzianum* preparation, it provided enhanced control of damping off of sugarbeet.

Papavizas (1985) proposed that, biological approach can be successful only if antagonists are compatible with fungicides and biopesticides. Mukhopadhyay and Chaturvedi (1986) obtained successful control of damping off of tobacco and egg plant by the application of *Trichoderma* preparation to soil and integrating it with metalaxyl seed treatment.

Alagarswamy and Sivaprakasam (1988) reported that pelleting of cowpea with *T. viride* Pers : Fr. either alone or in combination with carbendazim inhibited the growth of *Macrophomina phoseolina* (Tassi) Goid. Carbendazim did not show any adverse effect on the antagonists *viz., T. viride* and *T. harzianum* Rifai under *in vitro* and in pot culture studies.

Seed treatment @ 1.5 g/kg + soil drenching @ 0.05 per cent with carbendazim + soil application of *T. harzianum* was best integrated management practice for control of chickpea wilt (Patel, 1991). Kaur and Mukhopadhyay (1992) reported that chickpea wilt was effectively controlled by *T. harzianum* in combination with carboxin.

Sharma *et al.* (1992) reported that, biocides *viz., T. harzianum, T. viride* and *Gliocladium roseum* and fungicides MBC (methyl benzimidazole carbamate) and tridemefon reduced

the sclerotial germination of *Sclerotinia sclerotiorum* in pea and the combinations were more effective.

Vyas and Pathak (1995) observed that a mixture of mycelial extracts and culture wash of the antagonists *T. viride* along with Bavistin (500 ppm) rendered best results against the *Aspergillus* rot of mango. Carbendazim (0.2%) treated seeds of sunflower raised in *T. harzianum* infested soil followed by spray of carbendazim (0.2%) proved highly effective.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The foregoing investigations comprised of laboratory and green house experiments conducted during *kharif*, 1996-97 at the University of Agricultural Sciences, College of Agriculture, Dharwad. The details of the methodology employed in these investigations are described in the following pages.

3.1 General procedure

3.1.1. Glassware and cleaning

In all the experimental studies Corning glassware were used. The glasswares before use were kept in the cleaning solution containing 60 g of potassium dichromate, 60 ml. of concentrated sulphuric acid in one litre of water for 24 hrs. followed by washing repeatedly in tap water by using vim cleaning powder.

3.1.2 Sterilization

All the glassware were sterilized in an autoclave at 1.11 kg pressure per sq. cm for 20 min. The solid media and liquid media were sterilized at 1.11 kg pressure per sq. cm for 10 min. Soil used for the experimental purpose was sterilized in an autoclave for two hours at 1.33 kg pressure per sq. cm.

3.2 Preparation of potato dextrose agar (PDA)

In most of the experimental studies the potato dextrose agar (PDA) medium was used. The composition of PDA is as follows,

Peeled potato	200.00 g
Dextrose	20.00 g
Agar-agar	17 g
Distilled water	1000.00 ml (volume to make up)

Two hundred g of peeled potatoes were cut into small bits and boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose 20 g and agar-agar 17 g each were dissolved in the potato extract and the final volume was made upto 1000 ml with distilled water. Later, it was sterilized at 1.11 kg pressure per sq. cm for 20 min. and preserved for further use.

3.3 Isolation of the pathogen

The pigeonpea plants showing true vascular wilt symptoms were collected from the field for isolation of the pathogen.

Stem and root region exhibited different symptoms including white profused mycelial growth and also abundant sporulation of the fungus with wilting symptoms.

The pathogen was isolated by standard tissue isolation method. The infected stem and root of pigeonpea was

split longitudinally with the help of sterilized knife. The affected part showing brown discolouration of vascular tissue was cut into small bits of about 2-5 mm and washed well in running tap water. These bits were surface sterilized with 1:1000 mercuric chloride solution for 30 sec. Such bits were washed thoroughly in sterile distilled water thrice to remove traces of mercuric chloride if any and then aseptically transferred to sterile potato dextrose agar (PDA) slants. Further, such slants were incubated at 27 ± 1°C. After five days the growth of the pathogen was observed, indicating association of pathogen with the wilt symptom of pigeonpea plant.

The pure culture of this pathogen was obtained by hyphal tip isolation. The pure culture was maintained by subculturing once in a month and preserved in a refrigerator.

The identity of the fungal species associated was done by comparing the morphological characters and pathogenicity test.

3.4 Hyphal tip isolation

To get uniform and pure culture of species of *Fusarium udum* hyphal tip isolation method was followed.

In aseptic condition, a dilute spore suspension (8-10 spore/ml) of *F. udum* was prepared in sterile distilled water

in a test tube by taking spores from seven day old culture. One ml of dilute spore suspension was spread uniformly on two per cent water agar in sterilized Petriplates. The Petriplate was rotated well for uniform spread of spores on the medium. After 24 hrs, the plates were observed for spores under the microscope and located well isolated and germinated single spore. Such single spore (macroconodia) was then marked with ink. Such plates were incubated at room temperature (25±1°C) and periodically observed for germination of spores (Plate 1) under microscope. Hypha coming from the end cell of the single spore was traced and marked with ink. Tip of the hypha was then cut and transferred to potato dextrose agar slants and incubated for 15 days at room temperature (27±1°C). The slants with pure culture of the fungus was maintained for further studies.

3.5 Preparation of giant culture

Sand-corn meal medium was prepared in the proportion of 95:5 in order to get maximum inoculum of *Fusarium udum*. About 300 g of sand-corn meal medium was taken in 1000 ml conical flasks and watered to 20 per cent of its weight and were sterilized at 1.33 kg pressure per sq. cm. for one hour. Such flasks were inoculated with the fungal species of *F. udum* under aseptic condition and incubated at 27±1°C for 30 days. The flasks were shaken everyday to get uniform growth. The

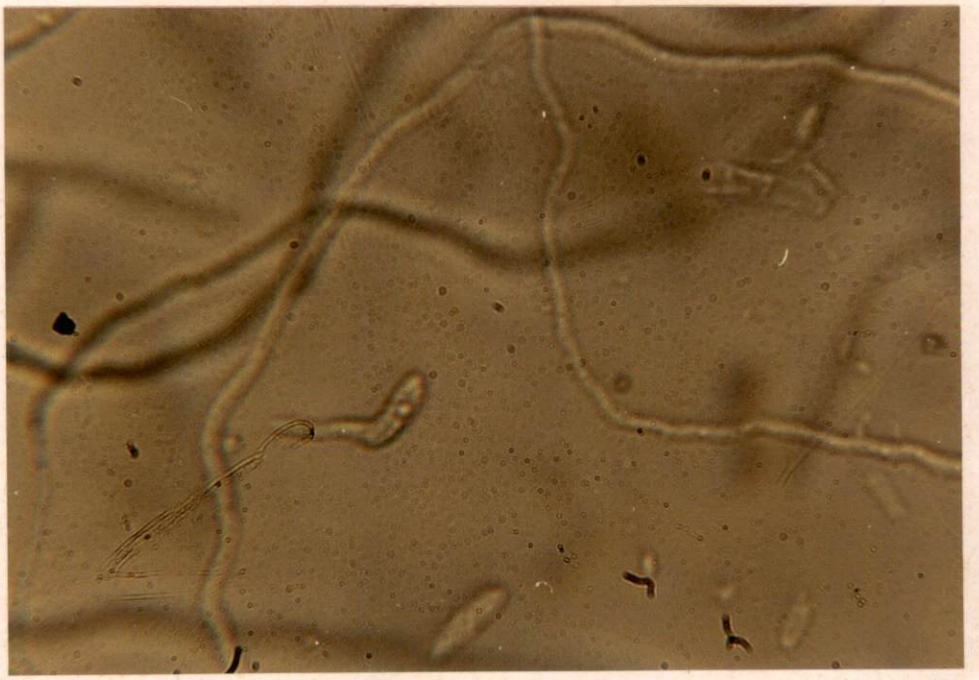
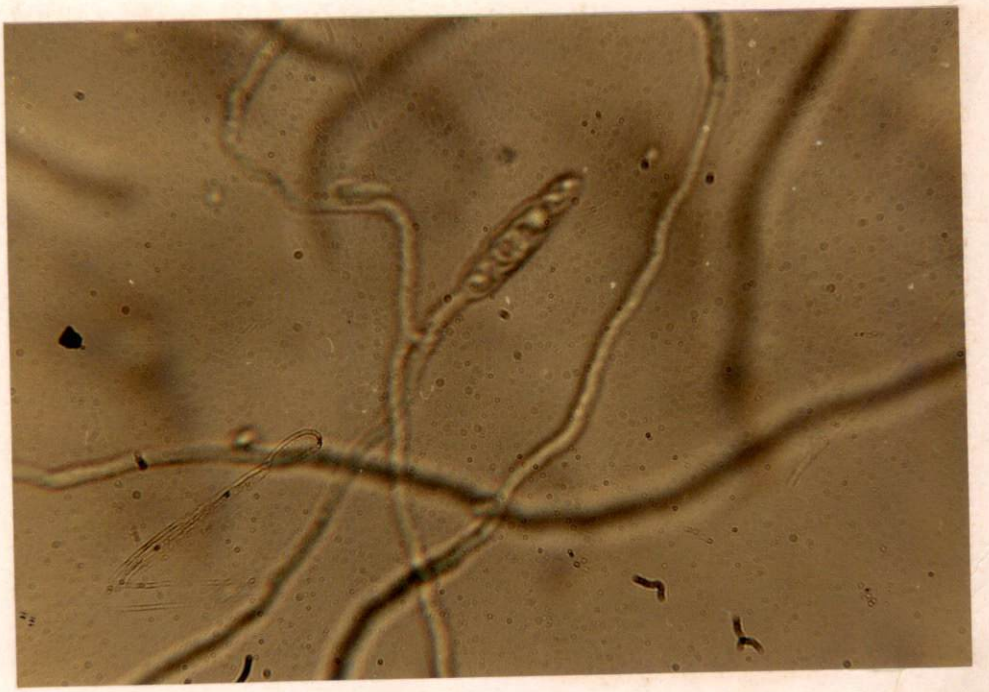


Plate 1. Photograph showing germination of *F. udum* spores



giant culture so obtained was used for proving pathogenicity of the fungus and for further studies.

3.6 Proving the pathogenicity

Five hundred g of sterilized soil was taken in earthen pots of the size 10 x 15 cm to which four per cent inoculum of *Fusarium udum* was added separately and mixed thoroughly. Seeds of pigeonpea were sown in these pots in inoculated soil. Control was also maintained to which no inoculum was added. The soil moisture was maintained at 30 per cent moisture holding capacity and then loss in moisture was maintained by adding water on weight basis. After 15 days, typical wilt symptoms were observed. The symptoms were identical to those observed in the field. The fungus was reisolated from the artificially inoculated seedlings and the cultures thus obtained were compared with the original.

3.7 Isolation of antagonists from the rhizosphere soil

Rhizosphere soil sample collected by uprooting the actively growing pigeonpea plant carefully without damaging the root system was shaken gently to remove the excess soil brought to the laboratory in sterile polythene bag. 10 g of soil sample was aseptically transferred to 90 ml sterile distilled water contained in 250 ml flasks and mixed thoroughly. One ml suspension was drawn with the help of sterile pipette and transferred to 9 ml sterile distilled water test tube blank.

The suspension was shaken for one minute, before it was further diluted, and also required dilutions were prepared. Dilution of 10^3 and 10^5 and 10^3 were used for fungi, bacteria and actinomycets respectively. Three replications were maintained to each dilution. One ml of suspension from each of the appropriate dilution was transferred aseptically into a sterile petriplate. About 15 ml of specific agar medium for fungi, bacteria and actinomycetes *viz.*, Martin's rose bengal agar, Nutrient agar and Kuster's agar were poured into plates and were rotated manually to let the suspension to distribute uniformly in the medium. The plates were inverted and incubated at 27±1°C.

Microbial enumeration was made on the fifth day for bacteria, on seventh day for fungi and on fifteenth day for actinomycetes. Counts were taken and recorded as colonies per g of soil. For further antagonistic studies the microbes so isolated were cultured on PDA and stored properly.

3.8 Maintenance of the culture

Fusarium udum and cultures of antagonistic microorganisms so obtained were grown for seven days at room temperature on PDA slants and subsequently stored at 6°C in a refrigerator and maintained by subculturing once in a month.

3.9 Media used for microbial enumeration

For bacteria,

Nutrient agar (Tuite, 1969)

Peptone	5.0 g
Beef extract	3.0 g
Agar-agar	18.0 g
Distilled water	1000.0 ml (to make up)
pH was adjusted to	7.0

For fungi

Martin's rose bengal agar medium (Martin, 1950)

Glucose	10.0 g
Peptone	5.0 g
Magnesium sulphate	0.5 g
Rose bengal	0.35 g
Agar-agar	18.00 g
Distilled water	1000.00 ml (to make up)
pH was adjusted to	6.00

One hundred ppm streptomycin sulphate was aseptically added just before pouring the medium.

For actinomycetes

Kuster's agar (Kuster and Williams, 1966).

Starch	10.0 g
Casein hydrolysate	0.3 g
Potassium nitrate	2.0 g
Dipotassium hydrogen phosphate	2.0 g
Calcium carbonate	0.2 g
Ferrous sulphate	0.01 g
Agar-agar	18.0 g
Distilled water	1000.00 ml (to make up)
pH was adjusted to	7.2

3.10 Effect of organic amendments on survival of *Fusarium udum*

Five hundred g of 2 mm sieved sterilized black soil was taken in earthen pots of size 20 cm X 15 cm. Four per cent inoculum of *Fusarium udum* was added to each pot. Different organic amendments *viz.*, groundnut oil cake, neem cake, farm yard manure and compost were evaluated against control (only with inoculation *F. udum*) for their effect on survival ability of the pathogen in artificially inoculated soil under the glass house condition. These amendments were powdered and separately added to soil in amount equal to 2 per cent of soil by weight and mixed thoroughly. After seven days of inoculation with giant culture (4% w/w) to soil, ten seeds of pigeonpea were sown in the pots. Four replications were maintained under each treatment. The soil moisture was maintained at 30 per cent of maximum water holding capacity by periodic watering.

Observations were made on 20 and 40 days after sowing for death of the seedling by counting the number. Per cent seed germination and per cent survival of the seedlings were recorded.

3.11.1 Assessment of microorganisms for antagonism against pathogen

The fungi, bacteria and actinomycetes isolated from rhizosphere soil were evaluated for their antagonistic effect in *in vitro* against *Fusarium udum*.

Fifteen ml of potato dextrose agar (PDA) medium was poured into sterile Petriplates. From eight day old actively growing culture of test fungus and antagonistic fungi, a mycelial disc of the fungal pathogen and antagonist were inoculated on opposite side of the same plate simultaneously. In case of bacteria, two discs of the test fungus were placed on opposite side of the plate simultaneously and after 48 hr bacterial antagonist streaking was done in between on PDA media poured plates. The actinomycetes were streaked in the centre and incubated. Later, discs of test fungus were placed at either side of the actinomycetes colony.

Trichoderma viride, *T. harzianum*, *Aspergillus niger*, *A. flavus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Penicillium* spp., and *Streptomyces* spp., were tried to test their antagonistic nature. Potato dextrose agar was poured into 75 mm diameter Petridishes and allowed to solidify. Five mm diameter of *Fusarium udum* was placed at one end of the Petridish. The antagonistic organism was inoculated on the opposite side. Such treatment was replicated thrice and the experiment was repeated twice for confirmation. The plates were incubated for seven days at 27±1°C. After the period of incubation, the colony diameter of *Fusarium udum* was recorded. The extent of activity of the antagonist, i.e., the growth after contact with the target fungi, their overgrowth and smothering of *Fusarium* colony and the zone of inhibition between *Fusarium* and antagonist colony were recorded.

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In another set of experiment, *Bacillus subtilis* and *Pseudomonas fluorescence* were tried as antagonistic organisms. Potato dextrose agar (PDA) was poured into Petridishes, allowed to solidify. Five mm. discs of *Fusarium udum* was placed on opposite sides of plate. After 48 hr. of incubation, bacterial suspension was prepared and streaked in the centre and incubated for five days at 28±1°C. Three replications were maintained and the experiment was repeated twice. A control was also maintained, wherein only the *F. udum* culture was inoculated. After the period of incubation, the growth of the *Fusarium* colony was recorded. The *Steptmyces* sp., was streaked in the centre of the Petriplate containing PDA medium and incubated for three days. Later, *Fusarium udum* five mm disc was placed at either side of the *Streptomyces* colony. In the experiments zone of inhibition was obtained. In other cases, where no distinct zones were produced, growth of the pathogen, its colony and their relation as overgrowth of the hyphae was observed.

Observations were recorded as per cent growth inhibition by poison food technique. In case of fungi the PDA medium was throughly mixed with the antagonist culture aseptically. Temperature of the medium was kept 40°C and this medium was poured into sterile Petriplates and 8 days old culture of pathogen was inoculated at the culture of the medium for bacterial antagonist potato dextrose agar medium was used

as test medium. Same procedure was repeated for other antagonist and replications were maintained. Appropriate control were maintained to compare the treatments.

The Petriplates were incubated at 27±1°C. The zone of inhibition was recorded in mm by measuring the clear distance between the margin of the test fungus and the antagonistic organism. Per cent inhibition was calculated by using the following formula (Vincent, 1927).

$$I = \frac{100\ (C-T)}{C}$$

Where,

I = Per cent inhibition
C = Growth in control
T = Growth in treatment.

The germination of the spores at the interaction zone and the centre of the *Fusarium* colony was studied. Five mm discs were cut randomly at the inteaction zone and a spore suspension was prepared. A drop was placed on a cover slip and inverted on to a cavity slide and sealed. The cavity slide was placed in a moist chamber. After 12 hr. the number of spores germinated over the total number of spores were counted for five microscopic fields. The per cent spores germinated was calculated. Similarly, it was done for the centre of *Fusarium* colony also.

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3.11.2 Effect of seed treatment with different antagonistic organisms on disease incidence

Seed treatment with antagonists was tried to evaluate their effect on the incidence of *Fusarium* wilt in the soil inoculated with *F. udum*.

Trichoderma viride Pers. ex. Fr., *T. harzianum* Rifai., *Aspergillus flavus* Link., *A. niger* Van Tiegh., *Bacillus subtilis* Cohn. Emend. Praz., *Pseudomonas florescence* Migula., and *Penicillum* spp., were tried for their antagonistic effect as seed treatment ones. Seven hundred g of 2 mm. sieved sterilized soil was taken in earthern pots of size 10 x 15 cm and inoculated with giant culture, so as to obtain four per cent inoculum in the infested soil.

Healthy surface sterilized seeds of pigeonpea were dipped in respective antagonistic cultures for 30 min. and ten treated seeds were sown in infested soil. Watering was done, so as to maintain about 30 per cent soil moisture of its moisture holding capacity. Untreated pots served as control. Each treatment was replicated thrice. The per cent plants affected were recorded.

3.11.3 Compatibility of antagonists with fungicides

To know the compatibility of antagonists with fungicides an experiment was conducted in the laboratory as

well as under glass house conditions. The experiment consisted of nine treatments inclusive of two antagonists *viz.*, *Trichoderma viride* and *T. harzianum* and a seed dresser *viz.*, Carbendazim and Captan, their combinations and a control.

Pelletting of pigeonpea seeds was done with fungicides *viz.*, Carbendazim @ 500 ppm and Captan @ 0.2 per cent of seed and with the antagonists *viz.*, *Trichoderm viride* and *T. harzianum* 4 g/kg of seeds individually and in combination with fungicides. In the combination treatments, seeds were first treated with fungicide and after 24 hr. fungicide treated seeds were treated with respective antagonists. After treatment seeds were shade dried and used in the experiments.

The effect of treatment were tested under *in vitro* conditions. The sterilized potato dextrose agar (PDA) medium inoculated with *Fusarium udum* was poured in the petridishes and the treated seeds were placed in the centre of the petridishes and these were incubated at room temperature. Three replication were maintained for each treatment. The inhibition zone was recorded on 6th day after planting.

The same treatments were also tried under pot culture conditions and each treatment was replicated thrice. The treated seeds were sown in the pots inoculated with *F. udum* as mentioned earlier. The number of germinated and infected plants were recorded.

Fungicides used in compability studies with their common and chemical names

Fungicide common name	Chemical name	Trade name with formulation	Concentration
I. Systemic fungicide			
Carbendazim	Methyl-2-benzimldazole Carbamate	Bavistin, 50% WP	500 ppm
II. Non-systemic fungicide			
captan	N-trichloromethyl thio-4-cyclo-hexane-1, 2-dicarboximide	Captan, 75% WP	0.2 %

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

4.1 Collection of diseased specimen and isolation of the pathogen from wilted pigeonpea plants

The wilted pigeonpea plants were collected from the field (Plate 2) of Agricultural College Farm, Dharwad, during *kharif* 1996-97. In order to assess the fungi associated with the wilted pigeonpea plants, tissue isolation was made as described in `Material and Methods'.

It was found that *Fusarium udum* was associated with wilted pigeonpea plants. The fungus was isolated even from the branches of the wilted plants.

4.2 Identification of pathogen isolated from host

The pathogen was identified as *Fusarium udum* based on morphological characters as mentioned below.

4.2.1 Culture on potato dextrose agar (PDA)

Growth of *Fusarium udum* was white cottony mass and appeared felted and wrinkled in old culture (Plate 3). Septate and hyaline hyphae (mycelium) were profusely branched. The fungus formed deep purple colour discoloration on PDA, produced three types of asexual spores *viz.*, macroconidia, microconidia (Plate 4) and chlamydospores. Microconidia produced in chains

Plate 2. Photograph showing the wilted and healthy pigeonpea plants in field conditions

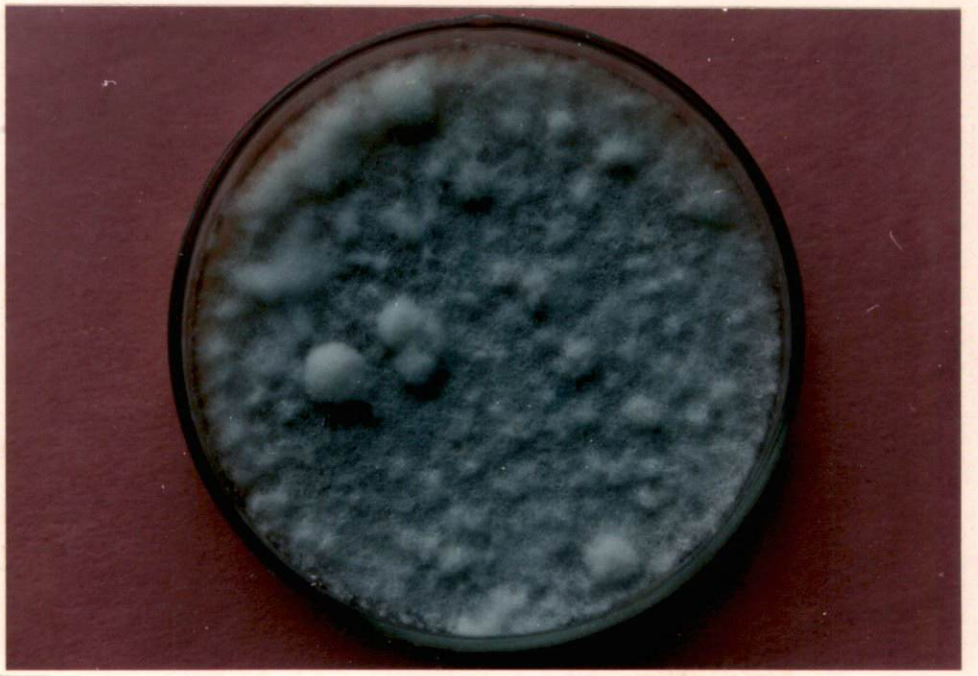
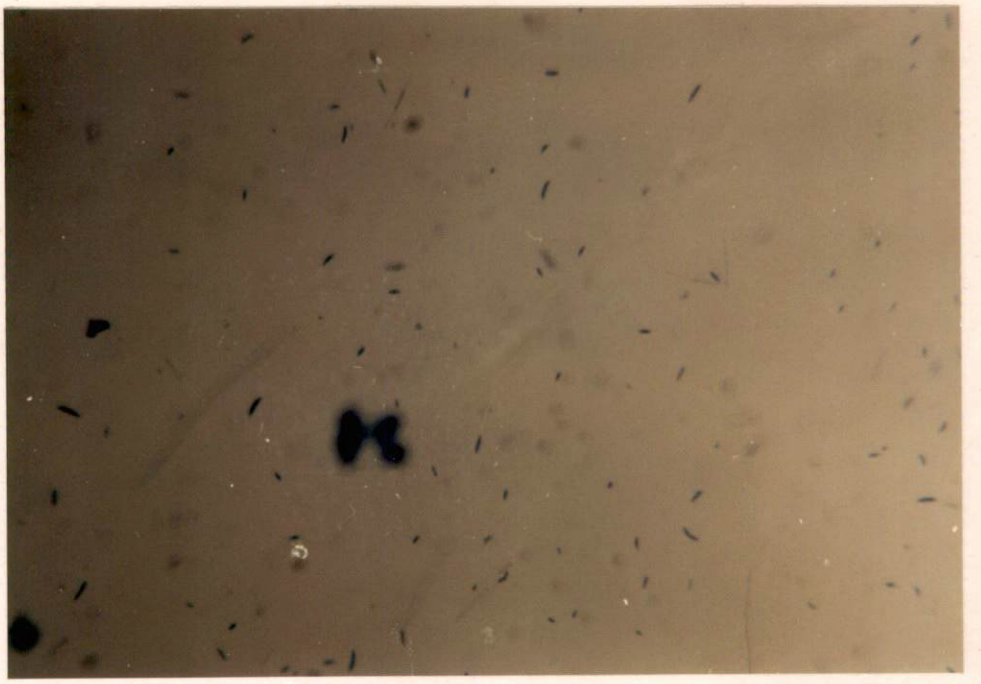
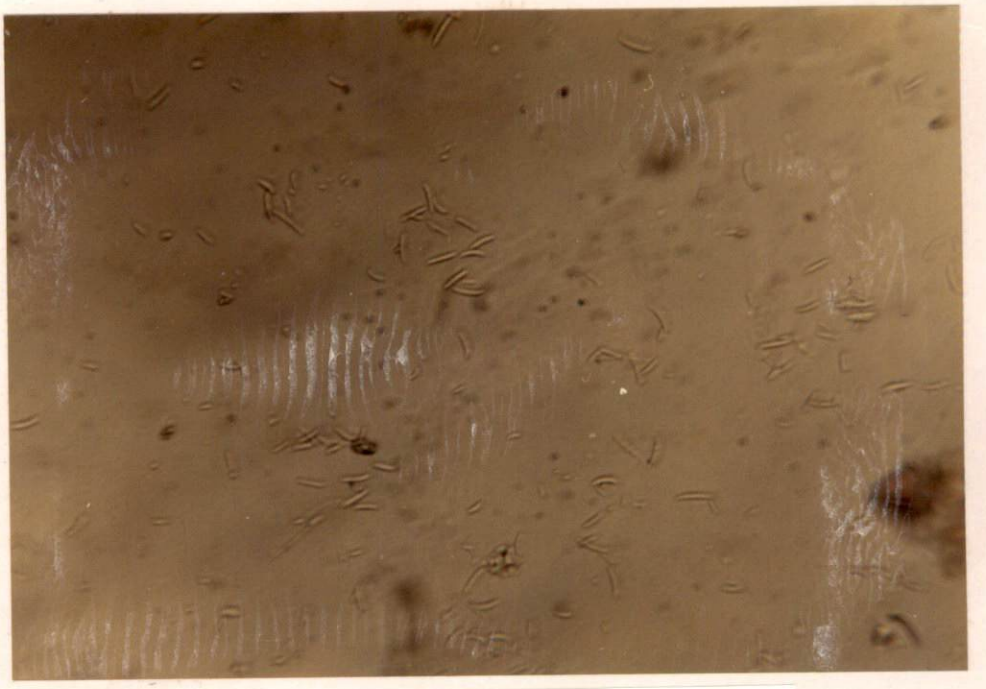


Plate 3. Photograph showing mycelial growth of *F. udum* after seven days



1. Microconidia at 10x

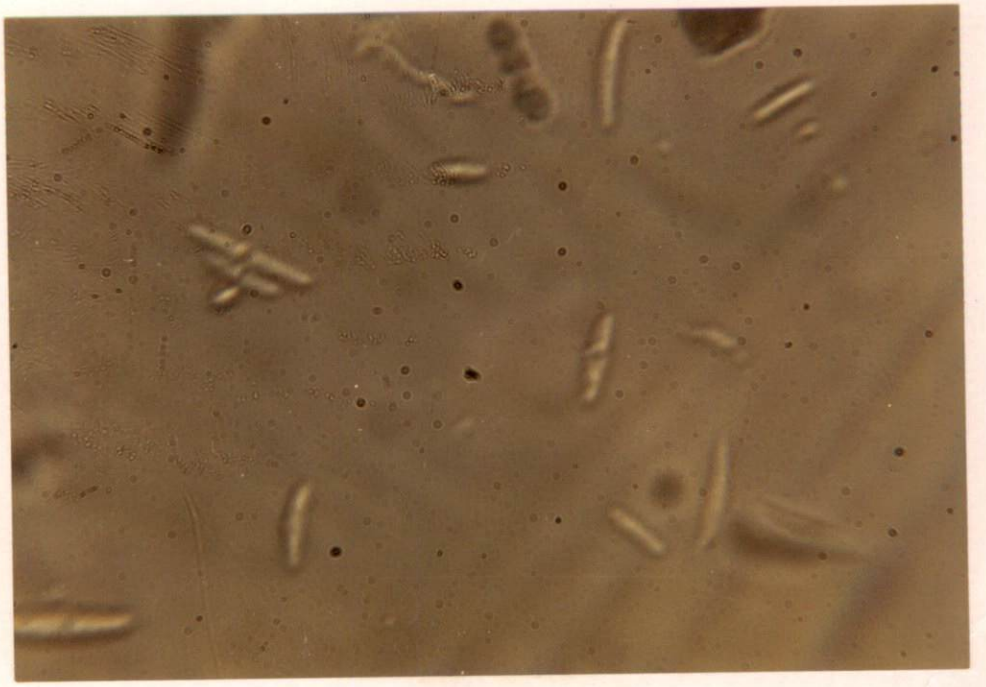


2. Macroconidia at (a) at 10x

Plate 4. Microphotograph showing spores of *F. udum*

2. Macroconidia at (b) at 40x

Plate 4. Photograph showing germination of *F. udum*



were later detached and were small elliptical or curved, unicellular or with one to two septa and measured 6-14 X 2.3-4.1 uM. Macroconidia were long curved (fusiod) pointed at the tip and knotched at the base, thin walled with three to five septa and measured 14-50 X 3.1-5.2 uM. Chlamydospores were spherical to oval, thick walled single, terminal intercalary or in chains. These characters were compared with the originals and identified as *F. udum*.

4.3 Pathogenicity

Pathogenecity test of *Fusarium udum* was carried out under glass house conditions as described in material and methods.

The wilting and death of pigeonpea plants due to *Fusarium udum* was observed at 25-30 days after sowing of seeds (Plate 5). Details of the above ground symptoms expressed on plants were recorded. Symptoms observed under glasshouse conditions were similar to wilt symptoms observed in the field.

4.4 Symptoms caused by the pathogen

Visual observations of various pigeonpea plants and the repeated isolations of the associated pathogen were made during the cropping season of 1996-97. Studies revealed that, wilted plants expressed typical symptoms of the disease (Plate 6). Such typical symptoms observed under field conditions were

Plate 5. Photograph showing the healthy and wilted pigeonpea under glass house conditions

H- Healthy, W-Wilted

Plate 6. Photograph showing symptoms of *Fusarium*

confirmed by the pathogenecity test under glasshouse conditions. The symptoms caused by *Fusarium udum* are described as below.

Vascular wilt symptoms were characterised by gradual or sudden yellowing, withering and drying of leaves followed by drying of entire plant or some of its branches. Wilted plants when pulled out and examined in the field, black streaks of varying size may be seen on the taproot and on the base of stem spreading upward. The main and lateral roots were rotten but it was dry rot. Partial wilting having one or two branches was also seen. Aerial growth of the fungus also occurred on the base of the stem near ground level as a whitish to pinkish mass. Loss of turgidity, drying and dropping of leaves was observed. Extensive wilting of the plants was noticed. It was difficult to uproot such affected plants. Root system showed brown vascular discoloration. Intracellular mycelium was observed in affected xylem vessels (Plate 7).

4.5 Reisolation

Reisolations from the wilted plant roots were made and pathogenic culture thus obtained was compared with the original culture and was found identical. All the morphological characters were compared and the identity confirmed.

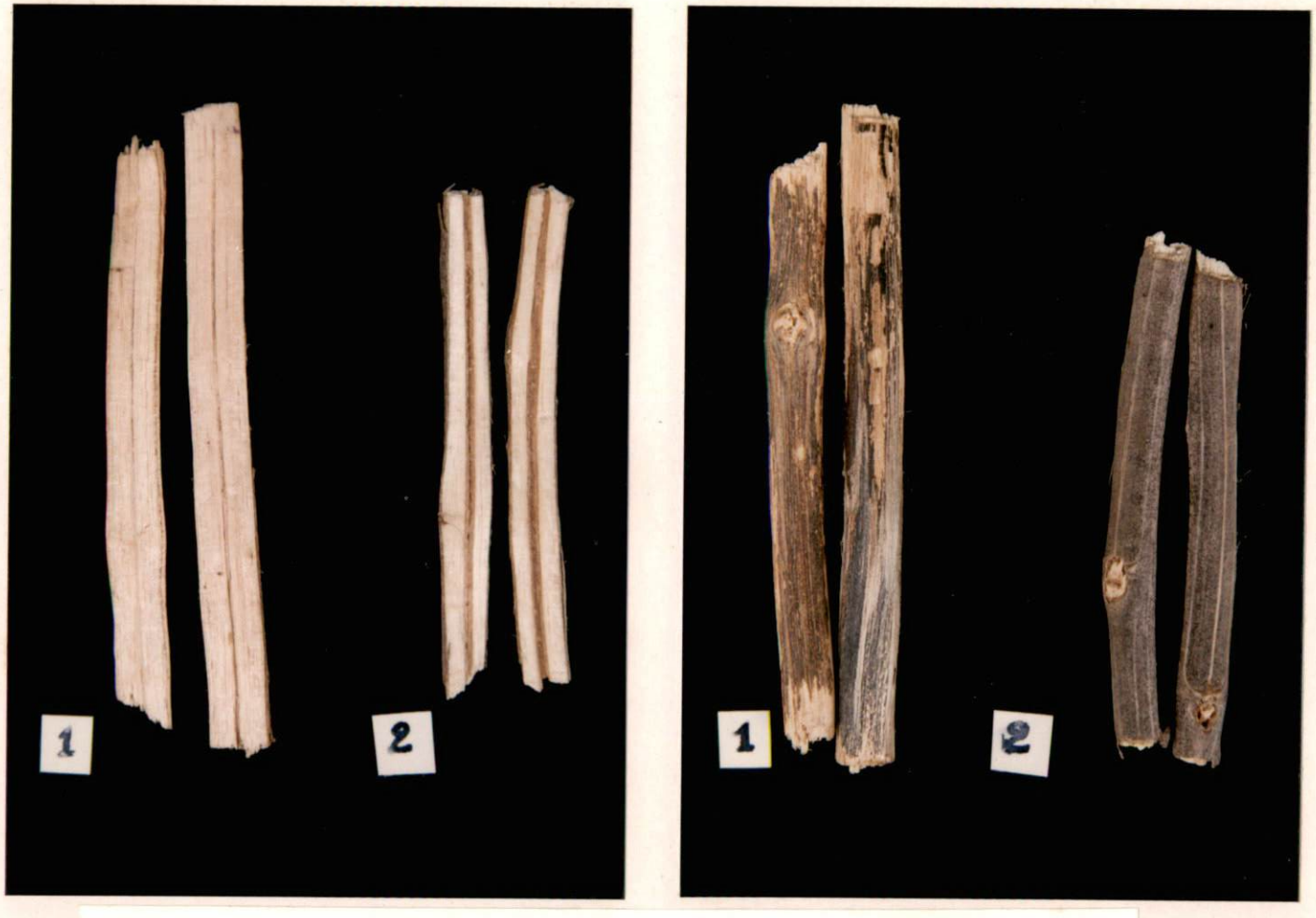
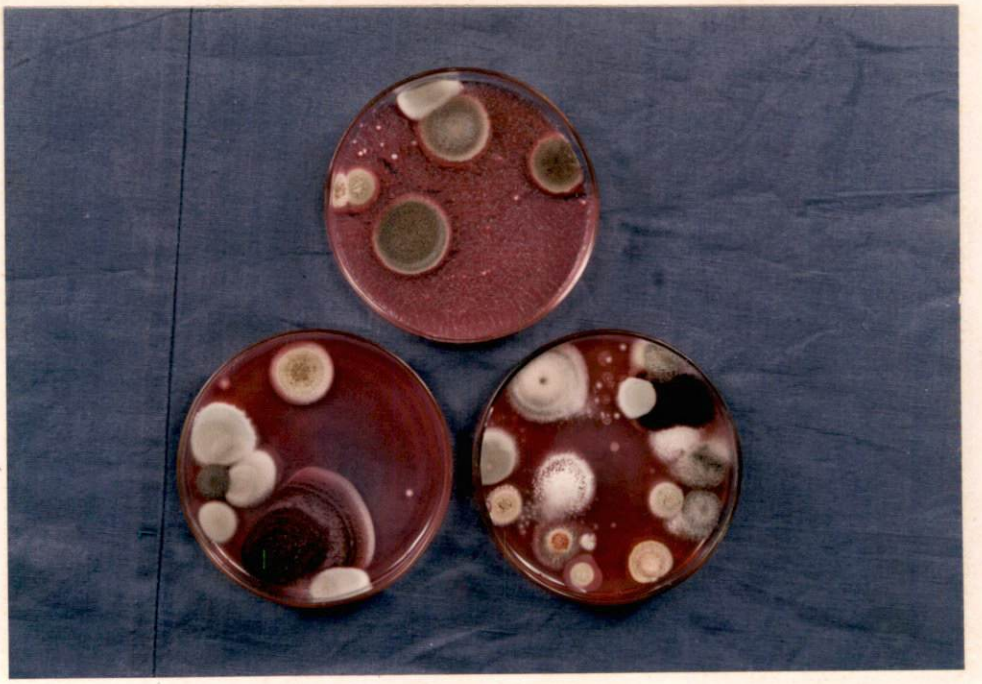


Plate 7. Photograph showing vasicular discoloration in wilted plants

2. Wilted stem

1. Healthy stem



a) Fungi,

Plate 8. Photograph showing rhizosphere microorganisms isolated from pigeonpea fields

4.6.1 Isolation of Microflora from rhizosphere of pigeonpea plants

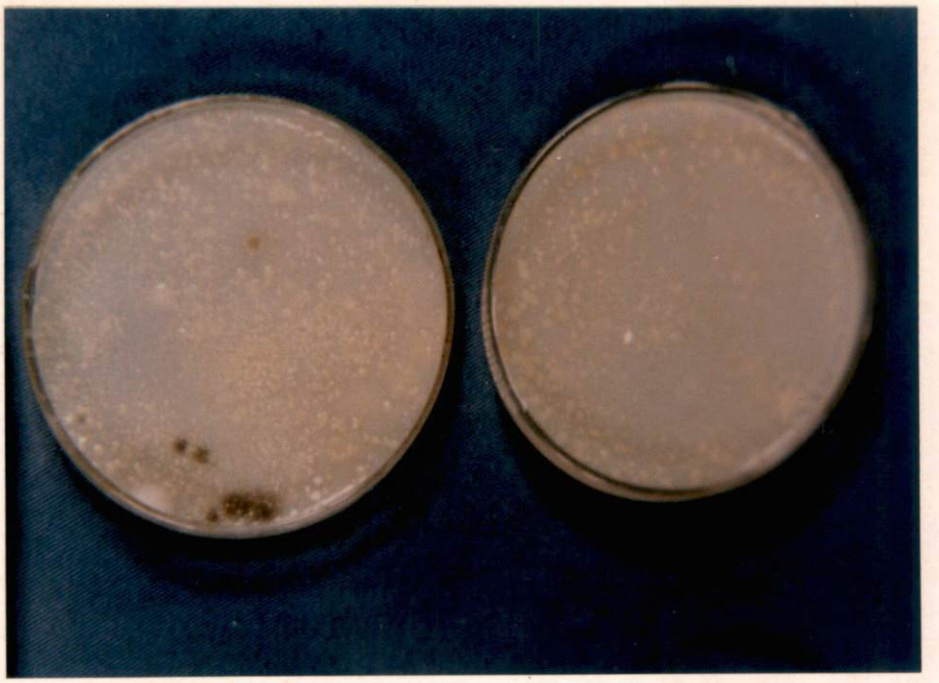
In order to isolate the antagonistic organisms from rhizosphere of pigeonpea, soil dilution plate technique was followed as described in `Material and Methods'.

The population of fungi, bacteria and actinomycetes from the rhizosphere of both wilted and healthy plants were enumerated and the data are presented in the Table 1, Fig 1 ; Plate 8.

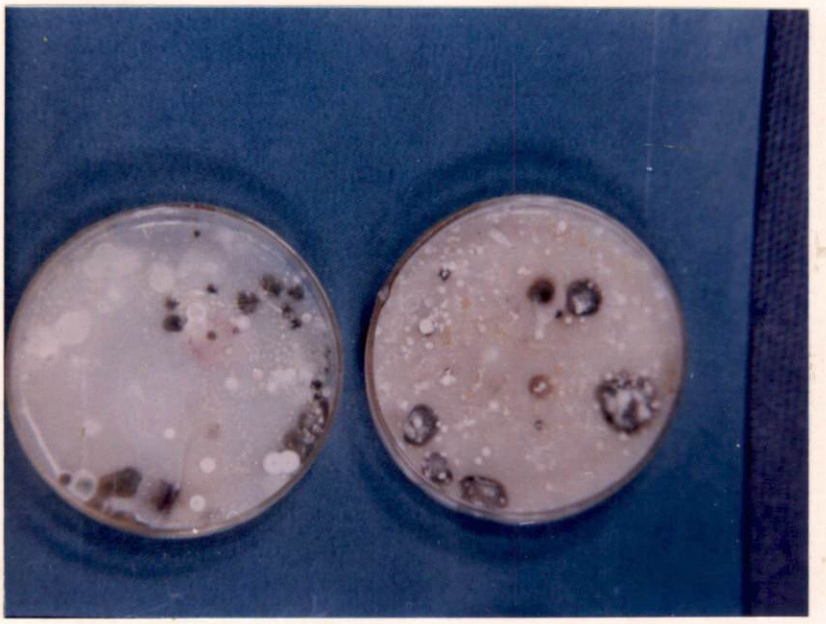
From the data, it is clear that, the population of fungi, bacteria and actinomycetes were more in rhizosphere of healthy plants than that of wilted plants.

The propagules of fungi was found to be maximum 34.14 X 10^3 per g of soil in case of rhizosphere of healthy plant as compared to 19.71 X 10^3 per g of soil in that of wilted plants. The bacterial population was recorded maximum in rhizosphere of healthy plants (63.71 x 10^5 per g of soil) compared to 16.86 x 10^5 per g of soil in wilted plants. On an average actinomycetes were found to be maximum (16.0 X 10^3 per g of soil) in rhizosphere soil of healthy plants compared to 8.71 X 10^3 per g of soil in that of wilted plants.

In the present study, the healthy plants showed significantly higher population of bacteria (63.71 x 10^5 per g



b) Bacteria



c) Actinomycetes

Plate 8. Photograph showing rhizosphere microorganisms isolated from pigeonpea fields

Table 1 : The microbial population of rhizosphere soil of pigeonpea plants (g^{-1} of soil).

	Rhizosphere of healthy plants	Rhizosphere of wilted plants
Fungi	34.14×10^3	19.71×10^3
Bacteria	63.71×10^5	16.86×10^5
Actinomycetes	16.00×10^3	08.71×10^3
SEm±	0.79	0.79
CD at 1% level	3.40	3.40

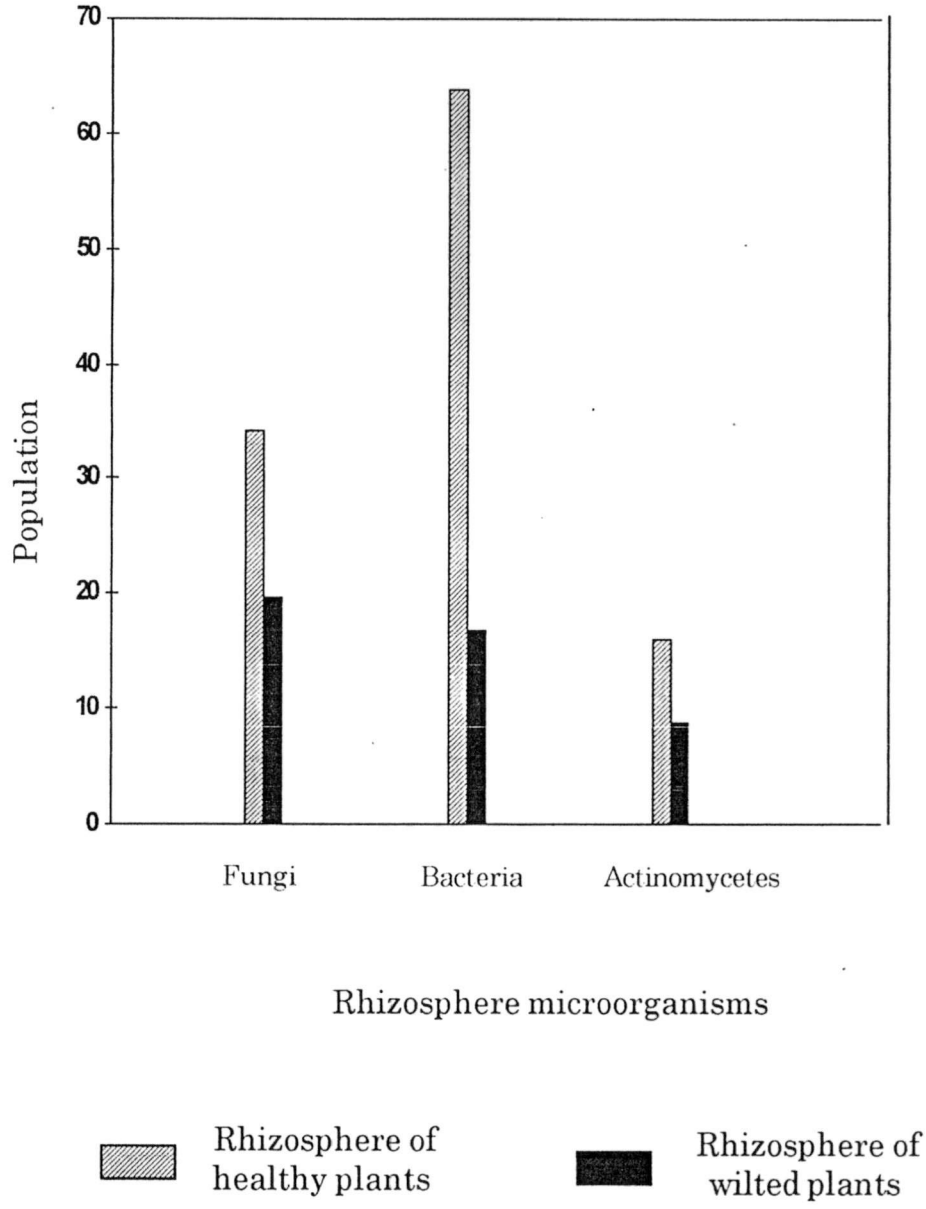


Fig. 1. Microbial population from rhizosphere soil of pigeonpea plants (g^{-1} of soil)

of soil) than fungi (34.14 X 103 per g of soil) and actinomycetes 16.0 X 10^3 per g of soil. Generally, in rhizosphere of the wilted host, the fungal propagules and bacteria were significantly higher (19.71 X 10^3 per g of soil and 16.86 X 10^5 per g of soil respectively) than that of actinomycetes (8.71 x 10^3 per g of soil).

4.6.2 Isolation of fungi from rhizosphere microflora

The antagonistic organisms from soils were assessed by soil dilution plate technique as described under material and methods.

The fungi isolated from rhizosphere of healthy plants includes *viz., Aspergillus flavus, A. niger A. terreus, Cladosporium* spp., *Penicillium* spp., *Fusarium udum, T. viride.* The population of fungi is given in the Table 2 ; Fig. 2. Maximum population of *A. flavus* was seen (17.00 X 10^3 per g of soil), followed by *Penicillium* spp., 9.0 X 10^3 per g of soil. Least population of *Aspergillus niger* 3.5 X 10^3 per g of soil was observed.

Major groups of fungi were isolated form rhizosphere of wilted pigeonpea plants *viz., Aspergillus terreus* Thom., *Fusarium udum* Butler and *Cladosporium* spp., population of fungi is given in the Table 2 ; Fig. 2. From the data it is clear that, population of *Fusarium udum* was maximum (82.50x 10^3 per g of soil), followed by *Aspergillus terreus* and *Cladosporium*

Table 2 : Per cent population of fungi from rhizosphere soil of healthy and wilted pigeonpea plants.

Fungi isolated	Rhizosphere of healthy plants		Rhizosphere of wilted plants	
Aspergillus flavus	17.00	(24.35)	00.00	(00.00)
A. niger	03.50	(10.78)	00.00	(00.00)
A. terrus	06.00	(14.18)	03.00	(09.97)
Cladosporium spp.,	06.50	(14.77)	03.00	(09.97)
Fusarium udum	05.00	(12.92)	82.50	(65.27)
Penicillium spp.,	09.00	(17.46)	00.00	(00.00)
Trichoderma viride	04.00	(11.54)	00.00	(00.00)
SEm±	1.35		0.59	
CD at 1% level	5.48		2.41	

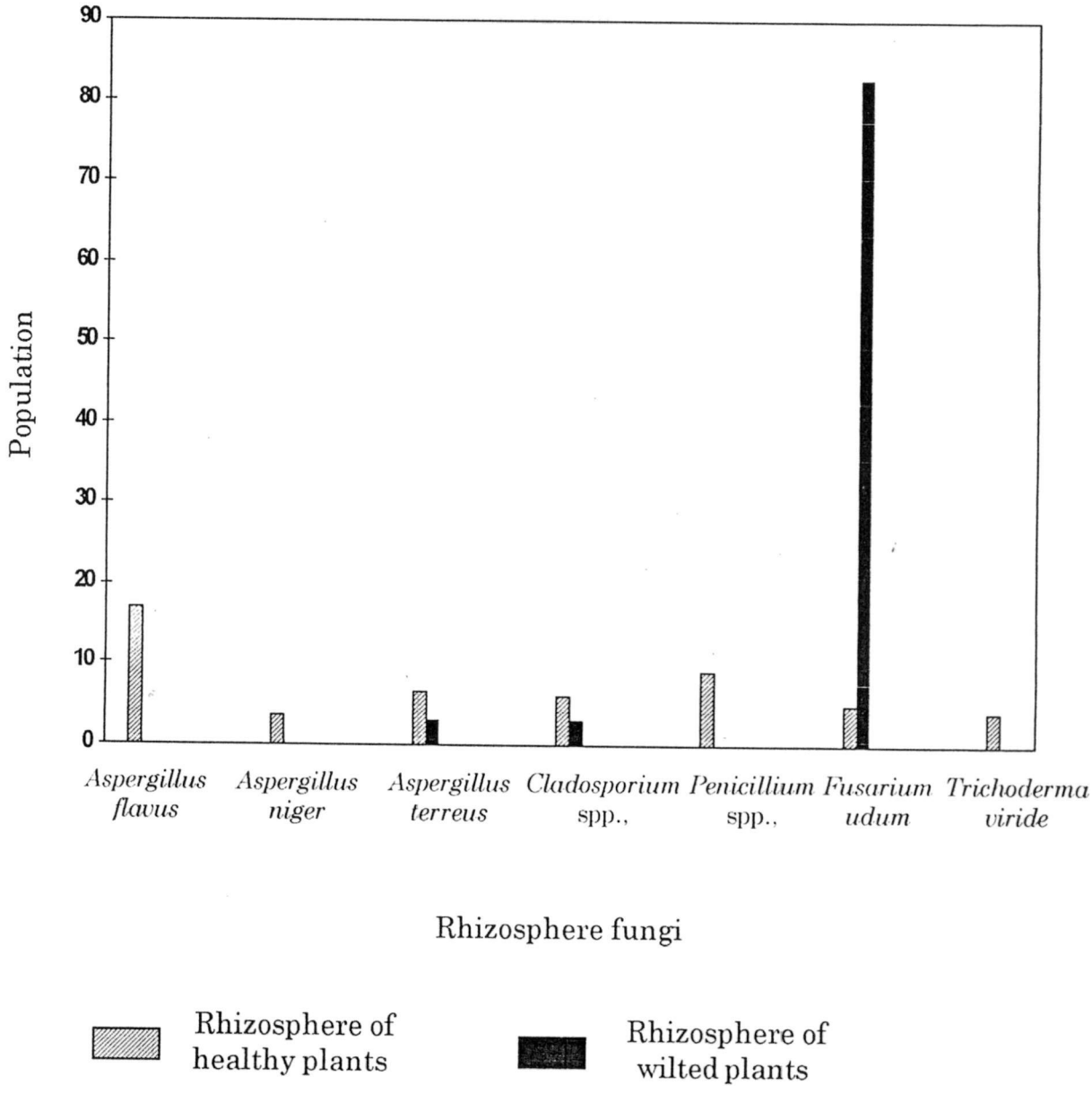


Fig. 2. Population of fungi from Rhizophere soil of healthy and wilted pigeonpea plants

spp., both accounted same population of 3.00 X 10^3 per g of soil.

4.7 Effect of different organic amendments on the survival of pigeonpea plants

Amending soil with agricultural waste and oil cakes have in many cases given good result in suppressing soil borne pathogens. In the present study, oil cake *viz.*, groundnut cake and neem cake along with compost and farm yard manure to see their effect on *Fusarium udum* and effect on seed germination further their antagonist enhancing effect under the glass house condition was evaluated as described under `Material and Methods'.

The effect of these amendments were tested on seed germination. 77.5 per cent germination occurred in control. The germination was 90 per cent in seeds sown in neem cake treated plots. 87.5 per cent in compost, 82.5 in farm yard manure and 85 per cent in groundnut cake. However, there was no significant difference in germination among all the treatments.

Pots treated with neem cake showed 27.50 per cent of wilt incidence, followed by compost (42.50%), FYM (60%) and groundnut cake (62.50%). There is significant difference over the control (75.00%). The data are presented in the Table 3 ; Fig.3.

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Table 3 : Effect of organic amendments on the incidence of fusarium wilt of pigeonpea.

Organic amendments	Per cent seed germinated	Per cent plants affected by wilt
Compost	87.50 (72.11)	42.50 (40.61)
Farm Yard Manure	85.00 (67.50)	60.00 (50.83)
Groundnut oil cake	82.50 (65.47)	62.50 (52.34)
Neem cake	90.00 (74.14)	27.50 (36.00)
Control	77.50 (60.11)	75.00 (60.10)
SEm±	4.15	2.51
CD at 5% level	--	7.72

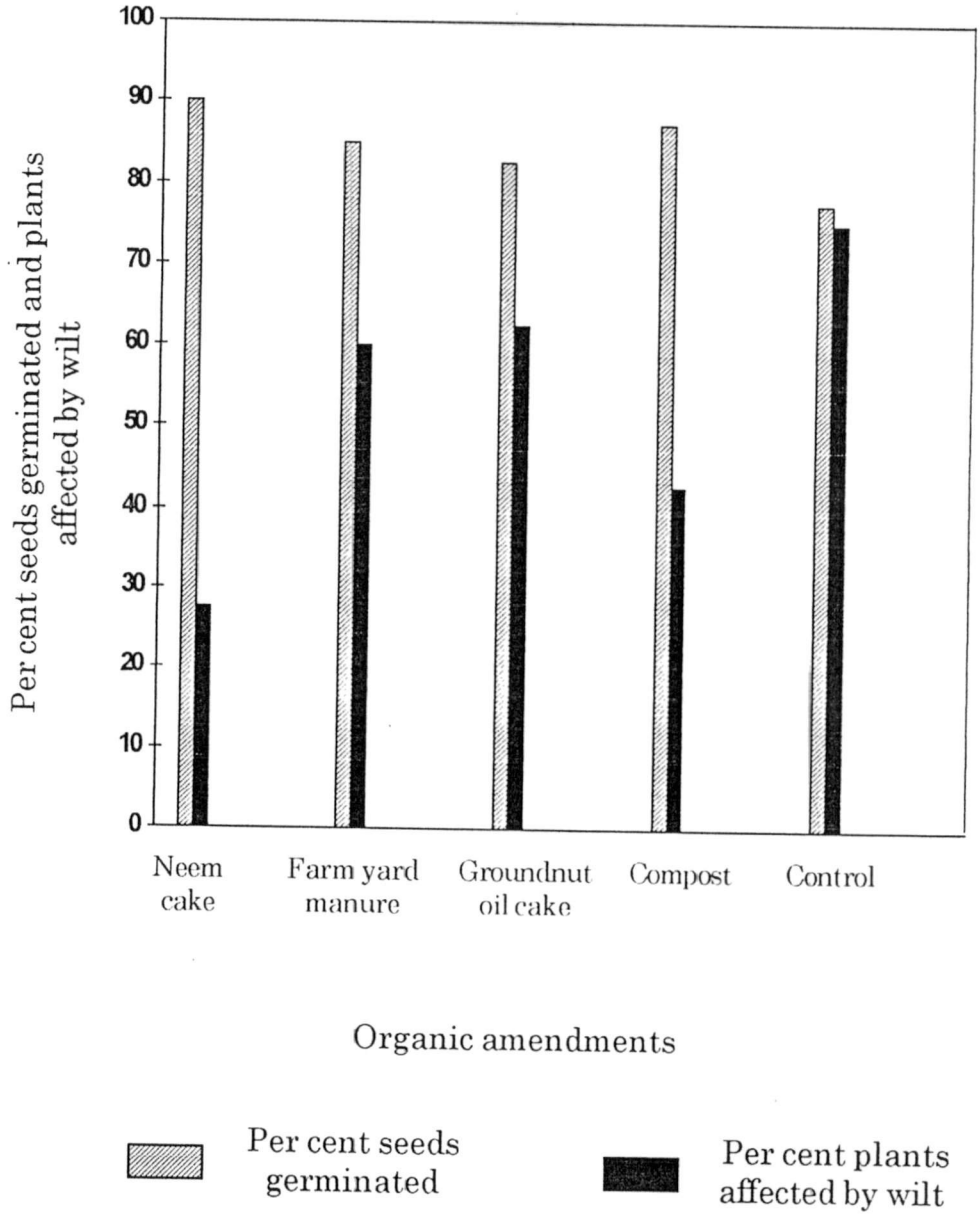

Fig. 3. **Effect of organic amendments on pigeonpea fusarium wilt incidence**

4.7.1 Plant stand at 20 days after sowing

Maximum per cent seedling survival was recorded in neemcake (85%), followed by compost (75%), groundnut cake (72.5%) followed by farm yard manure (65%) and this was significantly more than in the treatment without organic amendments (control, 57.5%). The data are presented in the Table 4 and Fig. 4.

4.7.2 Plant stand at 40 days after sowing

Maximum per cent seedling survival was recorded in neem cake (77.5%), followed by compost (67.5%), farm yard manure (60%) and groundnut cake (60%) and this was significantly more than in the control (50%). However, the survival was slightly reduced at 40 days compared to 20 days. The data are presented in the Table 4 ; Fig. 4.

4.8.1 *In vitro* evaluation of Antagonists against *Fusarium udum*

Five species of antagonistic fungi, two species of bacteria and one spp., of actinomycetes were tested against *Fusarium udum* in *in vitro* for their antagonistic effect as explained in `Material and Methods'.

Inhibition zone in mm was determined and the data are presented in the Table 5 ; Plate 9. The per cent inhibition was also calculated and are presented in Table 6 ; Fig. 5.

Table 4 : Survival of pigeonpea seedlings in different organic amendments.

Organic amendments	Per cent seedlings survived			
	in 20 days		in 40 days	
Compost	75.0	(60.64)	67.5	(55.50)
Farm Yard Manure	65.0	(53.78)	60.0	(50.83)
Groundnut oil cake	72.5	(56.95)	60.0	(50.83)
Neem cake	85.0	(67.50)	77.5	(62.15)
Control	57.5	(49.39)	50.0	(45.00)
SEm±	3.16		2.85	
CD at 5% level	9.72		8.79	

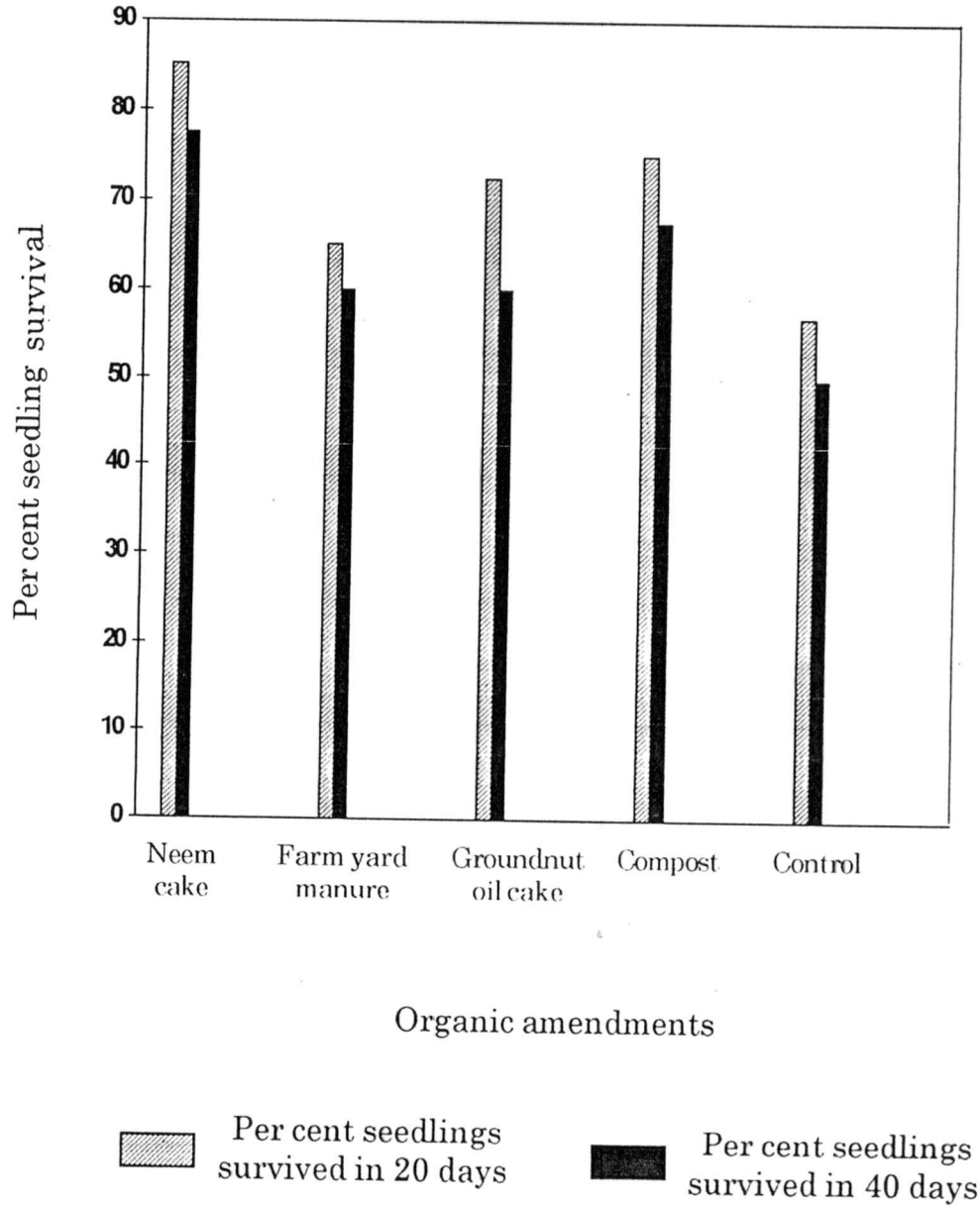

Fig. 4. Survival ability of pigeonpea in different organic amendments

Among the fungi tested, *Trichoderma viride* showed maximum inhibition zone of 3.4 mm. followed by *T. harzianum* (2.2 mm.) but later on, both overgrew and completely suppressed the test pathogen. *Aspergillus flavus, A. niger* and *Penicillum* sp., completely inhibited the *Fusarium udum*. Initially *Fusarium udum* grew towards antagonistic organisms. However, the growing edge of *Fusarium udum* ceased before it could make contact with growing colony of antagonistic organism.

Bacillus subtilis showed antagonism by producing inhibition zone of 3.5 mm and *Pseudomonas fluorescens* produced 7.5 mm inhibition zone against test fungus.

Streptomyces sp. was more effective antagonistic organism and produced an inhibition zone of 13.2 mm against test fungus.

Maximum inhibition was observed by *T. viride* (87.03%) followed by *T. harzianum* (85.40%), *Pseudomonas fluorescens* (81.87%), *Bacillus subtilis* (72.23%), *Aspergillus niger* (55.60%) and least (49.57%) inhibition was observed by *Aspergillus flavus*. Inhibition by *Trichoderma viride* and *Trichoderma harzianum* was significantly more than the inhibition by *Aspergillus flavus* and *Aspergillus niger*. In control there was no inhibition and complete growth of test fungus was observed.

Table 5 : *In vitro* evaluation of antagonistic fungi, bacteria and actinomycetes on *Fusarium udum*

Antagonists	Inhibition zone (mm)	Type of antagonist
Aspergillus flavus	Inhibited the *F. udum* but no zone was produced	Highly suppressive competitive saprophytic activity
A. niger	Inhibited the *F. udum* but no zone was produced	Highly suppressive competitive saprophytic activity
Bacillus subtilis	3.5 mm	Antagonism
Penicillium spp.,	Inhibited the *F. udum* but no zone was produced	Highly suppressive competitive saprophytic activity
Pseudomonas fluorescens	7.5 mm	Antagonism
Streptomyces spp.,	13.2 mm	Suppressive and competitive
Trichoderma harzianum	2.2 mm inhibition zone was observed lateron overgrew	Antagonism
T. viride	3.4 mm inhibition zone was produced but later on overgrew	Antagonism

Table 6 : Inhibition of *Fusarium udum* by antagonists isolated from rhizosphere of pigeonpea.

Antagonistic organisms	Per cent inhibition	
Aspergillus flavus	49.57	(44.75)
A. niger	55.60	(48.22)
Bacillus subtilis	72.23	(58.20)
Pseudomonas flourescens	81.87	(64.91)
Trichoderma harzianum	85.40	(67.55)
T. viride	87.03	(68.93)
SEm±	0.541	
CD at 1% level	2.425	

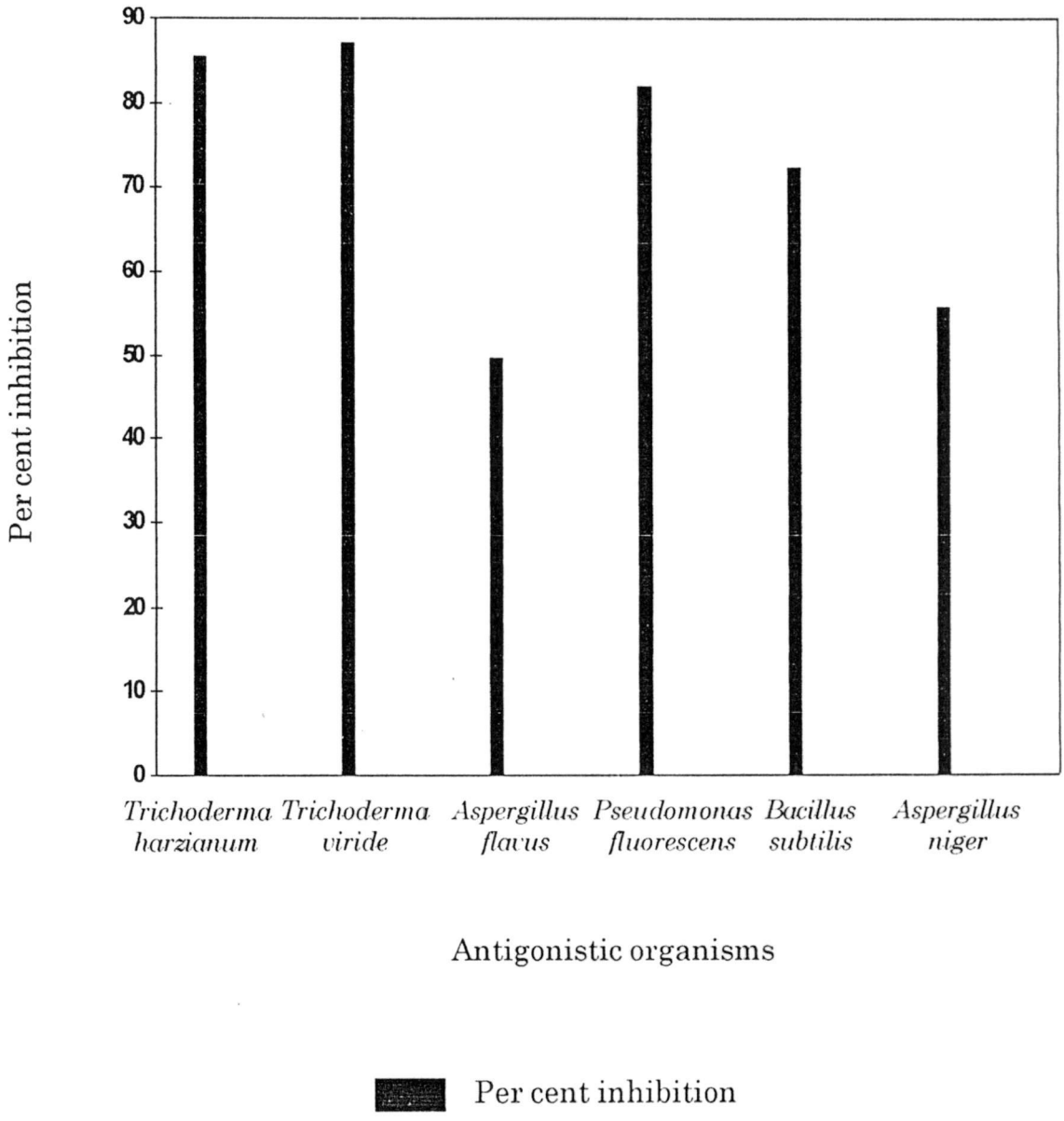

Fig. 5. Inhibition of *Fusarium udum* by antagonists isolated from rhizophere of pigeonpea plants

4.8.2 Effect of antagonistic microorganisms on germination and viability of *Fusarium udum*

This experiment was undertaken to known the amount of germination and viability of spores of *Fusarium udum* at the centre of *Fusarium* colony and at the interaction zone. The results obtained are recorded in Table 7 ; Fig. 6.

The results indicated that the germination and viability of spores reduced significantly in interaction zone in comparison to the centre of the *Fusarium* colony.

The per cent spore germination of *Fusarium* was not affected adversly in culture of *Bacillus subtilis* (63.67%). However, minimum per cent spore germination was recorded in cultures interacting with *T. viride* (10.19%) followed by *T. harzianum* (29.38%).

4.8.3 Effect of seed treatment with different antagonist on wilt incidence in pigeonpea

Five fungi and two bacteria were tested for their antagonistic effect as seed dresser against wilt caused by *F. udum* and the data are presented in Table 8 ; Fig. 7.

The least per cent wilt was recorded in case of *Trichoderma viride* (26.67%) followed by *T. harzianum* (30.00%), *Pseudomonas fluorescens* (36.67%), *Aspergillus niger* (56.67%), *Bacillus subtilis* and *Penicillium* spp., (60% and 63.33%

Table 7 : Effect of antagonistic microorganisms on spore germination of *F. udum*.

Antagonists	Centre of the colony per cent spore germination		Interaction zone per cent spore germination	
Bacillus subtilis	63.67	(53.08)	34.33	(35.79)
Pseudomonas fluorescens	48.00	(43.86)	25.67	(27.36)
Trichoderma harzianum	35.50	(36.54)	29.38	(32.64)
Trichoderma viride	22.05	(27.92)	10.19	(14.58)
Control	84.36	(69.25)	83.14	(68.24)
SEm±	3.21		5.48	
CD at 1% level	13.27		22.64	

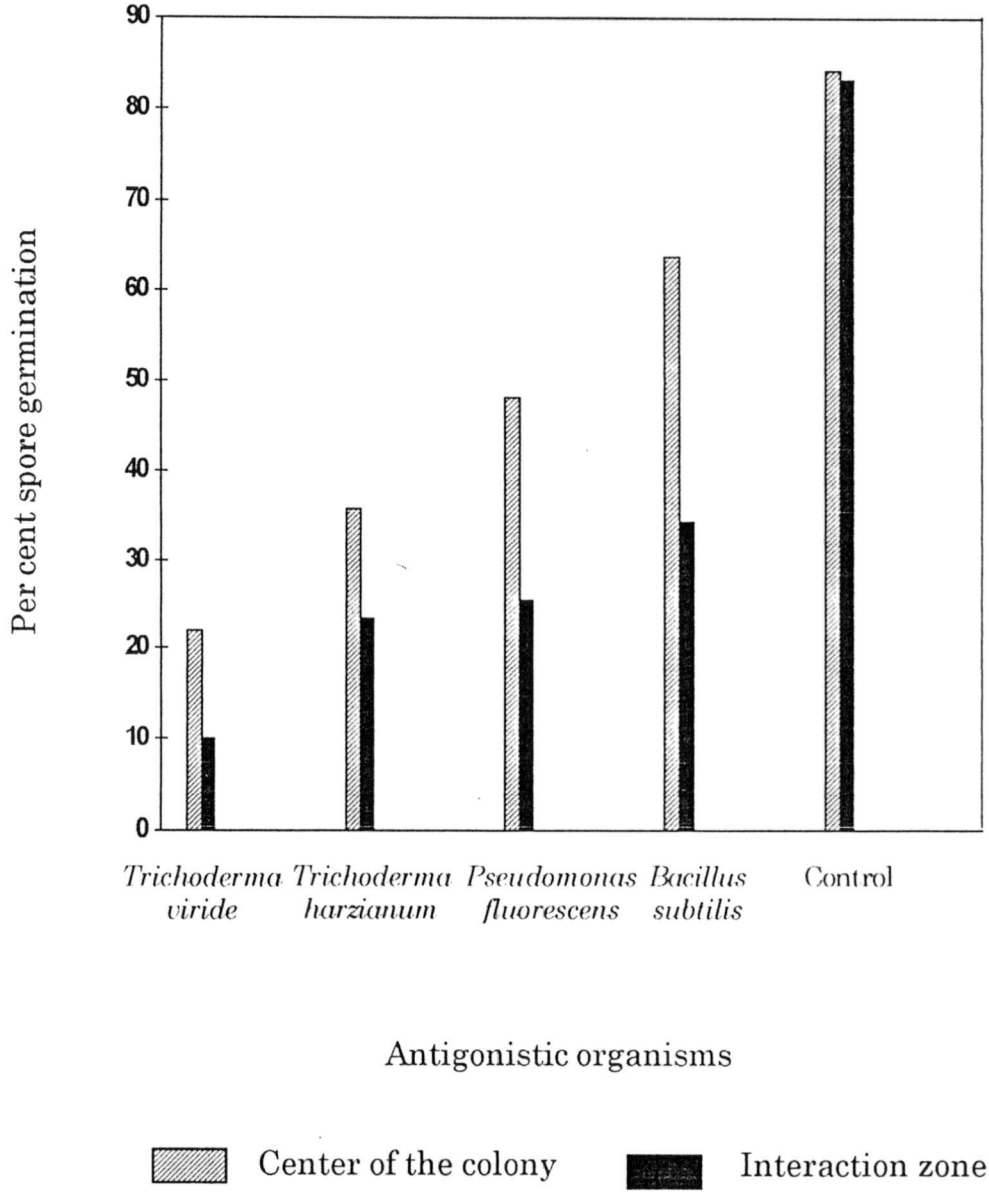

Fig. 6. Effect of antagonistic microorgansims on spore germination of *F. udum*

Table 8 : Effect of seed treatment with antagonists on pigeonpea wilt incidence

Antagonistic organism	Per cent fusarium wilt
Aspergillus flavus	66.67 (54.78)
A. niger	56.67 (48.85)
Bacillus subtilis	60.00 (50.85)
Penicillium spp.	63.33 (52.78)
Pseudomonas fluorescens	36.67 (37.22)
Trichoderma harzianum	30.00 (33.21)
T. viride	26.67 (30.90)
Control	80.00 (63.93)
SEm±	2.50
CD at 1% level	10.52

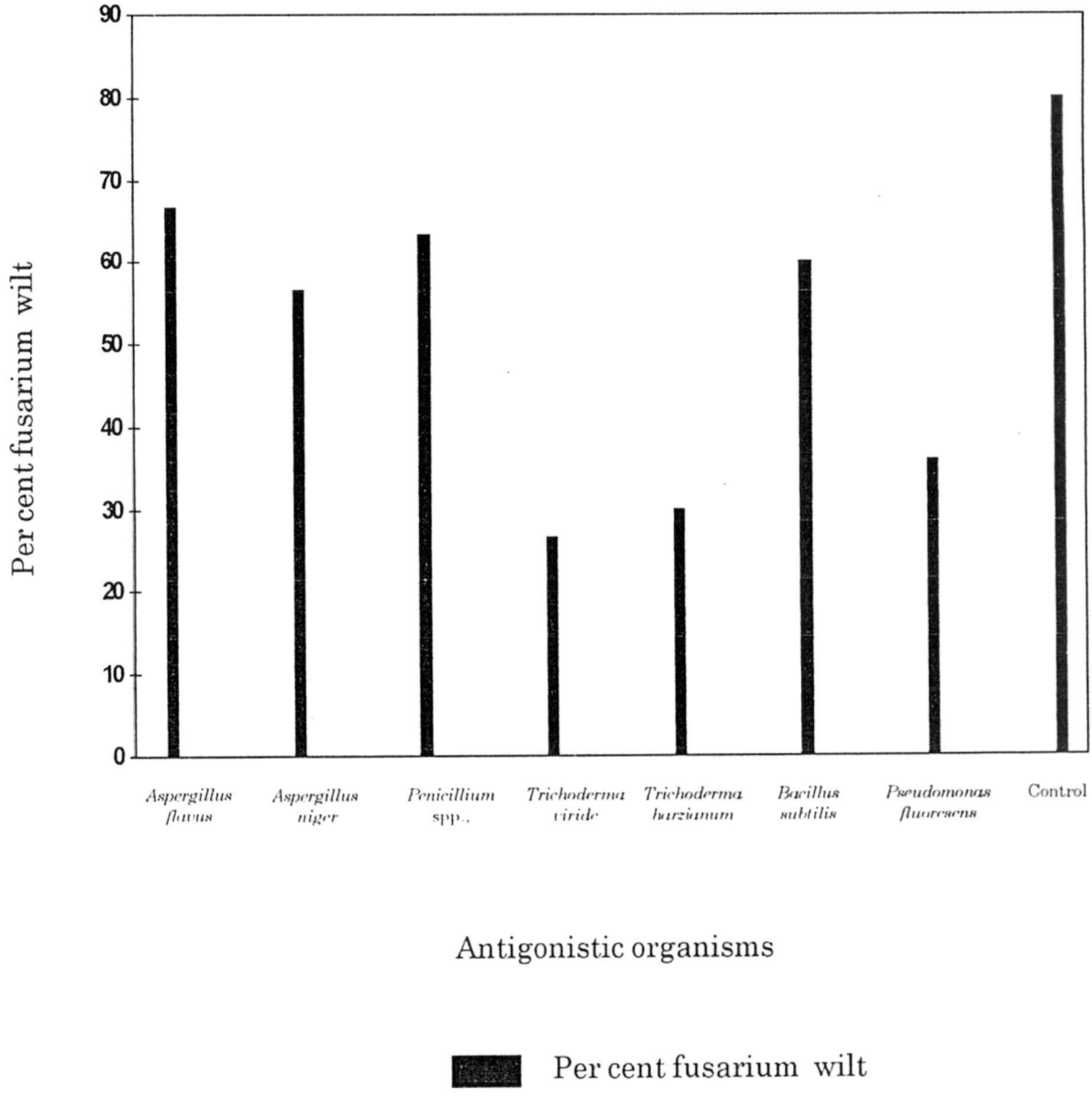

Fig. 7. Effect of seed treatment with antagonists on pigeonpea wilt incidence

respectively) and *Aspergillus flavus* (66.67%) as compared to control (80.00%). *Trichoderma viride* and *T. harzianum* differed significantly over other antagonists tested and control.

4.9 Compatibility effect of antagonists with fungicides

To assess the compatibility effect of antagonists with chemicals, an experiment was conducted in *in vitro* and as well as under glass house conditions. In this test pelleting of pigeonpea seeds was done with Carbendazim @ 500 ppm and Captan @ 0.2 per cent of seeds and with antagonists individually and also in combination as described in `Material and Methods'.

The *in vitro* study showed that, the antagonists were not affected by Carbendazim and Captan fungicides. The data are presented in Table 9 ; Fig. 8. Per cent inhibition in *Trichoderma viride* was 55.83 whereas, in *T. harzianum* caused 51.43 per cent inhibition of *F. udum* over control. In treatments of Carbendazim and Captan 52.63 and 44.80 per cent inhibition were observed respectively. Combined treatments showed more effective results than that of individual treatment. In treatments with Captan + *T. harzianum* 60.06 per cent inhibition, 60.80 inhibition observed by treatments with Captain + *T. viride*. Maximum inhibition of 63.03 was observed in the treatment of Carbadenzim + *T. viride* and 60.70 per cent in case of Carbendazim + *T. harzianum*. The results showed significant difference between combined treatment effects than individual treatment effect.

Table 9 : Inhibition of *Fusarium udum* by antagonists in combination with fungicides.

Treatments	Per cent inhibition
Carbendazim	52.63 (46.52)
Captan	44.80 (42.02)
Trichoderma harzianum	51.43 (45.82)
T. viride	55.83 (48.36)
Carbendazim + *T. harzianum*	60.70 (51.19)
Carbendazim + *T. viride*	63.03 (52.57)
Captan + *T. harzianum*	60.06 (50.81)
Captan + *T. viride*	60.80 (51.24)
SEm±	1.01
CD at 1% level	4.25

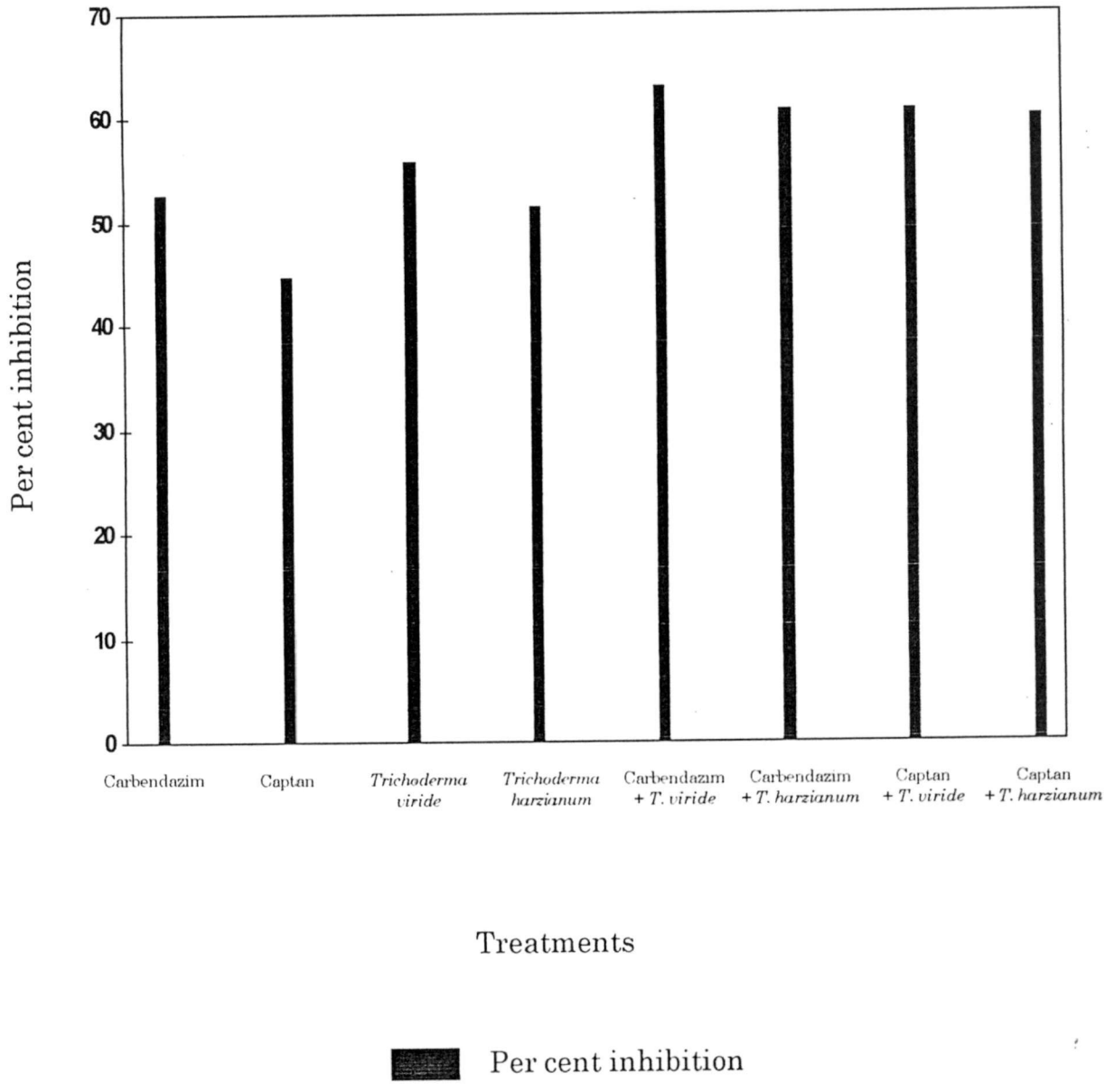

Fig. 8. Inhibition of *F. udum* by antagonists in combination with chemicals

4.9.2 Compatibility effect on pigeonpea wilt incidence

The germination was 90.00 per cent in seeds treated with Carbendazim and Carbendazim + *Trichoderma viride*, followed by 86.67 per cent in seeds treated with *T. viride*, 83.33 per cent in seed treated with Carbendazim + *T. harzianum* 83.33 per cent in seeds treated with *T. harzianum*, 80 per cent in seeds treated with Captan and Captan + *T. viride* and in seeds treated with Captan + *T. harzianum* seed germination was 76.67 per cent. The data presented in the Table 10 ; Fig. 8. 76.67 per cent germination in control. There was no significant difference in seed germination among all the treatments.

In treatments, least wilt incidence was noticed with Carbendazim + *T. viride* (16.67%) and Carbendazim + *T. harzianum* (20.00%) and varied significantly over control (73.33%). There was no significant results in the treatment of *T. viride* seed treatments and Captan + *T. harzianum*. The data (Table 10) clearly shows per cent wilt incidence was least in case of antagonist than in fungicides and that the combination treatments were effective.

Table 10 : Effect of antagonists in combination with the fungicides against incidence of pigeonpea wilt.

Treatments	Per cent seeds germination		Per cent plants wilted	
Carbendazim	90.00	(75.00)	50.00	(45.00)
Captan	80.00	(63.93)	60.00	(50.85)
Trichoderma harzianum	83.33	(66.14)	30.00	(33.21)
Trichoderma viride	86.67	(68.85)	26.67	(31.00)
Carbendazim + *T. harzianum*	83.33	(66.64)	20.00	(26.57)
Carbendazim + *T. viride*	90.00	(75.00)	16.67	(23.86)
Captan + *T. harzianum*	76.67	(61.72)	26.67	(31.00)
Captan + *T. viride*	70.00	(63.93)	23.33	(28.78)
Control	76.67	(61.72)	73.33	(56.60)
SEm±	4.72		2.59	
CD at 1% level	--		10.71	

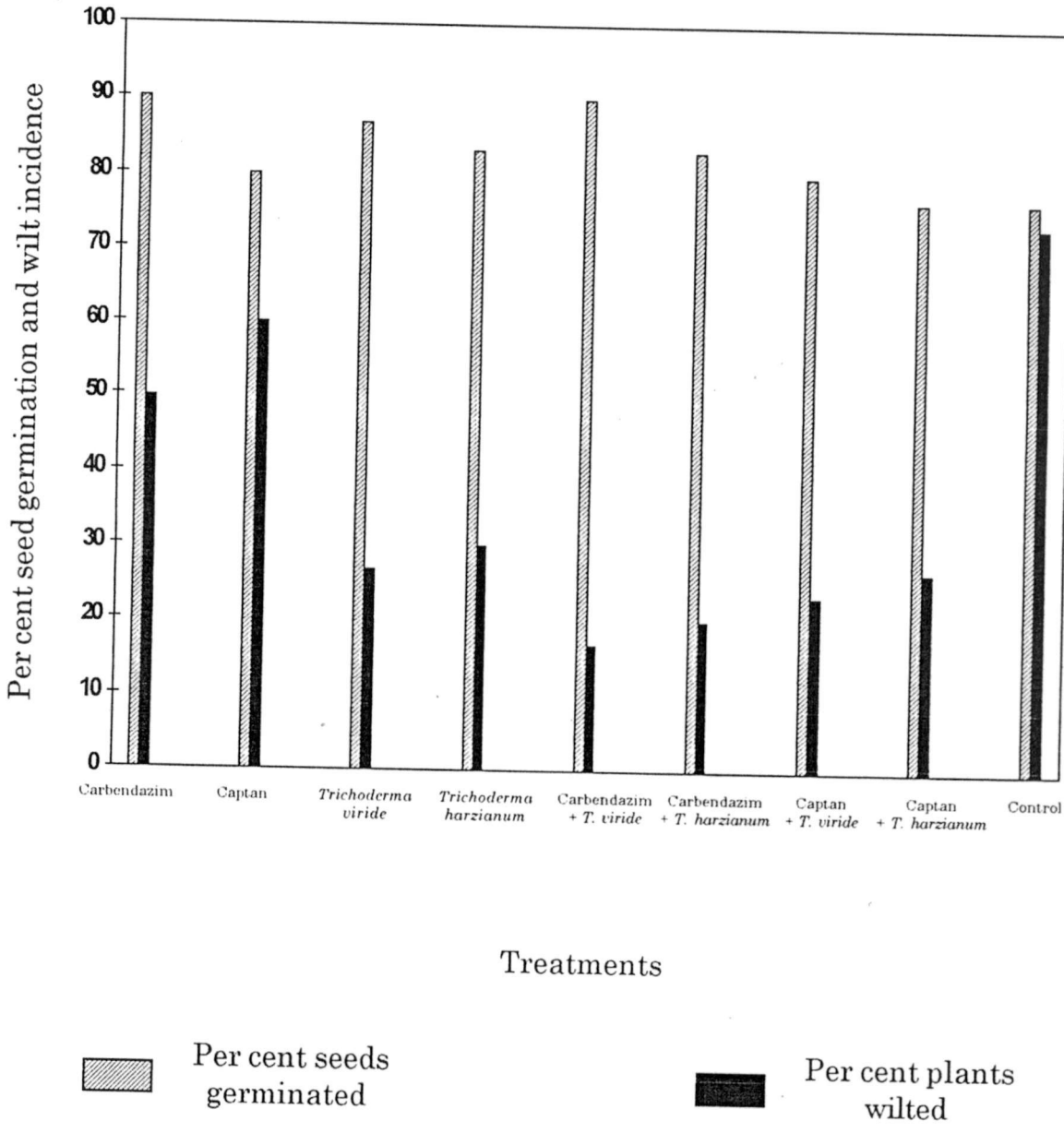

Fig. 9. Effect of antagonists in combination with the chemicals against pigeonpea wilt incidence

DISCUSSION

V. DISCUSSION

Pigeonpea is one of the most important pulse crops of India. It is a major source of protein to many people who largely or wholly depend on vegetarian diet. It improves the poor soils through the deep strong rooting system. Therefore, it is often called as the 'biological plough'. Several factors responsible for low production of pigeonpea have been recognised. High yields and stabilization are must for meeting demands of ever increasing population of India. Pigeonpea can be affected by more than 100 pathogens. Fortunately only few of them cause economic losses. Among them pigeonpea wilt is the most important soil borne pathogen and was first described in 1906 from Bihar state, India. Several investigators have reported *Fusarium udum* as cause of vascular wilt in pigeonpea. The pathogen being the soil-borne, is hard to achieve economic control of the resultant disease. Literature reviewed indicated, the need to take up the investigation on certain aspects of wilt of pigeonpea ultimately leading to the management of the disease. This includes the isolation of the pathogen and study of pathogenicity, isolation of antagonists from rhizosphere soil of healthy and wilted plants, assessment of their antagonistic effect, effect of organic amendments and compatibility of antagonists with fungicides. Such type of

work is lacking and also has direct bearing in devising suitable and economic management of the disease. Therefore, the investigations on these aspects were carried out and the results obtained are discussed herein.

The diseased specimens were collected from field and repeated tissue isolations were also made. It was found that, *Fusarium udum* was always associated with wilted pigeonpea plants. The present findings are in agreement with the earlier workers Butler (1910), Wollenweber and Reinking (1935) and Mohanty (1946).

The identification of fungal pathogen in the present study as *Fusarium udum* was based on the principal morphological and cultural characters described by Butler (1910), Padwick (1940) and Booth (1971). Pathogenecity test carried out under pot culture conditions, showed the wilting of seedlings after four weeks. The initial visible symptoms were loss in turgidity in leaves, slight interveinal clearing. Foliage showed slight chlorosis and bright yellow before wilting. Leaves were retained on wilted plants. Wilted plants after splitting showed brown vascular discoloration. Symptoms produced are in agreement with the descriptions given by Butler (1918), Chube (1968) and Sheldrake *et al.* (1978).

Microorganisms were also isolated from the rhizosphere soil of healthy and wilted pigeonpea plants. The

number of fungi, bacteria and actinomycetes in the rhizosphere soil of healthy plant recorded were 34.14 X 10^3, 63.71 x 10^5 and 16.00 x 10^3 respectively. But the number of fungi, bacteria and fungi reduced in the rhizosphere soil of wilted plant and number recorded were 19.71 x 10^3, 16.86 x 10^5 and 08.71 x 10^3 respectively. Upadhyay and Rai (1982) reported number of bacteria per g of soil were higher in the rhizosphere of healthy plants. The actinomycetes bacteria differed in their occurrence on roots of healthy plants compared with diseased ones. The probable reason was that the diseased roots supported a large population of *F. udum* , which might have competitively suppressed the bacteria and actinomycetes. Rhizosphere of healthy plant showed higher population of bacteria compared to that of fungi and actinomycetes. But in the rhizosphere of wilted plant fungal population was higher than bacteria and actinomycetes.

The rhizosphere soil of resistant cultivar mainly comprised viz., *Aspergillus flavus, A. niger, A. terreus, Penicillium* spp. and *Trichoderma viride*. About 90 per cent of the fungal population in rhizosphere of wilted plants accounts for *F. udum*. These findings are in agreement with the earlier work of Upadhyay and Rai (1982) and Gaur and Sharma (1991).

Use of organic amendments to control soil borne diseases of plants is a potential non-chemical method and al -so an indirect approach to control the pathogen. Organic

amendments not only reduced the disease intensity, but also increased the soil fertility and crop yields to considerable extent. In the present study, groundnut cake, neem cake, farm yard manure and compost were employed to work out the management practice on wilt of pigeonpea.

Oil cakes have been found to act by way of fungistasis and also production of toxic volatiles substances and gases. Treatment of the soil with oil cake caused quicker rise in pH compared with the control. Hence, the growth of the pathogen was inhibited, which thrives well at neutral to slightly alkaline pH (6.5-7.5).

Various biochemicals including antibiotics and phenols released during decomposition of lignin-containing materials, induce disease resistance on the root surface as well as in the tissue when absorbed. Certain substances, such as aldehyde are stimulatory for pathogens, which are destroyed by strong biological antagonism operating during the decomposition of organic amendments.

Excessive decomposition of organic amendments result in accumulation of large amount of CO_2 and scarcity of nitrogen in the soil and resulting into suppression of soil borne pathogens (Papavizas and Davey, 1960).

Organic amendments are being recommended as bio-control agents to reduce the incidence of several plant diseases especially in control of specialized parasites (Sanford, 1926 ; Nargund, 1981). Application of organic amendments to the soil promotes the biological antagonism leading to suppression of build-up of inoculum threshold or inoculum density. The inoculum density of pathogen is reduced by various mechanisms like lysis or antibiosis of survival structures or competition among the antagonistic microbes and the pathogen.

Organic amendments also favour biological protection of plant and there will be enhanced competition among the soil microorganisms for nitrogen, carbon or both. This may be expressed as germination of fewer propagules or less growth of the pathogen in the infection court.

Effectiveness of the amendment in decreasing the disease caused by soil inhabitating pathogen depends also upon their ability to change the microbial population in soil, so also, their C:N ratio. Organic amendments reduce the saprophytic activity and lower the population of pathogens by lysing the propagules or chlamydospores or sclerotia which are present in the soil.

Singh and Singh (1980) reported lysis of *F. udum* cells in soils amended with carbon materials and found

materials with low C:N ratio, which decomposed and encouraged microbial activity rapidly produced greater lysis than with materials having high C:N ratio.

Neem cake had shown highest per cent survival (85% and 77.5% at 20 and 40 days respectively). It may be due to improved soil physical properties such as pore size, aeration, temperature, water holding capacity, etc. These further might have helped in better solubilization of minerals which together with the nutrients released by decomposition helped in the rapid extension of the root system, better uptake of nutrients and finally better plant vigour. Singh (1983) stated that it is the decomposition of the organic matter that is the cause of disease suppression. The amendment itself may not contain the inhibitory factor for pathogens. Even if it does contain such substances their status in soil may change due to microbial action.

Next to neem cake, compost and groundnut oil cake showed highest per cent plant survival at 20 days. In 40 days it is found to be compost in which highest per cent plant survived. FYM was found to be least effective. It may be due to slow decomposition of FYM, as compared to other amendments. But per cent survival (60%) was more as compared to control (50%). Singh (1983) noted that, soil organic amendments help the plant to resist the attack of pathogen or replace the damaged root quickly by stimulating formatin of new roots.

The end result was better per cent plant survival and suppression of wilt pathogen.

Neem cake shows least wilt per cent (27.5%) followed by compost and significant results over control (75.00%). Punja (1985) reported that the addition of organic amendments such as compost or oat or corn straw to soil limits disease incidence, possibly due to the release of toxic ammonia (NH_3) or increase in the levels of resident antagonistic soil microflora.

In the present studies, saprophytic activity of the pathogen and disease severity reduced within 20 days following the addition of organic amendments. This effect is more in neem cake compared to other amendments. Hence, it is better to incorporate organic amendments, 15-20 days prior to sowing, so that, the activity of the pathogen could be suppressed by the enhanced activity of antagonistic microflora.

Among the fungi, *Trichoderma viride* and *T. harzianum* overgrew and completely inhibited the growth of *Fusarium udum*. Among bacteria *Pseudomonas fluorescens* produced an inhibition zone of 7.5 mm, while *Streptomyces* spp., produced an inhibition zone of 13.2 mm.

Among the fungi, *Trichoderma viride* exhibited maximum parasitic activity followed by *T. harzianum*. Similar studies

conducted on *F. oxysporum* f. sp. *fragriae* (Moon *et al.*, 1988) and *F. oxysporum* (Morshed, 1985) indicated the suppression of these fungi by *T. harzianum* and *T. viride* respectively.

The complete inhibition of growth of *F. udum* by *Aspergillus niger, A. terrus,* and *Penicillium* sp. may be due to the production of anti-fungal substances in the culture medium. *Trichoderma harzianum* and *T. viride,* both suppressed the growth of *F. udum* may be due to coiling and disintegration of hyphae of the test fungus and the loss of competitive saprophytic ability. The results of the present study is supported by the previous report by Gaur and Sharma (1991).

Godtfredsen and Vangedal (1965) reported trichodermin; Pyke and Dietz (1960) found dermadine, while Dennis and Webster (1971) reported, acetaldehyde as major volatile antibiotics produced by *Trichoderma* spp. which suppress several plant pathogens.

Pseudomonas fluorescens antagonise *Fusarium* by producing one or more metabolities that include antibiotics (Fravel, 1988) Siderophores (Leong, 1986) and cyanide (Voisard *et al.*, 1989). In addition to these characters, they also act as plant growth promoters (Surlow and Schroth, 1982).

The inhibition effect of *Streptomyces* spp., on *F. udum* in agar plates may be attributed to production of antifungal substances which might have diffused through the agar medium.

Antagonists were evaluated by following poison food technique against the *F. udum* . Maximum inhibition was observed by *Trichoderma viride* ((87.03%), followed by *T. harzianum* (85.40%).

Both the bacterial antagonists, *Pseudomonas fluorescens* (81.87%) and *Bacillus subtilis* (72.23%) showed antagonistic activity. Vasudeva (1949) noticed that *B. subtilis* inhibited growth and spore germination and caused lysis of the mycelium and germ tubes of *F. udum*. Sivamani and Gnanamanickam (1988) reported that *P. fluorescence* exhibited *in vitro* antibiosis towards isolates of *F. oxysporum* f. sp *cubense*.

Thus based on the studies of antagonistic microorganisms, it can be concluded that, *T. viride, T. harzianum* and *P. fluorescence* were effective against *Fusarium udum*. In many instances, viridin which is easily converted to viridiol is known to be phytotoxic (Howell and Stapanovic, 1984 ; Jones and Hancock, 1988). However the *T. viride* had no adverse effect on pigeonpea seedlings as well as seed germination. So it can be used as a biological agent for management of *F. udum* seed treatment with antagonistic organisms will help to check the incidence of the disease to a greater extent.

Minimum per cent wilt was recorded by treating the seed with *T. viride, T. harzianum* both showed 26.67 per cent

and 30.00 per cent wilt respectively. The effect of *T. viride* might be due to direct attack and lysis of the mycelium. This is in agreement with Gaur and Sharma (1991) and Kotasthane *et al.* (1987). Efficient biocontrol of damping-off caused by *Rhizoctonia solani* and *Phythium* spp., was achieved by treating seeds of peas and radish with conidia of *T. harzianum* (Harman *et al.*, 1989) and control of Fusarium crown rot of tomato by *T. harzianum* (Sivan *et al.*, 1987). Brain (1951) identified antagonistic substance as viridea in case of *T. viride* which is toxic to many pathogens.

P. fluorescens shows 36.67 per cent wilt incidence, which follows the Aspergillus spp., and penicillium sp., act through antibiosis or as a competition of the pathogen at the infection court and hence they decreased the disease intensity (Baker and Cook, 1979). It is presumed that, such a mechanism might have taken place in suppressing *F. udum* also. Seed bacterization with *P. fluorescens* gave effective control of the disease by producing siderophores which inhibit the pathogen. Similar information was obtained by Laha *et al.* (1992) in case of *R. solani* a causal agent of root rot of cotton.

Biocontrol agents were effective and they were compatible with modern production practices, so that their use can be integrated into the production system. In the present study, two antagonists *viz.*, *Trichoderma viride* and *T. harzianum* were tested individually and in combination with two

fungicides viz., Carbendazim and Captan. *In vitro* results showed antagonists were not affected by fungicides. Carbendazim + *T. viride* and Carbendazim + *T. harzianum* showed inhibition of 63.03 and 60.70 per cent respectively.

As per data 30.00 and 26.67 per cent plant were affected by the seed treatment of *T. harzianum* + *T. viride* respectively. However, only 16.67 and 20.00 per cent plants were affected in the combination treatment of Carbendazim + *T. viride* and Carbendazim + *T. harzianum* respectively. The benefit of antagonists in the suppression of disease symptoms has been widely reported (Baker and Cook, 1979 ; Papavizas and Lumsden, 1980). The effect of integration of *Trichoderma* with fungicides was also reported by Sharma *et al.* (1992) in controlling *Sclerotinia sclerotiorum* and Singh and Tripathi (1997) in the management of sclerotinia rot of sunflower. These results are in agreement with Alagarswamy and Sivaprakasam (1988).

In the present study, seed pelleting with *T. viride* either alone or in combination with Carbendazim reduced the per cent plant infection besides enhancing the growth. The change in soil reaction that was expected due to the increased activity of introduced *Trichoderma* might be one of the reasons for reduction of the disease besides production of some growth regulating substances by the antagonists (Papavizas and Lumsden, 1980).

Biological control through the use of antagonistic microorganisms is a potential, non chemical means for plant disease control by reducing the inoculum levels and activity of the soil borne pathogens. Such a management would help in preventing the pollution and also health hazards.

Future line of work

The present study has given an insight into the different aspects of biological control of pigeonpea wilt and has led to the following observations which may be looked into in future.

1. Study of different ecological factors affecting survival of *F. udum* and its application under field condition to combat the disease.

2. Studies on the interaction of Vesicular Arbuscular Mycorrhizae associated with pigeonpea and *F. udum*.

3. Effect of crop rotation and intercropping systems on the population dynamics of *F. udum*.

4. Integrated management of the disease by employing components of management practices under field conditions.

SUMMARY

VI. SUMMARY

Pigeonpea, one of the most important pulse crops said to be originated from Peninsular India. It is grown commercially in India, South-East Asia, Africa and Central America. Pigeonpea is known for its high nutritional values (seed protein content 21%). It also fixes atmospheric nitrogen. It ranks second after bengal gram and suffers from more than 100 diseases. Among them wilt is very important.

Pigeonpea wilt was first reported by Butler in 1910. Later on *Fusarium udum* was proved to be the cause of wilt. Considering the importance of the problem, investigations were carried out on the management of pigeonpea wilt by employing various methods *viz.*, isolation of microorganism from pigeonpea rhizosphere soil, effect of application of organic amendments, effect of application of antagonists and their compatibility effect with chemicals on *F. udum*.

Repeated tissue isolation was made from wilted pigeonpea plants collected from Main Research Stationm UAS Farm, Dharwad during *kharif* season revealed association of *F. udum* with the wilted plants. The pure culture of *F. udum* was obtained by hyphal tip isolation method. Pathogenecity test revealed the symptoms caused by *F. udum* as loss of turgidity in leaves, slight interveinal clearing. The foliage shows

slight chlorosis and becomes bright yellow before wilting. Leaves were retained on plants, wilted plants after splitting showed brown vascular discoloration. The pathogen was identified as *F. udum* on the basis of major/principal morphological characters.

The population of bacteria, fungi and actinomycetes were higher in rhizosphere soil of healthy plants compared to rhizosphere soil of wilted plants. From the rhizosphere soil of healthy plants, *Trichoderma viride* Pers. ex. Fr., *Aspergillus flavus* Link., *A. niger* Van Tiegh., *Penicillium* spp. were isolated and were found to be antagonistic to *F. udum*. But rhizosphere soil of wilted plants comprise mainly *Fusarium udum* (about 90% of fungal population).

There was no adverse effect on seed germination in soil amended with neem cake, compost, FYM, groundnut oil cake and non-amended soil. Among the various amendments tested, neem cake was most effective in reducing the wilt incidence and also per cent survival of the plants were higher and followed by compost. These treatments were followed by FYM amended soils and groundnut oil cake amended soils.

Efficacy of these amendments in reducing the disease incidence was increased, if they were incorporated 15-20 days before sowing of pigeonpea seeds.

Among the antagonists evaluated in *In Vitro* conditions, *T. viride* and *T. harzianum* over grew the test fungus. *Pseudomonas fluorescens* Migula., and *Streptomyces* spp., produced 7.5 mm and 13.2 mm inhibition zone respectively. Per cent inhibition was found to be more in *T. viride* (87.03%) followed by *T. harzianum, Pseudomonas fluorescens, Bacillus subtilis*.

The spore germination and viability reduced significantly in interaction zone in comparison to the centre of *Fusarium* colony. Minimum per cent spore germination recorded in culture interacting with *T. viride*.

Seed treatment with antagonists reduced the wilt incidence to a considerable extent by suppressing *F. udum*. Among antagonistic organisms tested, *T. viride, T. harzianum* and *Pseudomonas fluorescens* were effective as seed dresser. The investigations indicated the possible role of biological antagonism in suppression of wilt pathogen of pigeonpea.

In combined effect of antagonists and fungicides, the results revealed that antagonists were not affected due to fungicide treatment. *T. viride* and *T. harzianum* in combination with Carbendazim reduced the growth of *F. udum* in Petriplates.

Under pot culture conditions the combined effect of *T. viride* + Carbendazim reduced the wilt incidence to considerable extent followed by *T. viride* + Captan, Carbendazim + *T. harzianum* and *T. harzianum* + Captan treatment.

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VII. REFERENCES

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* Original not seen.

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