BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT IN TOMATO (*Lycopersicon esculentum* Mill.) CAUSED BY *Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) Snyder and Hansen

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Dedicated to

Beloved Parents

Father-in-law and Late Mother-in-law

Wife and Daughters

Department of Plant Pathology
University of Agricultural Sciences
Bangalore

Certificate

This is to certify that the thesis entitled "**BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT IN TOMATO (*Lycopersicon esculentum* Mill.) CAUSED BY *Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) SNYDER AND HANSEN**" submitted by Sri. Bhaskara Padmodaya, for the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) IN PLANT PATHOLOGY of the University of Agricultural Sciences, Bangalore, is a record of **bonafide** research work done 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.

March 21 1994

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INTRODUCTION

I. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular vegetables grown in the world next to potato. In India, it is grown in about 2,90,000 ha with an annual production of 46,00,000 metric tonnes. In Karnataka alone, the crop is grown in an area of 25,000 ha with an annual production of 5,00,000 metric tonnes(Anonymous, 1991).

Among the fungal diseases occurring on tomato early blight (*Alternaria solani* (Ell. and Martin) Jones and Grout), wilt (*Fusarium oxysporum* Schl. f. sp. *lycopersici* (Sacc.) Snyder and Hansen), septoria leaf spot (*Septoria lycopersici* Speg.), buck eye rot (*Phytophthora nicotianae* var. *parasitica* (Dast.) Waterh.), late blight (*Phytophthora infestans* (Mont.) de Bary and Fruit rots are considered problem diseases currently threatening tomato production. (Sokhi et al., 1991)

F. oxysporum f. sp. *lycopersici* is a soil-borne pathogen highly specific to tomato occurring world wide in distribution, wherever tomato is grown (Walker, 1969). It is primarily a soil inhabiting pathogen, once introduced it remains in the soil for very long time, attacks tomato at all stages of plant growth starting from nursery to fruiting. It causes damping-off in nurseries at pre-emergence stage, killing the germinating seeds and young seedlings before they emerge out of soil surface, and at post-emergence stage, attacks seedlings at or below ground level resulting in death of seedlings (Varma, 1954 and El Helaly *et al.*, 1962). Wilt is the most important disease caused by this pathogen and a limiting factor to tomato production (Sokhi, *et. al.* 1991). Two of the earliest symptoms on young plants are clearing of veinlets and drooping of the petioles. Later the entire plant wilts. In the field the disease appears at any time under favourable conditions. Lower leaves become yellow and die with continuation of symptoms on successive younger leaves. These symptoms may appear on the entire plant or on only a few branches. After the disease has advanced for a few weeks, browning of the vascular system in a cross section of the lower stem can be seen or by removing tissue near the base with a knife. Young plants wilt suddenly but older plants linger on for some time. The plant as a whole is stunted.Due to attack on roots, the laterals show a black rot type of condition, hastening drying of the plants. In wet weather the fungal growth can be seen on dead plants in the form of pinkish mycelial layers (Singh, 1985 and Agrios, 1988).

Biological control offers answers to many serious problems of modern agriculture and is an essential component in the development of a sustainable agriculture capable of

continuing without interruption or diminution. Intensive management systems, including higher use of nitrogen fertilizer would result in water stress favouring certain pathogens especially *Fusarium* spp. Biological control aimed directly at the pathogen or mediated through adjustments in the host offers unlimited opportunities to reduce losses associated with stress. Since 1960s, the public became aware of environmental pollution and of the increasing amounts of toxic chemicals in nature's food chains. Public opinion, once so favourable to the cheap and rapid control of pathogens and insects by chemicals, then moved towards the opposite extreme of imposing serious restrictions on their use. At the same time, plant pathologists realized that upsetting the biological balance using pesticides can lead to severe disease outbreaks and consequently more and more biological control procedures are included in their integrated control programmes. Most fungicides have only a temporary effect and therefore, require repeated applications. Besides, development of resistance to fungicides by plant pathogens is also posing a threat and forcing application of chemicals in combinations, intermittently or alternately in sequence to avoid selection for resistant strains. Biological control usually lasts longer. Replacing pesticides entirely by biological control is not an attainable or even a reasonable goal except perhaps under special circumstances like in green house operations. Biological control should be tried as a first rather than as a last strategy. Meeting the future challenge for a productive but sustainable agriculture will require the use of all available strategies that are effective, economical, safe and compatible. Biological control often works by gentler, more subtle methods to which there is little resistance. Moreover, disease control is more than merely killing pathogens (Cook and Baker, 1983). Biological control of soil-borne pathogens can be achieved by different methods; the addition of organic amendments to soil to stimulate resident antagonists (Papavizas and Lumsden 1980; Singh, 1983; Reddy, 1987; and Somasekhara, 1992); introduction of antagonists especially *Trichoderma* spp, (Papavizas, 1985) and bacterial antagonists (Campbell and Faull, 1979) into soil are some of the methods successfully employed.

Information regarding the occurrence and importance of the seedling disease and wilt caused by *Fusarium* spp. is very meagre in the state. Hence it was felt necessary to find out the status of this disease in the state, besides its control by biological methods. Keeping this in view, the present studies were undertaken with the following objectives.

1. Survey of the major tomato growing areas around Bangalore for assessment of economically important soil borne diseases especially damping-off/wilt induced by *Fusarium* spp.

2. Screening of plant residues/soil amendments for their effect on the control of damping-off/wilt.
3. Screening of fungal and bacterial antagonists against *Fusarium* spp. as biocontrol agents.
4. Screening of promising varieties of Tomato to damping-off/wilt disease and
5. To develop method of disease management practice which is economical and environment friendly and simple enough for the farmers to follow.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular vegetables due to its acid taste, yield and protective qualities. Lukyanenko (1991) stated that tomato is susceptible to more than 200 diseases resulting in 75-95 per cent yield loss. Further, he pointed out that Fusarium wilt casued by *F. oxysporum* f. sp.*lycopersici* had been the most devastating disease resulting in 10-50 per cent crop losses around the world. This disease is recognised as one of the most destructive diseases of cultivated crops posing considerable hazards universally. It is one of the typical wilt diseases most thoroughly studied all over the world (Beckman, 1987). *F. oxysporum* f. sp.*lycopersici* being soil-borne pathogen and soil-inhabitant, persists for longer periods in the soil. To maintain a low inoculum load by a continuous application of systemic fungicides or biocides alone is not practical for the control of wilt diseases. Most fungicides have only a temporary effect and therefore require repeated applications. In many fields the pesticid al costs can be prohibitive. Further, most of the popular tomato varieties grown in India are reported to be susceptible to Fusarium wilt (Chauhan, 1988 and Kapoor, 1988).

Compared to chemical means of control, biological control usually lasts longer as it often works by gentler, more subtle methods to which there is little resistance. Pimental (1981) reported that "Biological control using micro organisms (Protozoans, nematodes, bacteria, fungi, viruses) has not resulted in any known environmental problem." Agriculture must turn from the short term view and the "quick kill", to the long term view and sustainable procedures. Practices that promise long term effectiveness, even if, at a somewhat lower level than the short term procedures, are more likely to be sustainable, thus the need for a sustainable agriculture will be met atleast in part by wider use of biological control of plant pathogens. Biological control is defined as "The reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man" (Cook and Baker, 1983). Because of these reasons, biological control of soil-borne plant pathogens is gaining importance and is widely practised all over the world. In this review the work done on *F. oxysporum* f. sp. *lycopersici* or related pathogens on the aspects of disease survey, *Fusarium* spp. associated with wilt disease of tomato, their pathogenicity, use of organic amendments, antagonistic microorganisms in biocontrol and finally screening promising tomato varieties against the pathogen.

2.1 DISEASE SURVEY

Fusarium wilt of tomato is of worldwide importance and universal in distribution. Saccordo (1886) was the first to attribute it to *F. oxysporum* from Italy. Massee (1895) gave the first published description from Britain and referred it to as sleepy disease of tomato. Subsequently several investigators have reported it from different parts of the world viz., USA (Walker, 1969); Africa (Wager, 1933); Australia (Mills and Hutton, 1945); and India (Butler, 1918).

In India it was first reported from Bihar, Punjab and UP (Butler, 1918). Subsequently its occurrence was reported from Andaman Islands (Mitra, 1929); Bombay Presidency (Uppal *et. al.*, 1934); Assam (Chowdhury, 1948); Bhattacharya and Baruah, 1953 and Goswami and Baruah, 1956); Tamil Nadu (Varma, 1954; Nedumaran and Vidyashekharan, 1982); Andhra Pradesh (Vaheeduddin, 1955); Bihar (Chand, 1965; Chand and Thakur, 1969); Delhi (Sen and Kapoor, 1974) and Haryana (Banerjee *et. al.*, 1990).

A field survey revealed the prevalence of Fusarium wilt of tomato in 68 per cent of the fields examined in Utah inflicting losses from 7.87 to 85 per cent (Anonymous, 1926). White (1927) reported 10-100 per cent in Kansas state and also observed constant increase in disease incidence. In New Zealand losses upto 50 percent both in glass house and field conditions was observed (Chamberlain, 1933), the seriousness of the problem was indicated by Wager (1933) in South Africa and in Australia by Mills and Hutton (1946).

In India it was reported to occur in different parts of Bihar to an extent of 10-20 per cent some times upto 80 per cent in severe cases (Mishra, 1954). Kapoor (1988) reported that the disease was prevalent in Maharastra (18.5 per cent), Delhi (18.3 per cent) and Tamil Nadu (7.8 per cent). Further, he noticed that the disease was prevalent in germplasm / breeding material upto 30-100 per cent in IARI, 50-60 percent in Mahatma Phule Agricultural University and 25 per cent in Tamil Nadu Agricultural University. Banerjee *et. al.* (1990) reported that it occurred in an epidemic form during 1985-86 in Haryana.

All the commercial varieties of tomato were reported to be susceptible to Fusarium wilt (Chauhan, 1988, Kapoor, 1988 and Banerjee 1990). Root diseases of cucurbits, tomato and egg plants were more serious when grown as monoculture crops than when rotated with cereal crops (Williams, 1979). In Florida, race 2 caused serious damage to tomatoes grown on repeatedly cropped sandy soils (Jones *et. al.*, 1972 and Katan, 1974).

Tomatoes grew better when grown after wheat than after potatoes in *Fusarium* infested soil (Dorenda, 1975). The disease was found more prevalent in differnt parts of India where it was grown as a mono crop or grown after vegetables (Kapoor, 1988).

Fusarium wilt of tomato becomes serious during warm seasons in temperate regions of the world (Walker, 1971). Kapoor (1988), noticed that it was more prevalent during main tomato growing season (March / April) while in off-season (September / October) root / collar rots / stem rots caused by *F. solani; F. equeseti* and *Rhizoctonia bataticola* were more common. Damping off of tomato caused by *F. oxysporum* f. sp. *lycopersici* was found more common in the hot season in Egypt, with 30°C and soil moisture at 40 per cent water holding capacity (El Helaly *et. al.*, 1962).

2.2 SYMPTOMS OF THE DISEASE

The Fusarial wilt organism of tomato is a vascular pathogen invading the plants through roots. The earliest observable symptoms appear within 48 hours after the entry of the pahogen when the ultimate veinlets become translucent followed by epinasty of older leaves (Foster, 1946). The obvious symptoms of yellowing of leaves and discolouration of vascular tissue appear seven to ten days later. The oldest leaves turn yellow succeeded by younger petioles and stem and the whole plant slowly wilts. Leaves become chlorotic and desiccated and the plant withers and dies slowly (Dimond, 1955). Occasionally, adventitious roots may form on the stem keeping pace with yellowing of leaves (Fisher, 1935).

Yellowing, epinasty of leaves and adventitious root formation are connected with the liberation of ethylene and auxins. Vascular discolouration is brought about by oxidation of conjugated phenols into melanins, which in turn causes browning (Davis *et. al., 1953).* Wilting, the prominent and unique symptom, is caused by the failure of transport system to conduct adequate water. Withering is associated with polysaccharides and glycopeptides. These compounds, resulting from infection, are transported to the margin of leaves and they damage membranes in parenchyma cells (Dimond, 1970).

F. solani, produced lesions on the tap root or main roots with young foliage distorted, lightly mottled with necrotic spotting in tomato (Vawdrey and Peterson, 1988). An isolate of *F. moniliformae* and *F. oxysporum* produced toxin(s) causing necrotic lesions on leaves, petioles and blackening of lower stem (Kapoor, 1988).

2.3. PATHOGEN

Wollenweber and Reinking (1935) recorded various *Fusaria* causing either wilt or rot of tomatoes viz. *F. bulbigenum* v. *lycopersici; F. equiseti; F. scripi; F. scripi* v. *accuminatum; F. caeruleum; F. semitectum* and *F. culmorum*. Wellman (1943), found a new species *F. retusum* causing vascular wilt in tomato identical in symptoms with that of *F. bulbigenum* v. *lycopersici*. In 1940, the forma specialis specific to tomato was designated as *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen.

In India, Varma (1954) observed that wilt in tomato was caused by several species of *Fusaria*. *F. lateritium* Nees v.*uncinatum*Wr. was found to be the most virulent. Besides, all the isolates of *Fusarium* were found to cause preemergence and postemergence seedling disease. Though several species of *Fusarium* were reported to cause wilt in tomato, *F. oxysporum* f. sp.*lycopersici* is highly specific to tomato and produces typical vascular wilt. The description of the fungus is given below.

Aerial mycelium floccose, white to pink, stroma plectenchymatous, reddish violet to pale sometimes sclerotial, smooth, flat, later disappearing. Sporodochia minute, raised often coalescing to form pionnotal masses, flesh pink or light orange in colour, Microconidia one celled or with 1-2 septa, produced freely on the aerial mycelium. Macroconidia 3-5 septate, seldom 6-7 septate elongate, cylindrical to fusiform, falcate, hyaline. The conidia measuring ;

0 septate 8 x 2.5 µm; 1 septate 18 x 2.8 µm; 3 septate 37 x 3.4 µm; mostly 31-50 x 2.8-3.8 µm (25-66 x 2.3 to 4.3) µm and 5 Septate 49 x 3.5 mostly 43 to 56 x 3.4 (50-70 x 3-4.5) µm. Chlamydospores may be intercalary or terminal.

White (1927) found that the temperature optima for several strains of *F. lycopersici* was between 24—28^{0}c. Maximum growth of the fungus occurred at pH value of 4.5 to 5.3 followed by a minimum at pH 5.25 to 5.8, with a second maximum at pH 5.85 to 6.85 (Scott, 1925).

2.3.1. SURVIVAL

Once in the soil, the fusaria enter a new phase of existence in which they must compete for suitable growing niches and energy competing with many micro-organisms that are particularly adapted to saprophytic growth. Most forma speciales of *Fusarium*

oxysporum remain dormant and immobile as chlamydospores in decaying host tissues until stimulated to germinate. Invasion usually through the roots of a suspectible plant is followed by development of a systemic vascular wilt disease. In advanced stages of disease development, the fungus grows out of vascular system and produces conidia and chlamydospores (Beckman, 1987).Katan (1971) reported that several weeds viz., *Oryzopsis, Digitaria, Amaranthus* and *Malva* and wild or cultivated egg plants served as symptomless carriers for *F. oxysporum* f.sp.*lycopersici.* Abdullah and Ismail (1976) found seven plant species out of 18 they tried were colonized by *F. oxysporum* f.sp. *lycopersici* to different extent without exhibiting symptoms.

Microconidia of *F. oxysporum* f.sp. *lycopersici* stored in talc survived for about one month without changing their appearance or became thick walled, micro chlamydospores (Tello-Marquina and Alabouvette, 1984). A 2.5 month mid winter overseasoning period between crops was found to greatly reduce the incidence of Fusarium wilt. Further it was noticed that race 3 survived greater than race 2 which survived better than race 1 (Jones *et. al.*, 1990)

2.3.2. ROLE OF ENVIRONMENTAL CONDITIONS ON DISEASE DEVELOPMENT

A sound understanding of the inter relationships of host, pathogen, ambient temperature, soil nutrients, microflora and oxygen supply is necessary as they influnce infection pressure exerted by the pathogen upon the host and upon the capacity of the host to resist (Woltz and Jones, 1981). A better understanding of environmental factors leading to development of cultural practices adapted to local conditions which when combined with ho-st resistance would ensure better crop production with a minimum of damage.

Temperature :

Clayton (1923) was the first to report the effect of temperature on Fusarium wilt development in tomato under controlled conditions. He found that optimum soil temperature was 28°C. The disease was inhibited above 33°C and below 21°C. Air temperatures also had similar effect. Foster and Walker (1947), demonstrated that multigene type of resistance is conditioned by temperature while the single dominant I gene type of resistance is not at all conditioned by temperature. Tomato variety Red Currant having a single dominant I gene for resistance was found disease free at all temperatures while, Bonny Best was severely affected and multiple gene resistant cultivar Marglobe was less

affected. Besri (1980) observed that when both the *verticillium* and *Fusarium* occur simultaneously, spring and autumn seasons favoured *verticillium* infection compared to *Fusarium* though air temperatures were favourable for symptom development of Fusarium wilt. Thus, the situation in the field, when both the pathogens are present can become rather complex.

Light:

Foster and Walker (1947) demonstrated that light of low intensity (100 ft-c) or short duration (6 hr) promoted rapid disease development in the susceptible Bonny Best cultivar but less rapid development in the multiple gene resistant Marglobe and no symptoms in the I gene resistant Red Currant. Wilt caused by *F. oxysporum* f. sp *lycopersici* was common and destructive in spring crops maturing in May in Manatee-Ruskin area of France whereas its occurrence greatly reduced during the fall (Jones *et. al.*, 1982).

Soil Moisture and Aeration :

Soil moisture conducive to best vegetative growth of the host was most favourable to disease development (Clayton, 1923; Wellman, 1939 and Walker and Foster, 1946). The susceptible cultivar Beiju showed the highest disease even at saturation while resistant cultivar Shinhakan No. 2 did not (Endo *et. al.*, 1975). The degree of colonization of basal stem tissues and the degree of foliar symptoms were favoured by high soil moisture levels in Marmande variety by weakening resistance factors (Matta and Garibaldi, 1972). Frank and Bakker (1975) stated that plants grown with high soil moisture availability and poor aeration succumbed to the disease, whereas, plants held with sufficiently available water and good aeration remained tolerant to Fusarium wilt. They concluded that soil aeration was the decisive factor. Besri (1980) reported that the fungus *F. oxysporum* f. sp *lycopersici* grew at -140 bars. A reduction in water potential from -1.5 to -13.79 bars increased its development. The optimum growth of the fungus was found at -41.79 bars.

pH

Jones and Woltz (1969), observed that high soil pH reduced Fusarium wilt in tomato especially at pH 7.5 and 8.0 by making certain micronutrients unavailable for the pathogen.

Sherwood (1923) observed maximum growth at pH 7.6 to 8.2. White (1927) stated that 24 strains of *F. oxysporum* f. sp *lycopersici* exhibited a double maximum at pH 5-7.0 and above 7.0.

2.3.3. MORPHOLOGICAL AND CULTURAL CHARACTERS AND PATHOGENICITY

A major problem in working with the vascular wilt isolates of *F. oxysporum* is the maintenance of the original morphology and virulence of the organism. Starting from the sporodochial type, variation in general, proceeds either towards types producing abundant aerial mycelium but few macroconidia termed mycelial types or towards types producing little mycelium or no aerial mycelium but abundant macroconidia, termed pionnotal types (Nelson, 1981). The morphology of conidiophore was used as a primary basis for species identification (Booth, 1971). White (1927) has grouped 24 strains of *F. oxysporum* f. sp *lycopersici* into two groups based on morphology and cultural characters. In India, Varma (1954) identified 37 isolates of *Fusarium* on the basis of morphological and cultural characters and pathogenicity, causing wilt in tomato. Goswami and Baruah (1956) identified two strains of *F. oxysporum* causing wilt of tomato in Assam based on morphological and cultural characters.

Pathogenicity :

Pathogenicity of the *Fusarium* isolates is distinguished on the basis of the relative percentage of plants killed. White (1927) observed different degrees of virulence of 24 strains of *F. oxysporum* f. sp *lycopersici* towards tomato plants in glass house tests and divided them into two distinct groups. The existance of physiologic specialization in *F. oxysporum* f. sp. *lycopersici* was revealed by Gerdemann and Finley (1951). They identified two races with variation in pathogenicity. Forty two isolates of *F. oxysporum* were isolated from crown and root rot affected tomato plants produced similar symptoms and pattern of infection on four differential tomato lines but differed from that of *F. oxysporum* f. sp *lycopersici* race 1 and 2. The results from inoculation of 15 hosts indicated that legumes were moderately susceptible to crown and root rot while cucurbits, crucifers and cereals were not infected. It was concluded to place all these isolates into a new speciales of *F. oxysporum* (Randall and Rowe, 1980)

Variability in pathogenicity of strains of *F. oxysporum* f. sp. *lycopersici* were reported by several investigators (Edgerton, 1920; Clayton, 1923; White, 1927; Haymaker, 1928; Wager, 1933; Mills and Hutton, 1946; Varma, 1954 and Chand, 1965). More recently Chandra *et. al.*, (1983) observed that the isolates within *Fusarium* species differed in their potentiality to induce pre- and post-emergence killing of seedlings and wilt in mature

plants. Several isolates belonging to different species of *Fusarium viz., F. oxysporum; F. solani; F. semitectum; F. chlamydosporum and F. moniliforme,* were isolated from wilted tomato plants from different agroclimatic zones of India. The isolates within a species differed in their ability to cause pre and post-emergence killing and induce wilt in main crop (Kapoor, 1987).

Grouping of Isolates of *Fusarium* based on Morphological and Cultural Characters and Pathogenicity :

White (1927) classified 24 strains of *F. lycopersici* into two groups based on morphological and cultural characters and pathogenicity. He observed differences in pigmentation, mycelial characters, sporulation and the isolates differed markedly in inducing wilt. Varma (1954) grouped 37 isolates of *Fusarium* on the basis of morphological and cultural characters and pathogenicity into different species. Goswami and Baruah (1956) observed marked differences in morphological and cultural characters and pathogenicity between two strains of *F. oxysporum* occurring on tomato in Assam. Similar studies were made by Chand (1965) when he named two isolates of *F. oxysporum* f. sp. *lycopersici* into isolate A and isolate B. These two isolates were differentiated on the basis of mycelial growth, pigmentation, macroconidia, production of chlamydospore, size of macro and microconidia on PDA medium. Isolate A was found to be more virulent than isolate B. Chandra *et. al.* (1983) grouped isolates of *Fusarium* into two catagories on the basis of pathogenicity. The first category consisted of isolates capable of inducing pre-emergence killing of seedlings and wilting of mature plants of two varieties Marglobe and Pusa Ruby. The second category included isolates having different behaviour towards the two varieties. Various isolates belonging to five *Fusarium* species were grouped into three categories viz., a) vigorous pre-emergence killers; b) vigorous post-emergence killers and c) capable of causing equal pre and post-emergence damage (Kapoor, 1987).

2.3.4. FACTORS INFLUENCING DISEASE DEVELOPMENT

Soil type and Disease

Wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* race 2 was reported to be the major limiting factor of tomato production on sandy soils of Florida (Jones *et. al.*, 1972).

In India tomato is grown on a variety of soils from sandy loam to heavy clay, but it grows on well drained heavy loam soil. Fusarium wilt was observed mostly in sandy soils

and least in heavy clay soils. The percentage of infection increased with higher percentage of sand in soil (Chand and Thakur, 1969 and Kapoor, 1988).

Inoculum Density and Disease

Haymaker (1928) studied the relation of spore load to infection with two strains of *F. oxysporum* f. sp. *lycopersici* (A and B). Infecting power of strain A was found to be diminished by reducing the number of conidia while that of strain B was not affected. Inoculum densities, generally were known to be directly proportional to the disease severity (Baker,1968). Plant growth was substantially reduced and a clear indication of a heightened tempo of disease development was observed upon increasing the levels of inoculum from 30 to 50 per cent. Wilt symptoms appeared subsequently at all inoculum levels and the plant succumbed within a period of two and a half weeks. It was apparent that inoculum levels from 30 to 50 per cent are the most effective in causing a quick outset of the disease. It was also observed that a period of 5 weeks was needed before the complete death of plant occurs. It appeared that 20% inoculum and 80% sterilized soil was required to cause the death of the plant. (Padua and Quebral, 1972). The percentage of wilted tomato plants due to *F.oxysporum* and *F. solani* varied in proportion to the quantum of inoculum added into the soil. Warm weather favoured the disease severity (Kapoor, 1987). The inoculum levels at which 50 percent of the plants were infected were found to contain 300 chlamydospores g^{-1} fumigated soil, 900 chlamydospores g^{-1} soil and 6500 g^{-1} of fumigated soil with selected antagonists in case of *F. oxysporum* f. sp. *radicis lycopersici* in tomato (Marios *et al.*, 1981).

Tomato Varieties and Disease

A resistant variety is one of the best answers to sustain the attack of a soil inhabitant like *F. oxysporum* f. sp. *lycopersici* which defies other means of control. The first report on the development of varieties resistant to Fusarium wilt of tomato was by Essary in Tennessee as early as 1912. Bohn and Tucker (1939) were the first to report a high level of resistance against this disease found in Missouri accession 160 (PI 79532) of *Lycopersicon pimpinellifolium* Mill. They also reported that resistance in accession 160 is dependent on single dominant genetic character.

In India, host resistance to Fusarial wilt of tomato was studied by evaluating a collection of 134 cultivars, breeding lines and allied species of tomato. Of these genotypes eight possessed a high degree of resistance to race 1 of *F. oxysporum* f. sp. *lycopersici*

(Kesavan and Chowdhury, 1977). Chauhan (1988) studied the reaction of varieties/lines of tomato to Fusarium wilt at Hisar. None of the varieties/lines was immune or resistant. Nine varieties/lines were found moderately resistant (11-30 per cent). Of the remaining 44 varieties/lines 17 were susceptible and 27 were highly susceptible. Recently Banerjee *et. al.*, (1990) eavluated 48 entries consisting of promising cultivars, lines and wild species of *Lycopersicon* under disease stress conditions in early spring seasons of 1987 and 1989. Two accessions of *L. peruvianum*, 'EC 148897' and 'EC 148898' were near immune as there were no symptoms of vascular browning or wilt till 65 days after inoculation. However, 'EC 144975' and 'B6013' of *L. hirsutum* f. *glabratum* were highly resistant and *L. Pimpinellifolium* 'A 1921 and L. peruvianum; B6002/77' were resistant. *L. hirsutum* f. *glabratum* 'EC 144975 and *L. cheesamanii* 'EC 102193' showed no plant mortality but vascular browning. Of the cultivated varieties 'Walter' exhibited the minimum score for susceptibility and 'Columbia', 'Roma', 'HS 110', 'Homestead No.24', 'Flora-Dade', 'Ace', 'Sel-26', 'Sel 22 and 'Sel 30' had a low rating for wilt incidence.

Seedling Age and Disease.

Foster and Walker (1947) found that neither size nor vigour of the host as determined by particular variation in the environmental conditions or by age of the plants at the time of inoculation showed any consistent correlation with subsequent disease development. Retig *et al.*, (1967) reported that age of the seedling had no correlation with final percentage of diseased plants although it influenced the nature and time of appearance of disease symptoms. The disease appeared a few days later in the older plants than in the younger plants. Kesavan and Chowdhury (1977), found no significant difference in wilt indices among different ages of the seedlings. Stunting was more severe when seedlings with a single true leaf were inoculated. The disease progress was more rapid with younger seedlings. Young seedlings succumbed to the disease 5-10 days earlier which was attributed to their faster reaction resulting in the collapse of the young and developing root system.

Age of Inoculum and Disease :

The reports regarding the age of the fungal cultures to be used unanimously indicated that the culture must be old enough preferably 3-4 weeks old to cause wilt (Fisher, 1935; Wellman, 1939; Foster and Walker 1946; Gerdemann and Finley, 1951). This was later confirmed by Kesavan and Chowdhury (1977) who observed significantly higher wilt indices when cultures of 3 or more than 3 weeks old were used. Young cultures (one or

two weeks old) were found inefficient in producing wilt. The fact that the fungus *F. oxysporum* f. sp. *lycopersici* produced significant quantities of pectolytic enzymes (Gothoskar *et. al.*, 1955) and wilting toxins (Sanwal, 1956) at the end of 2nd week after seeding in liquid media might explain the above results. It was opined that the fungus could produce effective amount of propagules at this period to cause wilting.

Method of inoculation and Pathogenicity :

Various techniques were employed by different investigators for inducing wilt symptoms in plants. The techniques included mixing of 3 week old wheat kernel culture at the rate of 50 g to sterilized soil for each 4" pot (White, 1927); pouring a heavy spore suspension and mycelial fragment suspension to the plants grown in pots containing sterilized soil (Haymaker, 1928), dipping of injured roots of transplants in the mycelial suspension (Wellman, 1939); Inoculating the soil with fungus culture and transplanting the seedlings (Schroder *et. al.*, 1953); pouring suspension around the root zone (Henderson and Winstead, 1961); mixing of oat : soil meal culture (Varma, 1954); sand maize meal medium (Sen and Kapoor, 1974) and soil inoculation (Kesavan and Chowdhury, 1977). Haymaker (1928) observed no difference in pathogenicity when two strains of *F. oxysporum* f. sp. *lycopersici* were added to soil either as spore suspension or as wheat kernel culture.

Disease Assay :

The pathogen *F. oxysporum* f. sp. *lycopersici* causes a continuing grade of symptoms with epinasty and ending with wilting and withering. Hence assessment of disease severity enables us to find out the effectiveness of any management practice employed. Wellman (1939) advocated a system of numerical disease evaluation based on symptoms of Fusarial wilt in tomato. Plants were given numbers from 0-15 according to the severity of the disease. These 15 categories were condensed to four classes of resistance. Subsequently, Walker and Foster (1946) gave a detailed disease index which was accepted widely. Their system was composed of numbers ranging from 0-4, indicating plants in the range of healthy to dead. The number of plants in each class was multiplied by the respective class rating. The sum of the products thus obtained was multiplied by 100. This product was then divided by maximum rating x total number of plants tested to secure the disease index which can vary from 0-100 according to the severity of the disease. A similar method was adopted by several investigators (Henderson and Winstead 1961; Kesavan and Chowdhury, 1977; Kapoor, 1987; Banerjee *et. al.*,1990; Mandal and Sinha,

1992; Malhotra and Vashistha, 1992). The other method employed was to calculate the percentage of plants diseased or wilted. This was particularly useful in genetic studies as used by Kedar *et. al.*, (1967) and Retig *et.al.*, (1967).

2.4. INFLUENCE OF ORGANIC AMENDMENTS IN BIOCONTROL :

Biological control of plant diseases aims at reduction in inoculum density or pathogen activity. Among the several biological control methods soil amendment is one of the effective methods. Amendment in the form of plant debris, green manures, farm yard manure, compost, oil cakes and fertilizers are known to improve crop productivity by improving nutrient status and soil tilth. Diseases may be reduced by certain amendments due to suppressiveness. Addition of amendments to soils may increase microbial activities in soil to supress disease (Siva Prakasam, 1991). The use of organic amendments for the control of diseases of certain crops like corn, soybeans, wheat, potato and other major crops are generally prohibitively costly on large modern farms. Organic amendments are perhaps more practical for biological control of plant pathogens of smaller and diversified farms, especially for pathogens of vegetable crops and in orchards (Cook and Baker, 1983).

2.4.1. Plant Residue :

The earliest report on the use of organic amendments for biological control was by Sanford (1926), who reported control of potato scab by ploughing green rye under the soil. Even before this, utility of organic manure to improve crop production was realized nearly as old as farming itself. The role of plant residues in the biological control of soil-borne pathogens has been recognised, studied and made use of for decades (Baker and Cook, 1974; Singh, 1983). Population of *F. oxysporum* and *F. solani* responsible for seedling disease of soybean was reduced when artificially infested soils were amended with 1% plant and animal residues (Zakaria and Lockwood, 1980). Soybean, linseed and cotton seed meals incorporated into a loamy sand soil at 1% concentration reduced chlamydospore population densities of *F. oxysporum* and *F. solani* from 10^5 g^{-1} to 10^2 g^{-1} or less after four to six weeks of incubation in closed containers (Zakaria *et. al*, 1980). Soil amendments with crop residues of oats minimized wilt incidence of pigeon pea and number of propagules of *F. udum* in soil (Kotasthane and Om Gupta, 1986). Amendments like neem (*Azadirachta indica)* leaves and *Laucaena* leaves reduced root rot of soybean (Arjunan *et. al.*, 1987). Low fungal population and frequency values were observed in the rhizosphere soils amended

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with chopped leaves of neem and china berry / bakaim neem (*Melia azadiracht)* in tomato (Prakash *et. al.,* 1985). Neem oil at concentrations of 2.5 to five per cent inhibited radial growth of *F. moniliformae* by 57.8 and 66.7 per cent respectively and at ten per cent completely inhibited the fungus (Vir and Sharma, 1985). Seed treatment with crude neem oil improved germination and gave 13.2 per cent disease control in tomato against *F. oxysporum.* Seed treatment combined with root dip application provided 42.3 per cent disease control (Raj and Kapoor, 1993).

2.4.2. Oil Cakes

Oil cakes are used as organic manures. Neem cake at 0.5 to five percent level was stimulatory to vegetative growth of *F. oxysporum,* f. sp. *lini; F. oxysporum* f. sp. *udum* and *F. oxysporum* f. sp. *vasinfectum* (Singh and Singh, 1970). There are various reports on neem cake being used against plant pathogenic fungi. The oil cakes of castor, neem, mahua, peanut and mustard at 25 q/ha reduced disease intensity of black scurf of potato. Mustard cake provided the best control followed by peanut, neem and castor cakes (Singh, 1968). *Fusarium solani* disappeared in safflower, groundnut and neem cake amended soils (Khalis and Manoharchary, 1985). Crossandra wilt caused by *Fusarium solani* was reduced when neem cake was added as soil amendment. The pathogen population was also found to be reduced (Lakshmi and Jeyarajan, 1987). Suppresion of muskmelon wilt caused by *F. solani* was reduced by mustard cake (2%) groundnut (1%) and neem cake (2%) and saw dust (1%). Neem cake reduced wilt by 80 per cent followed by mustard cake (65%) and saw dust (45%). Compared to check, populations of soil fungi and bacteria were 26 and eight per cent higher respectively (Chakrabarti and Sen, 1991). Singh *et. al.* (1980) investigated the effects of seed treatment with certain oil cakes including neem cake or nematicides on tomato growth and rhizosphere population of fungi. Pot / seed treatment with neem cake powder was found to be effective in reducing population of fungi during early stages.

2.4.3. Composts and Farm Yard Manure (FYM)

Success or failure of compost treatment for the control of disease depends on the nature of the raw product from which it was prepared, the process of composting used and the maturity of the compost (Lakshmi and Jeyarajan, 1987). Brown rot of yam caused by *Fusarium* sp. was found decreased when composted larch bark was added to infested plots. This decrease was attributed to increase in the population of *Trichoderma* spp. and

not due to bacteria and actinomycetes. (Sekiguchi, 1977). FYM at 10 tonnes ha^{-1} reduced seedling disease of cotton by *Rhizoctonia solani* by increasing the soil microflora (Ramakrishnan and Jeyarajan, 1986). Addition of FYM at 10 tonnes ha^{-1} and inoculation of soil with *Trichoderma viridae* reduced post-emergence mortality and improved seed germination of cotton (Krishnamohan and Kanda Swamy, 1986). In pot and field experiments chicken manure reduced wilt incidence in tomato caused by *F. oxysporum* f. sp. *lycopersici* and it was observed that the disease reduction was due to reduction in pathogen population (Homma *et. al.*, 1979). Later it was found that reduction in Fusarium wilt in tomato was not due to reduction in pathogen propagules but due to inhibition of germination and further retardation of growth of the pathogen in rhizosphere soil and rhizoplane of tomato. However, Homma *et. al.*, (19 79) opined that increased activity of other microorganisms and antifungal substances is found to be the chief factor for the reduction in wilt of tomato.

2.4.4. Mechanism of Biocontrol by Organic Amendments :

Suppression of soil-borne pathogens by organic amendments operates through several mechanisms. Organic matter influences soil physical characters such as pore size, aeration, temperature, water retention capacity etc. These help in better solubulization of minerals together with the nutrients released by decomposing organic matter which in turn aid in rapid expansion of the root system, better uptake of nutrients and finally better plant vigour. In many diseases this can help the plant to resist attack by pathogens or replace the damaged roots quickly by new roots (Singh, 1983). Decomposition of organic amendments, being a biological process stimulates microbial activity both quantitatively and qualitatively, antagonistic to plant pathogens (Johnson, 1963; Papavizas, 1963, Bouhot, 1981). Plant residues/organic amendments also control soil borne plant pathogens by propagule germination lysis. Mitchell *et.al.* (1941) reported for the first time that germination lysis of sclerotia of *Phymatotrichum omnivorum* Duggar occured after the addition of organic amendments.Chinn *et. al.* (1953) observed that soybean meal stimulated conidial germination of *Helminthosporium sativum* Pam but germ tubes subsequently lysed and the propagules were killed. Nonvolatile and volatile substances released during the decomposition of organic amendments was found to have suppressive effect on many soil borne plant pathogens (Lewis and Papavizas, 1971; Linderman and Gilbert, 1975; Papavizas, 1976; Dwivedi and Dubey, 1986).

2.5. Compost Extracts Against Plant Pathogens.

Weltzein and Ketterer (1986) were the first to report the use of watery compost preparations against foliar disease like downy mildew of grape caused by *Plasmopara viticola*. Liquid compost preparations suitable for spray application on plants was developed using tap water as extractant. Ratios of 1:5 to 1:10 of compost to water were found most suitable. Mixture would be allowed to ferment for 5-8 days at 15-20°C. The extracts were filtered just before use through cheese cloth or milk filters to remove all larger particles to avoid clogging of spray nozzles. Horse, cattle, pig and goat manure composts were used to prepare extracts (Weltzein, 1991). The microbial composition of the extracts was studied by several workers. Ketterer (1990) observed the colony forming units (cfu) increased from extraction days 1-14 days and decreased afterwards. The highest count was 1.1×10^{11} per ml after 14 days from horse manure compost extract, an increase of 10^3 from day one. Stindt (1990) differentiatesd among the groups of micro-organisms on selective media and concluded that compost extracts contain very rich and varied microbial population, which could play an important role in disease suppression. Direct inhibition of zoospore release in case of *Phytophthora infestans* and *Plasmopara viticola* by compost extracts was attributed to unknown extract qualities (Ketterer, 1990). Eight day old Cattle manure compost extract completely inhibited growth of *Botrytis cinerea* when added (1:1 v/v) with water agar. It was concluded that direct inhibition of germination and mycelial growth was the main factor in *Botrytis cinerea* inhibition by compost extracts (Stindt, 1990). Weltzein and Ketterer (1986) observed reduction in infected leaf area by 95% compared to control, when horse manure compost extract was sprayed after an extraction period of three days. Winterscheidt *et. al.* (1990) achieved 85% reduction of cucumber downymildew with cattle manure compost extract after three days of extraction time. Thus for the first time compost extract age was considered as an important factor. Efficacy of compost extracts in field experiments was also proved effective with grapevine (downymildew), potato (Late blight) and Strawberry (Grey mold) (Ketterer, 1990, Stindt and Weltzein, 1988 and Stindt, 1990). Extracts of horse manure compost sterilized by filtration through a pore size of 0.2 µ and heat sterilized by autoclaving for 40 minutes at 120°C lost their activity against *Phytophthora infestans* completely (Ketterer, 1990). Similarly Sitndt (1990) also observed loss of activity of extract of horse manure compost against *Botrytis cinerea*. However, as extraction time increased, heat stability at medium temperatures decreased.

2.6. COW PRODUCTS IN THE MANAGEMENT OF CROP DISEASES :

Munshi (1964) in his monograph has given an account of cow products viz; milk, curds, ghee, dung and urine along with several kinds of plant products in the ancient agricultural practices followed in India. Importance of manure in crop cultivation was recognised in as early as 1500 BC in 'Rigveda'. The manure chiefly consisted of excreta of various animals mixed with litter which absorbed the urine and kept the animals clean, bones, flesh of animals, fish washings. Varahamihira (500 - 700 AD) in 'Brhat-Samhita' prescribed that seeds should be treated with ghee, soaked in milk, dried and repeated the process for 10 days, then seeds were rubbed with cow dung and sown followed by sprinkling with water mixed with milk.

According to Munshi (1964), various laws and instructions are quoted in 'Atharvaveda' (1500-500 BC) regarding protection of crops from insects, pests and diseases 'Vrksayurveda' describes the 'Science of medicine for plants', where treatment of plant diseases by cow products viz milk, ghee, curd, urine and dung was mentioned either individually or in combination of all the above with various plant products. The ailments of trees caused by vermin were reported to be cured by treating the affected trees with the paste of cow's urine, ghee, vidanga (*Embelia ribes*), mustard (*Brassica nigra*) and sesamum (*Sesamum indicum*) and watered with diluted milk. Treatment given with the bark of karanja (*Pongamia glabra*) pounded in cow's urine along with musta (*Cyperus rotundus*) and vidanga destroyed the vermin. Trees showing withering of branches either for want of watering or due to excessive watering recovered when watered with a mixture of ghee, vidanga and milk for just one week.

Similarly in 'Visva Vallabha' prescription of various kinds of medicines with offensive odour, acrid taste or magical diagrams used to protect the plants from pests and diseases are mentioned. Surapala in his book 'Vrksayurveda' suggested plastering of roots and tree branches at the time of transplanting with paste of ghee, usira (*Andropogon muricatus*), sesamum, honey, vidanga, milk and cowdung against diseases (as quoted by Munshi 1964).

The term 'panchagavya' according to the explanation of technical terms used in 'Ayurveda' includes exclusively cow's ghee, juice of cow dung, curds prepared of cow's milk, cow's milk and cow's urine. This preparation is being administered to consume orally as a act of purification to persons before certain religious rites since the ancient

times of 'Mahabharatha'. 'Panchagavya ghritha' is extensively used in Ayurveda to cure several human diseases such as epilepsy, jaundice, and fever. It is prepared by cooking cow's ghee by adding equal proportions of the juice of fresh cow dung, sour curds prepared out of cows milk, cow milk and cow urine. 'Maha panchagavya grhritha' contains many drugs besides panchagavya and is like ambrosia in the treatment of epilepsy, tumors, piles, amaemia, juandice and irregular fevers (Charaka Samhita, 100 - 500 AD).

Numerous obnoxious concoctions were used as early as in the 17th and 18th centuries for treatment of tree wounds. Treatment of fresh pruning wounds with a mixutre of cow dung and urine was advocated to prevent canker disease of apple (Austen, 1657). Application of a mixture of fresh cow dung, lime, wood ashes and sand to fresh tree wounds was recommanded by Forsyth (1791).

Jim Martin derived a colourless, odourless liquid called living water from sea water, cow manure and a few simple ingredients. The living water formed by fermentation of these substances possessed certain remarkable qualities. It could stimulate microbes that exist in nature to multiply rapidly and cleanse polluted water and soils, neutralize chemicals, eat sewage and sludge, even make deserts bloom. Martin demonstrated all these uses even as early as in 1950 but his work was ignored. Today at least six Texas companies are quitely peddling variations of Martin's seminal breakthrough, everywhere from Middle east to American garden shops. These substances have become a subject of study seriously by scientists impressed by their phenomenal abilities. Horticultural specialists and farmers were very much impressed by the power of these products, confirmed in controlled tests, to dramatically increase crop production and to stanch soil erosion. Others have noted capacity to reclaim salt stressed soils and allow crops to thrive with salt water irrigation. Martin used dung from milking cows for preparing formulations and expressed that it contained unidentified biological active principle which was responsible for enhancing crop growth (Curtis, 1992).

2.7. USE OF *Trichoderma spp.* IN BIOLOGICAL CONTROL

Trichoderma species represent the antagonistic activities against soil-borne plant pathogens, though excellent biocontrol agents, their use in biological control, for the most part, has been restricted to laboratory, green house and experimental field plots. Some successful biological control of soil borne plant pathogens like *Sclerotium rolfsii* inducing

root rot of several crops has been achieved (Chet, 1991 ; Elad *et. al.*, 1980), but only when applied at realtively high inoculum rates. *T. hazrzianum* has been shown to be an effective agent in suppressing *Rhizoctonia solani* (Chet and Baker, 1980). *Trichoderma harzianum* has shown potential to control diseases caused by *Fusarium* spp.A strain T-35 of *Trichoderma harzianum* controlled Fusarium wilt of cotton and melons caused by *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f.sp. *melonis* respectively, *Fusarium* seedling blight in wheat caused by *F. culmorum* all under natural soil conditions (Kempf and Wolf, 1989 and Sivan and Chet, 1986)

2.7.1 Occurrence and Distribution

Trichoderma is widely distributed all over the world (Domsch *et.al.*, 1980) and occur in nearly all soils and other natural habitats, especially in those containing organic matter. Individual species aggregates may be restricted in their geographic distribution. *Trichoderma* seems to be a secondary colonizer, as its frequent isolation from well decomposed organic matter indicates (Danielson and Davy, 1973). Danielson and Davy (1973) concluded that certain strains of *T. hamatum* and *T. pseudokoningii* were adopted to conditions of excessive soil moisture and that *T. viride* and *T. polysporum* were restricted to areas where low temperatures prevailed, whereas *T. harzianum* was most commonly found in warm climate regions and *T. hamatum* and *T. koningii* were widely distributed in areas of diverse climatic conditions.

2.7.2 Survival and Proliferation in Soil

Very little is known about the fate of conidia of *Trichoderma* added to soil or to any other environment. Davet (1979) reported that conidia added to soil decreased initially, then stabilized for two years to about one tenth the original number added. Papavizas (1981) found that some of the conidia of *T. harzianum* added to soil without nutrient supplying amendments survived between 110 and 130 days, but the length of survival depended on the isolate used. He presumed that most of the conidia either might be lysed without first germinating or they germinated in response to some nutrients released from organic matter and subsequently lysed in the absence of food bases adequate enough to sustain further growth and sporulation. Hyphae also survived in the soil (Papavizas *et. al.* 1984) Although the ecological importance of chlamydospores was disregarded in the past, there is now evidence of their importance in the survival of *Trichoderma* in soil. Caldwell (1958) was among the first to observe that chlamydospores survive in soil better than

conidia. Lewis and Papavizas (1984) showed that *Trichoderma* and other potential biocontrol fungi proliferate abundantly in various natural soils when added as young mycelia in intimate contact with food base (sterile moist bran inoculated with conidia and allowed to incubate for one to three days before addition to soil; but not as conidia with or without bran. Population of isolates of *T. viride* and *T. harzianum* increased about 10^4 and 10^3 -fold respecetively during the first three weeks of incubation. Population levels remained constant from nine to 36 weeks after the colonized food was introduced.

2.8. USE OF BACTERIAL ANTAGONISTS IN BIOCOTROL.

Microorganisms that can grow in the rhizosphere are ideal for use as a biocontrol agent, since, the rhizosphere provides the front line defence for roots against attack by pathogens. Pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and also during secondary spread on the root. Bacteria have been proved to have potential for biocontrol, occur in many genera and were found effective even under field conditions. A comprehensive review on the use of rhizosphere bacteria as biocontrol agents against soil-borne diseases has been made by Weller (1988). *Bacillus* spp. were found to be appealing candidates for biocontrol because of their ability to produce endospores to resist heat and desiccation. *Bacillus subtilis* Strain A13, isolated from lysed mycelium of *Sclerotium rolfsii* was reported to be inhibitory to several plant pathogens *in vitro,* (Broadbent, 1972; Yuen *et. al.* 1985). Currently, *Pseudomonas* spp. are receiving much attention as biocontrol agents in the studies initiated at the University of California, Berkeley during 1970s. Burr *et.al.* (1978) observed that strains of *P. fluorescens* and *P. putida,* applied to seed pieces, improved growth of potatoes. There is evidence that *Pseudomonas* have a role in the suppressiveness of certain soils to Fusarium wilt of flax, radish and cucumber (Scher and Baker, 1982). Weller (1988) in his review has stressed the importance of establishment and maintenance of a threshold population of bacteria on planting material or in soil and any drop in viability below that level may reduce the possibility of biological control. Many soil edaphic factors *viz;* soil temperature, soil moisture, soil pH and clay content, influence the survival and establishment of bacteria and their interaction with the pathogens. The way in which the bacteria are cultured and then processed will affect their viability and tolerance to adversse conditions once applied. Root colonization by the introduced bacteria is essential for biocontrol of root pathogens and that increasing the population of an introduced bacterium on the root should enhance disease control (Suslow, 1982). The ability of different rhizobacteria to colonize roots

offering control of several soil-borne pathogens has been reviewed recently (Weller, 1988, Schroth and Becker, 1990). *Bacillus* species which produce endospores can be easily made into commercial formulations for biocontrol of soil-borne pathogens (Kenny, 1981). Formulations with gram negative biocontrol agents were tried with peat and other carriers.A granular peat formulation of a *P. fluorescens* (Dagger^{TM}G showed promise for control of seedling pathogens of cotton (Weller, 1988). Entrapment of bacteria in polymer gels such as Xanthum gum or alginate also proved potential. (Fravel *et. al.* 1985 and Bashan, 1986).

2.9 INFLUENCE OF BIOCONTROL AGENTS AGAINST *Fusarium* spp. CAUSING WILT DISEASE IN TOMATO.

Smith (1957) reported that *Micromonospora* sp. had an inhibitory effect on *F. oxysporum* f. sp. *lycopersici* when grown together on PDA or when culture filtrate of *Micromonospora* was added to agar at 50 per cent concentration. Symptoms of Fusarium wilt were inhibited in tomato plants treated with *Micromonospora* prior to planting in *Fusarium* infested soil or inoculated by swirling the plant roots in broth cultures of *F. oxysporum* f. sp. *lycopersici.* A *Cephalosporium* sp. was found to protect tomato seedlings when treated to tomato seeds (Bhelwa *et.al.*, 1962). A *Cephalosporium* sp. previously described by Smith as a species of *Micromonospora* reduced symptom development of Fusarium wilt of Bonny Best seedlings. Of the methods tested inoculation of seedlings by agitating roots in a *Cephalosporium* culture for several hours resulted in a great retardation of Fusarium wilt symptoms. Increasing the time interval between inoculation of seedlings with the *Cephalosporium* sp. and inoculation with *F. oxysporum* f. sp. *lycopersici* resulted in greater mitigation of symptoms (Chisler *et.al.*, 1962). Costache *et.al.*, (1980) observed that in glass house tests Benlate 50wp (Benomyl) and Bavistin 50wp (Carbendazim) and Derosol 60wp (Carbendazim) at 0.05 - 0.1% were the most effective against *F. oxysporum* f. sp. *lycopersici. Trichoderma lignorum (T. viride)* retarded symptom development by 18-21 days. Panteleev (1971) reported that soil application of *T. viride* reduced tomato wilt from 49.5 - 6.15%. Yehia *et. al.* (1981) found that the antagonists *Trichoderma viride , Streptomyces griseus* and *Bacillus subtilis* effectively reduced damping-off of tomato seedlings caused by *Pythium debaryanum, Phytophthora nicotianae* var. *parasitica* and *F. oxysporum* f. sp. *lycopersici.* Marios and Mitchell (1981) found that when freshly fumigated with methyl bromide - chloropicrin, the nonaugmented soil resulted in 90 per cent infection of tomato cv. Bonny Best after two weeks at 20°C while only 25 per cent were infected by *F. oxysporum* f. sp. *lycopersici* in

similar but augmented soil with *T. harzianum*. Conidial suspensions of three fungal antagonists of *F. oxysporum* f. sp. *radicis lycopersici*, *T. harzianum* at 5 x 10^5 conidia ml^{-1} were applied to roots and crowns of tomato transplants at the time of planting. Pathogen was added at 0, 50, 500 and 5000 chlamydospores pl^{-1} at the time of planting at 10 cm apart from transplant. The incidence of disease increased with increase in inoculum density of the pathogen in fumigated soil not augmented with antagonists but not in augmented soils. The disease incidence was only seven per cent in soil augmented with antagonists while, it was 37% in soil not augmented. Successful cross protection in tomato against *F. oxysporum* f. sp. *lycopersici* with *F. oxysporum* f. sp. *dianthii* was reported by several workers (Wymore and Baker, 1982; Kroon *et. al.*, 1991 and Kroon and Elgersma, 1991). Callegarin and Cugudda (1986) observed that *F. arthrosporioides* and *a Fusarium* sp. isolated from suppressive soil reduced wilt incidence in tomato when transplants were root dipped in mixed conidial suspension of the above and *F. oxysporum* f. sp. *lycopersici*. Martinez and Garcia (1984) observed that inhibitory substances produced by *T. viride* at dilutions of 1:5 to 1:10, reduced *F. oxysporum* infection of tomato seeds by 40-60 per cent without affecting germination. Couteaudier *et. al.* (1985) reported that steamed suppressive soil when augmented with nonpathogenic *F. oxysporum* and *F. solani, the soil* behaved as pathogen suppressive soil. This soil when incorporated at eight per cent into soil heavily infested with *F. oxysporum* f. sp. *lycopersici*, a reduction in wilt was observed for three successive years. Further, a strain of non pathogenic *Fusarium* was multiplied on steamed peat by glucose amendment. Steam treated *F. oxysporum* f. sp. *lycopersici* infested soil, when augmented with peat at 1:10 (v/v) of the soil reduced wilt incidence considerably by the end of first year. Sivan and Chet (1986 and 1987) stated that *T. harzianum* isolated from rhizosphere of cotton plant was effective against *F. oxysporum* f. sp. *radicis lycopersici* on tomato. Application of antagonist either to soil as a wheat bran peat (1:1 v/v) based preparation or as a seed coating decreased disease incidence. D'Ercole *et. al.* (1988) reported that *Trichoderma* spp. could be potential bio-control agents against several soil-borne pathogens in Italy viz., *Fusarium* wilt and *verticillium* wilt of tomato. Monaco *et. al.* (1991) observed the effectiveness of *Trichoderma* spp. against *Fusarium* spp. and *Sclerotium rolfsii* when used as seed dressers. *T. koningii*, *T. harzianum* and *T. aureoviride* , isolated from tomato fields naturally infested with *Fusarium* spp and *Sclerotium rolfsii* effectively controlled these pathogens.

Scher and Baker (1980) observed that *Pseudomonas* sp. when added to conducive soil at 10^5 cells g^{-1} soil reduced Fusarium wilt of flax considerably. Further, they noticed that viability of this organism reduced drastically when the soil was treated with aerated steam at 54°C. In a second study, *Pseudomonas putida* isolated from suppressive soil and EDDHA or Fe EDDHA were added to conducive soil and it resulted in suppression of Fusarium wilts of flax, cucumber and radish. Experimental evidences showed that Fe EDDHA and siderophores produced by *P. putida* had higher Fe stability constants than siderophores produced by various pathogenic *Fusaria* (Scher and Baker, 1982). Laboratory inoculation tests showed that *Pseudomonas pseudoalcaligenes* and the C-3 str. of *Bacillus cereus* were best in inhibiting infection by *F. oxysporum* f. sp. *lycopersici* (Garcia, 1982). Turhan (1981) reported that immersion of seeds or roots and lower stems of seedlings into the homogenate of $C_{/2}$- 9 a new race of *Streptomyces ochraceuscloroticus* before planting in natural, inoculated soil gave protection against *F. oxysporum* on tomato upto 73 per cent. Podile *et. al.* (1985) investigated the antagonism of *Bacillus subtilis* against wilt causing pathogens. Bacillus subtilis appreciably inhibited cultures of *verticillium alboatrum, v. dahliae, F. udum, F. oxysporum.* f.sp. *lycopersici, F. oxysporum.* f.sp. *vasinfectum* and *Ceratocystis ulmi.* A nonpathogenic resistant mutant of *Pseudomonas solanacearum* detoxified fusaric acid and protected tomato cuttings from disease (Toyoda *et. al.* 1988). Kapoor and Kumar (1988) investigated antagonistic effects of soil microbes on *F. oxysporum.* f.sp. *lycopersici,* causing tomato wilt. *Bacillus* sp. showed greater inhibition of tomato wilt pathogen and produced antifungal compounds in culture. Culture filtrates as well as cell free filtrates from *Bacillus* sp. showed inhibitory effect, while *Azotobacter chroococcum* failed to inhibit the wilt pathogen. Peer *et. al.* (1990 and 1991) investigated the involvement of mechanisms other than competetion for iron in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. and found that induced resistance, and increased accumulation of phytoalexins were attributed to be responsible for reduction of disease by *Pseudomonas* sp.

2.10 MECHANISMS OF BIOCONTROL

An understanding of the mechanisms by which biological control of plant diseases occur is critical to the eventual improvement and wider use of biocontrol methods. These mechanisms are generally classified as competetion, parasitism/predation, and antibiosis (Baker, 1968).

2.10.1 Antibiosis in Biocontrol

Antibiosis is antagonism mediated by specific or nonspecific metabolites of microbial origin, by lytic enzymes, volatile compounds or other toxic substances (Jackson, 1965). The role of antibiotics in biocontrol has been studied by the generation of mutants that do not produce antibiotics. Panteleev (1975) reported that a strain of *Pseudomonas* reduced wilt incidence by producing antibiotics. *Pseudomonas fluorescens* is suppressive to *Gaeumanomyces graminis* var. *tritici*, the causal agent of take all of wheat. Production of an antibiotic, phenazine has been implicated in biocontrol (Gurusiddaiah *et. al.*,1986, Thomashow *et. al.*, 1986 and Weller 1985).

Use of Culture Filtrates to Demonstrate Antibiosis.

Bacteria

Autocalved culture filtrates of *Bacillus subtilis* applied 3 times a week controlled bean rust in the field significantly better than fungicide mancozeb applied once in a week. Bacterial antagonists (*Bacillus* spp.) showed greater inhibition of tomato wilt pathogen *F. oxysporum* f.sp. *lycopersici* and produced antifungal antibiotics in culture. Culture broth as well as cell free filtrates of four potent bacterial isolates (*Bacillus* sp.) showed inhibitory effect, whereas three strains of *Azotobacter chroococcum* failed to inhibit wilt pathogen (Kapoor and Kumar, 1988). Bacterial antagonist, *Bacillus* sp. (B_1) could reduce 70 per cent linear growth in four isolates of *F. oxysporum* and two of *F. solani* causing wilt in water melon, similarly culture filtrate of bacterial antagonist (B_1) also inhibited germination of microconidia of *F. oxysporum* (Naik, 1989).

Siderophores in Biocontrol

Siderophores are extracellular, low molecular weight compounds with a very high affinity for ferric ion. The literature on siderophores has been reviewed recently (Leong, 1986).

Scher and Baker (1982) suggested that synthetic chelator Fe EDDHA and siderophore produced by *Pseudomonas putida* had higher Fe stability constants. They also observed that *P. putida* did not produce siderophores in the agar containing 10^{-4} M Fe unless EDDHA or DTPA (2mg/ml) was added. These ligands created iron deficiency in the medium. The presence of EDTA in the agar medium inhibited production of fluorescent siderophores. It

was suggested that the management of Fe availability in the infection court through Fe competition can induce, suppressiveness to *Fusarium* wilt pathogens.

***Trichoderma* spp. :**

Hornby (1983) in his review article on suppressive soils, stated that "a major problem that besets the subject of microbial antagonism in soil is that many of the mechanisms discussed are presumptive and proof is difficult to come by." Hence the mechanisms of biocontrol by *Trichoderma* were also presumptive. Suggested mechanisms for biocontrol were antibiosis, lysis, competetion and mycoparasitism (Ayers and Adams, 1981; Papavizas and Lumsden, 1980; Cook and Baker, 1983).

Toxins :

Weindling and Emerson (1936) isolated an organic metabolite produced by *T. lignorum* in crystalline form which was highly toxic at high dilution to *Rhizoctonia solani* Kuhn. The metabolite was later named gliotoxin (Weindling, 1941). Brian and Mc Gowan (1945) described a second highly fungistatic antibiotic, viridin produced by *T. viridae.* Dennis and Webster (1971a) showed that *Trichoderma* spp. produced antibiotics different from gliotoxin and viridin, which were chloroform soluble antibiotics such as *Trichodermin* by *T. viride* and *T. polysporum* and other peptide antibiotics by *T. hamatan.* Papavizas *et. al.,* (1982) found that several UV-induced mutants of *T. harzianum* which produced two unidentified metabolites, one heat labile, the other heat stable. Upadhyay and Mukhopadhyay (1983) observed that culture filtrates of all the isolates of *T. harzianum* (except isolate 2) significantly reduced the growth of *Sclerotium rolfsii,* when compared with check. Per cent inhibition ranged from 5.67 to 50.67. Martinez and Garcia (1984) reported that maximum concentration of inhibitory substances were obtained with *T. viride* cultures in yeast extract medium after 60 days. Smith (1957) reported that when a 50 per cent concentration of crude culture filtrate of *Micromonospora* sp. was added to agar growth of *F. oxysporum* f. sp. *lycopersici* was inhibited and pigmentation intensified. Somasekhara (1992) studied the effect of culture filtrates of *Trichoderma* spp. on *Fusarium udum* observed that suppressive effect was more at low pH than in the ones adjusted to near neutral level. Increased antagonistic activity at pH 4.0 as compared to 6.5 was reported in many species of *Trichoderma* against several pathogens. The conidial germination of *F. udum* was greatly suppressed in culture filtrates of *Trichoderma* spp. In certain cases the culture filtrates were very effective even when diluted 1:4 with water.

Lytic Enzymes :

The relationship between mycolytic enzymes produced by *Trichoderma* and their significance in host cell degradation have been established by Cook and Baker (1983). Chet and Baker (1980 and 1981) showed that *Trichoderma* produced cellulose, β-(1-3)-glucanase, and chitinase and degrades the glucans in the walls of *Pythium* spp. and the walls of *Rhizoctonia solani.* Sivan and Chet (1986) studied the possible mechanisms for control of *Fusarium* spp. by *Trichoderma harzianum.* Under lab conditions *T. harzianum* did not parasitize colonies of *Fusarium* spp. when tested on agar. However, *T. harzianum* excreted β-1-3 glucanase and chitinase into the medium when grown in liquid medium supplemented with laminarin or chitin as sole source of carbon. The enzyme activity detected in dual cultures of both the mycoparasite and the soil-borne pathogens was suggested as the mechanism by means of which this parasite attacks its host. Fungal cell and sclerotial wall lysis and degradation was mainly due to key enzymes β-(1-3)-glucanase and chitinase (Elad *et. al.,* 1983; Jones *et. al.* 1974) .

Diffusible (Nonvolatile) Toxic Substances ;

Dennis and Webster (1971a) studied the production of non-volatile diffusible antibiotic substances by *Trichoderma* spp. by an agar layer technique. They noticed that many isolates produced non-volatile antibiotics active against a range of fungi. The ability to produce such substances varied between the isolates. The susceptibility of pathogenic fungi also varied widely. *Fomes annosus* was the most susceptible and *Fusarium oxysporum* the most resistant.

Volatile Inhibitory Substances

Antibiosis mediated by volatile substances has received less attention than antibiosis through the production of non-volatile antibiotics, bacteriocins or siderophores (Fravel, 1988). Dennis and Webster (1971b) studied in detail the production of volatile compounds by *Trichoderma* isolates and their inhibitory effect on other fungi. They reported that two isolates of *T. viride* and one isolate of *T. Koningii* inhibited the growth of *R. solani.* There was no relation between sporulation by the different isolates and their ability to produce volatile antibiotics. At no stage there was complete inhibition of test fungus. The effective isolates especially members of the *T. viride* presented a characteristic smell. The susceptibility varied with the *Trichoderma* isolate used. The active metabolites had the

same morphological effect on all the susceptible fungi resulting in stunted mycelial growth. The hyphae were more highly branched than normal hyphae. Acetaldehyde was identified tentatively as one inhibitory metabolite of *T. viride* . Studies on the effect of volatile compounds of *T. harzianum* on the growth of *Sclerotium rolfsii* revealed that, when *Trichoderma* isolates were grown for 3 days and *S. rolfsii* was exposed for 48 hrs vapour action, the growth of *S. rolfsii* was reduced in case of isolates 3 and 4 and stimulated in isolate 1 and 2. The vapour action of *T. harzianum* on 6th day was most inhibitory and thereafter it declined and reached zero on 15th day (Upadhyay and Mukhopadhyay 1983).

2.10.2. Mycoparasitism and Hyphal Interactions :

Following the discovery that *Trichoderma* has great potential for biocontrol (Weindling, 1934), many researchers dealing with *Trichoderma* noticed that hyphae of the antagonists parasitized hyphae of other fungi. Dennis and Webster (1971c) studied in detail the ability of a number of isolates from the different species groups of *Trichoderma*, to coil around or to penetrate hyphae of other fungi, when grown in dual culture. Hyphae of the majority of *Trichoderma* isolates coiled around hyphae of the majority of fungi. Penetration of hyphae seldom occurred. Of the 80 *Trichoderma* isolates tested, only 10 did not show coiling. Most isolates coiled around the hyphae of all test fungi, but some did not interact with *Fusarium oxysporum* Schlecht. ex. Fr. especially isolates belonging to the *T. polysporum* (Link ex pers.) Rifai and *T. piluliferum* Webster and Rifai species groups. The hyphae of test fungi nearest to the *Trichoderma* colony showed vacuolation and coagulation of cytoplasm in *R. solani* and *Fomes annosus*. The host fungus generally ceased growth soon after contact, whether or not the coiling fungus was an antibiotic - producing isolate. The *Trichoderma* isolates continued to grow and eventually covered the whole plate. Attempts to reisolate the test fungi generally failed, but very occasionally proved possible to reisolate *F. oxysporum*.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigations comprising laboratory, glass house, nylon net house and field experiments were conducted during 1991-1993 at the University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bangalore. The details of the methodology in these investigations are described.

1. STERILIZATION

Sterilization of glassware (wrapped in butter paper) and media was achieved by autoclaving at 15 lbs PSI(121.6°C) for 20 minutes.Sand and soil were sterilized by autoclaving at 15 lbs PSI for 1 hour for 3 consecutive days. The seeds and plant tissues were surface sterilized by 1% Sodium hypochlorite followed by three changes in sterile water. All cultural work and *in vitro* experiments were carried out under aseptic conditions under laminar flow chamber or isolation chamber. Using flame for sterilizing the tips of inoculation needle and foreceps.

2. ISOLATION AND MAINTENANCE OF THE PATHOGEN

Tomato plants showing typical symptoms of Fusarium wilt were collected from fields of cultivators during survey around Bangalore, plant material received from other places in Karnataka and UAS experimental fields. Isolations were made from the roots and the collar region of plant sample after a thorough surface sterilization with 1% Sodium hypochlorite followed by three washings with sterile water, on PDA. The isolates were purified by establishing single spore cultures by standard dilution plate method. The pathogens were identified with the help of descriptions of Subramanyam (1970) and Booth (1971) as *F. oxysporum* f.sp. *lycopersici* and *F. solani* groups. The cultures were maintained on freshly prepared PDA slants at 5°C and by periodically transferring to fresh medium.

3. TEST PLANT

Tomato (*Lycopersicon esculentum* Mill.) cv. Pusa Ruby was used in all the experiments unless mentioned other-wise Seeds of this variety were extracted from fully ripe healthy tomato fruits collected from fields by fermentation method in sufficient quantities and used for various experiments. The germination percent varied between 80-90 percent. Other Tomato varieties used in the investigations were Arka Vikas, Arka Saurabh, Arka Ashish, Arka Abha, Arka Alok, Arka Ahuti and Arka Vishal (obtained from IIHR, Bangalore). Pusa Early Dwarf, Roma, Best of All, Rupali, Rashmi, S-28, Punjab Chuhhara

Plate 1

a) Nylon net house employed for raising seedlings and conducting pot experiments.

b) Interior of nylon net house



a



b

PLATE 1

(all purchased from local markets) ATV-1 and ATV-2 obtained form Lathur, Maharashtra. Since the seeds of these varieties were treated with fungicide they were soaked in sterile tap water for one hour later drained the water and dried before using these varieties for experiments in order to reduce the fingicidal residue.

For the assessment of pre and post-emergence seedling mortality seeds were thoroughly surface sterilized with one per cent sodium hypochlorite for two minutes and washed three times in sterile water and air dried. Such seeds were sown in to pots containing infested soil.

Tomato seedlings were raised in cement pots (60 x 30 x 45 cm) containing sterilized sand. The pots were kept in nylon net house to prevent insect attack and viral diseases (plate 1). Seedlings were also given protective insecticidal sprays with Malathion (0.1 per cent), Nuvacron (0.05 per cent), Quinolphos (0.1 per cent).

4. PRODUCTION OF INOCULUM OF *Fusarium* spp.

Fusarium spp. were multiplied in sand maize meal medium at 27 ± 1°C for 3 weeks and used as soil inoculum. The medium was prepared by autoclaving 90 g dry sieved sand and 10 g maize meal with 40 ml of water in 500 ml Erlenmeyer flasks at 15 lbs PSI for half an hour for 3 consecutive days. The inoculum was added to the soil as per the required concentrations for various experiments by (w/w) basis and placed on the upper layers of the soil and allowed for 7 days to get infested (Sen and Kapoor, 1974).

Test pahogen was also multiplied on PDA in petriplates and after 7-10 days of growth conidia and mycelia were harvested by flooding the petriplate with sterile water and by camel hair brush to dislodge conidia and mycelial bits into water. This spore suspension thus obtained was used for experiments.

The third method followed was multiplying the pathogen in potato dextrose broth (PDB). One hundred ml of PDB was taken in to 250 ml Erlenmeyer conical flask and autoclaved at 15 lbs PSI for 20 minutes. A mycelical disc cut from the margin of young culture grown in petriplate was introduced in to PDB under aseptic conditions. The flasks were incubated at laboratory temperature at 27 ± 1°C for 15 days. The mycelial mats were collected after 15 days, washed with sterile water and conidia along with mycelium were extracted using waring blender and used for further experiments. The spore suspension was filtered through cheese cloth before use and spore load ml^{-1} was computed by using a haemocytometer.

5. SOILS

For all pot culture experiments red sandy loam soil (sand 75%, silt 9%, clay 16%, organic carbon 0.4%, water holding capacity 20%, pH 6.5 and EC 0.35) was used. Soil was collected from a fallow land without any crop for the previous 18 months. The *Fusarium* population was assessed on *Fusarium* selective medium (Nash and Snyder, 1962). After sifting the soil mixed with FYM (3 : 1) was sterilized by autoclaving at 15 lbs PSI for one hour for three consecutive days.

Five soil types were employed to assess the influence of soil type on disease incidence. The five types of soils and the analysis of soils is given below regarding their physical and chemical characteristics (Table 1).

Table 1. Physical and Chemical characteristics of soils used in the study.

Texture	Composition (Per cent)			pH	Electrical Conductivity m. mhos	Organic carbon
	Sand	Silt	Clay			
Sandy	79	11	10	7.2	0.72	0.35
Sandy Loam	75	9	16	6.5	0.35	0.40
Sandy Clay Loam	65	10	25	6.8	0.233	0.20
Sandy Clay	54	11	35	6.4	0.30	0.84
Clay	13	23	64	9.1	0.45	0.56

6. ESTIMATION OF *Fusarium* POPULATIONS FROM SOIL SAMPLES

Populations of *Fusarium* sp in different soils were estimated by dilution plate technique using the *Fusarium* selective medium (Peptone quinozene (PCNB) medium) of Nash and Snyder (1962). The composition of the medium is given in apendix.

7. ENUMERATION OF COLONY FORMING UNITS (CFU) OF *Trichoderma* spp.

Population densities of *Trichoderma* spp. from soil samples were estimated by dilution-plate technique using *Trichoderma* selective medium with captan (TSMC) developed by Elad *et al.*, (1981). The composition of the medium is given in apendix.

8 ENUMERATION OF SOIL MICROFLORA (FUNGI, BACTERIA AND ACTINOMYCETES) (Johnson and Curl, 1972)

The procedure given by Johnson and Curl (1972) for enumerating soil microflora was followed for both from rhizosphere and non rhizosphere soils using standard media, the composition of which is given in Appendix I. Ten grams of soil on oven dry weight basis from the sample was used for enumeration of soil microflora. The soil was transferred to a volumetric flask and volume made upto 100ml. This was serially diluted and plated to detect fungi, actinomycetes and bacteria. The dilutions used for fungi were 10^3 to 10^4 plated in Martin's rose bengal agar (MRBA). For actinomycetes dilutions of 10^2 and 10^3 were employed and the medium starch-casein agar was used. The bacteria were enumerated using soil extract agar (SEA) with 10^5 and 10^6 dilutions

Three replications were maintained for each organism under each dilution. The plates were incubated at 26±2°C. The colonies were counted three, five, and ten days after plating for bacteria, fungi and actinomycetes respectively and expressed as cfu per gram dry weight soil.

9 ORGANIC SOIL AMENDMENTS

Amendments used in the present study consisted of mainly crop residues. Fallen leaves of some fruit and forest trees, green leaf material, oil cakes, coffee and tea wastes. The list of amendments used with common names, botanical names and plant part used is presented in Table 2. For dry plant material, they were thoroughly sun dried, chopped to one cm bits and stored at room temparature in sealed polythene bags for further use. Green leaf material was also chopped to bits of 1 cm and used immediately for incorporation into the soil. The required quantities of amendments by weight were added to the soil in plastic trays and hand mixed thoroughly. The amount of amendments applied was expressed as percentage of air dried soil. The amended soils were transferred to 25 cm diameter earthern pots, moisture adjusted and maintained approximately at ten percent water holding capacity (WHC) by periodical watering. The pots were covered with polythene sheets and were kept in green house where the air temparatures varied between 22°-30° C during different periods as required in different experiments.

Table 2. List of Organic Amendments

S. No.	Botanical Name	Common Name	Plant Part used
1.	*Acasia auriculiformis* Cunn.	Acasia	Leaves
2.	*Arachis hypogaea* L.	Groundnut	Cake
3.	*Azadirachta indica* Juss.	Neem	Fresh Leaves
4.	*Azadirachta indica* Juss.	Neem	Dry Leaves
5.	*Azadirachta indica* Juss.	Neem	Cake
6.	*Camellia sinensis* (L) Kuntze.	Tea	Waste
7.	*Coffea arabica* L.	Coffee	Waste
8.	*Dolichos uniflorus* Lam..	Horse gram	Haulms
9.	*Eucalyptus* hybrid	Eucalyptus	Leaves(DL and FL)
10.	*Helianthus annus* L.	Sunflower	Cake
11.	*Madhuca induca* L..	Mahua	Cake
12.	*Mangifera indica* L..	Mango	Leaves (DL and FL)
13.	*Pongamia glabra* Vent.	Pongamia	Fresh Leaves
14.	*Pongamia glabra* Vent.	Pongamia	Dry Leaves
15.	*Pongamia glabra* Vent.	Pongamia	Cake
16.	*Ricinus communis* L.	Castor	Cake
17.	*Sesamum indicum* L.	Gingelly	Cake
18.	–	Farm Yard Manure (Cattle)	-

10. ISOLATION AND MAINTENANCE OF *Trichoderma* spp.

Trichoderma spp. were isolated from soil by dilution plate technique on selective medium (TSMC). The soil samples were collected from the rhizosphere of apparently healthy plants collected in between two wilted plants in the fields from Hebbal, and around Bangalore. Fungal cultures maintained in the Department of Plant Pathology, University of Agricultural Sciences, GKVK Bangalore were also used in the investigation. These isolates were maintained on PDA by periodical subculturing.

11 ISOLATION AND MAINTENANCE OF BACTERIAL ANTAGONISTS

Bacterial antagonists were isolated from rhizosphere soils of apparently healthy tomato plants between wilt affected plants by dilution plate technique on soil extract agar and kings B medium for non fluorescent and fluorescent bacteria respectively. Some isolates were obtained from the Departmentof plant Pathology, UAS, Bangalore. Isolates were maintained in Nutrient broth and Nutrient agar by periodical subculturing.

12. MODIFIED *Panchagavya* (MPG) MIXTURE

Three formulations of *Panchagavya* were prepared which were designated MPG-1, MPG-2 and MPG-3. The composition of the preparations is given in the Table 3. All the components were added one after another in to plastic cans and thoroughly mixed and their initial pH were determined. They were allowed to ferment in the closed plastic containers for seven to ten days. The crude preparation was diluted to ten times with water. All the three formulations were prepared separately. The diluted preparations were filtered through two layers of muslin/ cheese cloth to remove larger particles and the filtrate was used for seedling dip, soil drench in the experiments. The liquid formulation was also amenable for spraying. MPG-3 was used for all the experiments unless otherwise mentioned.

Table 3 Composition of Modified *Panchagavya* Mixture

S. No.	Item Per cent	pH	MPG - 1	MPG - 2	MPG - 3
1.	Ghee	6.9	20.0	5.0	2.0
2.	Curds	5.1	20.0	10.0	5.0
3.	Milk	7.0	20.0	10.0	5.0
4.	Fresh Cow dung	7.2	20.0	40.0	48.0
5.	Cow urine	8.2	20.0	35.0	40.0
	Total		100.0	100.0	100.0
6.	Common Salt	7.5	2.0	2.0	2.0
7.	Yeast		0.01	0.01	0.01
	pH on 0 day		6.8	7.2	7.3
	pH after 10 days		4.8	6.0	6.6

13. COMPUTATION OF DISEASE INDEX

The disease incidence was computed by adopting 0-4 scale to cover all broad symptomotological crieteria (Kesavan and Chowdhury, 1977)

0 = no symptoms.

1 = Slight drooping of leaves and/or vascular browning in root region and no plant mortality.

2 = Wilting of lower leaves and/or vascular browning extended in root region and no plant mortality.

3 = Severely wilted, all leaves wilted and withered except terminal buds.

4 = Dead plants.

Each of these symptoms was given due weightage as all the symptoms contributed for hindering the yield. Plants showing any of these symptoms were considered diseased. To quantify the disease severity, per cent plants infected (PPI); percent disease incidence (PDI) and coefficient of disease index (CODEX) were calculated by the below given formulae and classification of disease reaction is given in**Table** 4 (Banerjee et. al., 1990).

$$PPI = \frac{\text{Number of infected plants}}{\text{Number of plants observed}} \times 100$$

$$PDI = \frac{\sum \text{Total scores}}{\text{Maximum grade X total number of plants assessed}} \times 100$$

$$CODEX = \frac{PPI \times PDI}{100}$$

Pre-emergence and Post-emergence seedling disease were calculated by employing formulae given by Gangopadhyaya (1990).

$$\text{Pre-emergence Seedling Disease} = \frac{\text{Number of seedlings emerged in healthy soil - Number of seedlings emerged in sick soil}}{\text{Number of seedlings emerged in healthy soil}} \times 100$$

$$\text{Post-emergence Seedling Disease} = \frac{\text{Number of seedlings killed}}{\text{Number of seedlings emerged}} \times 100$$

Table 4. Classification of Disease Reaction of Tomato Varieties to Fusarium Wilt

Symptoms	Disease Grade	CODEX	Reaction
No Symptoms	0	0	NI
Slight drooping of leaves and/or vascular browning in root region and no plant mortality	1	1-5	HR
Wilting of lower leaves and/or vascular browing extended and no plant mortality	2	6-15	R
Severely wilted, all leaves wilted and withered except terminal buds.	3	16-25 26-50	MR S
Dead plants	4	51-100	HS

NI - Immune; HR - Highly Resistant; R - Resistant; MR - Moderately Resistant; S - Susceptible; HS - Highly Susceptible.

Percent reduction in shoot length was calculated using the formula given below.

$$\text{Reduction in shoot length (per cent)} = \frac{\text{MSL (healthy)} - \text{MSL (treated)}}{\text{MSL (healthy)}} \times 100$$

MSL = Mean shoot length

14 TEST PATHOGENS

Twelve isolates of *F. oxysporum* f. sp. *lycopersici* and two *F. solani* were obtained from the roots and collar regions of *Fusarium* wilt infected tomato plants collected from different places of Karnataka. They were desingnated as F_1 to F_{16} on comparison with the isolates of *F. oxysporum* f. sp. *lycopersici* (1322) and *F. solani* (3150) received from Indian Type Culture Collection of Fungi, IARI, New Delhi. Further, the most virulent isolate designated as F_1 was used throughout the investigations because of its superior performance over other isolates. The source and identity of the isolates used is given in Table 5.

TABLE 5. **Source and Identification of different isolates of *Fusarium* spp. isolated from Toamto**

Group	Isolate	Identity	Location
.A	F_1	*F. oxysporum* f. sp. *lycopersici*	Nelamangala
	F_2	*F. oxysporum* f. sp. *lycopersici*	Hebbal
	F_3	*F. oxysporum* f. sp. *lycopersici*	Chikkaballapur
	F_4	*F. oxysporum* f. sp. *lycopersici* (ITCC No. 1322)	IARI, New Delhi
	F_5	*F. oxysporum* f. sp. *lycopersici*	Chintamani
	F_6	*F. oxysporum* f. sp. *lycopersici*	Tumkur
	F_7	*F. oxysporum* f. sp. *lycopersici*	Belgaum
	F_9	*F. oxysporum* f. sp. *lycopersici*	Shiggon
	F_{10}	*F. oxysporum* f. sp. *lycopersici*	D. B. Pura
	F_{11}	*F. oxysporum* f. sp. *lycopersici*	Mugad
	F_{12}	*F. oxysporum* f. sp. *lycopersici*	Mysore
	F_{13}	*F. oxysporum* f. sp. *lycopersici*	Biligi
	F_{15}	*F. oxysporum* f. sp. *lycopersici*	Raichur
B.	F_8	*F. solani* Gadag	
	F_{14}	*F. solani* (ITCC No. 1350)	IARI
	F_{16}	*F. solani* Shimoga	

15. TEST ANTAGONISTS

An isolate of *Trichoderma viride* pers : ex. Fr. (IMI 317287) designated as A_4 (Hebbal Isolate) and a fluorescent *Pseudomonad* bacterial isolate designated as B_1 were selected as the test antagonists against the test pathogen *F. oxysporum* f. sp. *lycopersici* on the basis of their superior performances over other antagonists.

16. MULTIPLICATION OF ANTAGONISTS FOR SOIL APPLICATION

The test antagonists, *Trichoderma* spp. were multiplied in wheat bran, sand and tap water (1:1:2 w/w/v) medium. Wheat bran and sand medium was autoclaved for 1 hour in conical flasks and inoculated with spore suspensions of *Trichoderma* spp. and incubated at room temperature at 26 $\pm$ 2°C for varying periods for experimental use. Hereafter such cultures are referred to as wheat bran cultures (WBC) and were used for mixing with the

soil at required concentrations. Bacterial antagonists were multiplied in nutrient broth in conical flasks. Bacterial suspension was used for experiments as a medium for seedling dip and seed bacterization. Antagonists were also multiplied on sterilized cheaply available organic wastes viz. cottonwaste compost, FYM, biogas slurry and MPG - 3. Their efficacy against test pathogen was assessed.

17. CULTURE MEDIA USED

The Culture media used in our studies are potato dextrose agar (PDA), potato sucrose agar (PSA), peptone dextrose-rose bengal agar (MRBA) peptone quintozene agar (FSM) Trichoderma specific medium for fungi, starch-casein agar for actinomycetes, soil extract agar (SEA) and nutrient agar (NA), King's B medium for Bacteria were used with the composition given in appendix I.

3.1 DISEASE SURVEY

Survey of ten Taluks around Bangalore was undertaken to know the prevalence and distribution of Fusarium wilt of Tomato. Tomato crop was surveyed for three seasons during in September - October 1991, February - March 1992, and June - July 1992. Data sheets were sent to the Reseach stations of UAS, Bangalore and Dharwad and Departments of Agriculture of all the districts of Karnataka to ascertain information on the prevalence and distribution of the disease. Infected plant and soil samples were collected from various parts of Karnataka. *Fusarium* spp. were isolated from the roots and collar region of infected plants as described earlier. The data sheet given in appendix-II was used to gather all the details of this disease.

3.2 ISOLATION AND STUDY OF MORPHOLOGICAL, CULTURAL AND PATHOGENIC CHARACTERS OF *FUSARIUM* SPP.

3.2.1 Isolation

Sixty five plant samples showing typical Fusarium wilt symptoms were obtained from 13 districts of Karnataka. Plant tissue from collar and root region of such plants were taken, bark was removed and cut into one mm bits. They were thoroughly surface sterilized using one percent sodium hypochlorite solution for two minutes followed by washing in sterile water. Four bits were placed aseptically on PDA medium contained in sterile petriplates. The inoculated petriplates were incubated at 26 ± 2°C for seven days. Observations were taken for the development of *Fusarium* spp. in petriplates which were purified by single spore culture. A total of 38 plant samples out of 65 yielded *Fusarium* spp.

3.2.2. Morphological and Cultural characters

Thirty eight isolates of *Fusarium* spp. obtained on isolation from wilted tomato plants were compared for variation in morphological and cultural characters on two per cent potato sucrose agar (PSA). Twenty ml of medium was poured in to each sterilized petriplate and kept overnight. A five mm mycelial disc from actively growing seven day old cultures of each isolate of *Fusarium* spp. was inoculated at the centre of PSA medium and petri plates were incubated at 26 ± 2°C for seven days. Observations regarding colony colour, linear growth measurements wer recorded upto seven days. Spore measurements were taken with the help of filar micrometer. One hundred spores were assessed for each isolate. For comparison *F. oxysporum* f.sp. *lycopersici* (ITCC No. 1322) and *F. solani* (ITCC No. 1 350)cultures were obtained from Indian Type Culture Collection Centre for Fungi, IARI, New Delhi.

3.2.3. Pathogenicity

All the 38 isolates of *Fusarium* spp. were multiplied on sand maize meal medium for three weeks and mixed into sterilized soil seperately for each isolate at the rate of 15 per cent and allowed for one week for multiplication of inoculum.

Seedling Disease

Infested soil of each isolate was filled into a three kg pot (25 cm dia). Tomato seeds of cv. Pusa Ruby, a susceptible variety were surface sterilized with one per cent sodium hypochlorite solution for two minutes and washed with sterile water three times. Fifty seeds were sown into each pot and observations were recorded from seventh day to 28 days for pre-emergence and post-emergence seedling mortality. One hundred and fifty seeds were assessed for each isolate.

Wilt Disease

Four week old tomato seedlings raised on sterilized sand contained in cement pots were carefully removed and washed under gentle running tap water to remove soil particles. The roots of seedlings were slightly severed to ensure infection. Two seedlings were transplanted in each pot of 250 g capacity (10 cm dia.). Observations were recorded for wilt incidence (PDI) upto six weeks. Thirty plants were assessed for each isolate.

Invitro Pathogenicity Test

Seven isolates of *F. oxysporum* f. sp. *lycopersici* (F_1 to F_7) were tested for their virulence in *in vitro*. Tomato seeds, surface sterlized were soaked in spore suspensions ($4x10^6$ conidia ml^{-1}) of each isolate seperately for two hours. Twentyfive seeds of tomato treated with spores of pathogen were plated on moistened blotter papers contained in sterile petriplates and incubated at 26 ± 2^0C for 15 days. Control plates were maintained by plating seeds soaked in sterlie water. observations were recorded on seed germination, colonization of seeds and seedling mortality. Two hundred seeds were assessed for each isolate.

Rapid Pathogenicity - Vial Test

Four week old tomato seedlings were uprooted from cement pot by flooding water to loosen the sand avoiding root injury. The roots were washed gently under running water to remove as much sand as possible. Final washing was done with sterile water. The seedlings were then placed with roots immersed in distilled water in test tubes. Spore suspensions of isolates of *Fusarium* spp that proved to be pathogenic were prepared by flooding seven day old PDA culture plates with sterile tap water. For 100 ml spore suspension three petri-plate culture growth was used which accounted for spore concentration of 4 - 8 x 10^6 spores ml^{-1}, the resultant spore suspensions were strained through sterilized muslin cloth and 40 ml of such spore suspension was transferred into test tubes. The seedlings were then planted in each test tube such that the roots were immersed in spore suspension and cotton was placed around the plant at the mouth of the test tube. The test tubes were set in a tray containing sand and incubated at room temperature 27 ± 1^0C for seven days and graded on seventh day (Wensley and Mckeen, 1962) Repeated comparative trials have shown that the results obtained by vial test and by soil pot methods are highly consistant. It has the advantage of indicating the results faster, requires much less material, labour and bench space, therefore is more economical. Hence before conducting glass house trials in a pot or/and vial tests have been performed. For each isolate a minimum of 12 plants were tested. Because of limited number of large sized test tubes (12" x 1.5") each time 5 isolates were tested.

3.3. FACTORS INFLUENCING DISEASE DEVELOPMENT

3.3.1. Effect of Soil Types on the Incidence of Wilt.

Five types of soils as earlier mentioned were used viz., sandy, sandy loam, sandy clay loam, sandy clay and clay soil. Ten per cent of inoculum was added to pots containing sterilized soil thoroughly mixed in the upper layers (10 cm). Ten seedlings of Tomato of 4 weeks old were transplnated to each pot and observations were taken for 6 weeks for wilt incidence as per 0 to 4 scale mentioned earlier (Chand and Thakur, 1969).

3.3.2. Inoculum Density and Disease

Inoculum of the test isolate comprised three week growth in sand maize meal medium. Inoculam of the pathogen was diluted with sterile soil at the rate of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 ratio. For assessing pre and post-emergence seedling mortality fifty seeds surface sterilized with one per cent sodium hypoclorite and washed in sterile water were sown in each pot (25 cm dia) containing different densities of *F. oxysporum* f. sp. *lycopersici* (sick soil). Seedling mortality was recorded upto four weeks after sowing. Healthy controls were maintained by sowing seeds in pots containing sterilized soil. Two hundred seeds were assessed. Four week old Pusa Ruby tomato seedlings raised in sterilized sand were carefully uprooted, washed in running tap water and transplanted into pots containing infested soil of the above ratios. Control plants without pathogen were maintained. Disease incidence was noted periodically upto 6 weeks. Twenty plants were tested against each inoculum level. Propogules of *F. oxysporum* f.sp. *lycopersici* were estimated by plating the rhizosphere soil on oven dry weight basis on Fusarium specific medium (FSM)

3.3.3 Varieties and Disease

To screen some of the commercial varieties of tomato against wilt, seventeen available varieties viz., Arka Vikas, Arka Saurabh, Arka Ashish, Arka Abha, Arka Alok, Arka Ahuti, Arka Vishal (all from IIHR, Bangalore), Pusa Early Dwarf, Roma, Best of All, Rupali, Rashmi, S - 28, Punjab Chuhhara, and Pusa Ruby (All from local markets), ATV-1 and ATV-2 from Latur. Seedlings were raised in sterilized sand, after 4 weeks, plants were removed carefully, washed in tap water, roots slightly severed and planted in pots (25 cm dia) containing infested soil.

Observations for disease incidence were recorded upto 60 days. Ten plants were planted for each pot. Thirty plants were assessed for each variety. Three replications with one pot per replication were maintained and data analysed by Completely Randomized design (CRD).

3.3.4. Effect of Age of Seedlings and Disease

In order to find out the effect of age of tomato seedlings on the Fusarium wilt infection severity an experiment was conducted on similar lines of the above experiement. The variable factor was the age of the plant while the age of inoculum was three weeks old. Seeds of Pusa Ruby, were sown at periodic intervals so as to get seedlings differing in age from two weeks to six weeks old at the time of inoculation. The height of plants was measured in both the infected and healthy plants and percentage stunting in affected plant as compared to healthy was measured. Twenty plants were assessed for each treatment.

3.3.5. Effect of Age of Inoculum and Disease

An experiment to assess the effective age of inoculum to get maximum infection to continue further studies was laid out. The treatments were inocula of varying in age from one to six weeks. Inoculum differing in age was inoculated to sterilized soil at ten per cent and mixed thoroughly. The soil was allowed for seven days for the multiplication of the pathogen. Sixteen plants were assessed for each treatment along with suitable control plants. Inocula with different ages were obtained at one time by seeding the flasks containing sand-maize meal medium at weekly intervals (Kesavan and Chowdhury 1977).

3.3.6. Methods of Inoculation and Disease

The relative efficiency of different methods of inoculation was conducted using a susceptible tomato cultivar, Pusa Ruby. Fifteen four weeks old plants raised in sterilized sand were used for each treatment. Checks without pathogen were maintained for each treatment. Final disease index was calculated at the end of four weeks after inoculation. The following seven methods were employed.

1. Severed root-dip method

The fungus was cultured on PDB in 250 ml Erlenmeyer flasks, containing 100 ml of the medium. After a period of 2 weeks the axenic cultures were filtered to get the mycelial

mat. The fungal mat thus obtained was shredded in a waring blender at low speed for two minutes to get a suspension containing mycelial bits and spores. The spore concentration was adjusted with the help of haemocytometer to contain approximately 5×10^5 conidia ml^{-1}. The seedlings were removed carefully and their roots were washed free of soil particles in running tap water. The ter tiary roots were severed with sharp scissors and dipped in spore suspension for 30 minutes as suggested by Wellman (1939). They were planted in pots containing sterilized soil.

2. Unsevered root-dip method

The procedure as described above was followed excepting that the roots were not severed.

3. Split tap root and dip in spore suspension method.

The procedure is the same as in root dip method except that the roots were not severed. Instead, tap root was split at the tip and dipped in the spore suspension for half an hour.

4. Split tap root and insertion of mycelial culture

Tomato seedlings were carefully uprooted and washed in running tap water to remove soil particles. Tap root was split with a sharp razor blade and a mycelial culture, obtained from 7 day young culture was inserted. The plants were transplanted into pots containing sterilized soil. Control plants with split tap root inserted with only PDA medium were transplanted to pots with sterilized soil.

5. Pouring the spore suspension around the root zone of already established plants.

The fungus was multiplied on PDA medium under aseptic conditions in petriplates. When fully covered with fungal growth (seven days) the growth was harvested with the help of a sterile scalpel and washed with 100 ml of sterile water. The contents of each plate were filtered through a muslin cloth to get a spore suspension. The suspension was adjusted to have a concentration of 5×10^5 spores ml^{-1} of sterile water. Four holes were made in the soil around the collar region of each seedling grown (four weeks), in pot containing sterilized soil. The holes, 4-5 cm deep, each were made with the help of a small stick. Ten ml of the spore suspension was poured into the holes and covered so that spore could enter the plants through roots. Control plants were applied with sterilized tap water. (Kesavan and Chowdhury, 1977).

6. Point injury at the collar region and followed by pouring spore suspension

Four week old tomato plants already raised in pots containing sterilized soil were injured at the collar region with the help of a sterilized needle. A spore suspension (4×10^6 conidia ml^{-1}) at the rate of ten ml per plant was poured all around the collar region with the help of sterile cotton. Plants injured but applied with sterilized water served as control.

7. Planting in pre infested soil

As already mentioned fungus grown on sand maize meal medium for three weeks was incorporated to sterilized soil at the rate of ten per cent (w/w) and four week old tomato seedlings were transplanted. Plants transplanted into sterilized soil contained in pots served as control.

3.3.7. Influence of Age of Tomato Seedlings of Different Varieties and the Incidence of Seedling Disease due to *F. oxysporum* f.sp. *lycopersici*

Eleven varieties of Tomato viz; Arka vikas, Arka Saurabh, Arka Vishal, Arka Ahuti, Arka Alok, Arka Ashish and Arka Abha (All from 11HR), Rupali, Pusa Ruby, Pusa Early Dwarf and Roma (from local market) were employed. Seeds were washed in sterilized distilled water three times to wash off fungicide residues and sown in ice cream cups containing 150 g of sterilized soil. ten seeds per cup were sown and after germination only five seedlings were retained for each cup. Fifteen seedlings were assessed for each variety. At the collar region of each seedling soil was scooped with a sterile scalpel and a five mm mycelial disc cut from one week old culture was placed in close contact to collar region and covered with scooped soil. Then it was supplemented with two to three drops of 0.1 per cent glucose solution and observed for seedling mortality for one week. Control plants were treated with sterilized PDA disc without pathogen.

3.4. BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT OF TOMATO CAUSED BY *F. oxysporum* f.sp. *lycopersici* USING ORGANIC AMENDMENTS

Effect of organic amendments was studied in three stages. Initially, all the amendments were screened at one concentration and one incubation period. In the second stage, selected amendments were tested at three concentrations and two incubation periods. In

the final stage, selected amendments were studied in detail for their effect on seedling disease, pathogen population and changes in soil microflora (fungi, bacteria and actinomycetes).

3.4.1. Preliminary Screening of Organic Amendments Against Seedling Disease and Wilt in Tomato

A total of 22 amendments, consisting of seven oil cakes, nine dry amendments and six green leaf amendments were assessed. All the amendments were prepared and mixed with infested soil (10% inoculum) at a concentration of 0.1 per cent (w/w) for oil cakes, 1.5 per cent (w/w) for dry amendments and three per cent (w/w) for fresh green leaf material. The amended soil was filled into 25 cm diameter pots and moisture was adjusted to ten per cent of water holding capacity (WHC) and incubated in green house for a period of two weeks. Moisture level was maintained by periodical watering. Three replications for each treatment and a control where no amendment was added were maintained.

At the end of the incubation period, the soil in each pot was mixed and sown with 50 seeds of tomato and soil moisture was maintained at below 50 per cent water holding capacity by regular watering. Seed germination counts and post-emergence seedling mortality were recorded for a 4 week period. Final healthy seedling counts were taken four weeks after sowing and the data were analysed using CRD.

Four week old tomato seedlings were carefully removed, washed under gentle running water and transplanted into pots containing amended infested soil with organic amendments. Ten seedlings were planted to each pot.Three replictions were maintained. Observations for wilt were taken upto six weeks. Control pots were maintained without any amendment. Healthy control was also maintained without pathogen and amendment. Data were analysed by CRD.

3.4.2. Effect of Selected Amendments at Three Concentrations and Two Incubation Periods Against Seedling Disease and Wilt in Tomato.

Two oil cakes, one green leaf amendment and three dry amendments were selected from earlier experiments.Three concentrations of each amendment viz., 0.05, 0.10 and 0.15 per cent for oil cakes, 2.0, 3.0 and 4.0 percent for green leaf amendment, 1.0, 1.5 and 2.0 per cent for dry amendments were mixed to infested soil by (w/w). At each concentration influence of two incubation periods of two and four weeks were studied.

Tomato seeds were sown as in earlier method in three replicates. Soil moisture was maintained near 50 per cent WHC by periodical watering regularly. Seed germination, counts, post-emergence seedling mortality of seedlings and final count of healthy seedlings were taken four weeks after sowing and statistical analysis of the data was done. For wilt disease four week old tomato seedlings were transplanted at ten plants per pot (25 cm diameter). Observations were made for wilt incidence upto 60 days and data analysed by three factorial CRD.

3.4.3. Effect of Selected Amendments on Wilt Incidence, Population Dynamics of Microflora and Pathogen in Rhizosphere of Tomato

Six amendments selected from the earlier experiments were studied at a concentration of 0.1% (w/w) for oil cakes, three per cent for fresh amendments and 1.5% for dry amendments with incubation period of two weeks. Mixing, incubation and planting of tomato seedlings was done as explained. Before planting, soil samples were enumerated for *F oxysporum*. Total fungi, bacteria and actinomycetes populations were enumerated on tenth day after planting by dilution plate technique as described earlier. Shoot length was taken at the termination of experiment on 60th day. Population of pathogen, bacteria, fungi and actinomycetes were taken at ten day interval upto 40 days and data were analysed statistically by CRD.

3.5. EVALUATION OF EFFICACY OF MODIFIED *PANCHAGAVYA* (MPG) FORMULATIONS ON THE INCIDENCE OF SEEDLING DISEASE AND WILT CAUSED BY *F. oxysporum* f.sp. *lycopersici* IN TOMATO

Effect of several combinations of modified *panchagavya* (MPG) on the incidence of seedling disease and wilt was studied. Initially MPG formulations were screened at four dilutions at one incubation period (14 days). In the second stage selected formulation was tried at different incubation periods, later the selected formulation was studied in the field

3.5.1. Preliminary screening of MPG Formulations at Different Dilutions for their Efficacy on the Incidence of Seedling Disease and Wilt.

Three formulations MPG - 1, MPG - 2 and MPG - 3 were tried at four levels 0, 10, 100 and 1000 dilutions. Tomato seeds were soaked in these formulations for two hours and later dried. Twenty five seeds were sown per each pot containing infested soil and observations on germination and seedling mortality were taken for four weeks. At the end

of four weeks percent healthy seedlings were counted. For each treatment 200 seeds were tested. Control was maintained by sowing tomato seeds soaked in sterile water. For comparison seeds treated with 0.1 per cent carbendazim and surface sterilized seeds with one per cent sodium hypochlorite sown in healthy sterile soil were maintained. Observations were taken as above. Data were statistically analysed by CRD.

For wilt incidence, four week old seedlings, thoroughly washed were used. A set of 16 plants were dipped in MPG formulations at different dilutions for half an hour and planted four seedlings into pots (one kg capacity) containing infested soil. After planting ten ml of these formulations were drenched all around the plant making hole with a stick, so that the solution will be placed at root zone. Again ten and 20 days after planting MPG was drenched around the base of the plants. Observations were taken for wilt incidence upto eight weeks. At the end of eight weeks shoot lengths were taken. Data were analysed statistically.

3.5.2. Influence of Ageing on the Efficacy of MPG - 3 on the Indicidence of Wilt

The performing MPG - 3 formulation was tested against wilt incidence at different ageing periods ranging from 0 to 18 days at two day intervals. Four week old tomato seedlings thoroughly washed were dipped in the MPG - 3 for half an hour and transplanted to pots (one kg capacity) containing infested soil. A set of 16 plants were tested against each treatment having four plants per pot. Observations were taken for wilt incidence upto six weeks. MPG - 3 was drenched around the base of the plant immediately after planting and followed by two applications at ten day intervals. Data were analysed statistically.

3.6. BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT OF TOMATO CAUSED BY *F. oxyspours* f.sp. *lycopersici* USING MICROBIAL ANTAGONISTS

Several experiments were conducted to know the antagonistic activity of *Trichoderma* and bacterial isolates against *F. oxysporum* f.sp. *lycopersici in vitro* and the details of each are presented.

3.6.1. Preliminary Screening of Antagonists Against *F. oxysporum* f.sp. *lycopersici*

Nineteen isolates of *Trichoderma*, and five isolates of bacteria were screened for their antagonistic activity in dual culture on PDA in petridishes. Both the antagonist and pathogen were inoculated at the same time. Discs of five mm cut from the margin of

young vigorously growing cultures were placed at the opposite periphery of the plates four cm apart from each other. The plates were incubated at room temperature 26 ± 2° C. *Trichoderma* isolates were selected for their potential after contact with the test pathogen on the basis of their overgrowth and smothering of *Fusarium* colony. Types of interaction were studied on the fifth day when check showed full plate growth (Porter, 1924, Jackson *et. al.* 1991 and Sharma and Sen, 1991). Five bacterial antagonists, three obtained from the Department of plant pathology and two isolated from rhizosphere of apparently healthy tomato plants between two wilted plants were employed. Bacterial antagonists were screened against *F. oxysporum* f. sp. *lycopersici in vitro* by dual culture (Buxton and Richards, 1955). Each pair was streaked 40 mm apart in parellel lines and incubated at 26 ± 2°C. Observations on the types of interactions were made on the 5th day. Two bacterial antagonists were selected and designated as B_1 and B_2.

3.6.2. Interaction of Selected *Trichoderma* Isolates with *F. oxysporum* f.sp. *lycopersici* in Dual Culture

Ten isolates of *Trichoderma* were selected from preliminary screening for their antagonistic potential in dual culture. *Trichoderma* isolates and the test pathogen were inoculated as mentioned earlier and observations were made on the rate of smothering *Fusarium* colony. Hyphal interactions were studied in dual culture by growing them on a cellophane membrane placed over solidified PDA in petridishes. After both the fungi came in contact with each other, the contact zone was cut using a sharp blade and taken out along with cellophane. It was washed gently with water, mounted under lactophenol - cottonblue over a clean glass slide and observations were made under microscope.

3.6.3. Effect of Volatile Inhibitory Substances Produced by *Trichoderma* Isolates Against *F. oxysporum.* f. sp. *lycopersici*

For this study, methods adopted by Dennis and Webster (1977 b) were followed. The petriplates containing PDA were inoculated with *Trichoderma* isolates by placing 5 mm disc cut from actively growing cultures and incubated at room temperature. After this period, the lid of each dish was replaced by a bottom containing PDA inoculated with the test pathogen. The two dishes were sealed together mouth to mouth with adhesive tape. The control plates were set up in triplicate. After 1 and 2 days of incubation, the colony diameter of the test pathogen was measured and compared with that of control plates. The data pertaining to radial growth of test pathogen was recorded upto 7 days and analysed.

3.6.4. Production of Diffusible Inhibitory Substances by Isolates of *Trichoderma* Against *F. oxysporum.* f. sp. *lycopersici.*

The method employed for the study was the same as adopted by Dennis and Webster (1971 a). Twenty ml of PDA medium, pH adjusted to 7.0 was poured into petriplates. A single sterile cellophane disc (25 mm Thick) was placed aseptically over PDA in each dish and left overnight. Discs (5 mm) cut from the margin of a three day old culture of each of *Trichoderma* isolates were placed in the centre.

The plates were incubated at room temperature (25 ± 2^0c) for two days. Then the cellophane along with adhering fungus were removed carefully. A 5 mm disc of the test pathogen was placed immediately on the medium at the centre. For each isolate 3 plates were maintained. After 1 and 2 days colony diameter of the test pathogen was recorded. The data were analysed statistically.

3.6.5. Interaction of Antagonists with *F. oxysporum* f. sp. *lycopersici.*

Conidial suspension of test pathogen was added to luke warm PDA and uniformly mixed. Twenty ml of this medium was poured in to petriplates and allowed for 12 hours for solidification and settlement. Actively growing five mm agar mycelial disc of *Trichoderma* isolates were placed at the centre of the PDA. The colony growth/lysis zone was recorded daily for seven days. Two bacterial antagonists selected from the previous study B_1 and B_2 were grown in nutrient agar for 24 hours. Spore suspension of the test pathogen was added to PDA and poured into sterile petriplates at the rate of 20 ml and incubated for 12 hours. At the end of 12 hours a loopful of each bacterial antagonist was placed at the centre separately. The petriplates were incubated at room temperature 26 ± 2 ^{0}C and observations were noted for one week. Control plates with inoculated antagonists were maintained.

3.6.6. Production of Inhibitory Substances by Isolates of *Trichoderma* in Liquid Culture

Weindling medium (Weindling, 1941) suitable for extraction of antibiotics was used here. The composition of the medium is given in Appendix I. Flasks containing 50 ml medium were inoculated with five mm mycelial discs of each isolate of *Trichoderma* and incubated for one week at room temperature (26 ± 2^0c). Three replicates were maintained for each isolate.

At the end of incubation period, culture liquid was filtered through Whatman No.1 filter paper and pH was measured using pH meter. The filtrate was divided into two sets. one set was autoclaved at 15 Ibs PSI for 15 min. and another was passed through millipore filters (0.45 μ pore size). The filtrates were mixed with molten PDA medium containing double the concentration of the solids in a ratio of 1:1 (v/v) and then poured into petridishes. Sterile tap water was substituted in place of filtrate for control. A five mm mycelial disc of test pathogen was placed at the centre of the solidified medium in each plate and incubated at room temperature. Radial growth of the test fungus was recorded upto seven days of incubation. In another experiment, the pH of the culture filtrates from different *Trichoderma* isolates were recorded and adjusted to pH 6.5 using 0.1 N sodium hydroxide solution. Then the filtrate was heat sterilized or filter sterilized through millipore filters and assayed for inhibitory action against the test pathogen as mentioned in the previous experiment. Statistical analysis of the data was done.

3.6.7. Effect of Culture Filtrates of Antagonists and MGP-3 on the Growth of F. oxysporum f.sp. lycopersici

Heat sterilized

Liquid culture filtrates of isolates of Trichoderma (A_1 to A_{10}) grown in weindling medium, bacterial antogonists (B_1 and B_2) grown in nutrient broth for one week, and MPG-3 (14 day old preparation) were obtained by passing through whatman No.1 filter paper. These filtrates were divided into two sets. One set of 15 ml. was added to 15 ml potato dextrose broth (PDB) containing double the concentration of the solids in the ratio of 1:1 (v/v) into 100 ml Erlenmeyer conical flasks seperately. Two fungicides carbendazim (500 ppm) and Emisan (1000 ppm) were added to 30 ml PDB. Weindling medium and sterile water added to PDB (1:1 v/v) served as control - 1 and control -2. The contents were autoclaved for 15 minutes at 121°C and after cooling the flasks were inoculated with a five mm mycelial disc of *F. oxysporum* f.sp. *lycopersici*. The flasks were incubated for 15 days at 26±2°C and at the end of 15 days mycelial mats of test pathogen were collected and dried in oven at 105°C for 24 hrs. Mycelial dry weights were recorded until constant weights are obtained for two consecutive days. Statistical analysis of the data was done by CRD.

Filter Sterilized

Another set of culture filtrates were passed through millipore filters (0.45 μm) and

the filtrates were added to PDB aseptically in the ratio of 1:1 (v/v). The flasks were inoculated with the test pathogen as above and observations on mycelial dry weights were recorded at the end of 15 days of inocubation period.

3.6.8. Effect of Culture Filtrates of Antagonists, MPG - 3 and Bavistin on Conidial Germination of *F. oxysporum* f.sp. *lycopersici.*

Culture filtrates of ten *Trichoderma* spp and two bacterial antagonists, MPG-3, carbendazim (500 ppm) solution and distilled water were tested for their effect on conidial germination of *F. oxysporum.* f. sp. *lycopersici.* 100 conidia were observed under microscope for each treatment. Three replications were maintained. A drop of culture filtrate of antogonists, MPG-3 and carbendazim solution was placed over cover slip and a drop of conidial suspension was added. The cover slip was placed over a 3 ml vial containing sterile distilled water for 24 hours and conidial germination was observed.

3.6.9. Effect of heat sterilized Culture Filtrates of Selected Antagonists, MPG-3 and Fungicides on the growth of *F. oxysporum* f.sp. *lycopersici*

Culture filtrates of *T. viride* (A_4), *Pseudomonas* (B_1), *Bacillus subtilis* (B_2) and MPG-3 were added to PDA (double strength) in the ratio of 1:1 (v/v), carbendazim (500 ppm), emisan (1000 ppm) were added to PDA. Thus amended PDA was autoclaved and poured at the rate of 20 ml in to petriplates and kept overnight. PDA without culture filtrates or fungicides served as control. A five mm disc of *F. oxysporum* f.sp. *lycophersici* was inoculated at the centre of the medium and plates were incubated for seven days. Observations regarding radial growth were taken upto seven days and statistical analysis was done.

3.6.10. *In vitro* Screening of Antagonists and MPG - 3 for their Effect on Seed Germination.

Spore suspension of ten isolates of *Trichoderma,* bacterial suspension of 24-48 hour growth on nutrient broth, MPG-3, carbendazim (500 ppm) and distilled water were tried for testing their effect on seed germination. Seeds were soaked in the above for 2 hours and dried. Twentyfive seeds were placed in petridishes containing sterile moistened blotter papers and incubated at room temparature (26 ± 2^0C) for 10 days. Germination was recorded upto 10 days.

3.7 Efficacy of Antagonists against Seedling Disease and Wilt in Tomato in Pot Culture.

Ten isolates of *Trichoderma* and two bacterial antagonists were evaluated for their efficacy to control *F. oxysporum.* f. sp. *lycopersici* in pot culture experiments.

3.7.1. Effect of Antagonists Against Seedling Disease

Seed Treatment

Trichoderma isolates grown on PDA were flooded with water to prepare spore suspensions. Spore load was adjusted to 5-6x10^6 conidia ml^{-1}. Tomato seeds were soaked in spore suspension for two hours and dried. Twentyfive seeds were sown for each pot (10 cm dia) containing infested soil. Control pots were sown with seeds soaked in sterile water. Data were collected on germination, post-emergence seedling disease and healthy seedling stand at the end of fourth week. Statistical analysis was done. One hundred and fifty seeds were tested against each isolate.

Bacterial antagonists grown in nutrient broth medium for 24 hours were used to evaluate their efficacy in controlling seedling disease and wilt. Tomato seeds, surface sterilized, washed in sterile water and air dried were soaked in bacterial suspension for two hours and air dried. Twenty five seeds were sown for each pot (10 cm dia) containing 250 g infested soil. One hundred and fifty seeds were evaluated for each isolate. Observations regarding pre and post-emergence seedling mortality and healthy seedling stand at the end of four weeks were taken.

Soil Application

In another study *Trichoderma* isolates multiplied in wheat bran sand medium were used at the rate of 0.2 percent (w/w). The mixture of antogonist and infested soil was placed in ten cm diameter earthen pots separately for each isolate. Moisture was adjusted to ten percent and incubated for three days. These were covered with a polythene sheet. After incubation, twentyfive tomato seeds were sown in each pot and watered regularly and maintained near 50 percent water holding capacity. Germination counts, post-emergence seedling mortality and healthy seedling stand were taken upto four weeks. Data were analysed statistically.

In another study, bacterial suspension was added to the pots (10 cm dia) containing infested soil at the rate of 20 ml and 25 seeds were sown. Observations regarding pre and post-emergence seedling mortality and healthy seedling stand was noted.

3.7.2. Effect of Antognists Against Wilt

Root Dip

Four weeks old Tomato plants were uprooted, washed and roots were dipped in spore suspensions of isolates of *Trichoderma* for half an hour. A set of 12 plants were tested against each isolate. Four plants per replication were maintained. Plants were transplanted to pots (10 cm) containing infested soil. Data for wilt incidence were recorded for eight weeks and compared with control. Plants were transplanted into sick soil. Data were statistically analysed.

For wilt incidence roots of four week old seedlings were steeped in bacterial suspension for half an hour and transplanted to pots (10 cm dia) containing infested soil, at the rate of 1 seedling per pot. 4 plants were kept for each replication. Data were recorded for wilt incidence upto 60 days and statistically analysed.

The WBC of Isolates of Trichoderma were added to Fusarium infested soilcontained in parts at the rate of 0.5% and incubated for one week. Four week old tomato seedlings thoroughly washed were transplanted in to 10 cm diameter pots at the rate of one plant per pot. Twelve plants were tested against each isolate. four plants were kept for each replication. Control plants were transplanted into pots containing only the test pathogen. Data were recorded for wilt incidence upto 60 days and statistically analysed.

The Antagonistic bacterial suspensions were added to the Fusarium infested soil contained in the pots at the rate of 10 ml and four week old tomato plants were transplanted. One plant was maintained for each pot and observations for wilt incidence were taken upto 60 days. Tomato seedlings planted in Fusarium infested soil alone served as control.

3.7.3. Mass Multiplication of *Trichoderma viride* (A_4) and Bacterial Antagonist (B_1)

In order to facilitate economical ways of using A_4 and B_1 antagonists in biocontrol three cheaply available materials and MPG - 3 were tried. The materials used were farm yard manure (FYM), biogas slurry, well decomposed cotton waste and MPG - 3. Fifty grams of each of these was taken into 250 ml Erlenmeyer conical flask and 10 percent

water was added to the first three items. The flasks were autoclaved for 15 minutes at 15 lbs PSI, Then inoculted five mm disc of actively growing A_4 and B_1 culture and incubated at room temperature (26±2°C) for ten days. In another experiment the same material was supplemented with one per cent jaggery and inoculated with A_4 and B_1 and incubated for ten days at room temperature. At the end of ten days the contents of the flasks were thoroughly mixed and 10 g from each was analysed for colony forming units on *Trichoderma* specific medium and King's B medium. For these, controls were maintained by inoculating *Trichoderma* to unsterilized flasks containing these material, whose *Trichoderma* and bacterial population was determined prior to experiment. Final *Trichoderma* and bacterial counts were determined by assessing colony forming units on *Trichoderma* specific medium and King's B medium. four replications were maintained and data were statistically analysed.

3.7.4. Methods of Application of the Antagonists

The antagonists *Trichoderma viride* (A_4) and fluorescent *Pseudomonas* (B_1) were applied in different ways with or without carriers to develop an effective method to carry the antagonists to the soil. The experiment was conducted in sick soil in pots of 25 cm diameter.

Direct application

Both A_4 and B_1 were grown on PDB and nutrient broth respectively, in 250 ml flasks for 15 days at 26 ± 2°C, the mycelial mat of A_4 antagonist was separated by muslin cloth, macerated in sterile water in a waring blender and final suspension adjusted with sterile water to give one flask growth equivalent to 50 ml suspension. The B_1 suspension was prepared by thorough shaking of the growth, added with sterile water to give one flask equivalent to 50 ml suspension. The suspension was added at the rate of 100 ml pot^{-1}.

Seed Soaking

Tomato seeds were soaked in the antagonist spore suspensions (5-6 x 10^6 spore, heavy bacterial cells) for two hours and dried. The seeds soaked in sterile water served as control.

Root Dipping

Four week old seedlings were taken out, washed thoroughly and then dipped in antagonist suspensions of 6x10^6 spores or heavy bacterial cells.

Carboxy Methyl Cellulose (CMC) Pelleting

Seeds were dipped in one per cent CMC solution for one munite, removed and immersed in suspensions of a well sporulating antagonist cultures (5 x 10^6 or bacterial cells). The coated seeds were then rolled on dry CMC powder to remove excess moisture and to form pellets and these pelletes were stored in poly propylene bags at room temperature for 24 hours. The seeds pelleted with CMC alone served as control.

Carriers

Earlier work was done using farm yard manure (FYM) and saw dust as carriers for delivery of antagonists (Naik, 1989). In the present study FYM and biogas slurry were mixed individually with water 1:1 v/v basis and autoclaved in 250 ml flasks by tyndalization. Flasks containing carriers were inoculated with five ml of antagonist suspensions and incubated for three weeks at 26 $\pm$ 2^0C, and shaken frequently for uniform growth.

The carriers containing antagonists were added to the pots containing infested soil with test pathogen at the rate of 0.5 percent. Carriers containing test pathogen were added to pots containing sterilized soil at the rate of ten per cent. Seeds were sown three days after adding antagonists directly or through carriers for the study of seedling disease. For wilt study four week old seedlings were dipped in the antagonist suspension for half an hour and planted. The pots were regularly watered. Observations regarding germination, seedling mortality and healthy seedling stand were taken for four weeks. Wilt incidence was observed for eight weeks.

3.8. Biological Control of Seedling Wilt in Tomato caused by F. oxysporum f.sp. lycopersici in pot Culture using Organic Amendments, Antagonists and MPG-3

A pot culture study was taken up with the most effective treatments in the previous experiments. Neem Cake, *Trichoderma viride* (A_4), fluorescent *Pseudomonas* (B_1) and modified *panchagavya* (MPG-3) were tested individually and in combinations. A comparison was made with carbendazim, an effective fungicide presently used against fusarial wilts and a healthy check.

Seedling Disease

Neem cake was added to infested soil at 0.1 per cent and incubated for two weeks,

test antagonists and MPG-3 were applied as seed dressers. Combinations were obtained by mixing the cultures required and soaking the seed in the preparations for two hours air dried and sown in the pots containing infested soil with test pathogen.Observations on germination, post-emergence seedling mortality and healthy seedling stand were recorded upto four weeks. Data were analysed statistically .

Wilt (PDI)

Four week old tomato seedlings were used as earlier mentioned. Seedlings were dipped in test antogonist culture preparations, MPG-3 and their combinations for half an hour and transplanted. Neem cake was applied to the infested soil two weeks before planting. The soil was drenched with MPG-3 at the time of planting and at ten day interval twice subsequently, seedlings were dipped in carbendazim (0.05%) solution for half an hour, drenched the soil containing test pathogen as in case of MPG-3. Control was maintained by planting tomato seedlings in infested soil without any treatment. A healthy check was maintained by planting seedlings in sterilized soil contained in pots. Ten seedlings were planted for each pot (25 cm diameter) and three replications were maintained. Observations on wilt incidence and *Fusarium* population at the beginning, at the time of wilting and at the termination of experiment after eight weeks were recorded.

3.9. MANAGEMENT OF FUSARIUM WILT OF TOMATO UNDER FIELD CONDITIONS.

A field study was taken up to observe the efficacy of neem cake (0.1%) and MPG - 3 individually and in combinations in comparison with uninoculated check plants, bavistin and inoculated infested soil as checks. One square metre plots were prepared. For each plot, inoculum of the test pathogen multiplied in sand maize meal was applied at the rate of 600 g, and allowed for stabilization. One crop of tomato with susceptible tomato variety was taken for 60 days and the plants were incorporated into the soil to facilitate multiplication of the test pathogen and initial population of the test pathogen and microflora was enumerated before the experiment. A healthy control plot was not added with inoculum. Four replications were maintained for each treatment. The experiment was laid out in RCBD (6x4 m). A minimum of 12 four week old seedlings were maintained $plot^{-1}$. Neem cake was applied at the rate of 250 g $plot^{-1}$ two weeks before the experiment and ten percent moisture was maintained. MPG-3 was prepared and a 14 day old preparation was used. Seedlings were dipped in MPG-3 solution for 30 minutes and

planted. The soil around the plant was drenched after planting and at ten day interval twice subsequently. Seedlings were dipped in 0.1 percent carbendazim (500ppm) solution for minutes and planted followed by drenching around the plant as in the case of MPG-3 and the process was repeated twice at an interval of ten days. Plants were planted in uninoculated plots without any treatment. Observations were recorded on wilt incidence, shoot length, root length, enumeration of microflora, test pathogen and fruit yield, during kharif season from July to October, 1993. The data were analysed statistically.

3.10. Effect of Management Practices on Survival of *F. oxysporum* f.sp. *lycopersici*

Infested soil with *F. oxysporum* f. sp. *lycopersici*, whose propagules g^{-1} of dry wt soil were pre determined was filled into 25 cm diameter pot at the rate of two kg each. Flooding of the pots with water was done by closing the drainage hole prior to filling the soil, and the standing water was allowed for three weeks. Antagonists were applied at the rate of 500 mg *T.viride* and (A_4) and 5 ml of *Pseudomonas* sp.(B_1) culture multiplied on wheat bran and nutrient broth respectively, drenching with bavistin (0.1%) and MPG was done. Control without any treatment was maintained. Four replications were maintained and propagules g^{-1} were counted by dilution plate technique for three weeks at weekly intervals.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

4.1 DISEASE SURVEY

Tomato is one of the most important vegetables grown intensively in the state. *Fusarium* wilt was found to be more common wherever tomato is grown. A survey of 10 areas around Bangalore where tomato is grown intensively was conducted in different seasons and crop growth stages with a view to assess the intensity of the disease. Besides this, data sheets were sent to all the Assistant Directors of Agriculture in Karnataka, and to the Research stations of University of Agricultural Sciences, Bangalore and Dharwad, to furnish information and to send the wilt infected tomato plant and infested soil samples. Thirteen districts were covered under the survey and information on the occurrence of the disease was recorded. The data obtained are presented in the Table 6 and Fig. 1. Bacterial wilt infected plant samples were identified by positive ooze test and discarded (Plate 2).

From the Table 6, it is found that out of 65 infected plant samples 38 gave *Fusarium* spp on isolation from roots and collar regions. Further, 17 samples yielded *Rhizoctonia* spp. and *sclerotium* spp. Ten samples yielded none of the pathogens.

From the results presented in the Table 7, it can be seen that per cent plants wilted is comparatively low in North eastern dry zone (1.5) than Northern Transition zone (7.8). Northern dry zone and Eastern dry zone had 7.0 and 7.1 per cent wilted plants respectively. Frequency occurrence of *Fusarium* spp. in root was highest in Northern Transition Zone and Southern Transition Zone (100 per cent) followed by Northern Dry Zone (75 per cent). Propagules of *Fusarium* spp. in soil was the highest in North Eastern Dry Zone (39.5×10^3 cfu g^{-1} dry wt of soil) followed by Northern Dry Zone (38.5×10^3 cfu g^{-1} dry wt of soil). Least number of propagules was found in Central Dry Zone (30.8×10^3 cfu g^{-1} dry wt of soil). The data are presented in Table 7 and Fig. 2.

The highest per cent wilted plants was recorded in Bijapur district (10.0) followed by Bangalore Urban (9.75) and Kolar (8.97). The least percent of wilted plants was noticed in Mysore, Mandya, Raichur and Chitradurga districts. Per cent frequency occurrence of *Fusarium* spp. in roots was the highest in Bijapur, Belgaum and Shimoga districts (100) while the least in Bangalore urban (33.1). *Fusarium* population in soil was the highest in Hassan district (42.3×10^3 cfu g^{-1} dry wt of soil) followed by Bijapur (41.10×10^3 cfu g^{-1} dry

Plate 2 Symptoms of Fusarium wilt of Tomato

a) Complete wilting

b) Partial wilting and yellowing of the aerial parts of tomato.



a



b

PLATE 2

Table 6. Distribution of Fusarium Wilt of Tomato in Different Districts of Karnataka

District	Sample No.	Taluk	Propagules of *Fusarium* spp. (cfu. $g^{-1}x10^3$)	Isolation of Fungi from roots		
				Fusarium	Others	None
Bangalore (R)	56	Channapatna	35.3	+	-	-
"	57	"	35.3	+	-	-
"	4	Devanahalli	33.3	+	-	-
"	5	"	34.3	-	+	-
"	6	"	34.0	-	-	-
"	32	Doddaballapur	41.0	+	-	-
"	33	"	32.3	-	-	-
"	34	"	36.3	-	+	-
"	1	Nelamangala	45.0	+	-	-
"	2	"	34.6	+	-	-
"	3	"	36.6	+	-	-
"	30	"	37.0	-	+	-
"	31	"	39.3	+	-	-
Bangalore (U)	13	Anekal	31.6	-	+	-
"	55	"	30.6	-	+	-
"	64	"	33.3	-	-	-
"	7	Bangalore North	38.3	-	+	-
"	8	"	34.3	+	-	-
"	9	"	34.0	-	+	-
"	35	"	32.6	+	-	-
"	36	"	41.0	+	-	-
"	37	"	32.3	-	-	-
Belgaum	43	Hukkeri	39.0	+	-	-
Bijapur	61	Biligi	39.6	+	-	-
"	62	"	42.6	+	-	-
Chitradurg	26	Hiriyur	36.3	-	+	-
"	27	"	43.3	+	-	-
Dharwad	52	Dharwad	38.3	+	-	-
"	53	Gadag	36.0	+	-	-
"	54	"	37.3	-	+	-
"	63	Shiggon	37.3	+	-	-
Hassan	21	Arsikere	41.6	+	-	-
"	22	"	42.3	+	-	-
"	65	"	43.0	-	+	-
Kolar	15	Bagepalli	34.0	+	-	-
"	16	"	37.3	-	+	-
"	17	"	34.0	-	+	-
"	18	"	34.0	-	+	-

Contd.

Table 6. contd..

District	Sample No.	Taluk	Propagules of *Fusarium* spp. (cfu. $g^{-1}x10^3$)	Isolation of Fungi from roots		
				Fusarium	Others	None
"	19	Chikkaballapur	48.3	+	-	-
"	20	"	32.6	-	+	-
"	49	"	46.6	-	-	-
"	50	"	30.6	+	-	-
"	51	"	36.3	-	+	-
"	14	Chintamani	36.3	+	-	-
"	46	"	41.0	-	-	-
"	47	"	34.3	+	-	-
"	48	"	35.3	+	-	-
"	10	Malur	34.0	+	-	-
"	11	"	35.3	-	-	-
"	12	"	33.6	-	+	-
Mandya	41	Nagamangala	35.3	+	-	-
"	42	"	37.0	-	-	-
Mysore	24	Mysore	34.0	-	-	-
"	25	"	39.0	+	-	-
Raichur	44	Raichur	43.3	+	-	-
"	45	"	35.6	-	+	-
Shimoga	39	Shimoga	33.6	+	-	-
"	40	"	36.3	+	-	-
Tumkur	23	Gubbi	42.6	+	-	-
"	38	"	40.0	+	-	-
"	60	Kunigal	33.3	+	-	-
"	58	Madhugiri	42.6	+	-	-
"	59	"	33.3	-	-	-
"	28	Pavagada	34.3	+	-	-
"	29	"	36.3	+	-	-
13	**65**	**65**		**38**	**17**	

Others : *Rhizoctonia* spp., *Sclerotium* spp.

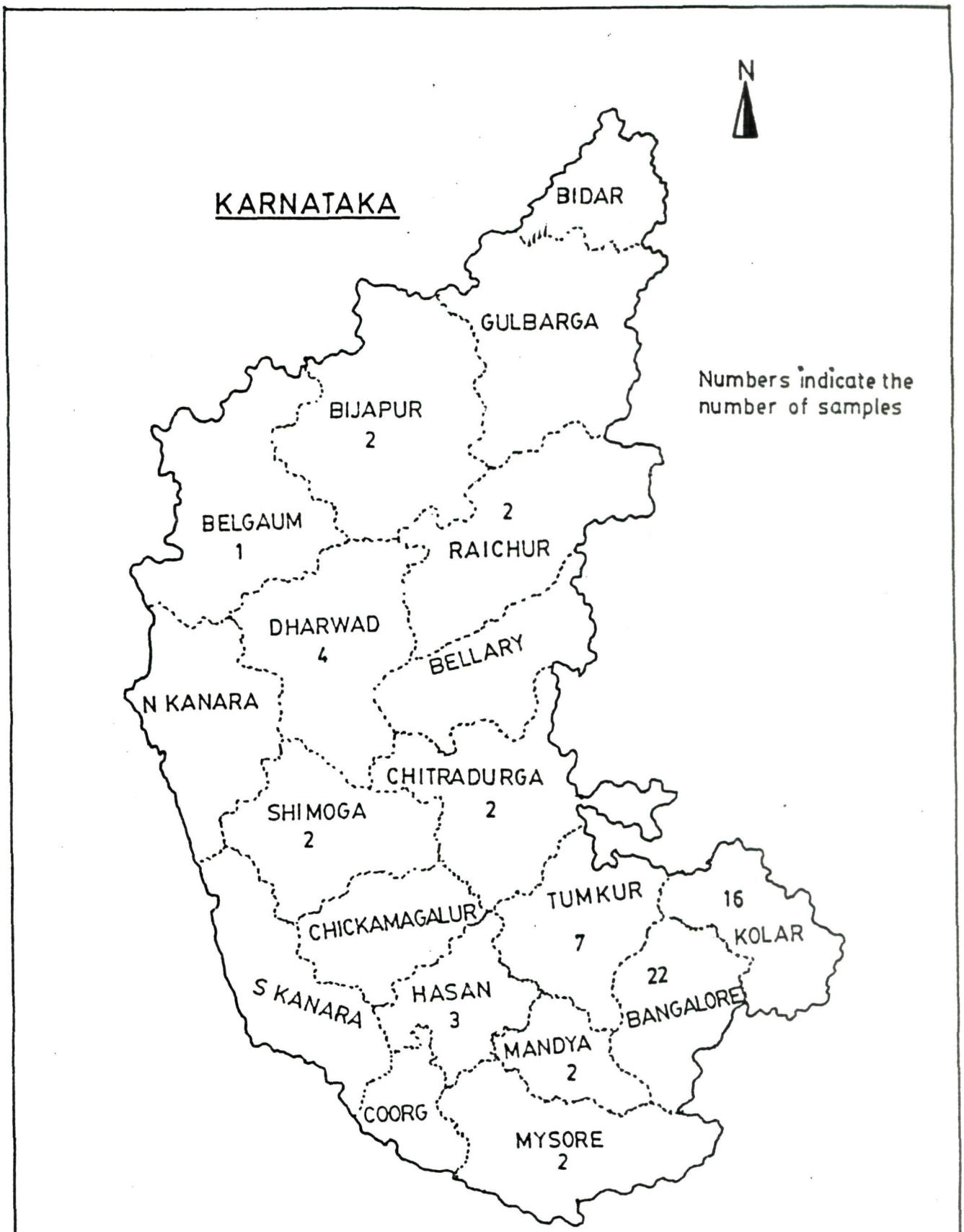


Fig. 1. Number of samples collected from different districts of Karnataka to know the incidence of Fusarium wilt in tomato.

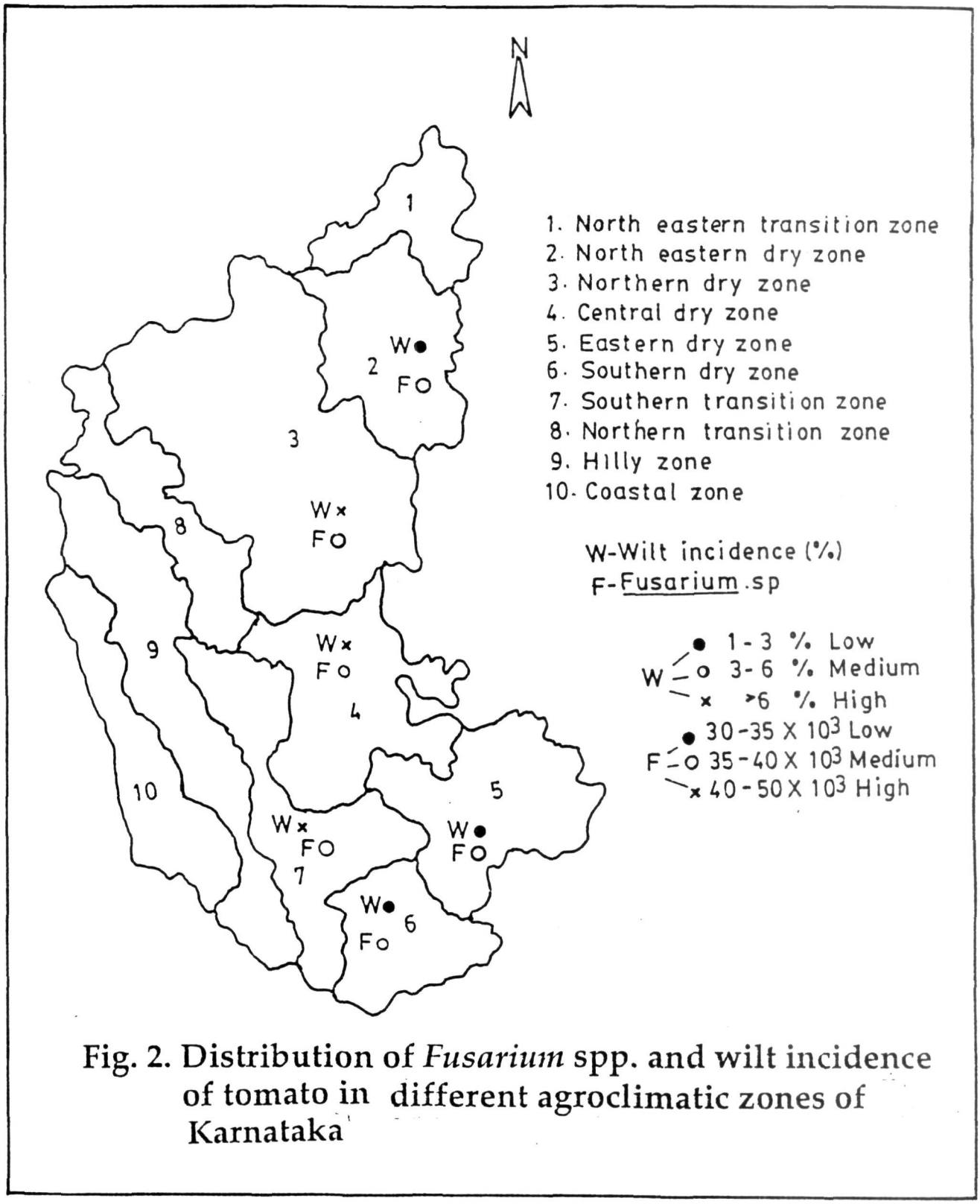


Fig. 2. Distribution of *Fusarium* spp. and wilt incidence of tomato in different agroclimatic zones of Karnataka

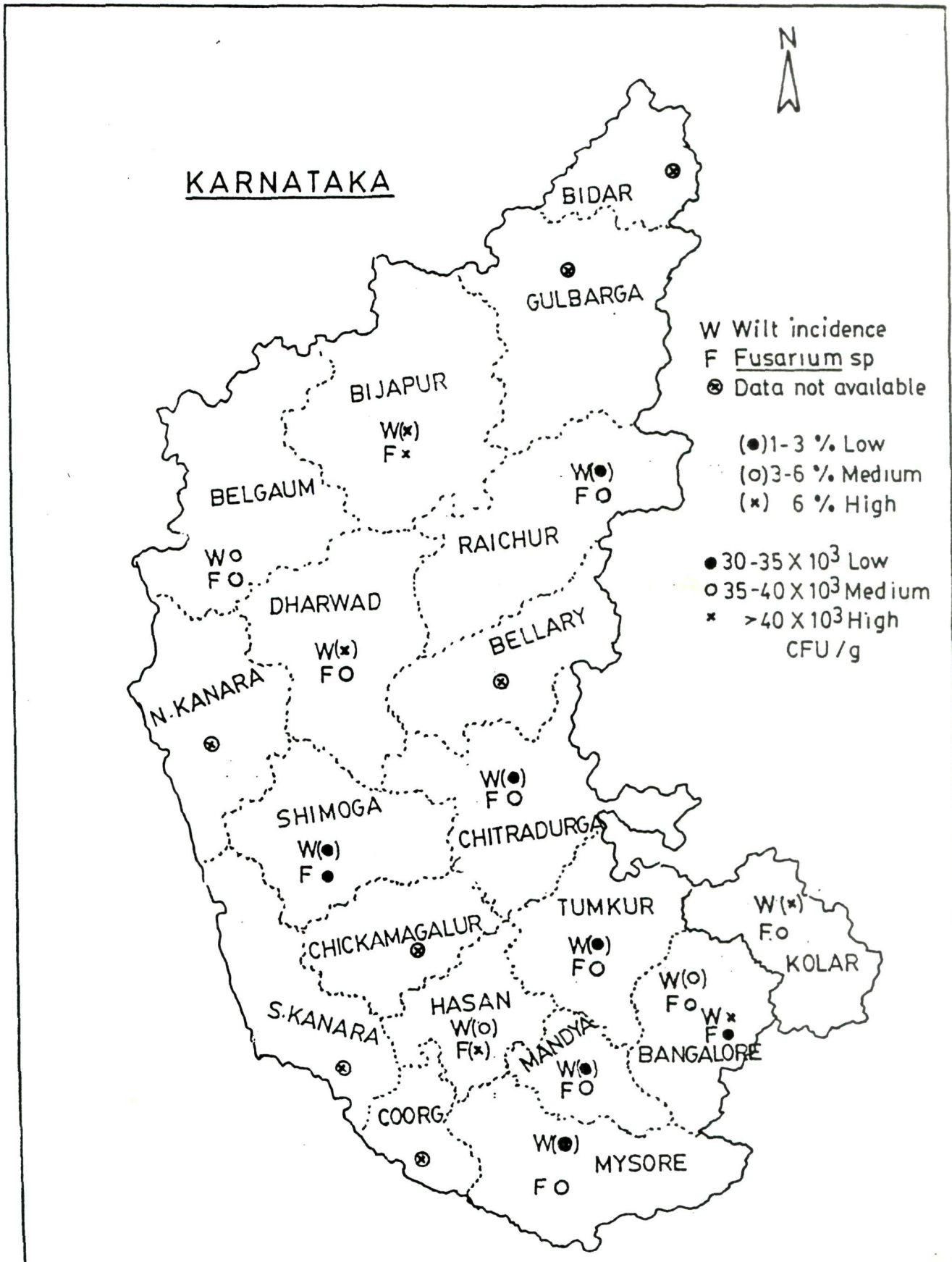


Fig. 3. Distribution of *Fusarium* spp. and wilt incidence of tomato in different districts of Karnataka

Table 7. Incidence of Fusarium Wilt of Tomato in Different Agro Climatic Zones of Karnataka

Zone	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	Per cent diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
North Eastern Dry Zone	2	50.0	1.5	39.5
Northern Dry Zone	4	75.0	7.0	38.5
Central Dry Zone	9	66.6	3.2	30.8
Eastern Dry Zone	40	50.0	7.1	36.2
Southern Dry Zone	5	60.0	2.4	36.3
Southern Transition Zone	2	100.0	3.0	35.0
Northern Transition Zone	3	100.0	7.8	38.3
	65			

Table 8. Incidence of Fusarium Wilt of Tomato in Different Districts of Karnataka

Districts	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
Kolar	16	43.5	8.97	34.20
Bangalore(R)	13	61.5	4.55	36.40
Bangalore(U)	9	33.1	9.75	34.74
Tumkur	7	85.7	2.78	37.48
Dharwad	4	75.0	7.00	37.40
Hassan	3	66.6	3.50	42.30
Mysore	2	50.0	1.50	36.50
Mandya	2	50.0	1.50	39.50
Raichur	2	50.0	1.50	39.50
Bijapur	2	100.0	10.00	41.10
Chitradurg	2	50.0	1.50	39.80
Shimoga	2	100.0	3.00	34.95
Belgaum	1	100.00	3.50	39.50
13	**65**			

Table 9. Effect of Soil Types on the Incidence of Fusarium Wilt in Tomato in Karnataka

Soil Type	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
Red Sandy Loam	34	50.00	7.20	35.40
Sandy Loam	14	71.43	4.68	36.90
Sandy Clay Loam	12	50.00	3.40	36.30
Red Clay Loam	5	80.00	8.80	39.30
4	65			

Table 10. Reaction of Tomato Varieties to the Incidence of Fusarium wilt in Karnataka

Variety	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
Local	16	62.50	3.00	39.80
Rashmi	13	38.50	7.50	36.30
Pusa Ruby	10	90.00	4.40	34.50
Hybrids	8	62.50	3.00	38.20
Rupali	6	50.00	11.90	36.00
Arka Vikas	3	66.60	3.20	33.40
Pusa Early Dwarf	2	-	1.50	31.80
Sonalika	2	100.00	10.00	41.10
Karnataka Hybrid	1	100.00	7.50	35.30
Bhagyalakshmi	1	-	32.50	30.60
Summer Shakti	1	-	17.50	31.60
Real Shakti	1	-	12.50	33.30
Preethi	1	100.00	7.50	37.30
	65			

Table 11. Effect of Stage of Tomato Crop on the Incidence of Fusarium Wilt in Karnataka

Stage of the Crop	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
Nursery	8	75.00	12.50	35.70
Vegetative Phase	9	88.90	2.08	36.60
Flowering	25	48.00	5.90	37.00
Fruiting	23	52.20	5.60	37.30
	65			

Table 12. Effect of Cropping Pattern on the Incidence of Fusarium Wilt of Tomato in Karnataka

Name of the Previous Crop	Total no. of Samples	frequency occurrence of *Fusarium* spp. in root (%)	diseased plants (%)	Propagules of *Fusarium* spp. in soil (cfu. $g^{-1} \times 10^3$)
Ragi	18	55.60	5.30	36.70
Vegetables	13	69.20	4.40	37.60
Maize	8	62.50	7.00	38.00
Cabbage	4	-	3.00	38.00
Paddy	4	100.00	8.10	33.10
Bhendi	3	66.60	1.25	33.30
Brinjal	2	50.00	2.50	35.00
Onion	2	50.00	17.50	41.00
Beans	2	50.00	7.00	38.30
Carrot	2	50.00	7.00	35.50
Sunflower	1	-	2.50	37.00
Cucurbits	1	100.00	1.50	39.30
Chillies	1	100.00	2.50	34.30
Beetroot	1	-	1.50	33.60
Potato	1	100.00	35.00	34.30
Garlic	1	-	9.00	32.60
Banana	1	100.00	5.00	33.30
Tomato	1	100.00	17.50	41.00
	65			

wt of soil) and the least in Kolar district (34.20 x 10^3 cfu g^{-1} dry wt of soil). The data are presented in Table 8 and Fig. 3.

Effect of soil types on the disease incidence revealed that highest incidence of wilt was observed in red clay loam soil (8.8%) followed by red sandy loam soil (7.2%). The frequency occurrence of *Fusarium* spp. was highest in red clay loam (80%) followed by sandy loam soil (71.43%). Population of *Fusarium* spp. was also high in red clay loam (39.3 x 10^3 cfu g^{-1} soil). Sandy clay loam soil recorded the least incidence of wilting plants (3.4%). The frequency occurrence of *Fusarium* spp. in root was (50%) and population of *Fusarium* sp (36.3 x 10^3 cfu g^{-1} of soil), Table 9.

Reaction of tomato varieties to wilt incidence revealed that the variety Bhagyalaxmi recorded highest (32.5 per cent) followed by Summer Shakti (17.5 per cent) and Real Shakti (12.5 per cent). Among high yielding varieties Pusa Ruby recorded highest per cent of wilted plants (4.4). Pusa Early Dwarf recorded the least percentage of wilted plants(1.50). Varieties such as Sonalika, Preethi, Karnataka Hybrid recorded the highest frequency occurrence of *Fusarium* spp. (100 per cent) followed by Pusa Ruby (90 per cent). Least was found in the variety Rashmi. (38.50%). Population of *Fusarium* spp in soil was maximum in Sonalika (41.1 x 10^3 cfu g^{-1} of soil) and least in Bhagyalakshmi (30.6 x 10^3 cfu g^{-1} of soil). From the Table 10 it can also be seen that on the number of samples analysed Pusa Ruby and Local varieties recorded 4.4 and 3.0 per cent wilted plants while percent frequency occurrence of *Fusarium* spp. was 90 and 62.5 respectively.

From the results of survey (Table 11) it can be seen that wilt incidence is comparatively less in vegetative phase (2.08 per cent) than in nursery (12.5 per cent). Flowering and fruiting stages recorded 5.9 and 5.6 per cent wilted plants respectively. Per cent frequency occurrence of *Fusarium* spp. was the highest in vegetative phase (88.9) followed by Nursery stage (75). Population was the maximum at fruiting stage (37.3 x 10^3 cfu g^{-1} of soil) and least in nursery (35.7 x 10^3 cfu g^{-1} of soil).

Data on cropping pattern (Table 12) on the incidence of wilt indicated that growing tomato after bhendi recorded the lowest per cent of wilted plants (1.25) followed by that of cucurbits and beet root (1.50). Percent diseased plants was the highest in tomato after potato (35) followed by tomato after onion (17.5).

Sample No.	Morphological and Cultural Characters on 2% PSA	Micro Conidia	Macro Conidia	Stem Vascular Discolouration	Root Discolouration	Pathogenic Reaction	Disease			Location
							Pre	Post	PDI	
2, 19, 32, 14, 43, 44	Sparse to abundant mycelium, initially white, cottony, fluffy, turned to pale pink to purple, chlamydospores round to slightly elliptical single or two celled, both terminal and intercalary 10 (9-11) µ sporulation moderate to profuse, colony diameter 45-90 mm.	Oval, round to fusiform, 0-1 septate, borne singly, 12.13 (9.2 - 16.4) µ x 3.4 (2.9 - 4.0) µ	Fusiform with 1 - 5 septa mostly 3 septate 31.6 x 3.4 µ (20.9 - 35.7) µ	Vascular discolouration above collar region present.	Yellowish brown discolouration of roots with brown vascular discolouration.	Pathogenic	34.4 (22.6to 47.6)	48.15 (34.0to 63.8)	60.63 (44.2to) 81.66)	Nelamangala Chikkaballapur Doddaballapur Chintamani Raichur Belgaum
35, 23, 63, 52, 24, 62	Moderate to abundant aerial mycelium, cottony, fluffy, white to pale pink colony diameter 55 -90 mm,sporulation poor to profuse, chlamydospores terminal or intercalary, one or two celled circular to oval, 8.4 to 10 µ	Oval to fusiform 0 to 1 septate borne singly, 15.8x5.2µ (11.1-15.4 µ to4.5-5.2)	1 - 5 septate, more common are 3 septate 31.0 x 5.3µ (23.8 - 38 to 4.76 - 5.24)µ	Vascular discolouration above collar region prsent.	Pale yellowish brown discolouration with brown vascular discolouration.	Pathogenic	23.7 (4.30to 37.90)	42.00 (12.60to 56.00)	59.23 (45.00to 75.80)	Bangalore North Tumkur (Gubbi) Shiggon Mugad Mysore Biligi
39, 53	Moderate to abundant aerial mycelium, cottony, white to buff colony diameter 65 - 85 mm, sporulation moderate chlamydospores terminal or intercalary, two to three celled circular 9.5 to 11.0 µ	Oval to fusi form 0 to 1 septate borne singly, 6.4 - 12.5 X 2.4 - 3.6 µ	fusiform 1 - 6 septate, more common are 3 - 5 septate 25.4 - 45.0 x 3.6 - 4.5 µ		Brown discolouration of roots with brown vascular discolouration.	Pathogenic	37.9 (28.2to 47.60)	53.3 (35.40to 55.00)	47.35 (43.00to 51.70)	Gadag Shimoga
1, 3, 4 10, 31 36, 56 57	Abundant aerial mycelium, cottony, fluffy, white to pale pink colony diameter 75 - 90 mm, sporulation moderate to profuse, chlamydospores terminal or intercalary, 1 or 2 celled circular to oval, 9.0 to 10 µ	Oval to fusi form 0 to 1 septate borne singly, 12.9 (8.4 - 16.8) x4.48(4.2-6.3) µ	fusiform 1 - 5 septate, more common are 3 septate 32.3 µ (25 - 56) x4.5(4.2-4.6) µ	—	—	Pathogenic	10 - 12.5%	25.50to 40 80	—	Nelamangala Devanahalli Channapatna Malur Bangalore North
,21,22, 7,28 9,17 8,40 1,47 8,50 8,60 2	Moderate to abundant aerial mycelium, cottony, fluffy, white to buff colony diameter 80-90 mm, sporulation moderate to profuse, chlamydospores terminal or intercalary, 1 or 2 celled circular to oval, 10.0 to 12 µ	Oval to fusi form 0 to 1 septate	fusiform 1 - 6 septate,	Non Pathogenic	—	—	—	—	—	Arsikere Channapatna Chintamani Bagepalli Chikkaballapur Madhugiri Pavagada Shimoga Gubbi Kunigal Nagamangala Biligi Hiriyur

or	Sporulation	2-3 x 105 ml -1	Poor
dium		3-5 x 105 ml -1	Moderate
od		> 5 x 105 ml -1	Profuse

Pre = Per cent pre-emergence seedling disease
Post = Per cent post-emergence seedling disease

Table 14. Pathogenic Reaction of Isolates of *Fusarium* spp. in Tomato

Isolate No.	Location	pre-emergence seedling disease (%)	post-emergence seedling disease (%)	Per cent disease incidence (PDI)	Days taken for appearence of first wilt symptoms
F_1	Nelamangala	33.80	63.80	81.66	14
F_2	Hebbal	39.52	56.00	74.20	14
F_3	Chikkaballapur	22.60	54.20	65.80	16
F_4	IARI (ITCC 1322)	40.30	54.10	65.00	18
F_5	Chintamani	42.70	36.60	44.20	21
F_6	Tumkur	37.90	53.20	75.80	16
F_7	Belgaum	47.00	52.30	68.30	14
F_8	Gadag	28.20	51.60	51.70	15
F_9	Shiggon	18.60	51.50	46.70	21
F_{10}	Doddaballapur	30.60	48.80	45.80	20
F_{11}	Mugad	24.00	26.00	45.00	18
F_{12}	Mysore	4.30	12.60	51.70	15
F_{13}	Biligi	36.00	54.00	54.10	18
F_{14}	IARI (ITCC 1350)	47.50	35.40	50.50	24
F_{15}	Raichur	29.00	34.00	56.20	21
F_{16}	Shimoga	47.00	55.00	43.00	18

PDI = Measurment of Wilt

4.2. ISOLATION OF *Fusarium* spp. AND STUDY OF MORPHOLOGICAL, CULTURAL AND PATHOGENIC CHARACTERS.

Fusarium spp. associated with wilt were isolated from infected tomato plant samples received from 13 districts of Karnataka. All the isolates were grown on two per cent potato sucrose agar (PSA), their morphological and cultural characters were studied in comparison with typical *F.oxysporum* f.sp. *lycopersici* (ITCC No 1322) and *F. solani* (ITCCNo 1350) cultures. Pathogenicity tests were conducted with all the isolates of *Fusarium* spp. and results are given below.

4.2.1. Isolation

Isolation from the roots and collar region of wilted tomato plant samples collected during survey yielded 38 isolates of *Fusarium* spp. These isolates were named after the place from which the plant samples were collected. Individual single spore cultures established from all the isolates were maintained on PDA by periodic transfers to fresh medium.

4.2.2. Morphological and Cultural characters.

Morphological and cultural characters of all the 38 isolates along with *F.oxysporum* f.sp. *lycopersici* and *F. solani* were studied on two per cent PSA. The results are presented in table 13.

4.2.3. Pathogenicity

Among the 38 isolates of *Fusarium* spp. 22 isolates along with *F.oxysporum* f.sp. *lycopersici* and *F. solani* induced seedling disease and 16 isolates were non pathogenic. Fourteen isolates of *Fusarium* spp. induced wilt disease in tomato and the data are presented in table 13.

4.2.4. Grouping of Isolates of *Fusarium* spp. Based on Morphological and Cultural Characters and Pathogenicity.

Thirty eight isolates belonging to *Fusarium* spp.were obtained from the roots and collar regions of infected tomato plant samples. On the basis of morphological and cultural characters of the isolates studied on two per cent potato sucrose agar together with pathogenicity on tomato (Table 13) the 38 isolates of Fusarium spp. were classified in to five groups of which the first three were pathogenic to tomato plants causing

Table 15. Effect of Different Isolates of *F. oxysporum* f.sp. *lycopersici* and *F. solani* on Disease parameters in Tomato

Isolate No.	Mean Shoot Length (cm)	reduction in shoot length (%)	stunted plants (%)	wilted plants (%)	Total Diseased Plants (%)
F_1	28.40	44.90	10.00	83.33	93.33
F_2	29.10	43.60	16.60	76.60	93.20
F_3	33.20	35.60	26.60	60.00	86.60
F_4	31.60	38.75	30.00	63.30	93.30
F_5	35.30	31.59	50.00	30.00	80.00
F_6	30.30	41.30	16.60	76.60	93.20
F_7	31.20	39.50	33.30	60.00	93.30
F_9	40.30	21.90	46.60	40.00	86.60
F_{10}	38.20	26.00	46.60	33.33	79.93
F_{11}	33.80	34.50	56.60	33.33	89.93
F_{12}	41.10	20.30	56.60	36.60	93.20
F_{13}	35.40	31.40	43.30	43.30	86.60
F_{15}	32.80	36.43	46.60	46.60	93.20
F_8	42.60	17.40	33.30	40.00	73.30
F_{14}	36.50	29.26	26.60	51.30	77.90
F_{16}	34.80	32.60	43.30	33.33	76.60
Healthy	51.60				

F_1 to F_{15} - *F. oxysporum* f.sp. *lycopersici*

F_8 F_{14} and F_{16} - *F. solani*

F_4 and F_{14} - Received from IARI New Delhi

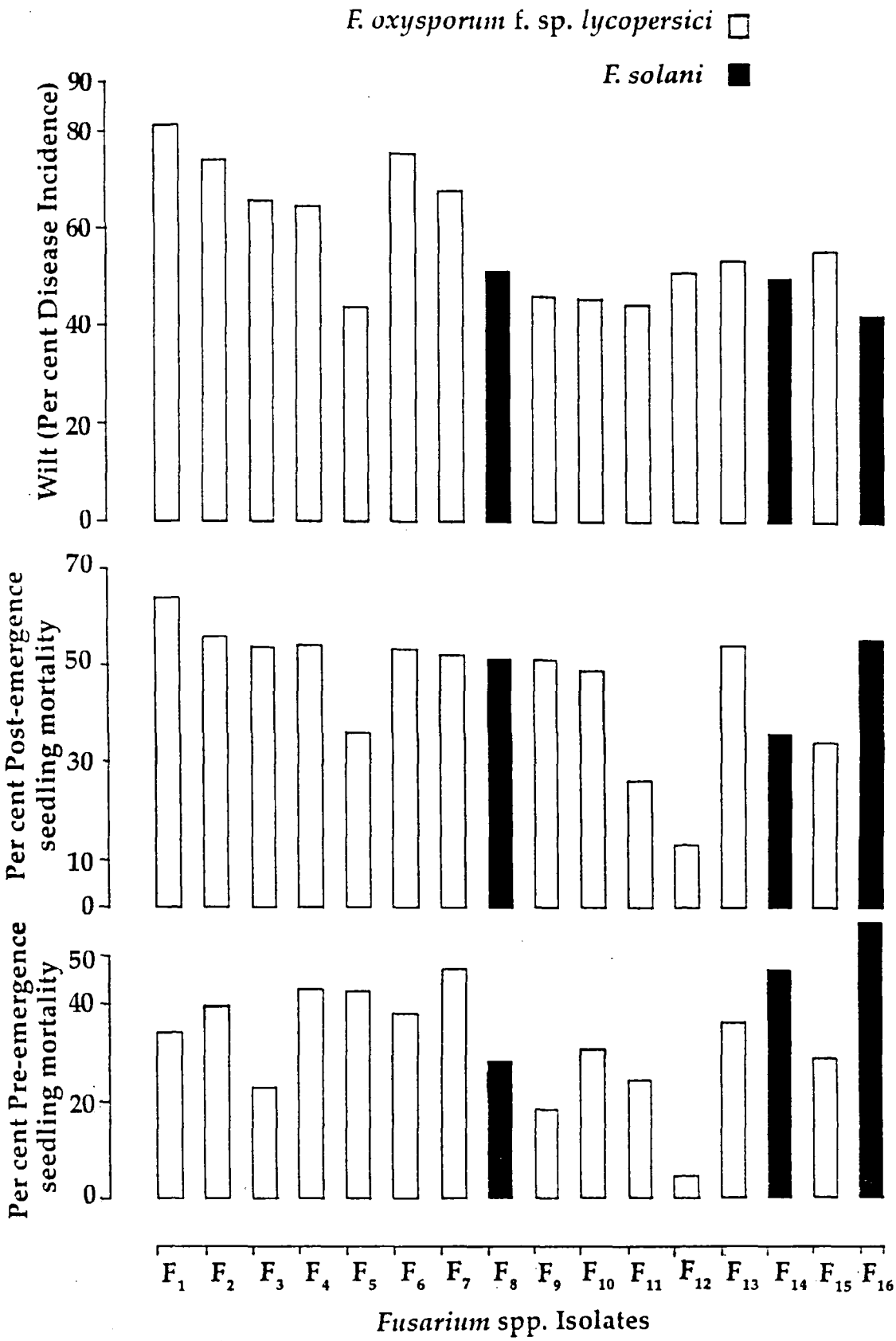


Fig. 4. Pathogenic Reaction of Isolates of Fusarium spp. in Tomato cv Pusa Ruby

Plate 3 *a & b* Seedling disease induced by different isolates of *F. oxysporum* f.sp. *lycopersici* (F_1 - F_6)

H : Healthy



a



b

PLATE 3

Plate 4

a) Seedling mortality due to isolate of *F.oxysporum* f.sp. *lycopersici* (F_7)
H. Healthy

b) Tomato seedlings showing typical girling at collar region due to *F.oxysporum* f.sp. *lycopersici*
H : Healthy



a



b

PLATE 4

Plate 5 Tomato plants cv Pusa Ruby showing wilt due to different isolates of *F.oxysporum* f.sp. *lycopersici* (F_1 to F_7)
Control : Healthy seedling.





PLATE 5

Plate 6 Transverse section of infected tomato roots showing mycelium of *F.oxysporum* f.sp. *lycopersici* in the xylem vessels.

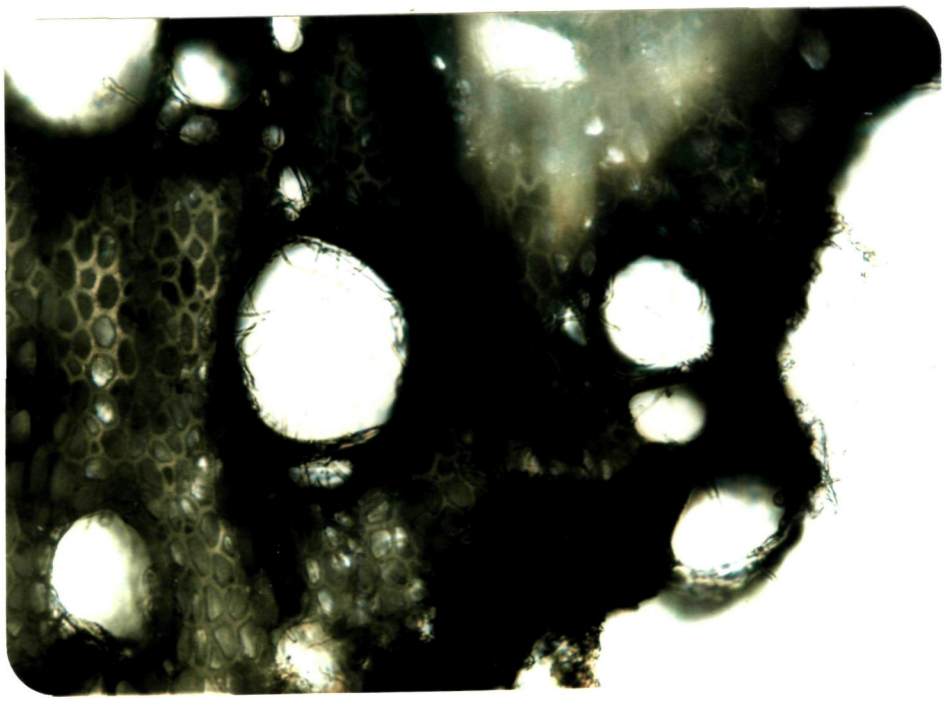


PLATE 6

pre-emergence and post-emergence seedling mortality and wilt of transplanted plant. The fourth group was able to cause only seedling disease and failed to induce wilt symptoms. Isolates belonging to group five did not cause any infection in tomato plants. The isolates belonging to Group I and II were identified as *F. oxysporum* f. sp. *lycopersici* and Group III as *F. solani* as per Subramanyam (1970) and Booth (1971).

4.2.5. PATHOGENIC REACTION OF ISOLATES OF *Fusarium* spp.

Among the pathogenic isolates twelve belonged to *F. oxysporum* f. sp. *lycopersici* and two *to F. solani.* These isolates along with cultures of *F. oxysporum* f. sp. *lycopersici* and *F. solani* obtained from IARI were designated as F_1 to F_{16}; Pathogenic reaction of these isolates on seedling disease and wilt revealed that isolate F_1 is more virulent compared to all other isolates as it produced the highest post-emergence seedling disease (63.8 per cent) and wilt (81.66 per cent) and produced symptoms by 14 days after planting. Isolates F_6 and F_2 were the next best virulent by producing post-emergence seedling disease (53.2 and 54.2 per cent respectively) and wilt (75.8 and 74.2 per cent respectively). Wilt symptoms appeared 16 and 14 days after planting respectively. Isolates of *F. solani* isolates (F_8, F_{14} and F_{16}) were found to be more pathogenic at seedling stage as compared to adult stage of plants. All the three isolates produced wilt incidence in the range of 43.0 to 51.7 per cent. Symptoms appeared 15 to 24 days after planting (Table 8). Reisolation from diseased plants consistently yielded the respective isolates of *Fusarium* spp. (Table 14; Fig 4 and Plate 3, 4 and 5).

4.2.6. SYMPTOMOTOLOGY

Seedling Disease

All the isolates exhibited more or less similar symptoms on young seedlings. Initially water soaked areas developed at the collar region which soon appeared as a girdling symptom resulting in seedling collapse. Foliage of such seedling turned yellow and plants exhibited wilt symptoms and died slowly.

Studies revealed that the disease development involved a chain of sy-mptoms. The extent and degree of the disease varied depending upon the virulence of the pathogen and susceptibility of host. The common symptoms caused by *F. oxysporum* f. sp. *lycopersici* and *F. solani* are given below.

Table 16. Computation of Disease Index with 16 Isolates of *Fusarium* causing Wilt in Tomato

Isolate No.	Disease Grade				Mean Disease Grade	Per cent Disease Incidence
	1	2	3	4		
F_1	1	2	7	18	3.27	81.66
F_2	2	3	11	12	2.96	74.20
F_3	1	7	8	10	2.63	65.80
F_4	5	4	11	8	2.60	65.00
F_5	8	7	5	4	1.76	44.20
F_6	2	3	9	14	3.01	75.80
F_7	4	6	6	12	2.83	68.30
F_8	6	4	3	9	2.06	51.70
F_9	9	5	8	4	1.87	46.70
F_{10}	10	4	3	7	1.83	45.80
F_{11}	12	5	8	2	1.80	45.00
F_{12}	8	9	7	4	2.07	51.67
F_{13}	7	6	6	7	2.50	54.10
F_{14}	3	5	10	6	2.23	55.80
F_{15}	10	4	6	8	2.27	56.60
F_{16}	8	5	6	4	1.73	43.30

Thirty plants were assessed for the purpose of arriving at the per cent disease incidence (PDI)

Disease Grade

1 = Slight drooping of leaves 2 = Wilting of lower leaves

3 = Severely wilted, all leaves wilted and withered except terminal buds

4 = Dead plants

Stunting.

There was a reduction in plant shoot length in all the cases wherever infection occurred. Depending upon the virulence of the isolate the mean shoot length of diseased plants varied from 28.4 cm to 42.6 cm. while the mean shoot length of healthy plants was 51.60 cm (Table 15)

Yellowing

The next prominent symptom in the disease development was yellowing of leaves starting from lower leaves progressing upward. The plant showing yellowing symptoms remained as such throughout or developed other symptoms of wilting.

Leaf Necrosis

As the disease progressed the chlorotic leaves showed marginal necrosis, which either remained static or advanced to next stage.

Wilting

This was the ultimate stage of the disease in which the whole plant dried. Wilting sometimes was gradual, following yellowing and necrosis, resembling slow wilting. The lower leaves turned yellow followed by marginal leaf necrosis leading to complete drying of leaves resulting in death of plants.

Among the underground symptoms reduction of root growth, loss of tap root system was frequent. All the isolates of *F. oxysporum* f. sp. *lycopersici* caused pale yellow to yellowish browning of roots. Vascular browning, discolouration above collar region was the prominent symptom observed with all isolates of *F. oxysporum* f. sp. *lycopersici.* Isolates of *F. solani* produced a brown sunken lesion, following girdling of the plants at the collar region ultimately leading to the collapse of seedlings.

4.2.7. COMPUTATION OF DISEASE INDEX

The wilt disease was first measured on the basis of the number of the stunted and wilted plants in each of the treatment populations. The results are presented in Table15. The mean shoot length (MSL) in healthy population was 51.6 cm while it ranged from 28.4 to 42.60 cm in treated population. In this case per cent stunted and wilted plants together represented diseased plants which ranged from 73.3 (F_8) to 93.3 (F_1, F_4 and F_7). In cases

Table 17. *In Vitro* Pathogenic Reaction of Isolates of *Fusarium* on Tomato Seedlings

Isolate No.	seedling mortality (%)	
	Pre-emergence	Post-emergence
F_1	29.50 (32.68)	82.25 (65.49)
F_2	34.10 (35.49)	67.92 (55.49)
F_3	42.73 (40.57)	75.78 (60.58(
F_4	39.73 (39.05)	65.85 (54.24)
F_5	10.70 (18.48)	21.48 (27.50)
F_6	27.25 (31.34)	77.03 (61.37)
F_7	41.84 (44.54)	69.40 (56.35)
CD at 5%	(7.44)	(3.91)

Figures in parentheses are transformed 'Arc Sine' Values.

Table 18. Effect of Different Soil Types on the Incidence of *Fusarium* Wilt in Tomato

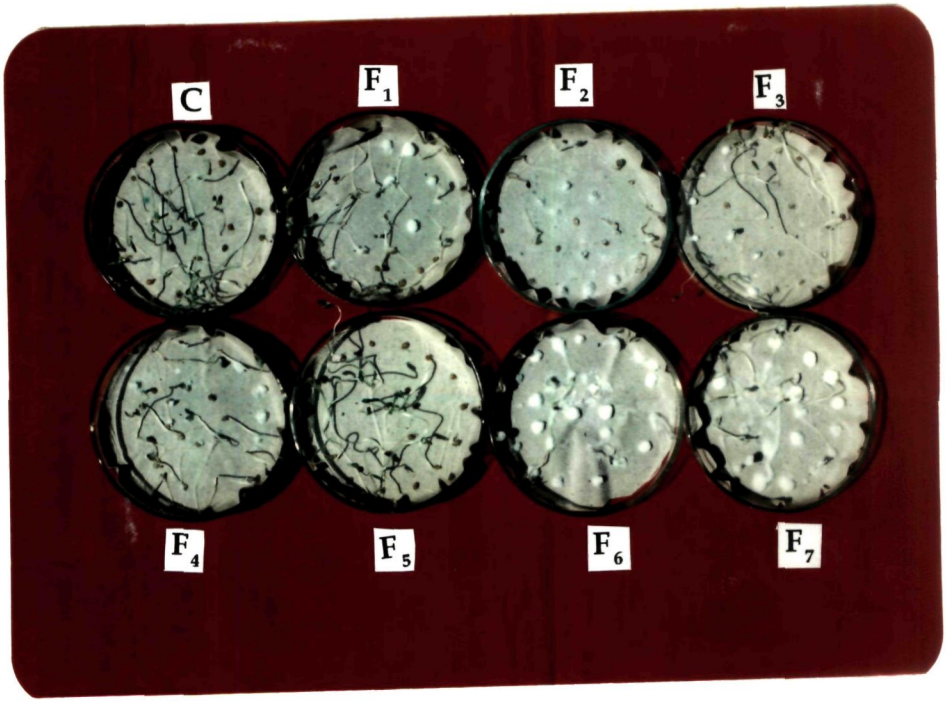
Soil Type	PDI	Propagules cfu g^{-1} Soil $x10^2$
Sandy Soil	84.00 (66.49)	55.80 (3.75)
Sandy Loam	80.50 (63.82)	46.40 (3.67)
Sandy Clay Loam	65.00 (53.80)	35.20 (3.55)
Sandy Clay	43.50 (41.23)	24.80 (3.39)
Clay	37.50 (37.64)	18.80 (3.27)
CD at 5%	(11.87)	(0.050)

Figures in parentheses are transformed 'Arc Sine" values for PDI and Logarithmic transformation values for propagules.

Plate 7

a) Isolates of *F. oxysporum* f.sp. *lycopersici* (F_1 to F_7) causing pre and post emergence damping-off of tomato seeds.

b) Wilting of tomato plants (B) induced by rapid pathogenicity test. A-Control (Healthy plant)



a



b

PLATE 7

where there was high percentage wilting (F_1, F_4, F_7, F_2 and F_6) the per cent average reduction in shoot length was also found to be high to the tune of 38.75 (F_4) to 44.90 (F_1). Per cent stunted plants was found to be low due to high percent wilting in isolates F_1, F_2 and F_6

The assessment of the disease only on the basis of stunting /wilting do not give complete picture of the impact of the disease on the plants. Hence it was felt necessary to take into consideration all the symptoms produced on the above ground parts of plants which are associated with Fusarium wilt. The data are presented in Table 16. The index values ranged from 1.73 to 3.27 for different isolates. The index values were grouped into three categories indicating low (1.73 to 2.23) medium (2.27 to 2.83) and high (2.96 to 3.27). Accordingly, isolates F_{16}, F_5, F_{11}, F_{10}, F_9, F_8, F_{12} and F_{14} recorded values falling under low disease index. In such cases the advanced stages of symptoms viz. necrosis and wilting were less. Isolates F_{15}, F_{13}, F_4, F_3 and F_7 were categorized under medium disease index showing all types of symptom grades with low to moderate frequency. In high disease grades (isolates F_2, F_6, and F_1) the frequency of wilted plants was always more comparatively less number of plants were found to be under in advanced stage of symptoms. When the disease index values were converted to percentage the highest percent disease incidence (PDI) of 81.66 was recorded in F_1 followed by F_6 and F_2 with 75.8 and 74.2 respectively. The least PDI was observed in F_{16} with 43.3 followed by F_5 with 44.2 (Fig. 4)

4.2.8 *In vitro* Pathogenicity of Isolates of *Fusarium.*

F_1 to F_7 isolates of *F. oxysporum* f. sp. *lycopersici* were tested for their virulence *in vitro*. Data were presented in Table 17. The highest per cent pre-emergence seedling mortality was observed with F_3 (42.73) followed by F_7 (41.84) and least in F_5 (10.70). Per cent post-emergence seedling mortality was highest in case of F_1 (82.25) followed by F_6 (77.03) and the least in F_5 (21.48) and (plate 7a).

4.2.9. Rapid Pathogenicity - Vial test

Four week old tomato seedlings were removed and roots severed with sharp blade and immersed in spore suspension of isolates of *Fusarium*. F_1 isolate proved more virulent by killing the plants by fourth day. After inoculation first wilt symptoms were noticed 36 hours after keeping the plants in spore suspension contained in test tube. (Plate.7b)

Table 19. Effect of Inoculum Density on the Incidence of Seedling Disease and Wilt in Tomato

Inoculum Density 1	Propagules cfu g^{-1} soil x 10^2 2	Germination (%) 3	pre-emergence seedling disease (%) 4	post-emergence seedling disease (%) 5	Per cent disease incidence (PDI) 6
1 : 1	477.25 (4.68)	34.50 (35.91)	59.00 (50.14)	82.70 (65.46)	91.25 (72.94)
1 : 2	373.50 (4.57)	44.00 (41.54)	47.70 (43.71)	78.30 (62.31)	90.00 (71.85)
1 : 4	146.75 (4.17)	47.50 (43.61)	43.70 (41.33)	71.40 (57.74)	83.75 (66.27)
1 : 8	66.25 (3.82)	59.00 (50.20)	30.20 (33.28)	66.35 (54.61)	81.25 (64.47)
1 : 16	41.75 (3.62)	63.50 (52.84)	24.70 (29.81)	63.81 (53.05)	80.00 (63.52)
1 : 32	21.50 (3.33)	72.50 (58.47)	14.20 (22.04)	32.40 (34.60)	31.25 (33.84)
1 : 64	7.75 (3.03)	75.50 (60.41)	12.30 (20.29)	18.30 (25.20)	17.50 (24.45)
Healthy		84.50 (66.90)			
CD at 5%	(0.18)	(4.11)	(5.65)	(4.62)	(5.46)

Figures in the parentheses are transformed 'Arc Sine' values (Columns 3, 4, 5, 6) and logarithmic transformation values (Column 2) for propagules.

PDI = Measurement of Wilt

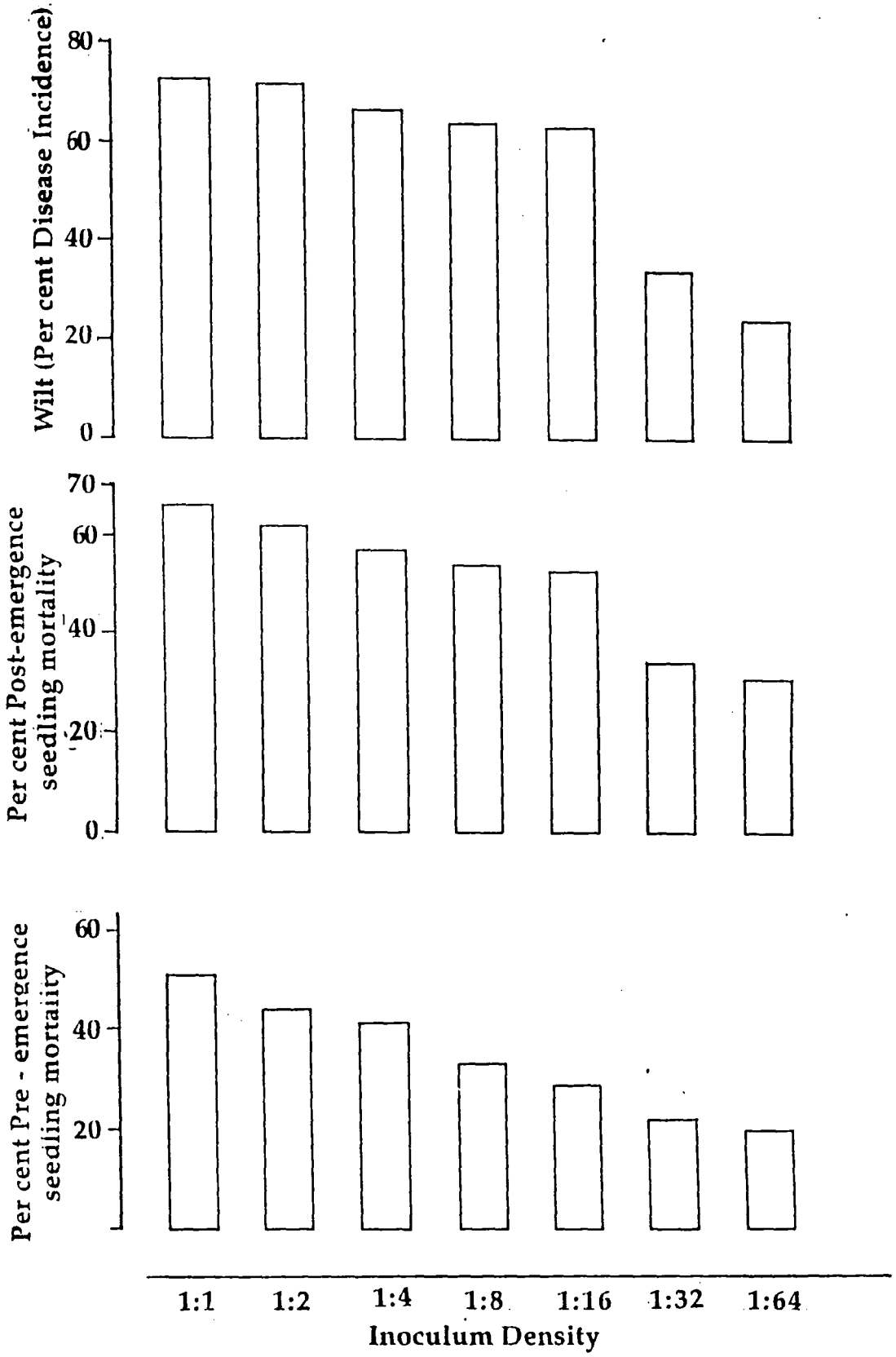


Fig. 5. Effect of Inoculum Density on Seedling Disease and wilt in Tomato

4.3. FACTORS INFLUENCING DISEASE DEVELOPMENT

4.3.1. Soil Types and Disease

Effect of five different soil types as given in Table 1, sandy soil to clay on disease development was studied. The per cent disease incidence recorded and the population of the propagules in each of these soils are presented in the Table 18. .

The highest PDI of 84.0 was recorded in sandy soil followed by sandy loam with 80.50 which did not differ significantly from each other. The least PDI of 37.5 was observed in clay soil followed by sandy clay with 43.5 which had no significant difference from each other. When the number of propagules in soil was estimated to know the survival and build up of the inoculum, a significantly higher number of propagules ranging from (55.8 - 46.4 x 10^2 g^{-1} soil)respectively were recovered from sandy soil and red sandy loam as compared to (18.8 - 24.8 x 10^2 g^{-1} soil) in clay and sandy clay soils respectively.

4.3.2. Inoculum Density and Disease

Influence of inoculum density on pathogen propagules, seed germination and disease development was determined. The different inoculum densities in the proportions of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 of inoculum and soil was used and the results are depicted in Table 19. From the data it can be seen that as the dilution level increased from a ratio of 1 : 64 to 1 : 1 there was an increase in number of propagules of pathogen in soil from 7.75 to 477.25 x 10^2 g^{-1} soil which was statistically significant. The germination of seeds decreased as the inoculum level increased ranging from 75.5% at 1 : 64 to 34.5% at 1 : 1 level.

Further, the incidence of seedling disease and wilt showed an increasing trend with an increase in the inoculum density. The perusal of the data revealed that 1:16 ratio as the infection threshold optimum for disease development. Correspondingly the inoculum in the soil was found to be 41.75 x 10^2 g^{-1} soil. It can be concluded that a minimum of 4200 cfu g^{-1} soil is required to get 50 per cent and above post-emergence seedling mortality and wilt.

4.3.3. Varieties and Disease

Seventeen varieties of tomato were evaluated under pot cuture studies in glass house

Table 20. Varietal Response of Tomato to Fusarium Wilt

Variety	PPI	PDI	CODEX
Arka Vikas	70.00 (57.00)	54.17 (47.40)	38.33 (38.16)
Arka Saurabh	73.30 (59.01)	50.00 (45.00)	36.83 (37.30)
Arka Ashish	66.60 (54.78)	50.83 (45.48)	33.92 (35.59)
Arka Abha	73.30 (59.22)	54.17 (47.42)	40.71 (39.39)
Arka Alok	63.30 (53.07)	51.71 (45.99)	34.58 (35.44)
Arka Ahuti	76.70 (61.22)	52.50 (46.44)	35.42 (35.99)
Arka Vishal	73.30 (59.01)	50.83 (45.48)	37.42 (37.56)
Pusa Early Dwarf	83.30 (66.15)	64.21 (53.27)	53.42 (46.95)
Roma	63.30 (52.78)	50.00 (45.00)	31.75 (34.26)
Best of All	80.00 (63.93)	65.83 (54.34)	53.21 (46.88)
Rupali	63.30 (52.86)	44.21 (41.59)	28.75 (32.07)
Rashmi	66.70 (55.08)	45.83 (42.22)	32.83 (33.93)
ATV-1	73.30 (59.01)	53.33 (46.93)	39.42 (38.81)
ATV-2	80.00 (63.43)	53.33 (46.92)	42.70 (40.76)
S-28	76.70 (61.22)	59.21 (50.34)	45.70 (42.47)
Punjab Chuhhara	66.70 (54.78)	43.33 (41.17)	28.92 (32.49)
Pusa Ruby	86.70 (68.85)	77.50 (62.02)	67.42 (55.38)
CD at 5%	(8.89)	(9.51)	(11.34)

Figures in parentheses are transformed 'Arc Sine' values.

PPI = Per cent Plants Infected
PDI = Percent Disease Incidence
CODEX = Co-efficient of Disease Index

Table 21. Effect of Age of Tomato Seedlings on The Incidence of Fusarium Wilt.

Age of seedlings (days)	PDI	Nu-mber of Days taken for appearence of first wilt symptoms
14	100.00 (89.43)	4-7
21	90.00 (71.76)	6-10
28	81.30 (64.44)	14-21
35	47.50 (43.50)	24-30
42	31.30 (33.50)	24-30
CD at 5%	(6.82)	

Figures in parentheses are transformed 'Arc Sine' values.

Table 22. Effect of Age of Culture of *F. oxysporum* f. sp. *lycopersici* on the Incidence of Wilt in Tomato

Age of the Culture	PDI
1 Week	53.10 (46.83)
2 Weeks	62.50 (52.29)
3 Weeks	81.30 (64.52)
4 Weeks	82.80 (66.09)
5 Weeks	85.30 (68.41)
6 Weeks	84.40 (66.84)
CD at 5%	(6.78)

Figures in parentheses are transformed 'Arc Sine' values.

Table 23. Effect of Different Methods of Inoculation of F.oxysporum f.sp. lycopersici on Incidence of Wilt in Tomato

Method of Inoculation	Number of Days taken for appearance of first wilt symptoms	PDI
Severed root dip in spore suspension	8-10	83.30 (66.15)
Unsevered root dip in spore suspension	11-12	78.30 (62.30)
Spilt tap root and dip in spore suspension	7-9	93.30 (75.24)
Split tap root and insertion of mycelial culture	15-16	55.00 (47.88)
Pouring spore suspension at root zone of established plant	14-18	41.71 (30.13)
Point injury at collar region followed by pouring spore suspension	20-21	35.00 (36.24)
Planting in pre infested soil	14-18	81.70 (64.70)
CD at 5%		(5.82)

Figures in parentheses are transformed 'Arc Sine' values.

during 1992-93 for their reaction to wilt. Four week old seedlings of different varieties raised in sterilized sand were washed with tap water followed by sterile water. The roots (≤ 2 mm) were severed with sharp razor blade and immersed in spore suspension (10^6 to 10^7 conidia ml^{-1}) for 30 minutes for inoculation as suggested by Wellman (1939) and planted into 25 cm dia.pots at the rate of 10 plants per pot and recorded wilt incidence upto 60 days. The data are presented in Table No. 20. The results indicated that coefficient of infection (CODEX) varied from 28.92 to 67.42. All the 17 varieties tested were found susceptible. However, the least CODEX was observed with Rupali (28.75) followed by Punjab Chuhhara (28.92) and the highest CODEX with Pusa Ruby (67.42).

4.3.4. Age of Seedlings and Disease

With a view to determine the optimum age of seedlings at which the plants are highly susceptible to infection, tomato plants of different ages from 14 days to 42 days were inoculated (Table 21). The highest PDI was observed at 14 days with 89.67 followed by 21 days with 90. PDI differed significantly among all the treatments with least incidence at 42 days with 31.3. Time taken for the first appearance of symptoms ranged from four to seven days after planting in case of 14 day old seedlings while 24-30 days after planting in case of 35 and 42 day old seedlings. The disease progress was quick with younger seedlings which succumbed to the disease within ten days where as older seedlings could withstand fungal attack by another five to ten days.

4.3.5. Age of Culture of *F. oxysporum* f. sp. *lycopersici* and Disease.

The influence of age of inoculam of the pathogen on the disease development was studied and the data are presented in Table 22. Pathogen inoculum differing in age from one to six weeks was inoculated to sterilized soil in pots at the rate of ten per cent and allowed one week for multiplication. Four week old Pusa Ruby tomato seedlings were transplanted in pots containing infested soil. It was observed that inoculam of three weeks old and above resulted in PDI ranging from 81.3 for 3 week old culture to 84.4 for 6 week old culture. Young cultures (one or two week old) produced significantly lower PDI of 53.1 and 62.5 respectively. It can be concluded that cultures aged 3 weeks and above were efficient in producing the wilt. Only such cultures were used in further experiments.

4.3.6. Method of Inoculation and Disease

The efficiency of different inoculation methods in producing wilt was compared and

Table 24. Influence of Age of Tomato Seedlings of Different Varieties on Seedling Disease Induced by *F. oxysporum* f. sp. *lycopersici*

Variety	seedling mortality (%) Age of the seedlings (days)					
	5	10	15	20	25	30
Arka Vikas	100.00	100.00	73.30	60.00	26.70	0.00
Arka Saurabh	100.00	100.00	86.60	60.00	33.30	0.00
Arka Vishal	100.00	100.00	53.30	53.30	40.00	0.00
Arka Ahuti	100.00	100.00	62.60	53.30	46.60	0.00
Arka Alok	100.00	100.00	80.00	66.60	60.60	13.30
Arka Ashish	100.00	100.00	80.00	60.00	53.30	6.60
Arka Abha	100.00	100.00	80.00	66.60	53.30	0.00
Rupali	100.00	80.00	60.00	46.60	33.30	0.00
Pusa Early Dwarf	100.00	100.00	100.00	73.30	66.60	26.60
Roma	100.00	100.00	60.00	33.30	33.30	0.00
Pusa Ruby	100.00	100.00	100.00	93.30	73.30	40.00

Average of 15 plants for each stage of the crop.

assessed. The methods employed and the incidence of wilt are presented in the Table 23.

The results showed that dipping of split tap root in suspension consisting of conidia and mycelial bits produced the highest PDI of 93.30 differing significantly over all the other treatments. This is followed by severed root dipped in spore suspension (83.30) and planting in preinfested soil (81.7). The later two methods were at par with each other. The least PDI of 35.0 was observed in case of point injury at collar region followed by pouring spore suspension at the collar region of established plant. Among the seven methods tested, planting in preinfested soil was found to be appropriate method as it simulates natural situation where transplanting of seedlings is practiced though compared to two other methods with minimum root injury.

4.3.7. Varietal Reaction at Different Ages of Plants and Disease

Eleven popular varieties of tomato were tested for their reaction to seedling disease caused by *F. oxysporum* f. sp. *lycopersici*.The age of seedlings ranged from 5 to 30 days at 5 day interval. The pathogen was introduced at the collar region by scooping the soil around collar region and placing a 5 mm mycelial disc and covering with soil without injuring the collar and it's roots. Later it was supplemented with two drops of 0.1 per cent glucose solution and observed for disease development for one week. The results of disease incidence on different varieties and ages are presented in the Table 24. Perusal of the data indicated that seedling mortality was 100 per cent in all the varieties except in Rupali up to 10 day old seedlings. Rupali showed 80 per cent mortality. Pusa Ruby followed by Pusa Early Dwarf showed 73.3 and 66.6 per cent seedling mortality respectively at 25 days of age and 40 and 26.6 per cent at 30 days. Rupali showed least incidence with 46.6 per cent at 20 days after sowing. It can be concluded that Pusa Ruby, Pusa Early Dwarf are highly susceptible compared to all other varieties tested and Rupali is the least affected among the varieties tested.

4.4. BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT OF TOMATO CAUSED BY *F. oxysporum* f. sp. *lycopersici* USING ORGANIC AMENDMENTS

Effect of 22 amendments on seedling disease and wilt in pots was studied in 3 stages. Initially all the amendments were tested at one concentration. In the second stage, six selected amendments were tested tried at three levels with two incubation periods. In the final stage, the six selected amendments were studied in detail for their effect on pathogen

Table 25. Preliminary Screening of Organic Amendments Against Seedling Disease and Wilt in Tomato

Amendment	healthy seedlings %		Wilt (PDI)	
Cakes (0.1%)				
Neem Cake	68.70	(55.96)	47.50	(43.60)
Pongamia Cake	62.33	(52.34)	50.80	(45.50)
Gingelly Cake	31.33	(34.01)	66.70	(54.80)
Groundnut Cake	22.70	(28.42)	66.70	(54.80)
Castor Cake	46.70	(43.09)	56.70	(48.84)
Mahua Cake	32.70	(34.83)	69.20	(56.32)
Sunflower Cake	26.70	(30.65)	65.80	(54.30)
Dry Amendments (1.5%)				
Horse gram Haulms	51.33	(46.15)	55.71	(48.33)
Mango Leaves	31.33	(33.90)	67.50	(55.30)
Eucalyptus Leaves	52.00	(46.15)	51.71	(46.00)
Acasia Leaves	38.00	(38.05)	66.71	(54.80)
Neem Leaves	51.33	(45.80)	60.83	(51.30)
Pongamia Leaves	59.33	(50.39)	50.83	(45.50)
Coffee Waste	43.33	(41.17)	58.30	(49.80)
Tea Waste	45.33	(42.71)	59.17	(50.30)
Farm Yard Manure (FYM)	66.70	(54.74)	51.71	(46.00)
Fresh Leaves (3%)				
Mango	36.00	(36.87)	60.80	(51.30)
Eucalyptus	49.30	(44.62)	54.20	(47.39)
Acasia	36.70	(37.25)	60.00	(50.80)
Neem	56.00	(48.45)	53.30	(46.91)
Pongamia	62.30	(52.14)	48.30	(44.04)
Horse Gram	52.60	(46.53)	55.00	(47.90)
Control (No amendment)	23.30	(28.85)	80.00	(63.46)
Healthy (uninoculated)	85.30	(67.55)	-	-
CD at 5%	(3.28)	(2.91)		

Figures in parentheses are transformed 'Arc Sine' values

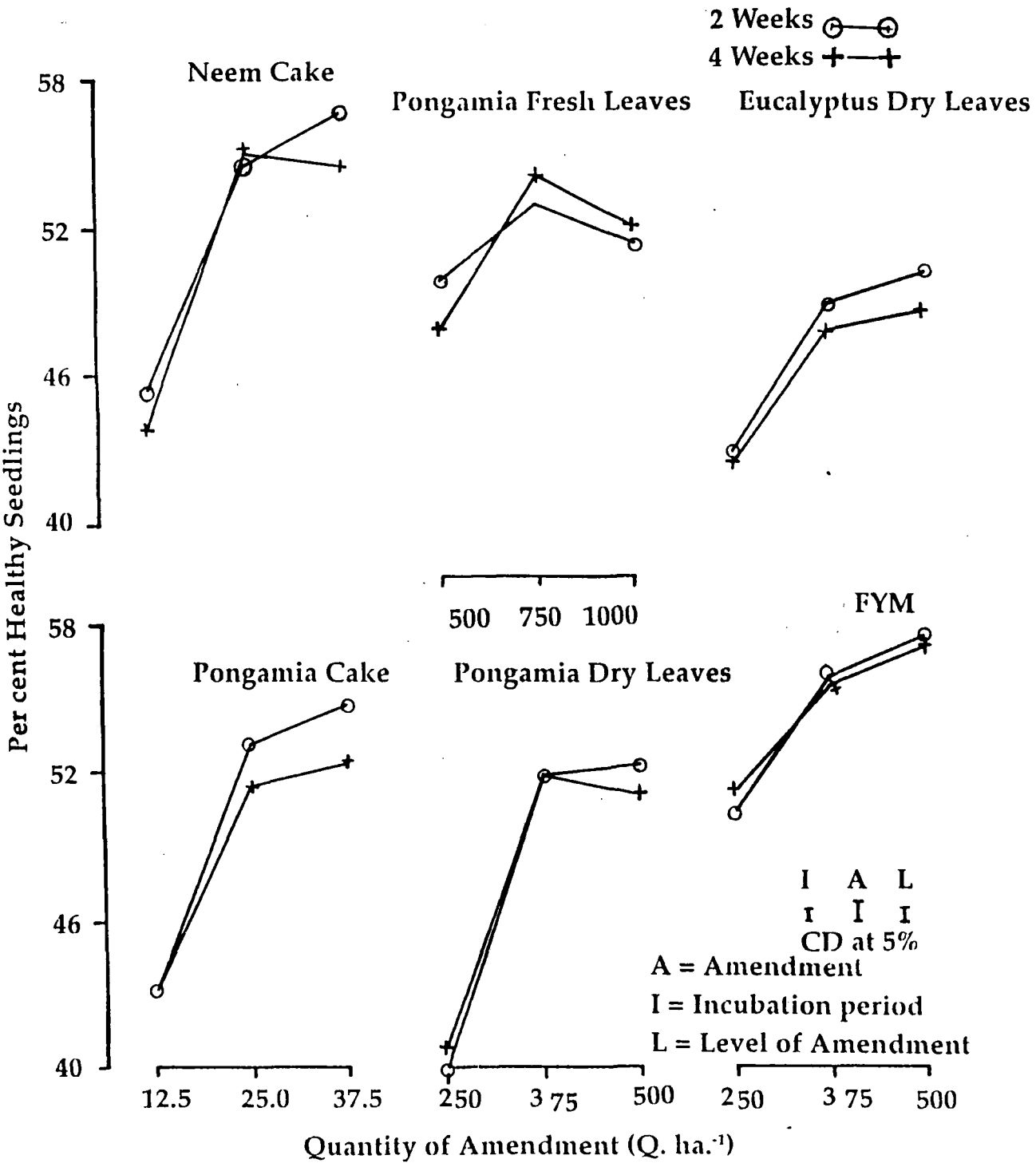


Fig. 6. Efficacy of Selected Amendments at Different Levels and Incubation Periods on the Healthy Seedling Stand in Tomato.

Table 26. Effect of Selected Amendments at Different Levels and Incubation Periods on the Seedling Stand in Tomato

Amendment	Quantity Q/ha	healthy seedlings (%)		Mean healthy seedlings (%)
		Incubation Period		
		2 Weeks	4 Weeks	
Neem Cake	12.50 (0.05%)	50.66 (45.38)	48.00 (43.85)	
	25.00 (0.1%)	66.66 (54.74)	67.33 (55.14)	
	37.50 (0.15%)	70.00 (56.81)	66.66 (54.75)	61.55 (51.78)
Pongamia Cake	12.50 (0.05%)	46.60 (43.09)	46.60 (43.11)	
	25.00 (0.1%)	64.00 (53.14)	61.33 (51.55)	
	37.50 (0.15%)	67.33 (54.74)	62.66 (52.34)	58.03 (49.67)
Pongamia Fresh Leaves	500 (2%)	58.66 (50.00)	55.33 (48.06)	
	750 (3%)	64.00 (53.14)	66.00 (54.34)	
	1000 (4%)	61.33 (51.55)	62.66 (52.34)	61.33 (51.57)
Pongamia Dry Leaves	250 (1%)	41.33 (40.01)	42.66 (40.78)	
	375 (1.5%)	62.00 (51.95)	62.00 (51.95)	
	500 (2%)	62.66 (52.34)	60.66 (51.17)	55.22 (48.03)

continued.....

Table 26. Contd ..

Eucalyptus Dry Leaves	250 (1%)	46.66 (43.11)	46.00 (42.70)	
	375 (1.5%)	57.33 (49.22)	55.33 (48.06)	
	500 (2%)	59.33 (50.39)	56.66 (48.83)	53.55 (47.05)
FYM	250 (1%)	59.33 (50.40)	60.66 (51.16)	
	375 (1.5%)	65.33 (55.97)	68.00 (55.58)	
	500 (2%)	71.33 (57.63)	70.66 (57.21)	65.88 (54.65)
Mean of incubation periods		59.67 (50.75)	58.84 (50.16)	

Figures in parentheses are transformed 'Arc sine' values for seedling stand and others are per cent levels of amendments

CD at 5%

Between incubation periods	;	0.459
Among amendments	:	0.795
Interaction among incubation period Amendments and levels	;	Not Significant
Among levels of amendments	;	0.562

Table 27. Effect of Selected Amendments applied at Different Levels on Healthy Seedling Stand.

Amendment	Healthy Seedlings (%)		
	Levels of Amendments		
	L_1	L_2	L_3
Neem Cake	49.33 (44.62)	66.99 (54.94)	68.33 (55.77)
Pongamia Cake	46.60 (43.11)	62.71 (52.34)	64.83 (53.54)
Pongamia Fresh Leaves	56.99 (49.03)	65.00 (53.74)	61.99 (51.95)
Pongamia Dry Leaves	41.99 (40.39)	62.00 (51.95)	61.66 (51.75)
Eucalyptus Dry Leaves	46.33 (42.91)	56.33 (48.64)	57.99 (49.61)
FYM	59.99 (50.77)	66.71 (55.77)	70.99 (57.42)

Figures in parentheses are transformed 'Arc Sine' values.
CD at 5%
Among levels of amendments (1.38)
Among amendments (0.79)
L_1 = 0.05% Oil cakes, 1% Dry amendments 2% Fresh amendments
L_2 = 0.1 % " 1.5% " 3% "
L_3 = 0.15% " 2% " 4% "

population, wilt incidence, shoot length and their influence on microflora. The results obtained are given below.

4.4.1. Preliminary Screening of Organic Amendments Against Seedling Disease and Wilt in Tomato

A total of 22 dry and green plant material including FYM, coffee and tea wastes were screened for their efficacy in suppressing the seedling disease and wilt. The final count of healthy seedlings and PDI for wilt were taken at 28 days after sowing and 60 days after planting respectively, and the results are presented in the Table 25. It was found that neem cake and pongamia cake, pongamia fresh leaves, FYM were on par with each other with regard to their suppressive action against the disease followed by Pongamia dry leaves with healthy seedling stand ranging from 68.70, 62.33, 66.7 and 59.33 per cent respectively. The least per cent healthy seedlings were observed in groundnut cake (22.7). Neem cake was found to be significantly superior to all other amendments in reducing PDI (47.50) followed by pongamia fresh leaves (48.30) and pongamia cake (50.80) which were at par with neem cake. Pongamia dry leaves with PDI 50.83 eucalyptus dry leaves and FYM with 51.71 were the next best against wilt incidence. The least effective one was mahua cake with PDI 69.20.

4.4.2. Effect of Selected Oganic Amendments at Different Concentrations and Incubation Periods Against Seedling Disease and Wilt in Tomato.

From the previous study, it was found that neem cake, pongamia cake, pongamia fresh and dry leaves, eucalyptus dry leaves and FYM were effective against seedling disease and wilt. These amendments were tested at 3 levels and two incubation periods for their efficacy on suppressing the development of seedling disease and wilt in tomato. The results are presented in Tables 26 to 31. The data presented in Table 26 and Fig.6 showed that per cent healthy seedling stand increased with increase in the level of the selected amendments except in pongamia fresh and pongamia dry leaves at four week incubation period. Better per cent healthy seedling stand was observed at two week incubation period with 59.67 than at four week incubation period with 58.84 which differed significantly. Among the six amendments, FYM was found significantly superior to all other amendments with the highest per cent healthy seedling stand (65.88) followed by neem cake (61.55) and pongamia fresh leaves (61.33). Interaction among incubation period, amendments and levels of amendments had no effect on per cent healthy seedling

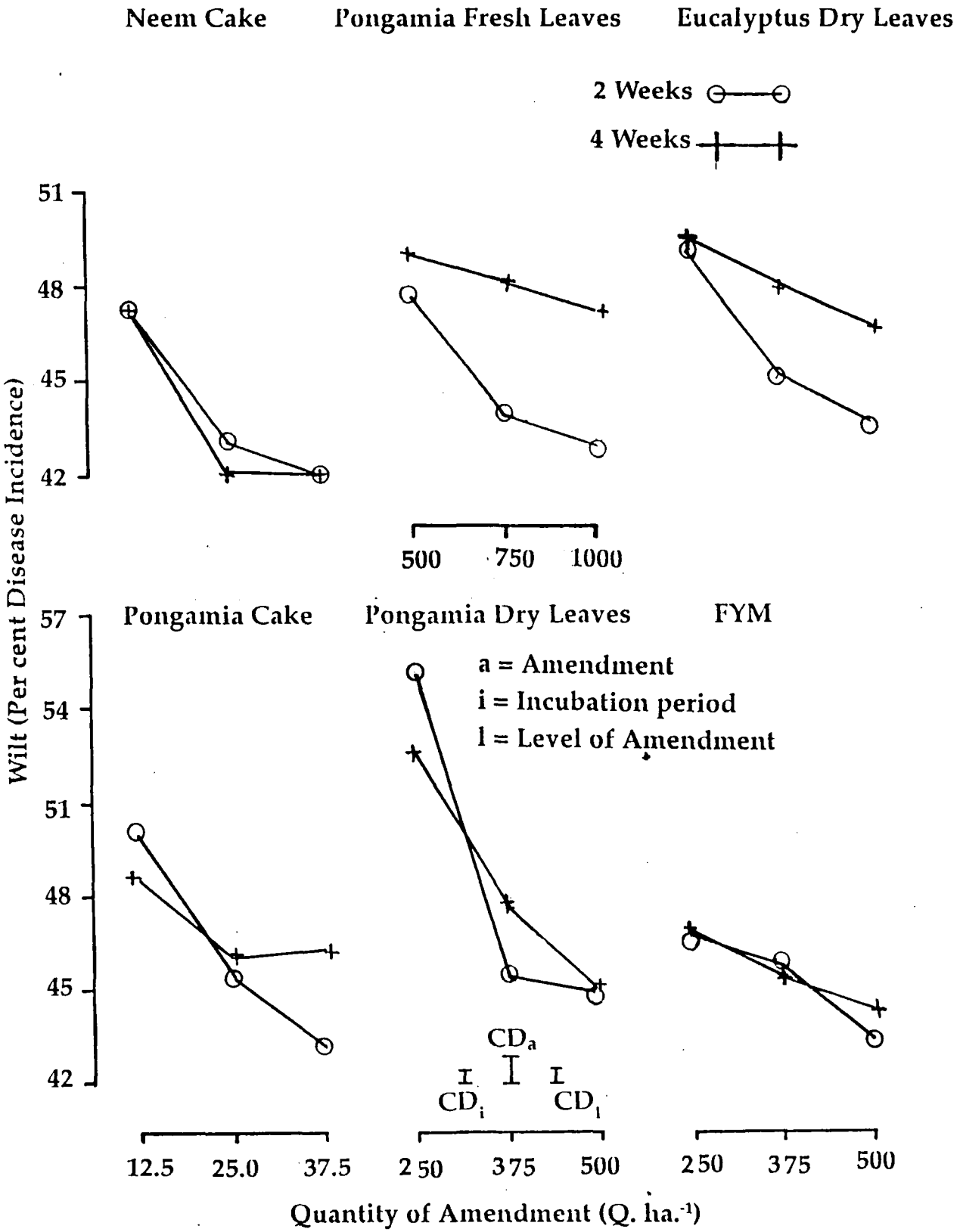


Fig. 7. Efficacy of Selected Amendments at Different Levels and Incubation Periods on Wilt Incidence in Tomato.

Table 28. Effect of Selected Amendments at Different Levels and Incubation Periods on Wilt Incidence in Tomato

Amendment	Quantity Q/ha	PDI		Mean PDI
		Incubation Period		
		2 Weeks	4 Weeks	
Neem Cake	12.5	54.17	54.20	
	(0.05%)	(47.39)	(47.39)	
	25.0	47.50	45.00	
	(0.1%)	(43.57)	(42.13)	
	37.5	45.83	45.83	48.76
	(0.15%)	(42.61)	(42.61)	(44.28)
Pongamia Cake	12.5	59.17	56.66	
	(0.05%)	(50.29)	(48.83)	
	25.0	50.80	53.33	
	(0.1%)	(45.48)	(46.91)	
	37.5	47.50	54.08	53.59
	(0.15%)	(43.57)	(47.39)	(47.08)
Pongamia Fresh Leaves	500	55.00	58.33	
	(2.%)	(47.87)	(49.81)	
	750	48.30	55.83	
	(3%)	(44.04)	(48.35)	
	1000	46.71	54.33	53.08
	(4%)	(43.09)	(47.49)	(46.77)
Pongamia Dry Leaves	250	67.50	65.00	
	(1%)	(55.25)	(53.74)	
	375	50.83	55.00	
	(1.5)	(45.48)	(47.87)	
	500	50.00	51.66	56.67
	(2%)	(45.00)	(45.95)	(48.88)

Table 28. contd..

Eucalyptus Dry Leaves	250 (1%)	57.50 (49.41)	58.33 (49.81)	
	375 (1.5%)	51.71 (45.96)	56.66 (48.83)	
	500 (2%)	49.17 (44.52)	55.83 (47.87)	54.87 (47.73)
FYM	250 (1%)	53.33 (46.82)	53.83 (46.91)	
	375 (1.5%)	51.71 (45.96)	50.83 (45.48)	
	500 (2%)	47.50 (43.57)	49.17 (44.52)	50.97 (45.54)
Mean of incubation periods		51.90 (46.10)	54.08 (47.32)	

Figures in parentheses are transformed 'Arc sine' values for PDI and others are per cent levels of amendments

CD at 5%

Between incubation periods	:	0.547
Among amendments	:	0.948
Interaction among incubation period, amendment and levels	:	Not Significant
Among levels of amendments	:	0.670

Table 29. Effect of Selected Amendments as Influenced by the Incubation Period in relation to the Incidence of Wilt of Tomato

Amendment	PDI Incubation period	
	2 Weeks	4 Weeks
Neem Cake	49.17 (44.52)	48.34 (44.04)
Pongamia Cake	52.49 (46.45)	54.69 (47.71)
Pongamia Fresh Leaves	50.00 (45.00)	56.16 (48.55)
Pongamia Dry Leaves	56.11 (48.58)	57.22 (49.21)
Eucalyptus Dry Leaves	52.79 (46.63)	56.94 (48.84)
FYM	50.85 (45.44)	51.11 (45.64)

Figures in parentheses are transformed 'Arc Sine' values.

CD at 5%
Between Incubation periods (1.34)
Among Amendments (0.95)

Table 30. Effect of Selected Amendments Applied at Different Levels on Wilt Incidence in Tomato

Amendment	PDI Levels of Amendments		
	L_1	L_2	L_3
Neem Cake	54.19 (47.39)	46.25 (42.85)	45.83 (42.61)
Pongamia Cake	57.92 (49.56)	52.07 (46.21)	50.79 (45.48)
Pongamia Fresh Leaves	56.67 (48.84)	52.07 (46.21)	50.52 (45.29)
Pongamia Dry Leaves	66.25 (54.51)	52.92 (46.68)	50.83 (45.48)
Eucalyptus Dry Leaves	57.92 (49.61)	54.19 (47.39)	52.50 (46.20)
FYM	53.33 (46.84)	51.27 (45.72)	48.60 (44.04)

Figures in Parentheses are transformed in 'Arc Sine' values

CD at 5%
Among Levels of Amendments (1.64)
Among Amendments (0.95)

L_1 = 0.05% Oil cakes, 1% Dry amendments 2% Fresh amendments
L_2 = 0.1 % " 1.5% " 3% "
L_3 = 0.15% " 2% " 4% "

Table 31. Interaction Between Incubation Period and Levels of Amendments on Incidence of Wilt in Tomato

Level of Amendment	(PDI) Incubation period	
	2 Weeks	4 Weeks
L_1	57.81 (49.51)	57.64 (49.42)
L_2	50.14 (45.08)	52.78 (46.61)
L_3	47.79 (43.73)	51.82 (46.00)

Figures in parentheses are transformed 'Arc Sine' values.

CD at 5%
Between Incubation Period (0.95)
Among Levels of Amendments (0.94)

L_1 = 0.05% Oil cakes, 1% Dry amendments 2% Fresh amendments
L_2 = 0.1 % " 1.5% " 3% "
L_3 = 0.15% " 2% " 4% "

Table 32. Effect of Selected Amendments on Wilt Incidence and Population Dynamics of *F. oxysporum* f. sp. *lycopersici* in Tomato

Amendment	PDI	Shoot length (cm)	Increase in Shoot length over inoculated control (%)	Propagules of *F.o.l.* (cfu g^{-1} x 10^2)			
				10 DAT	20 DAT	30 DAT	40 DAT
Neem Cake (0.1%)	46.87 (43.21)	57.45	83.10	14.00 (3.15)	17.25 (3.24)	21.25 (3.33)	24.25 (3.39)
Pongamia Cake (0.1%)	51.25 (45.72)	53.13	69.30	20.75 (3.32)	21.00 (3.32)	25.50 (3.40)	26.75 (3.42)
Pongamia FL (3%)	48.75 (44.21)	44.98	43.30	23.00 (3.36)	27.75 (3.44)	24.75 (3.39)	24.75 (3.40)
Pongamia DL (1.5%)	51.25 (45.72)	51.50	64.10	23.25 (3.37)	27.25 (3.44)	30.75 (3.49)	31.00 (3.51)
Eucalyptus DL (1.5%)	50.62 (45.35)	46.70	48.80	22.50 (3.35)	26.00 (3.41)	32.50 (3.51)	34.50 (3.54)
FYM (1.5%)	51.87 (46.07)	51.98	65.90	20.00 (3.30)	19.25 (3.28)	22.50 (3.351)	27.25 (3.43)
Inoculated control	81.98 (64.86)	31.38	-	37.25 (3.58)	41.25 (3.61)	46.75 (3.67)	43.50 (3.65)
Healthy Check	-	58.68	87.00				
CD at 5%	(1.86)	2.09		(0.07)	(0.06)	(0.06)	(0.06)

DL = Dry Leaves FL = Fresh Leaves DAT = Days After Transplanting

Initial Population of *F. o. l.* : 41.50 x 10^2 cfu g^{-1} soil

Figures in parentheses are transformed 'Arc Sine' values for PDI and Logarithmic transformation values for propagules.

stand. The highest healthy seedling stand of 71.33 per cent was observed with FYM (2%) at two weeks incubation period differing significantly over all other amendments, followed by neem cake with 70 per cent at 0.1% concentration at two week incubation period. Next best were pongamia cake (67.33%) and pongamia dry leaves (62.66%).

At four week incubation period FYM (2%) recorded the highest per cent healthy seedling stand of 70.99 followed by neem cake at 0.15% with 68.33 and neem cake (0.1%) with 67 respectively. Interaction between amendments and their levels indicated that at level two neem cake (0.1%) and FYM (1.5%) were on par with each other resulting in 66.99 and 66.71 per cent healthy seedlings followed by pongamia fresh leaves (3%) with 65. At level 3, FYM (2%) gave the highest per cent healthy seedling stand with 70.99, significantly superior compared to all other amendments followed by neem cake (0.15%) with 68.33 (*vide* Table 27 and Fig. 7). The results presented in Table 28 revealed that incubation period of two weeks is significantly superior to four weeks of incubation period in reducing wilt incidence with PDI of 51.90 and 54.08 respectively. Among the amendments, neem cake was found to be significantly superior with PDI of 48.76 followed by FYM with 50.97. Interaction between incubation periods and amendments on wilt incidence, presented in Table 29, indicated that at two week incubation period, neem cake, pongamia fresh leaves and FYM were at par among them selves recording PDI of 49.17, 50 00 and 50.85 respectively. At four week incubation period, neem cake was significantly superior to other amendments, having PDI of 48.34 followed by FYM (51.11).

The results of interaction between amendments and their levels on wilt incidence are presented in Table 30. It was observed that at lower levels of amendments, the PDI was significantly more than at higher levels of amendments. Neem cake at level two and three was found significantly superior to other amendments with PDI of 46.25 and 45.83 respectively.

Interaction between incubation periods and levels of amendments presented in Table 31 indicated that higher levels of amendments with two weeks of incubation period proved to be the best in reducing wilt compared to four weeks of incubation period.

4.4.3 Effect of Selected Amendments on Wilt Incidence, the Population Dynamics of *F.oxysporum* f.sp. *lycopersici* and other Microflora in Rhizosphere of Tomato.

Results of the six amendments on wilt incidence, shoot length in tomato, on population of *F. oxysporum* f.sp. *lycopersici* are presented in Table 32. The results showed that neem

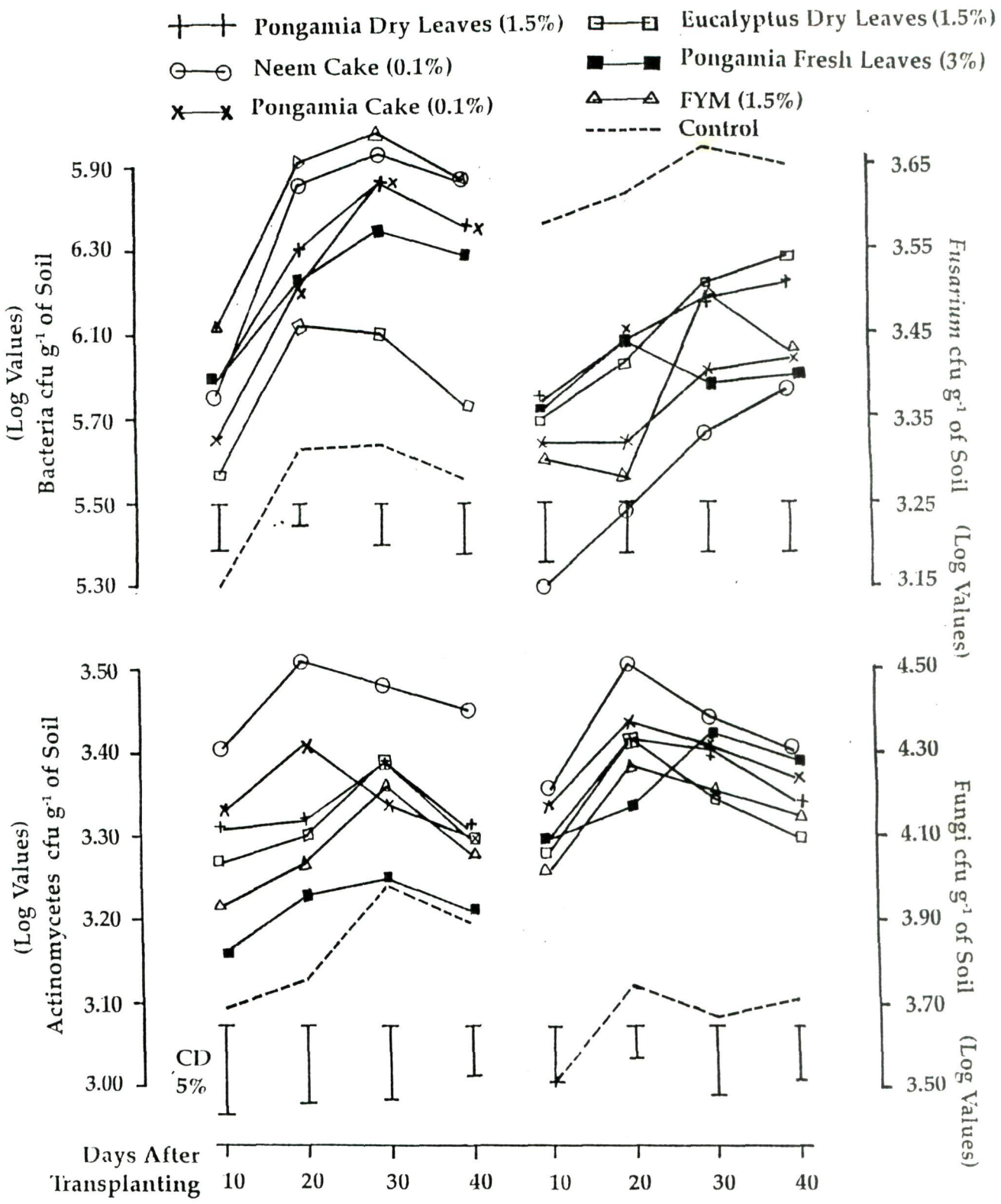


Fig. 8. Effect of Selected Amendments on the Population of Microflora and Pathogen Propagules in the Rhizosphere Soil of Tomato

Table 33. Effect of Selected Amendments on the Rhizosphere Microflora of Tomato

Amendment	Colony forming units g^{-1} soil															
	10 DAT				20 DAT				30 DAT				40 DAT			
	B	A	F	F'	B	A	F	F'	B	A	F	F'	B	A	F	F'
Neem Cake (0.1%)	5.75 (5.76)	25.25 (3.40)	16.25 (4.21)	14.00 (3.15)	18.25 (6.26)	32.75 (3.51)	21.5 (4.51)	17.25 (3.24)	21.5 (6.3313)	30.50 (3.48)	24.25 (4.38)	21.25 (3.33)	18.25 (6.26)	28.25 (3.45)	20.5 (4.30)	24.25 (3.38)
Pongamia Cake (0.1%)	4.50 (5.65)	21.50 (3.33)	14.75 (4.17)	20.75 (3.32)	12.75 (6.10)	23.75 (3.41)	18.75 (4.37)	21 (3.32)	18.25 (6.2608)	22.00 (3.34)	20.50 (4.31)	25.50 (3.40)	14.50 (6.16)	20.5 (3.30)	17.5 (4.24)	26.75 (3.42)
Pongamia DL (1.5%)	6.25 (5.79)	20.50 (3.32)	12.50 (4.09)	23.25 (3.37)	11.5 (6.06)	21.25 (3.32)	20.25 (4.33)	27.25 (3.44)	22.50 (6.35)	24.50 (3.39)	20.75 (4.30)	30.75 (3.49)	16.00 (6.20)	20.75 (3.31)	15.25 (4.18)	31.00 (3.51)
Eucalyptus DL (1.5%)	3.75 (5.57)	19.00 (3.27)	11.75 (4.07)	22.50 (3.35)	8.25 (5.92)	21.25 (3.33)	14.75 (4.33)	26 (3.41)	8.00 (5.90)	24.50 (3.39)	15.50 (4.19)	32.50 (3.51)	5.50 (5.73)	20.75 (3.30)	12.75 (4.10)	34.50 (3.54)
F Y M (1.5%)	8.25 (5.92)	16.75 (3.22)	10.50 (4.02)	20.00 (3.30)	20.50 (6.31)	18.50 (3.27)	16.50 (4.27)	19.25 (3.28)	24.00 (6.38)	22.75 (3.36)	16.75 (4.21)	22.50 (3.35)	18.75 (6.27)	19.25 (3.28)	14.50 (4.15)	27.25 (3.43)
Pongamia FL (3%)	6.25 (5.79)	14.50 (3.16)	12.25 (4.09)	23.00 (3.36)	10.75 (6.03)	17.25 (3.23)	19.25 (4.17)	27.75 (3.44)	14.00 (6.15)	18.00 (3.25)	22.50 (4.35)	24.75 (3.40)	12.50 (6.10)	16.25 (3.21)	18.75 (4.28)	24.75 (3.40)
Control	2.00 (5.30)	12.50 (3.10)	3.25 (3.50)	37.25 (3.59)	4.25 (5.63)	13.75 (3.14)	5.50 (4.14)	41.25 (3.61)	4.50 (5.64)	17.50 (3.24)	4.75 (3.66)	46.75 (3.67)	3.75 (5.56)	15.75 (3.20)	5.25 (3.71)	43.50 (3.65)
CD at 5%	(0.11)	(0.12)	(0.14)	(0.07)	(0.05)	(0.10)	(0.08)	(0.06)	(0.10)	(0.09)	(0.17)	(0.06)	(0.12)	(0.15)	(0.13)	(0.065)

NS = Not Significant

B = Bacteria (x 10^6), A = Actinomycetes (10^2), F = Fungi(10^3). F' = *Fusarium spp.* (x10^2), DAT = Days after Transplanting

Figures in parentheses are Logarithmic Transformation Values

Initial Population of F. o. l. = 45 x10^2

Plate 8 Response of tomato plants in *Fusarium* infested sick soil amended with different organic amendments

NC - Neem cake
PC : Pongamia cake
PDL - Pongamia dry leaves.

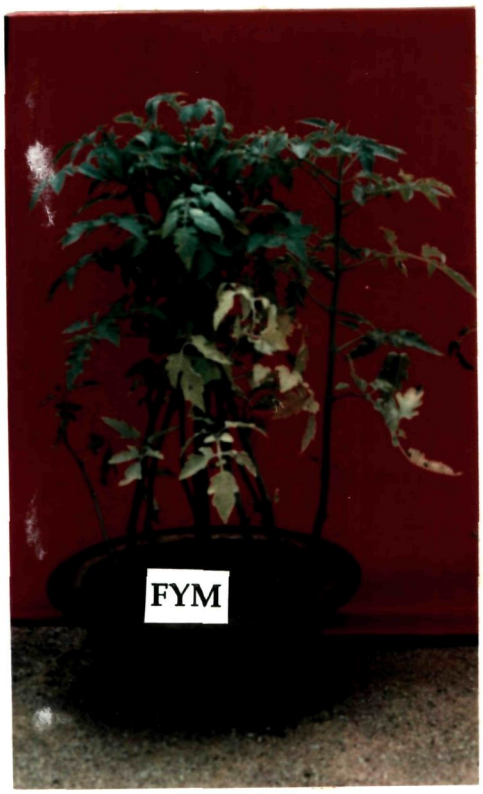




PLATE 8

Plate - 9 Response of tomato plants in *Fusarium* sick soils amended with different organic amendments.

EDL - Eucalyptus dry leaves
FYM - Farm yard manure
C - Control (no amendment)



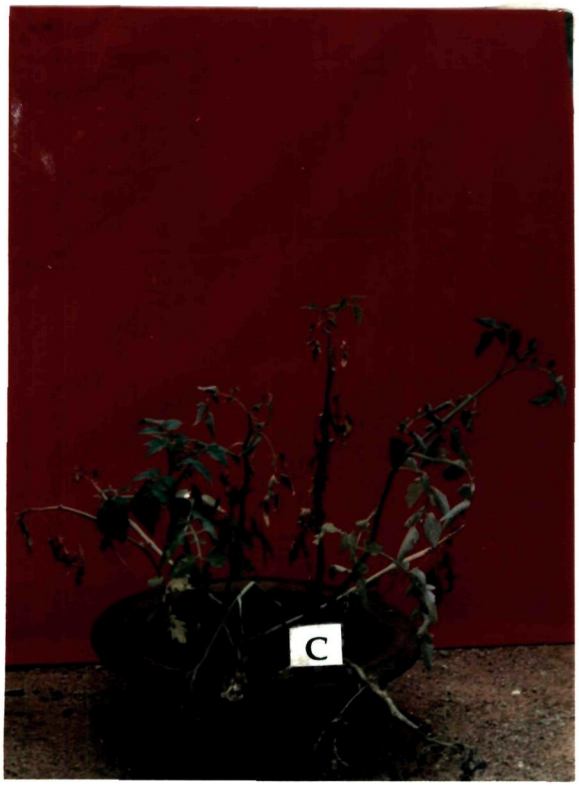


PLATE 9

cake (0.1%) and pongamia fresh leaves (3%) were at par, with PDI of 43.21 and 44.21 respectively. Among dry amendments, eucalyptus dry leaves (1.5%) was the next best recording PDI of 45.35. Inoculated control recorded the highest PDI of 81.98. Neem cake (0.1%) recorded the highest per cent increase in shoot length (83.10) followed by pongamia cake (69.30) (Plate 8 and 9). The propagules *F. oxysporum* f. sp.*lycopersici* g^{-1} soil increased with days after transplanting (DAT) from 10 to 40. Neem cake (0.1%) recorded the lowest population up to 30 DAT and significantly superior to all other treatments followed by FYM (1.5%) . Inoculated control recorded the highest population. At 40 DAT, neem cake (0.1%), pongamia cake (0.1 %), pongamia fresh leaves and FYM (1.5%) were at par.

The effect of these amendments on the rhizosphere microflora was assessed from 10 to 40 DAT and data are given in Table 33. Perusal of the data showed that there was a gradual increase in rhizosphere microflora up to 30 DAT and later on, decreased. Bacterial population was the highest in FYM (1.5%) ranging from 8.25 to 24 X 10^5 cfu g^{-1} soil followed by pongamia cake (0.1%) with 4.5 to 18.25 X 10^5 cfu g^{-1} soil. Actinomycetes population was the highest in neem cake (0.1%) ranging from 25. 25 X 10^2 cfu g^{-1} soil (10DAT) to 28.25 X 10^2 cfu g^{-1} soil (40 DAT) followed by pongamia cake (0.1%) with 21.50 to 24.50 X 10^2 cfu g^{-1} soil. All the amendments showed significantly higher microflora compared to control (Fig 8). Fungal population was also found to be higher with neem cake (0.1%) and pongamia cake (0.1%)

4.5. EVALUATION OF EFFICACY OF MODIFIED *Panchagavya* (MPG) FORMULATIONS ON THE INCIDENCE OF SEEDLING DISEASE AND WILT.

Three formulations out of various combinations of MPG, were found to be effective in reducing both the seedling disease and wilt incidence. Hence the 3 formulations were employed at four levels to choose the most effective formulation in giving protection against the disease and this formulation was used in all further studies.

4.5.1. Preliminary Screening of Three MPG Formulations at Different Dilutions for their Efficacy on the Incidence of Seedling Disease and Wilt.

Three MPG formulations designated as MPG - 1, MPG - 2 and MPG - 3 were used in four dilutions *viz.* 0, 10, 100, 1000 parts of water for one part of MPG. The results are presented in the Table 34. It was observed that there was no significant difference in per cent healthy seedling stand among 0 and 10 dilutions of MPG-3 and 10 dilution of MPG-2 with 79, 77 and 78 respectively. Even carbendazim (1000ppm)treatment (78 per cent) was

Table 34. Efficacy of MPG Formulations at Different Dilutions on the Incidence of Seedling Disease and Wilt in Tomato

Treatment	Dilu-tions	healthy seedlings (%)	Wilt (PDI)	appearence of first wilt symptoms (DAT)	Mean shoot length (cm)	Shoot length increase over control (%)
MPG - I	0	71.00 (57.54)	39.06 (38.62)	24-31	38.38	49.60
	10	64.00 (53.20)	34.37 (35.78)	24-34	37.80	47.20
	100	53.00 (46.75)	57.81 (49.56)	16-28	28.13	9.50
	1000	40.00 (39.18)	75.56 (61.08)	16-20	25.91	0.80
MPG - 2	0	71.00 (57.54)	21.86 (27.83)	24-28	36.93	43.80
	10	78.00 (62.14)	23.43 (28.91)	24-26	36.89	43.70
	100	50.00 (45.00)	57.81 (49.52)	18-20	31.43	22.40
	1000	46.00 (42.70)	75.00 (60.09)	16-20	30.64	19.30
MPG - 3	0	79.00 (62.79)	20.31 (26.59)	24-30	44.03	71.50
	10	77.00 (61.45)	23.44 (28.91)	24-35	45.75	78.15
	100	49.00 (44.42)	62.50 (52.27)	20-30	32.93	28.20
	1000	45.00 (42.12)	76.56 (61.09)	16-30	29.89	16.40
Inoculated Control	-	27.00 (31.18)	81.25 (64.50)	14-20	30.19	-
Carbendazim (1000 ppm)	-	78.00 (62.06)	53.12 (46.80)	20-32	25.68	17.56
Healthy Check	-	84.00 (66.50)	-	-	45.95	78.90
CD at 5%		5.04	5.09			1.303

Figures in parentheses are 'transformed 'Arc Sine' values.

Plate 10

a). Tomato plants raised in *Fusarium* sick soil treated with modified *Panchagavya* (MPG) formulations MPG-2 and MPG-3.

b) Tomato plants raised in *Fusarium* sick soil treated with carbendazim (1000 ppm) (bavistin) and control (plants without treatment).



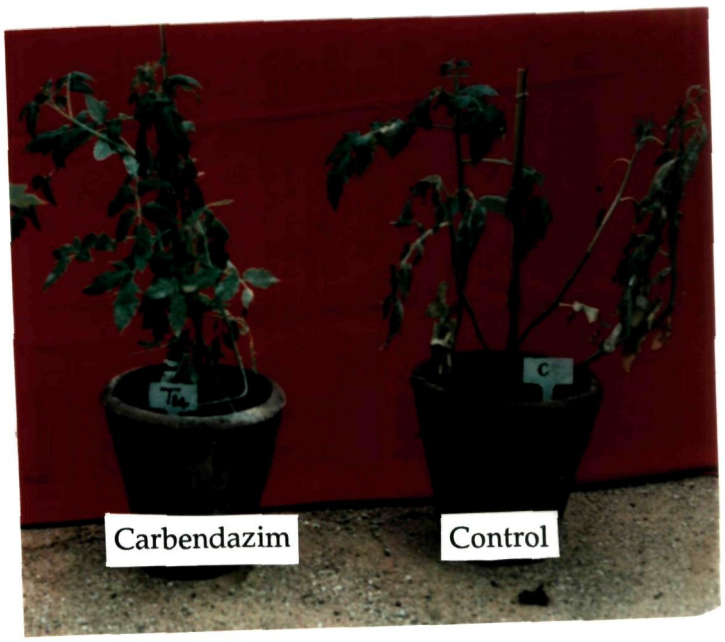


PLATE 10

Table 35. Effect of Ageing on the Efficacy of MPG-3 Formulation on the Incidence of Wilt in Tomato

Ageing (days)	PDI	Microflora at different ageing periods in cfu ml^{-1}	
		Bacteria (x 10^5)	Fungi (x 10^3)
0	79.70 (63.23)	17.33	-
2	76.60 (61.15)	36.00	-
4	70.30 (57.06)	38.66	4.33
6	53.10 (46.80)	30.33	8.66
8	40.60 (39.53)	25.66	12.00
10	32.80 (34.87)	23.00	14.33
12	26.60 (30.99)	23.00	18.66
14	21.90 (27.81)	20.66	21.33
16	50.00 (44.99)	18.00	25.33
18	64.10 (53.19)	18.00	24.33
Control	81.30 (64.59)	-	-
CD at 5%	(4.92)		

Figures in parentheses are transformed 'Arc Sine' values.

on par, with the dilutions of MPG-2 and MPG-3. MPG - 1 and MPG - 2 were at par at 0 dilution with regard to per cent healthy seedling stand with 71. As the dilution increased, the efficacy decreased. All the treatments showed significant differences in per cent healthy seedling stand over control (27). MPG - 3 and MPG - 2 at 0 and 10 dilutions were at par recording the lowest PDI thus they were significantly superior over other treatments. The lowest PDI (20.31) was observed in case of 0 dilution of MPG - 3 followed by 0 dilution of MPG - 2 (21.86). These two formulations were also at par at 10 dilution, recording 23.44 and 23.43 PDI respectively, while inoculated control exhibited the highest PDI (81.25). It was also observed that there was a delay in the appearance of wilt symptoms by about 2 to 10 days in all the treatments over control.

The highest per cent shoot length of 78.15 was observed in case of 10 dilution of MPG-3, followed by 71.50 with 0 dilution and significantly they were superior over all other treatments. It can be concluded that MPG - 3 formulation at 10 dilution was superior over all other formulations in reducing both the seedling disease and the wilt and increasing shoot length (Plate 10 A and B). Hence this formulation was continued for further experimentation.

4.5.2. Influence of Ageing on the Efficacy of MPG - 3 on the Incidence of Wilt.

The effect of age of the MPG formulation on final disease development was studied ranging from 0 to 18 days at two day interval. Roots of four week old tomato seedlings were dipped in MPG-3 formulation for 30 minutes and planted in pots containing infested soil. The soil was also drenched with MPG-3 three times at 10 day interval. The results are given in Table 35. It was found that the PDI decreased gradually from 79.70 to 21.90 with increase in ageing from 0 to 14 days and only slightly increased during the further ageing, indicating that the MPG - 3 formulation is most effective when used from 10 to 14 days of ageing.

Microflora of MPG - 3 was estimated on soil extract agar for bacteria and martins rose bengal agar for fungi at different periods of ageing from 0 to 18 days. The bacterial population increased from 0 days (17.33×10^5 ml $^{-1}$) to 14 days (31.33×10^5 ml $^{-1}$). Fungal population appeared on 4th day and gradually increased up to 16 days and remained almost constant up to 18 days.

Table 36. Antagonists used Against *F. oxysporum* f.sp. *lycopersici*

Isolate No.	Antagonists	Source
A_1	*Trichoderma viride* Pers.: Fr.	Andhra Pradesh
A_2	*Trichoderma harzianum* Rifai	Andhra Pradesh
A_3	*Trichoderma harzianum* Rifai	Uttar Pradesh
A_4	*Trichoderma viride* Pers.: Fr.	IMI 317287 (Hebbal)
A_5	*Trichoderma harzianum* Rifai	IMI 317288 (Hebbal)
A_6	*Trichoderma koningii* Oudem	IMI 317291 (Shimoga)
A_7	*Trichoderma koningii* Oudem	IMI 317293 (Shimoga)
A_8	*Trichoderma viride* Pers.: Fr.	Mandya
A_9	*Trichoderma* sp.	Doddaballapur
A_{10}	*Trichoderma* sp.	GKVK
B_1	*Pseudomonas* sp.	Doddaballapur
B_2	*Bacillus subtilis*	GKVK

Table 37. Effect of Isolates of Trichoderma spp. on the growth of *F. oxysporum* f. sp. *lycopersici*

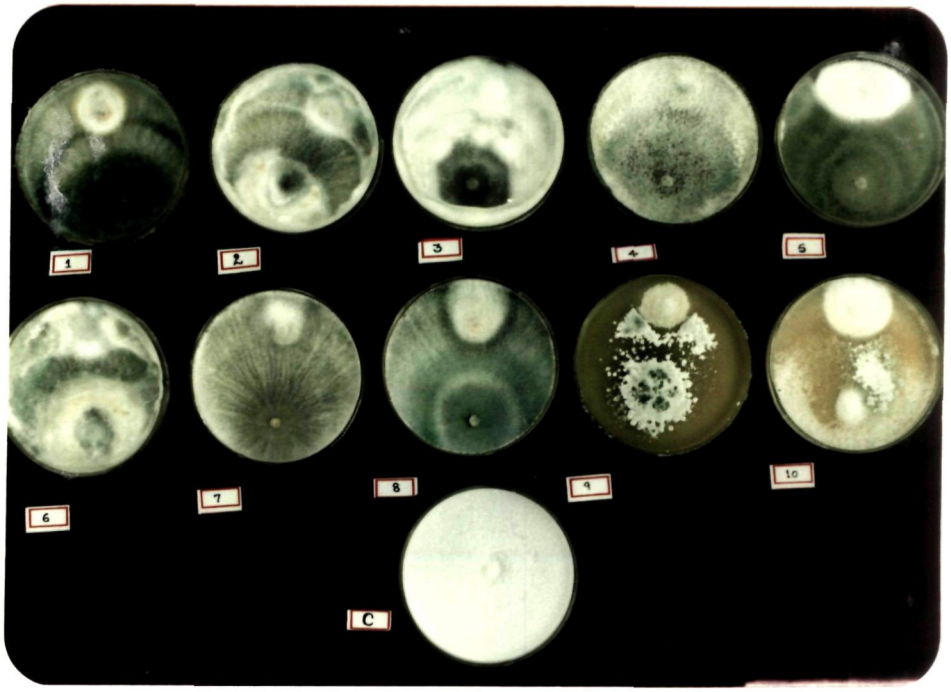
Tricchoderma Isolate No.	Per cent inhibition of radial growth			Non volatile Compounds	
	Dual Culture 7th Day	Volatile Compounds		Radial Growth after 48 hrs. (mm)	Per cent reduction over control
		3rd Day	7th Day		
A_1	33.30 (35.21)	75.40 (60.29)	75.30 (60.23)	29.30	16.30
A_2	42.20 (40.52)	70.00 (56.79)	68.10 (55.59)	31.60	9.70
A_3	36.10 (36.71)	65.50 (54.01)	66.40 (54.98)	34.60	1.10
A_4	44.80 (41.99)	75.40 (60.29)	78.80 (62.57)	28.00	20.00
A_5	22.10 (27.91)	31.80 (34.28)	45.10 (42.17)	33.30	4.80
A_6	33.30 (35.24)	40.80 (39.68)	63.82 (53.04)	34.30	2.00
A_7	19.30 (26.03)	47.20 (43.41)	23.00 (28.63)	32.60	6.90
A_8	15.50 (23.10)	51.60 (45.90)	53.60 (47.06)	33.30	4.80
A_9	26.30 (30.81)	75.40 (60.29)	72.00 (58.04)	29.30	16.30
A_{10}	27.50 (31.60)	50.00 (45.00)	57.00 (49.05)	33.60	4.00
Control				35.00	
CD at 5%	4.07	7.86	2.92	1.90	

Figures in parentheses are transformed 'Arc Sine' values.

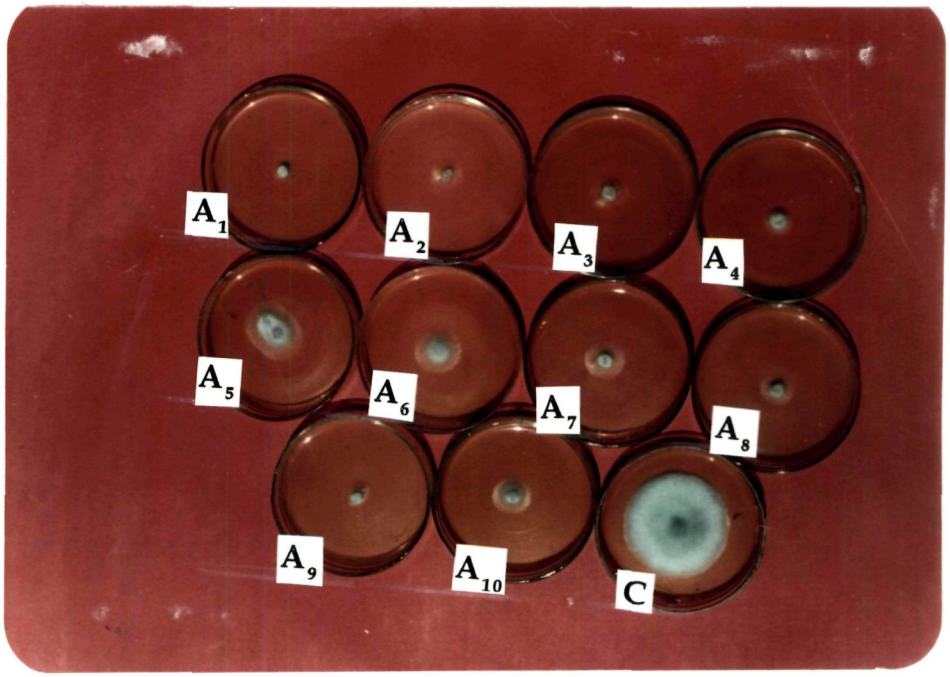
Plate 11

a) Dual culture interaction studies with isolates of *Trichoderma* spp. (1-10) with *F.oxysporum* f.sp. *lycopersici*

b) Effect of volatile compounds of different isolates of *Trichoderma* spp. (1-10) on radial growth of *F.oxysporum* f.sp. *lycopersici* (7 days after inoculation).



a



b

PLATE 11

4.6. BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT OF TOMATO CAUSED BY *F. oxysporum* f.sp. *lycopersici* USING ANTAGONISTIC MICRO ORGANISMS

Trichoderma and bacterial antagonists obtained from the Dept. of Plant Pathology and isolated from rhizosphere of tomato plants were used in the study. Several laboratory experiments were conducted to know the antagonistic potential of *Trichoderma* and bacterial isolates against *F. oxysporum* f. sp. *lycopersici in vitro* and the results are given below.

4.6.1. Preliminary Screening of Antagonists.

Nineteen isolates of *Trichoderma* and five bacterial isolates obtained from different sources were screened for antagonistic activity against *F. oxysporum* f. sp. *lycopersici* in dual culture. Of these, ten isolates of *Trichoderma* and two isolates of bacteria that suppressed the growth of *F. oxysporum* f. sp. *lycopersici* were selected. These isolates were used for further studies *in vitro* and for pot culture experiments *in vivo*. The details of the isolates *viz.* source and identity are presented in Table 36.

4.6.2. Interaction of *Trichoderma* Isolates with *F. oxysporum* f. sp. *lycopersici* in Dual Culture.

A reduction in the growth of *F. oxysporum* f. sp. *lycopersici* was noticed when it was paired with antagonists four cm apart on PDA. Differences in the effectiveness of *Trichoderma* isolates against *F. oxysporum* f. sp. *lycopersici* were observed after their contact with the pathogen. Within four days of inoculation, majority of the *Trichoderma* isolates (A_4, A_2 and A_3) over grew *F. oxysporum* f. sp. *lycopersici* mycelium (Plate 11a). The microscopic examination at the zone of contact of antagonist and test pathogen revealed excessive vacuolation, hyphal bulging and chlamydospore formation in the test pathogen. The results are presented in the Table 37. Perusal of data indicated that *T. viride* (A_4) was the most effective followed by *T. harzianum* (A_2) in reducing the radial growth of *F. oxysporum f. sp. lycopersici* with 44.80 and 42.20 per cent respectively over control. A_3 (36.10%), A_1 and A_6 (33.30%) were the next best. A_8 was the least effective (15.50%).

4.6.3. Effect of Volatile Inhibitory Substances Produced by Isolates of *Trichoderma* Against *F. oxysporum* f. sp. *lycopersici*

Radial growth measurement of the test pathogen as affected by volatile inhibitory substances produced by *Trichoderma* isolates are presented in Table 37. Among the 10

Table 38. Inhibitory Effect of Antagonists on *F. oxysporum* f. sp. *lycopersici*

Isolate No.	Lysis of *F.oxysporum* f.sp. *lycopersici* colony (mm)	
	3rd Day	7th Day
A_1	9.66	12.33
A_2	13.66	18.66
A_3	14.00	19.33
A_4	24.00	33.33
A_5	16.66	24.00
A_6	8.66	0.00
A_7	16.00	24.33
A_8	19.66	29.00
A_9	31.00	39.33
A_{10}	22.00	30.66
B_1	46.00	48.00
B_2	24.66	26.00
CD at 5%	2.66	2.24

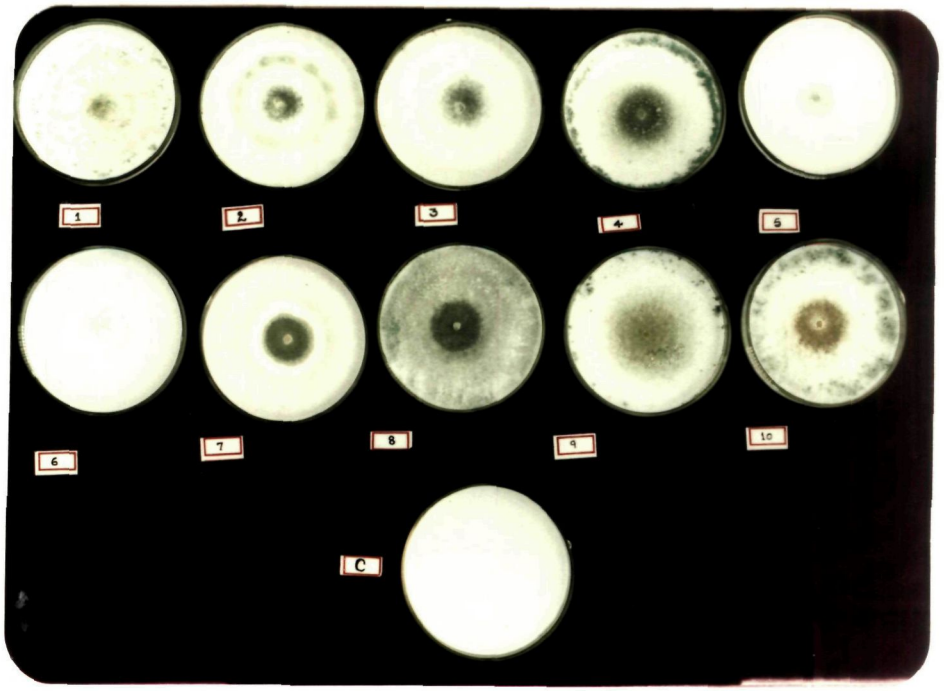
Table 39. Efficacy of Culture Filtrates of *Trichoderma* spp. on the Growth of *F. oxysporum* f. sp. *lycopersici*

Trichoderma Isolate No.	pH of the Culture filtrate	Radial growth of *F. oxysporum* f. sp. *lycopersici* (mm)			
		pH not adjusted		pH adjusted to 6.5	
		Heat sterilized	Filter sterilized	Heat sterilized	Filter sterilized
A_1	4.76	75.00	57.00	70.33	70.33
A_2	4.83	83.33	63.00	72.33	72.00
A_3	5.10	76.33	73.33	66.00	73.67
A_4	4.90	64.00	56.00	63.67	64.33
A_5	4.23	82.0	75.00	69.67	71.00
A_6	4.33	73.33	67.33	72.00	63.00
A_7	3.96	70.00	62.67	65.33	70.67
A_8	4.73	78.00	70.00	71.00	73.67
A_9	4.70	75.67	58.33	70.67	66.67
A_{10}	4.73	71.67	68.00	70.00	70.00
Control	6.46	75.67	79.33	75.33	77.33
CD at 5%	0.29	2.46	2.84	2.25	2.92

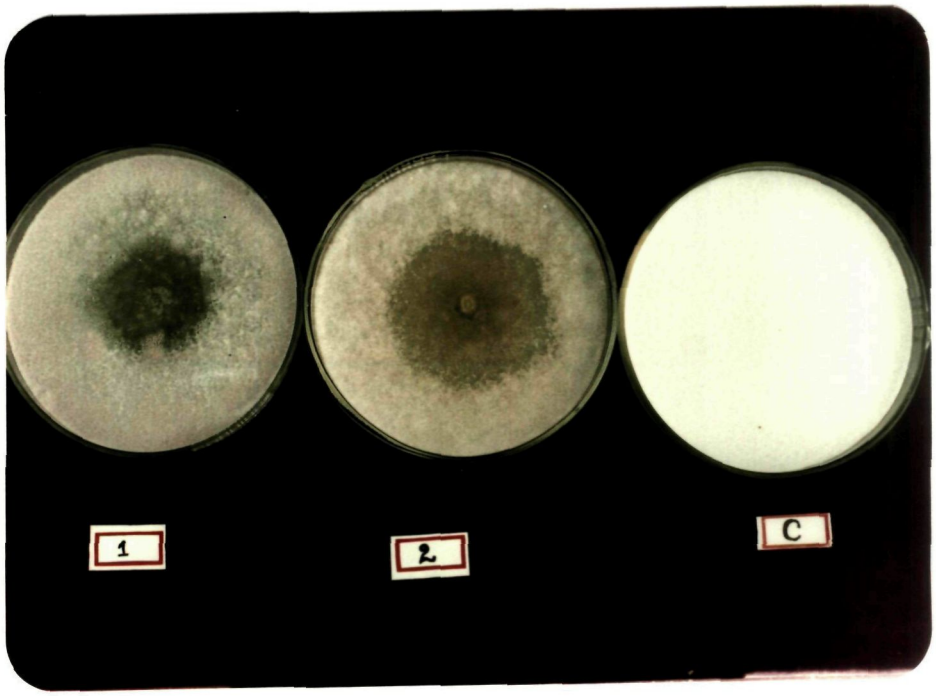
Plate 12

a) Mycelial growth inhibition of *F.oxysporum* f.sp. *lycopersici* by isolates of *Trichoderma* spp. (1-10) (7 days after inoculation).

b) Mycelial growth inhibition of *F.oxysporum* f.sp. *lycopersici* by isolates of *Bacillus subtilis* and *Pseudomonas* (2) (7 days after inoculation)



a



b

PLATE 12

Trichoderma isolates , the highest per cent inhibition of the test pathogen radial growth over control was observed in case of A_1, A_4 and A_9 with 75.40 followed by A_2 with 70.00 which were at par on 3rd day. The least effective was A_5 with 31.80 per cent. On 7th day also A_4 was consistantly found to be the most effective with 78.80 per cent reduction in radial growth over control, and was significantly superior to all other isolates. A_1 with 75.30 per cent was the next best and A_7 was the least effective with 23.00 per cent (Plate 11b).

4.6.4. Effect of Diffusible Inhibitory Substances produced by Isolates of *Trichoderma* against *F. oxysporum* f. sp. *lycopersici*

The data regarding the radial growth of the test pathogen on the medium previously occupied by isolates of *Trichoderma* separated by cellophane membrane in 48 hours are presented in Table 37. A_4, A_1 and A_9 were at par in respect of radial growth of 28.00 and 29.30 mm respectively and were significantly superior compared to all other isolates which were at par with control (35.00 mm).

4.6.5 Interaction of Antagonists with *F. oxysporum* f. sp. *lycopersici*

Ten isolates of *Trichoderma* (A_1 to A_{10}) and two bacterial (B_1 and B_2) antagonists were placed over PDA seeded with *F. oxysporum* f. sp. *lycopersici* after 12 hours. Area of lysis by antagonists was recorded on 3rd and 7th day and data are presented in Table 38 and Plate 12a and 12b. Among the isolates of antagonists B_1 was significantly superior to all other antagonists with the highest lysis area of *F. oxysporum* f. sp. *lycopersici* (46 mm) followed by B_2 (24.66 mm) and A_4 (24.00 mm) on 3rd day. On 7th day also B_1 was the highest with 48 mm and significantly differed from all other isolates followed by A_9 and A_4 with 39.33 and 33.33 mm respectively. The least effective antagonist was found to be A_6 with 8.66 mm on 3rd day and 0.0 on 7th day.

4.6.6 Production of Inhibitory Substances by Isolates of *Trichoderma* in Liquid Culture

The effect of culture filtrates of *Trichoderma* isolates on *F. oxysporum* f. sp. *lycopersici* as indicated by radial growth measurements are given in Table 39.

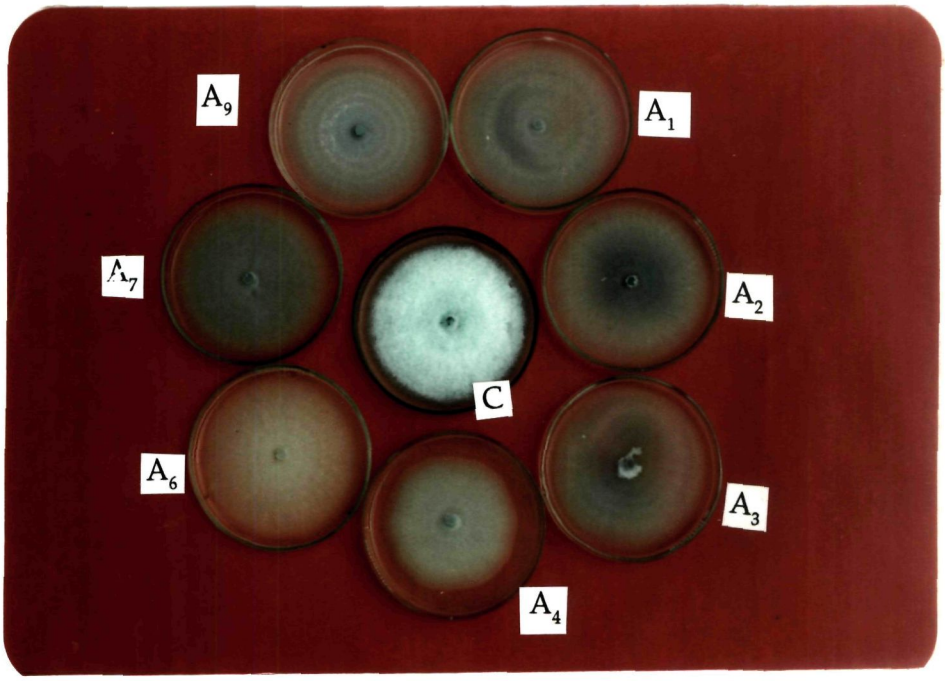
pH of the Culture Filtrate

It can be seen from the data presented in Table 39, that the *Trichoderma* isolates could

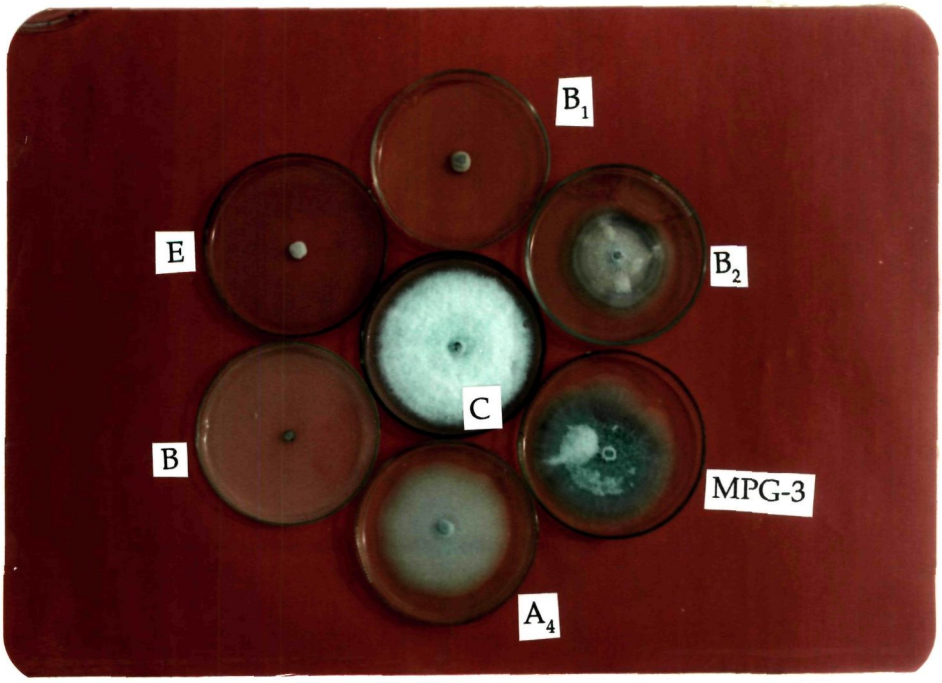
Plate13

a). Effect of culture filtrates of different isolates of *Trichoderma* spp. (A1 to A9) on radial growth of *F.oxysporum* f.sp. *lycopersici* (7 days after inoculation)

b) Effect of culture filtrates of *T. viride* (A_4) *Pseudomonas* sp. (B_1), *Bacillus subtilis* (B_2) in comparison with carbendazim (B) and Emisan (E) at 500 ppm on the radial growth of *F.oxysporum* f.sp. *lycopersici* (7 days after inoculation)



a



b

PLATE 13

alter the pH of the medium, ranging from 3.96 (A_7) to 5.10 (A_3) from initial pH of 6.5, at the time of inoculation within a week of incubation.

Heat Sterilized Culture Filtrates (pH not adjusted)

A_4 recorded the least radial growth of 64 mm and significantly superior to all other isolates followed by A_{10} with 71.67. A_2, A_3, A_5 and A_8 recorded radial growth more than control (75.67 mm)

Heat Sterilized Culture Filtrates (pH adjusted to 6.5)

Culture filtrates of A_4, A_1 and A_9 were at par with 56, 57 and 58.33 mm and were significantly superior to other isolates. A_7 was the next best with 62.67 mm. Least effective was A_5. Control showed radial growth of 79.33 mm.

Filter Sterilized Culture Filtrates (pH not adjusted)

None of the culture filtrates of *Trichoderma* isolates completely inhibited radial growth of F. *oxysporum* f. sp. *lycopersici*. However, culture filtrates of A_4 and A_7 recorded lower radial growth of 63.67 and 65.33 mm against 75.33 mm in control without culture filtrate (Plate13a).

Filter Sterilized Culture Filtrates (pH adjusted)

Here also none of the isolates completely inhibited the *F. oxysporum* f. sp. *lycopersici* Least growth of 63 mm was observed in case of culture filtrate of A_6 followed by A_4 (64.33) which did not differ significantly between each other. Next best was A_9 (66.67) which was at par with A_4 and significantly superior over all other isolates. The least effective isolates were A_1 (73.33 mm), A_3 and A_6 (73.67 mm), while the growth in control was 77.33 mm. Though there was no complete reduction in the radial growth the aerial growth of colony was found to be reduced in all the isolates, in all these experiments. However, growth reduction could not be clearly assessed by visual method hence culture filtrates were added to PD broth and test pathogen was inoculated and incubated for 15 days and growth was measured by testing mycelial dry weight. The results are presented in the Table 39.

Heat Sterilized Culture Filtrates of Antagonists (pH not adjusted)

Results showed that there is a marked reduction in the dry weight of mycelium of *F.*

Table 40. Effect of Culture Filtrates of Antagonists and MPG -3 on Growth and Conidial Germination of *F. oxysporum* f. sp. *lycopersici*

Treatment	Mycelial Dry Weight (mg)		Inhihition of conidial Germination (%)
	Heat sterilized	Filter sterilized	
A_1	49.00	51.67	63.33 (52.73)
A_2	58.33	53.33	52.66 (48.45)
A_3	149.00	103.67	42.33 (40.59)
A_4	45.33	46.00	84.33 (66.79)
A_5	98.67	89.00	25.00 (29.99)
A_6	51.67	74.67	67.00 (54.95)
A_7	98.00	92.33	62.33 (52.14)
A_8	86.33	94.67	50.33 (45.19)
A_9	50.00	52.33	79.00 (62.73)
A_{10}	50.67	63.33	66.33 (54.53)
B_1	45.00	46.67	83.33 (62.78)
B_2	108.33	103.33	52.00 (46.15)
MPG -3	62.33	66.67	63.33 (51.16)
Control -1	143.30	165.00	-
Control -2	161.60	176.60	-
Carbendazim 1000 ppm	0.00	0.00	87.00 (68.93)
Emisan 1000 ppm	0.00	0.00	-
CD at 5%	7.88	4.59	2.57

Figures in parentheses are transformed 'Arc Sine' values.

A1 to A10 - Isolates of Trichoderma
B1 to B2 - Isolates of Bacteria
MPG-3 - Modified Panchagavya Formulation 3
Control 1 - PDB amended with weindling medium
Control 2 _ PDB amended with sterile water

Table 41. Effect of Culture Filtrates of Antagonists, Fungicides and MPG -3 on the Growth of *F. oxysporum* f. sp. *lycopersici*

Treatment	Radial growth of F. o.l (mm) after 7 Days
Bavistin (1000 ppm)	0.00
Emisan (1000 ppm)	0.00
B_1	0.50
B_2	72.75
MPG - 3	76.25
A_4	64.50
Control	81.25
CD at 5%	1.72

Observations recorded after 7 days

oxysporum f. sp. *lycopersici* with the culture filtrates of *Trichoderma*, Bacterial antagonists MPG - 3, carbendazim and emisan compared to controls. B_1 and A_4 recorded the lowest mycelial dry weight of 45.00 mg and 45.33 mg respectively followed by A_1 (49.00 mg), A_9 (50.00 mg), A_{10} (50.67 mg), MPG - 3 recorded 62.33 mg dry weight. Carbendazim and emisan treated PDB completely inhibited the fungus growth (Table 40).

Filter Sterilized Crude Culture Filtrates (pH not adjusted).

Among the culture filtrates of antagonists A_4 (46.00 mg), and B_1 (46.67 mg), were significantly superior to all other isolates in inhibiting the growth of *F. oxysporum* f. sp. *lycopersici* MPG - 3 reduced the growth of (66.67 mg) significantly over controls (165 and 176.6 mg respectively). Here also bavistin and emisan completely inhibited growth of the pathogen.

Effect of Culture Filtrates on Conidial Germination of *F. oxysporum* f. sp. *lycopersici*

The effect of culture filtrates of the antagonists, MPG - 3 and fungicides was studied in comparison with control and the results obtained are given in Table 40. The highest per cent inhibition of conidial germination of *F. oxysporum* f. sp. *lycopersici* was recorded in case of A_4 (84.33) and B_1 (83.33) respectively, which were significantly superior to all other culture filtrates. The next best was A_9 with 79. MPG-3 resulted in 63.33 per cent inhibition of conidial germination. Carbendazim recorded the highest inhibition of conidial germination with (87%).

Effect of Heat Sterilized Culture Filtrates of Antagonists. MPG - 3 and Fungicides on Radial Growth of *F. oxysporum* f. sp. *lycopersici*

The results presented in Table 41 indicated that the culture filtrate of B_1 was at par with fungicides bavistin and emisan in inhibiting radial growth of the pathogen. All other treatments permitted the pathogen to grow. However growth was significantly lower than control (plate13b)

4.6.7. Efficacy of Antagonists Against Seedling Disease and Wilt in Pot Culture Studies.

Ten isolates of *Trichoderma* (A_1 to A_{10}) and two bacterial (B_1 and B_2) were assessed for their antagonistic potential against seedling disease and wilt in pot culture.

Table 42. Efficacy of Antagonists Against Seedling Disease and Wilt Incidence in Tomato

S. No	Antagonist	Per cent Healthy Seedlings		Wilt (P D I)	
		Seed treatment	Soil application	Root Dip	Soil Application
1	A_1	54.60 (47.68)	57.30 (49.22)	59.72 (50.60)	54.86 (47.80)
2	A_2	56.60 (50.77)	50.00 (45.00)	63.20 (52.66)	56.94 (49.03)
3	A_3	52.00 (46.15)	44.00 (41.55)	64.60 (53.51)	65.97 (54.29)
4	A_4	64.60 (53.59)	63.30 (52.80)	49.40 (44.75)	48.60 (44.20)
5	A_5	46.00 (40.78)	41.30 (40.01)	68.07 (55.60)	65.28 (53.92)
6	A_6	52.60 (46.53)	40.60 (39.61)	62.50 (52.25)	57.63 (49.39)
7	A_7	44.60 (41.93)	46.60 (43.09)	61.82 (51.85)	58.34 (49.84)
8	A_8	59.30 (50.77)	51.30 (45.76)	60.43 (51.05)	54.17 (47.41)
9	A_9	62.00 (51.95)	60.30 (51.16)	61.80 (51.82)	49.98 (44.99)
10	A_{10}	60.60 (51.16)	56.60 (48.83)	61.80 (51.86)	61.11 (51.45)
11	B_1	63.30 (52.73)	62.00 (51.94)	51.40 (45.81)	57.63 (49.39)
12	B_2	48.00 (43.85)	53.30 (46.91)	65.29 (53.91)	65.96 (54.34)
13	Control	27.30 (31.52)	28.60 (32.37)	78.84 (62.61)	80.56 (63.88)
14	Healthy	82.00 (64.91)	83.30 (65.96)		
CD at5%		(2.03)	(2.11)	(4.21)	(3.89)

Figures in parentheses are transformed 'Arc Sine' values.
A_1 to A_{10} - *Trichoderma* spp. B_1 - *Pseudomonas* B_2 - *Bacillus subtilis*

4.6.7.1. Effect of Antagonists Against Seedling Disease

Antagonists were tested for their efficacy by two methods viz. seed treatment and soil application for assessing seedling disease and root dip in antagonist suspension and soil application before planting for wilt studies. The data are presented in Table 42. Data indicated that the highest per cent healthy seedling stand was observed with A_4 (64.60 followed by B_1 (63.3) which were significantly superior to all other antagonists. The least per cent healthy seedling stand was noticed with A_7 (44.6) while in control it was 27.3.

In case of antagonists (WBC) applied to the infested soil prior to sowing tomato seeds also indicated that A_4 with 63.3 and B_1 with 62 per cent healthy seedling stand proved significantly superior to others followed by A_9 (60.3). A_6 with 40.6 recorded the lowest per cent healthy seedling stand among treatments while in control it was 28.60. All the antagonists were significantly superior over control.

4.6.7.2. Effect of Antagonist Against Wilt Incidence

Perusal of the data presented in Table 42 showed that the lowest PDI was found in case of *T. viride* (A_4) with 49.40 followed by *Pseudomonas* sp. (B_1) with 51.40 which were at par with each other. All other antagonists were significantly superior over control in case of root dip method of application of antagonists.

Antagonists multiplied on wheat bran were introduced to the soil infested soil and 4 week old seedlings were transplanted and observations were taken regularly for wilt incidence. The data are presented in Table 42 which shows that antagonists A_4 and A_9 were at par by inducing PDI of 48.6 and 50.0 respectively. A_1 (54.9) and A_8 (54.2) were at par with A_4 and A_8 and did not differ significantly. B_1 was the next best with 57.6 significantly superior over control (80.6).

4.6.8. Mass Multiplication of Antagonists *T. viride* (A_4) and *Pseudomonas* (B_1)

Antagonists were multiplied on cheap and easily available organic wastes viz. well decomposed cotton waste, FYM biogas slurry and MPG-3 for mass multiplication. The perusal of the data in Table 43 indicated that FYM supplemented with one per cent jaggery supported both the fungal and bacterial antagonists. Population of A_4 was the highest in FYM with jaggery (1%) with 70.7 x 10^3 cfu g^{-1} followed by FYM alone and bio-gas slurry with jaggery with 50.7 x10^3 cfu g^{-1}. Least number of propagules were found in MPG-3 with 34.7 x10^3 cfu g^{-1} on *Trichoderma* specific medium.

Table 43. Effect of Organic Materials on the Multiplication of Antagonists

Treatment	Population (cfu g^{-1})	
	(10^3) *T. viridae* (A_4)	(10^5) Pseudomonas(B_1)
Cotton Waste (CW)	41.00 (4.6117)	16.33 (6.2289)
Farm Yard Manure (FYM)	68.00 (4.8337)	29.33 (6.4648)
Biogas Slurry (BGS)	50.70 (4.7154)	21.70 (6.3308)
MPG - 3	34.70 (4.5388)	7.22 (6.0944)
CW + 1% Jaggery	44.70 (4.6487)	13.33 (6.1231)
FYM+ 1% Jaggery	70.70 (4.8323)	30.00 (6.4742)
BGS+ 1% Jaggery	48.00 (4.6968)	26.33 (6.4200)
MPG - 3 + 1% Jaggery	36.30 (4.5601)	13.33 (6.1735)
CD at 5%	0.0614	0.1105

Figures in parentheses are Logarithmic transformation values.

Observations recorded after 21 days

B_1 population was assessed on King's B medium. The highest cfu g^{-1} was observed in case of FYM supplemented with one per cent jaggery. Addition of jaggery to the organic wastes did not enhance the population significantly. However population between different organic wastes differed significantly. FYM was significantly superior to other carriers.

Population of A_4 and B_1 did not increase significantly in unsterilized organic wastes. The population of *Rhizopus, Aspergillus* spp. *and Mucor* sp. were predominent. Hence un sterilized organic materials were not used for further studies.

Effect of Antagonists and MPG-3 on Germination of tomato seeds *in vitro*

Germination test was conducted in order to find out any deleterious or stimulative effect of antagonists on seeds. The seeds were soaked in the suspensions of antagonists (1 x 10^6 spores ml^{-1} or 1 x 10^7 cells ml^{-1}) and 10^1 dilution of MPG-3 formulation for two hours air dried. Such seeds were incubated on sterile petriplates containing moist blotters at the rate of 25 seeds $plate^{-1}$ and germination counts were taken up to ten days. There was no deleteriors effect upon the germination of seeds by either of the antagonist or MPG-3 as compared to uninoculated control. There was more than 80 per cent germination of seeds treated with antagonists and MPG as was the case with control.

4.6.9. Methods of Application of the Antagonists to Soil.

Both the antagonists A_4 (*T. viride*) and B_1 (*Pseudomonas* sp) were applied in five different ways with or without carriers to find an efficient method to deliver the antagonists to the soil. The efficiency of the method of application was judged based upon the per cent healthy seedling stand at the end of 28 days after sowing and results are presented in Table 44 and Fig.9a. The healthy seedling stand in treatment, carboxy methyl cellulose (CMC) pelleting was the highest with 55.11 per cent and was significantly superior to all other treatments followed by seed soaking (52.89 per cent) and biogas slurry (51.11 per cent) which were at par with each other. The remaining two methods viz., direct application and through FYM had the lower per cent healthy seedling stand of 47.11 and 49.55 which were statistically at par. However, the seedling stand in all the methods differed significantly from that of check when they were treated with antagonists. Bacterial antagonist B_1 when applied on seed with CMC pelleting gave the highest healthy seedling stand with 63.33 per cent and significantly differed from other methods. Seed soaking and through biogas slurry were the next best with 62, 62.66 per cent respectively which were statistically at par with each other. With A_4 (*T. viride*) antagonist also CMC pelleting

Table 44. Effect of Method of Application of Antagonists on Seedling Disease in Tomato

Method of Application	Per cent healthy seedlings			Mean
	F. o . l. (F)	F + A_4	F + B_1	
Direct Application	27.33 (31.52)	60.66 (51.16)	53.33 (48.83)	47.11 (43.84)
Seed Soaking	28.00 (31.94)	68.66 (55.96)	62.00 (51.94)	52.89 (46.62)
Biogas Slurry	26.00 (30.65)	64.66 (53.53)	62.66 (52.34)	51.11 (45.51)
CMC Pelleting	28.00 (31.94)	74.00 (59.35)	63.33 (54.74)	55.11 (48.68)
F Y M	28.66 (32.35)	64.00 (53.14)	56.00 (48.46)	49.55 (44.65)
Mean	27.59 (31.68)	66.40 (54.63)	46.80 (51.26)	

Figures in parentheses are transformed 'Arc Sine' values.

CD at 5%

Methods	1.25
F vs Antagonists	0.97
Interaction	
Methods X F vs Antagonists	2.16

A4 - *T. virade* B1 - *Pseudomonas* sp.
F.o.l.(F) - *F.oxysporum* f.sp *lycopersici*

Table 45. Effect of Method of Application of Antagonists on Wilt Incidence in Tomato

Method of Application	PDI			Mean
	F. o. l. (F)	F + A_4	F + B_1	
Direct Application	60.00 (50.77)	47.50 (43.56)	53.25 (46.91)	53.58 (47.08)
Root Dip	83.83 (66.32)	45.00 (42.13)	51.67 (45.96)	58.26 (51.47)
Biogas Slurry	62.50 (52.25)	50.83 (45.48)	50.00 (45.00)	54.44 (47.57)
F Y M	64.17 (53.25)	47.50 (43.57)	50.00 (45.00)	53.91 (47.27)
Mean	65.62 (55.65)	47.58 (43.68)	51.23 (45.72)	

Figures in parentheses are transformed 'Arc Sine' values.

CD at 5%	
Methods	1.75
F vs Antagonists	1.52
Interaction	
Methods X F vs Antagonists	3.03

A4 - *T. viradе* B1 - *Pseudomonas* sp.
F.o.l.(F) - *F.oxysporum* f.sp *lycopersici*

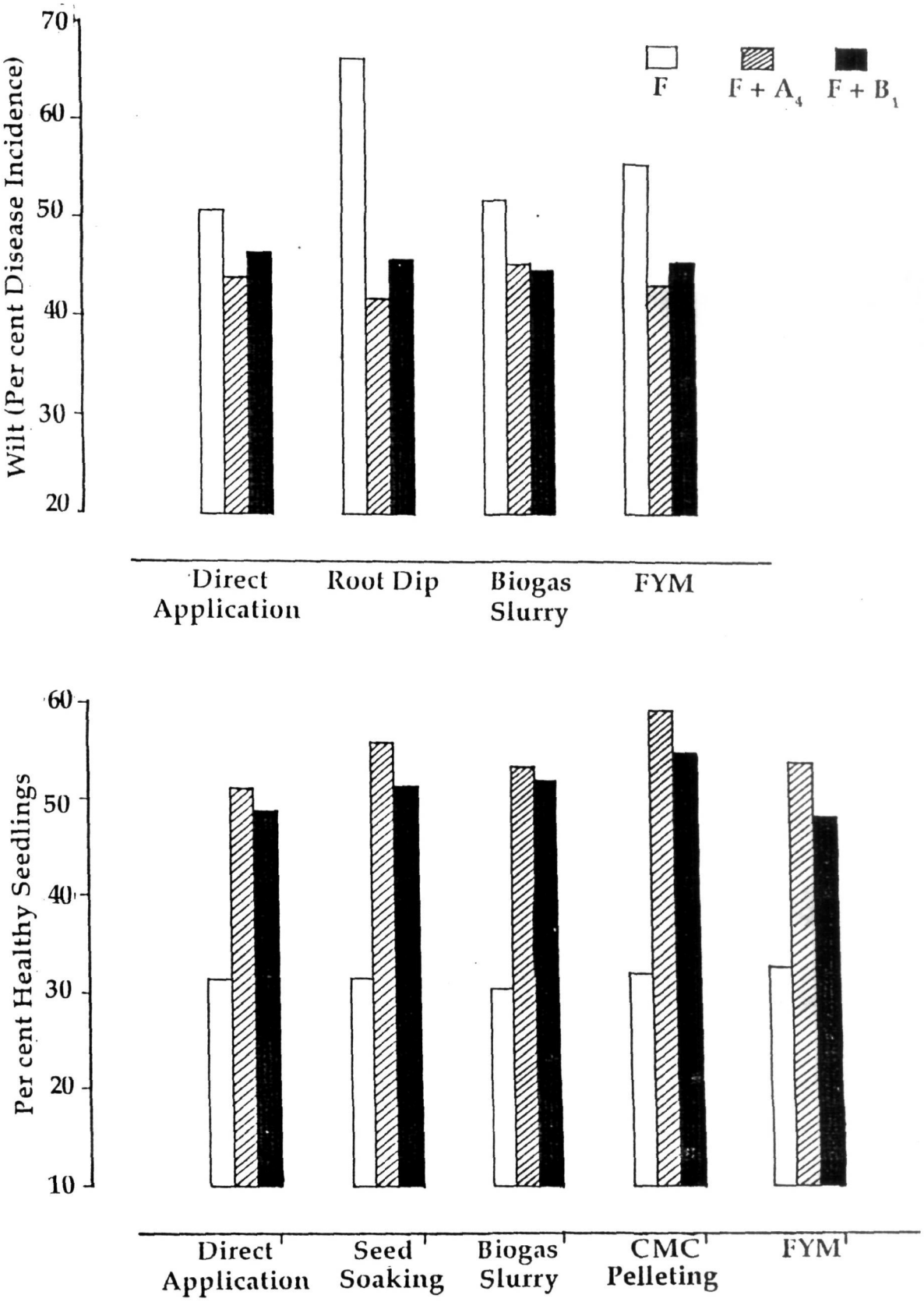


Fig. 9. Effect of Method of Application of Antagonists on Healthy Seedling Stand and Wilt Incidence.

Plate 14

a) Efficacy of various management partices on tomato seedling stand in *Fusarium* sick soil.

T_1 : Carbendazim (1000 ppm)
T_2 : Neem Cake (0.1%)
T_3 : Modified *Panchagavya* (MPG -3)
T_4 : *T.viride* (A_4)
T_5 : *Pseudomonas* sp.(B_1)
T_{17} : Inoculated Control

b) T_6 : Neem cake + MPG-3
T_9 : MPG-3 + T.viride
T_{18} : Healthy check (uninoculated)



a



b

PLATE 14

resulted in the highest per cent healthy seedlings 74.00 and differed significantly from other methods followed by seed soaking with 68.66 per cent.

Similarly the effect of method of application of antagonists on wilt incidence in tomato was studied by 4 methods. The data pertaining to disease incidence (PDI) has been given in Table 45 and fig 9 . Application of *T. viridae* (A_4) by different methods showed significant differences in disease reaction as compared to check. The average PDI with the use of A_4 by different methods ranged from 45 to 50.83 as compared to check with 60 to 83.83. The least PDI was observed when A_4 was applied as root dip (45.00) followed by direct application and through FYM (47.50). When *Pseudomonas* (B_1) was applied by different methods disease reduction was significantly higher compared to check. While the check recorded a PDI ranging from 60 to 83.83, the treatments with antagonist recorded PDI of 50 to 53.25. The least PDI was observed when bacterial antagonist was added through FYM and biogas (50.0) followed by root-dip (51.67) which were at par with each other. Direct method of application had the highest PDI with 53.25. The per cent disease incidence in treatments viz; direct application through FYM and biogas slurry ranged from 53.58 to 54.44 and statistically at par with each other. Root dip method showed the highest PDI and significantly higher than other methods. The PDI in all the methods differed significantly from that of check when they were treated with antagonists.

4.7. BIOLOGICAL CONTROL OF SEEDLING DISEASE AND WILT IN TOMATO CAUSED BY *F. oxysporum* f. sp. *lycopersici* IN POT CULTURE.

Neem cake, MPG-3, *T. viridae* (A_4) and *Pseudomonas* sp. (B_1) were selected from the results obtained from previous experiments and applied individually and in combination to find out the efficacy in controlling seeding disease and wilt in tomato. A comparison was made with bavistin and healthy check. The results are given below in Table 46.

Perusal of the data indicated that T_6 with 80.00 per cent healthy seedlings was significantly superior to all other treatments. Next best treatments were T_3 (76.00) and T_1 (74.66) which were statistically at par. Treatment 9 and T_7 were at par with each other and recorded 72.66 and 70.66 per cent healthy seedlings respectively. The least per cent healthy seedlings was observed in case of control with 26. All the treatments were significantly superior to control. However among the treatments the lowest per cent healthy seedlings was noticed in T_5, T_{11} and T_{16} with 58.00 (Plate 14a and 14b).

Table 46. Control of Tomato Seedling Disease and Wilt Through Different Treatments in Pot Culture

Treatment	healthy seedlings (%)	Wilt (PDI)	Inhibition of population of *F. o. l.* (%)	
			30 DAT	60 DAT
1. Bavistin (B)	74.66 (59.81)	50.80 (45.47)	36.50 (37.15)	28.20 (31.96)
2. Neem Cake (NC)	66.00 (54.33)	36.71 (37.25)	48.20 (43.90)	39.50 (38.84)
3. M P G -3	76.00 (60.67)	25.00 (29.92)	41.80 (40.25)	34.10 (35.69)
4. A_4	61.33 (51.56)	49.21 (44.52)	41.10 (39.84)	46.70 (43.13)
5. B_1	58.00 (49.61)	51.66 (45.96)	42.60 (40.68)	50.20 (45.11)
6. NC + M P G - 3	80.00 (63.45)	19.21 (25.89)	64.10 (53.22)	57.40 (49.25)
7. NC + A_4	70.66 (57.23)	33.33 (35.24)	55.00 (47.87)	56.80 (48.91)
8. NC + B_1	63.33 (52.74)	42.50 (40.68)	58.30 (49.76)	49.10 (44.50)
9. M P G - 3 + A_4	72.66 (58.48)	30.00 (33.11)	60.90 (51.33)	55.00 (68.02)
10. M P G - 3 + B_1	64.00 (53.13)	35.00 (36.21)	53.70 (47.15)	60.50 (51.08)
11. A_4 + B_1	58.00 (49.61)	36.71 (37.22)	50.40 (45.23)	61.70 (52.74)
12. NC + M P G - 3 + A_4	68.00 (55.56)	28.33 (32.13)	52.20 (46.32)	58.90 (50.17)
13. NC + M P G - 3 + B_1	61.33 (51.55)	36.71 (37.25)	58.90 (50.17)	54.20 (47.49)
14. NC + A_4 + B_1	60.00 (50.77)	41.71 (40.21)	54.30 (47.46)	46.10 (42.74)
15. MPG-3 + A_4 + B_1	62.00 (51.95)	36.71 (37.24)	49.10 (44.48)	52.40 (46.39)
16. NC+MPG-3 + A_4+B_1	58.00 (49.99)	35.00 (36.26)	60.90 (51.35)	60.20 (50.97)
17. Inoculated Control	26.00 (30.65)	80.33 (64.07)	-	
18. Healthy Check	87.30	- (69.16)	-	-
CD at 5%	(2.14)	(4.46)	(4.33)	(6.72)

DAT = Days After Transplanting
Figures in parentheses are transformed 'Arc Sine' values.
Initial propagules 45 x 10^2 g^{-1} soil

Plate 15 Effect of various treatments against *Fusarium* wilt in tomato

T1 : Carbendazim (1000 ppm)

T2 : Neem cake (0.1%)

T3 : Modified *Panchagavya* (MPG-3)

T4 : *T. viride*


T1


T2


T3


T4

Plate 15 contd...

T5 : Psedomonas sp.
T6 : Neem cake + MPG-3
T18 : Uninoculated check
T17 : Inoculated control


T5


T6


T17


T18

Table 47. Comparitive efficacy of Different Control Measures on the Incidence of Fusarium Wilt in Tomato Under Field Conditions

Treatment	PDI	Mean shoot length (cm)	Mean root length (cm)	Shoot dry weight (g)	Root dry weight (g)	Total plant dry weight (g)	Fruit yield tons per ha.	C:B Ratio
T1 Un Inoculated Control	--	104.0 (64.5)	26.2 (59.1)	38.4	2.6	41.1 (121.8)	14.1 (83.3)	-
T2 Carbendazim	34.4 (35.94)	94.3 (49.2)	23.7 (44.0)	31.4	2.2	33.6 (81.5)	10.3 (32.9)	1:4
T3 Neam Cake(NC)	25.0 (29.87)	95.0 (50.2)	25.5 (54.5)	35.4	2.6	37.9 (105.0)	11.6 (49.9)	1:1.1
T4 MPG -3	20.3 (26.72)	97.9 (54.8)	25.3 (53.4)	33.8	2.4	36.2 (95.6)	12.3 (59.7)	1:21.3
T5 NC + MPG -3	7.8 (15.93)	105.0 (66.1)	29.9 (81.5)	38.3	3.1	41.4 (123.4)	16.7 (116.6)	1:2.4
T6 Inoculated Control	55.7 (48.26)	63.2	16.5	17.5	1.1	18.5	7.7	-
CD at 5%	(4.82)	3.86	2.76	9.36	0.68	-	1.92	

Figures in parentheses are transformed 'Arc Sine' values for PDI and others are per cent increase over control

C:B Ratio = Cost Benefit Ratio

In case of wilt study T_6 and T_3 with PDI of 19.21 and 25.00 were significantly superior to all other treatments and were at par with each other. T_{12}, T_9, T_7, T_{10} and T_{16} were at par among themselves with PDI ranging from 28.33 to 35.00 (Plate 15 and 16). All the treatments were significantly superior over control (80.83).

All the treatments significantly differed over control in per cent reduction of propagules of *F. oxysporum* f. sp. *lycopersici* Per cent reduction of propagules ranged from 36.50 in T_1 to 64.1 in T_6 at 30 days after planting. At 60 days after planting per cent reduction of propagules ranged from 28.2 in T_1 to 61.7 in T_{11}.

4.8. MANAGEMENT OF FUSARIUM WILT OF TOMATO UNDER FIELD CONDITIONS.

Pot culture studies revealed that neem cake, MPG-3 and their combination could be exploited for the management of Fusarium wilt of tomato under field conditions. A field experiment was laid out in RCBD with neem cake (0.1%), MPG-3 (10^1) dilution individually and in combination as main treatments, bavistin treatment, inoculated control and uninoculated healthy plots as checks. The data were presented in Table 47. The PDI was the highest in inoculated control with 55.73 under field conditions. All the treatments differed significantly among themselves with PDI ranging from 7.8 (NC + MPG-3 combined treatment) to 34.40 (Carbendazim). The growth and vigour in T_5 was found as good as in case of uninoculated healthy check followed by T_4 and T_3

Increase in mean shoot length was the highest with T_5 (66.1%) which was at par with T_1 (64.5%) but differed significantly over other treatments. All other treatments were at par with each other in increase in mean shoot lebgth ranging from 49.3 to 54.8%.

In case of root length also T_5 (29.93) differed significantly over all other treatments. Other treatments T_2, T_3 and T_4 were at par with mean root length ranging from 23.74 to 25.48 cm while in control it was 16.49 cm.

There was no significant difference among treatments in shoot dry weight which varied from 31.43 to 38.44 g pl^{-1} All the treatments showed significant difference over control (17.45 g). T_5 was significantly superior to other treatments with root dry weight of 3.11 g pl^{-1}. Among treatments root dry weight ranged from 2.16 to 2.56 g pl-1. All the treatments were significantly superior to control with 1.06 g pl-1.

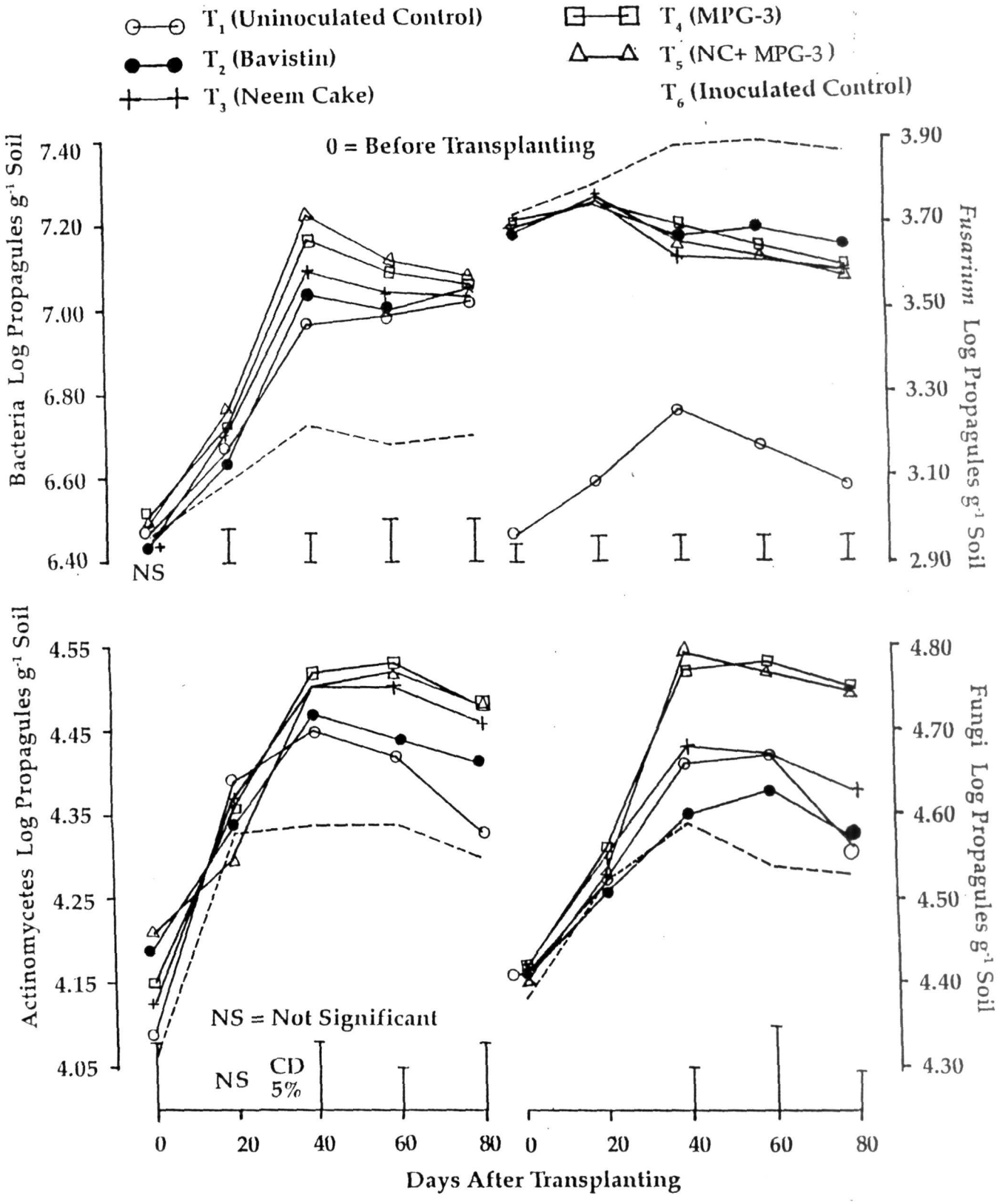


Fig. 10. Effect of Control Measures on the Population Dynamics of Microflora and Pathohgen in Rhizosphere Soil of Tomato cv Pusa Ruby

Table 48. Comparative Efficacy of Different Control Measures on the Population Dynamics on Rhizopheric Microflora of Tomato

Treatment	cfu g^{-1}				Colony forming units g^{-1} dry wilt soil (Rhizosphere soil)															
	Before Planting in Soil				20 DAT				40 DAT				60 DAT				80 DAT			
	B	A	F	F'	B	A	F	F'	B	A	F	F'	B	A	F	F'	B	A	F	F'
Uninoculated control (T1)	2.98 (6.47)	12.38 (4.09)	25.88 (4.41)	9.20 (2.96)	4.80 (6.67)	24.50 (4.39)	33.50 (4.52)	12.50 (3.09)	9.52 (6.97)	28.23 (4.45)	45.75 (4.66)	18.50 (3.26)	9.80 (6.73)	26.20 (4.41)	47.30 (4.67)	14.88 (3.17)	10.55 (7.02)	21.63 (4.33)	36.50 (4.56)	12.05 (3.08)
Bavistin (T2)	2.80 (6.44)	15.68 (4.19)	25.63 (4.41)	49.20 (3.69)	4.30 (6.64)	21.75 (4.34)	32.25 (4.51)	58.25 (3.75)	11.08 (7.04)	29.45 (4.470)	39.50 (4.60)	47.25 (3.67)	10.13 (7.00)	27.80 (4.44)	42.33 (4.63)	48.65 (3.69)	11.23 (7.05)	25.90 (4.41)	37.05 (4.57)	44.80 (3.65)
Neem Cake (T3) (NC)	2.75 (6.44)	13.20 (4.12)	26.33 (4.42)	48.20 (3.68)	5.15 (6.71)	23.25 (4.37)	35.50 (4.55)	58.00 (3.76)	12.58 (7.10)	31.8 (4.50)	47.50 (4.68)	42.25 (3.63)	11.03 (7.04)	31.98 (4.50)	46.3 (4.67)	40.95 (3.61)	10.8 (7.03)	28.78 (4.46)	42.45 (4.63)	39.80 (3.59)
MPG III (T4)	3.25 (6.51)	14.13 (4.15)	26.23 (4.42)	50.62 (3.70)	5.40 (6.73)	23.25 (4.36)	36.25 (4.56)	55.75 (3.75)	14.81 (7.17)	33.07 (4.52)	59.00 (4.77)	50.50 (3.70)	12.63 (7.10)	33.70 (4.53)	60.30 (4.78)	45.20 (3.65)	11.55 (7.06)	29.88 (4.48)	56.22 (4.750)	40.23 (3.60)
NC + MPG 3 (T5)	3.10 (6.49)	16.38 (4.21)	24.95 (4.40)	48.95 (3.69)	5.95 (6.77)	20.25 (4.30)	34.00 (4.53)	57.00 (3.76)	17.07 (7.23)	31.98 (4.50)	61.50 (4.79)	45.50 (3.66)	13.30 (7.12)	33.12 (4.52)	59.30 (4.77)	41.80 (3.62)	12.38 (7.08)	30.48 (4.48)	55.48 (4.74)	38.55 (3.59)
Inoculated Control (T6)	3.00 (6.47)	11.48 (4.06)	23.63 (4.38)	52.48 (3.72)	3.98 (6.60)	21.50 (4.33)	33.00 (4.52)	62.00 (3.80)	5.51 (6.73)	21.80 (4.34)	39.00 (4.59)	75.75 (3.88)	4.80 (6.68)	21.83 (4.34)	34.87 (4.54)	76.95 (3.89)	5.13 (6.70)	19.98 (4.23)	34.40 (4.53)	74.20 (3.87)
CD at 5%	NS	(0.08)	NS	(0.035)	(0.08)	NS	NS	(0.06)	(0.07)	(0.04)	(0.05)	(0.06)	(0.09)	(0.05)	(0.10)	(0.06)	(0.10)	(0.08)	(0.44)	(0.06)

NS = Non Significant

B = Bacteria (x 10^6), A = Actinomycetes (10^3), F = Fungi(10^3). F' = *Fusarium spp.* (x10^2), DAT = Days after Transplanting

Figures in parentheses are Logarithmic Transformation Values

Table 49. Effect of Management Practices on the Survival of *F. oxysporum* f. sp. *lycopersici*

Treatment	Propagules of *F. o. l.* (cfu g^{-1} soil $x10^2$)		
	1 Week	2 Weeks	3 Weeks
Control	45.23 (3.6553)	43.10 (3.6342)	37.75 (3.5743)
Flooding	9.65 (2.9827)	9.50 (2.9767)	9.00 (2.9539)
Carbendazim Drench (1000 ppm)	27.73 (3.4413)	28.81 (3.4578)	27.75 (3.4416)
MPG-3 Drench	33.23 (3.5211)	25.58 (3.4036)	24.00 (3.3782)
A_4 (Soil Application)	26.71 (3.4260)	21.71 (3.3339)	20.75 (3.3164)
B_1 (Soil Application)	32.51 (3.5108)	25.91 (3.4122)	22.00 (3.3406)
CD at 5%	(0.0578)	(0.0702)	(0.0715)

Initial Population of *F.o.l* 48.10 x 10^2 g^{-1} soil
Figures in parentheses are Logarithmic transformed values.

A_4 - *T.viride*
B_1 - *Pseudomonas sp.*

Fruit yield ha-1 was the highest with T_5 (16.70 tons ha^{-1}) differing significantly over other treatments T_3 (11.56 tons ha^{-1}) and T_4 (12.31 tons ha^{-1}) were at par with each other T_1 (10.25 tons ha^{-1}). All the treatments were significantly superior to control with 7.71 tons ha^{-1}. Cost : Benefit ratio was the highest with T_4 (1 : 21.3) followed T_1 (1 : 4).

Comparative efficacy of different control measures on the population dynamics of total microflora and the pathogen in the rhizosphere was assessed and the data are presented in Table 48 and Fig 10. Before seedling transplanting the population of field microflora and that of *F. oxysporum* f. sp. *lycopersici* were assessed in the soil using standard isolation media. Bacterial and total fungal population was non significant ranging from 2.75 to 3.25 x 10^6 cfu g^{-1} soil for bacteria and 23.63 to 26.33 x 10^3 g^{-1} soil for total fungi. Actinimycetes population ranged from 11.48 x 10^3 in uninoculated (T_6) control to 16.38 x 10^3 cfu g^{-1} soil (T_5). T_1, T_6 and T_3 were at par while all other treatments differed significantly. *Fusarium* propagules was the highest with T_6 (52.48 x 10^2 cfu g^{-1} soil) followed by T_4 (50.62 x 10^2 cfu g^{-1} soil). Bacterial population in rhizosphere increased from 20 DAT to 40 DAT and showed a decreasing trend from 60 DAT except in healthy check, where increasing trend was observed. T_5 showed highest bacterial population from 20 DAT to 80 DAT followed by T_4. Actinomycetes population also increased from 20 DAT to 40 DAT and showed a decreasing tendency from 60 DAT. T_4 showed the highest actinomycetes population from 20 DAT to 60 DAT followed by T_2 at 20 DAT and T_6 at 40 and 60 DAT. At 80 DAT, T_6 showed highest actinomycetes population. Total fungal population was highest with T_4 and T_5 without significant difference between themselves up to 80 DAT. Fusarium population increased initially upto 20 DAT and decreased in all the treatments from 40 DAT to 80 DAT; while there was an increase in uninoculated check (T_6). In general it was found that all the treatments were superior in controlling the microbial population and showed less of *Fusarium* population.

4.9. EFFECT OF MANAGEMENT PRACTICES ON SURVIVAL OF *F. oxysporum* f. sp. *lycopersici.*

A study was taken up to understand the survival capacity of *F. oxysporum* f. sp. *lycopersici* when subjected to certain management practices viz., flooding, drenching with bavistin, MPG-3, application of antagonists A_4 and B_1. The study was carried out up to 3 weeks after imposing treatment. The data were presented in the Table 49. From the perusal of the data it is observed that flooding had drastically reduced the propagules of *F. oxysporum* f. sp. *lycopersici* from 48.1 x 10^2 cfu g^{-1} soil to 9.0 x 10^2 g^{-1} by 3rd week

followed by *Trichoderma viridae* (A_4) with 2.75 x 10^2 g^{-1}. The population of *F. oxysporum* f. sp. *lycopersici* decreased from 48.10 to 37 x 10^2 g^{-1} soil in control. *Trichoderma viridae* was found effective in reducing propagules of *Fusarium* compared to all other treatments after flooding.

DISCUSSION

V. DISCUSSION

Fusarium wilt of Tomato caused by *F. oxysporum* f.sp. *lycopersici* is a serious disease wherever tomato is grown and is one of the limiting factors in tomato production. The pathogen being soil-borne, it is difficult to achieve economic control of the resultant disease by chemical control.

In the present investigations disease survey, distribution of disease in Karnataka and various aspects of disease such as inoculum density, influence of soil types, effect of age of pathogen culture, age of seedlings in development of disease were studied. In the disease management studies, several aspects were taken up to obtain economic management methods with environment friendly methods like use of organic amendments, antagonistic organisms such as *Trichoderma* spp. and *Pseudomonas*. Further, the unique method studied in the investigation is the application of a modified vedic practice of *panchagavya* in the management of disease, a simple environment friendly method which can be easily practiced by any farmer. Further, after identifying biological agents, studies were made on delivery system of the inputs for management of the disease and also the effect of different methods on the bioenvironment of soil being treated.

The disease, *Fusarium* wilt is reported to occur in different parts of the country wherever tomato is grown especially as a mono crop resulting in 10- 80 per cent losses (Mishra, 1954, Chand 1965, Chand and Thakur, 1969 and Kapoor, 1988). The disease is reported to occur more on warm sandy soil (Chand and Thakur, 1969) and also whenever tomato is grown with other vegetables (Kapoor, 1988). In the present studies out of 65 wilt infected plant samples received from different parts of state, nearly 50% of samples yielded *Fusarium* spp. (Table 6). The disease was found to be prevalent in all the districts, belonging to seven agroctimatic zones with the incidence ranging from 1.5 - 7.8 per cent (Table 7 and 8) Disease incidence was found more prevelent in red clay loam (8.8 per cent) followed by sandy loam 7.2 per cent. (Table 9). In the nurseries around 15 per cent incidence was observed while under field conditions the crop suffered up to 5.9 per cent plant stand at flowering stage followed by fruiting stage (5.6 per cent). All the commercial varieties were found to be affected by the disease (Table 10 and 11).

Several species of *Fusarium* were reported to cause either wilt or rot of tomato (Wollenweber and Reinking, 1935; Varma, 1954, Chandra *et al*, 1983 and Kapoor, 1987). The isolates of *Fusarium* causing wilt were divided in to groups based on morphological

and cultural characters and pathogenicity by several investigators (White 1927; Haymaker, 1928; Chandra *et al.*, 1983 and Kapoor, 1987 and 1988).

In the present studies 38 isolates of *Fusarium* spp. were obtained. They were clasified into five groups based on their morphological, cultural characters and pathogenicity. The first two (I and II) Groups were identified as the members of *F. oxysporum* f. sp. *lycopersici* and III group as *F. solani* in accordance with the descriptions given by Subramanyam 1970 and Booth, 1971 and their proved pathogenicity on tomato. Besides, these isolates were compared with identified cultures of *F. oxysporum* f. sp *lycopersici* (ITCC No. 1322) and F. *solani* (ITCC No 1350) obtained from IARI for morphological and cultural characters on 2% PSA

Symptoms produced are varied. In the field, the characteristic symptoms of wilt observed included stunted growth, yellowing and drooping of older leaves proceeding upwards, and complete or partial wilting in some cases. Infections in young plants resulted in sudden wilting. When the affected stems were cut open at the collar region prominent dark brown to slightly pinkish discolouration of vascular bundles was observed in most of the cases and the most consistantly isolated predominent pathogen was found to be *F.oxysporum*. f. sp. *lycopersici*. In some plants browning was seen only in roots, such roots also exhibited slightly to moderate rotting. Pathogen associateed in such cases was found to be *Fusarium solani* (Booth,1970).

Damping - off of tomato seedlings by *Fusarium* spp in general and *F. oxysporum* f. sp. *lycopersici* in particular has been reported by several workers (Wollenweber and Reinking 1935, Singh and Srivastava 1953; Varma, 1954; EI Helaly *et al.*, 1962; Chandra *et al.*, 1983 and Kapoor 1987.) In the present studies isolates of *F. oxysporum* f. sp. *lycopersici* and *F. solani* also were found to cause both pre and post - emergence seedling disease to varying degrees, suggesting its importance right from the seedling stage upto crop maturity. Hence selection of healthy seedlings and avoidance of infection especially in nurseries play an important role to avoid speedy distribution of the pathogen in the mainfield and minimize the lossess.

The present studies on the pathogenicity of 13 isolates of *F.oxysporum* f.sp. *lycopersici* and 3 isolates of *F. solani* on tomato showed variability in producing both the seedling disease and wilt. Isolate F_1 was found to be the most virulent followed by F_6 and F_2 isolates (Table 14), with post - emergence seedling disease of 63.8, 53.2 and 54.2 per cent respectively

and wilt incidence of 81.66, 75. 8 and 74.2 Percent respectively. Wilt symptoms were observed as early as within 14 days in case of F_1 and F_2 isolates while in F_6 it was 16 days. Similar observations were reported by earlier workers (Edgerton, 1920; Clayton, 1923; White, 1927; Haymaker, 1928; Wager, 1933; Mills and Hutton, 1946; Varma, 1954; Goswami and Baruah, 1956; Chand, 1965; Chandra *et al.*, 1983 and Kapoor 1987).

In vitro studies on the pathogenicity showed F_1 isolate (82.25 per cent) as the most virulent followed by F_6 (77.03 per cent) with post - emergence seedling mortality. This finding is in agreement with those reports of Chandra *et al.*, (1983) and Kapoor (1987) who evaluated several isolates of *Fusarium* spp. *in vitro* for their virulence in producing pre and post emergence seedling mortality in tomato. (Table 17)

The symptoms in artificially inoculated plants under glass house conditions agreed with those observed in nature. The above symptoms were found to be in agreement with earlier reports (Wellman, 1943; Foster, 1946; Dimond, 1955 and El Helaly *et al.*, 1962).

Symptoms are the qualitative changes that distinguish diseased from healthy plants and may be expressed in numbers or any characteristic that is consistently altered by infection. The sequence of symptoms constantly encountered were quite distinctive with stunting of plants, leaf yellowing and drooping, necrosis and subsequently gradual or sudden wilting. A diverse and complex symptomotology of wilt caused by *Fusarium* spp. had been described in tomato (Massee, 1895; Fisher, 1935; Wellman, 1941; Foster, 1946; Davis, 1953; Dimond, 1955; Goswami and Baruah, 1956; Chand and Thakur, 1969 and Kapoor, 1987).

In the present investigation, it was found that disease upto any intermediate level could result in partially diseased plants. Though the wilted plants are undoubtedly a matter of grave concern with total loss to the farmers, the partially diseased plants also lead to economic losses to the farmers.

In the earlier approaches, the varied and diversified symptoms which were consistently encountered both in the field and pot culture experiments, were completely ignored for computation of the disease. They considered only per cent mortality or wilting in to account, which was felt inadequate for evaluation of magnitude of disease thus forcing for an appropriate disease indexing method.

The results presented on assessment of the disease taking into account differential grades of symptoms reveal that infection with any pathogenic isolate of *F. oxysporum* f.sp. *lycopersici.* and *F. solani,* the broad nature of symptom development was almost common in tomato. Using per cent mortality as a sole criterion for disease evaluation for important investigations like loss appraisal, pathogenicity, race situation, screening for resistance, host range and for management of the disease had been used by several workers (Miller, 1945, Armstrong and Armstrong 1970). Such methods although simple and less laborious would not account for the comprehensive disease situation, and in many cases would give a partial picture resulting in incomplete understanding or wrong perception of the magnitude the losses. It is evident from the results (Table 15) that tomato wilt disease could be measured as per cent plants showing only mortality or wilting. Consideration for only wilting means neglecting of plants showing stunting and other symptoms of infection, as stunting was common and consistently present. Further, evaluation method of summing the proportion of stunted and wilted plants would not give a correct picture as it may not depict the respective importance of stunting and wilting in the result. The disease development was characterized by disease syndrome such as stunting, yellowing, slightly wilted leaves, moderately wilted plants with yellowing of older leaves showing extended vascular browning, severely wilted with all leaves wilted and withered except the tip portion and dead plants, any one of which remaining static or leading to a sudden or progressive wilting. Thus, the plants at intermediate stages of disease development could also contribute for pathological parameters like host susceptibility, virulence of the pathogen (Table 16) and for the final stand of the crop.

Considering all these facts, an attempt was made to find a quick disease computation method that can fit well both under glasshouse and field conditions. Such an indication with a numerical value is of immense importance as it quantifies the disease incidence giving due emphasis to each of stable symptoms. Another advantage of the method of disease computation is in minimizing the possible errors in human judgement in arbitrary rating of disease and thus enabling the comparison of disease severity in plant populations (Table 14 and 15).

Fusarium wilts in general, are disease of warm soils being most destructive around 28^{0}C. Serious losses due to wilt disease in tomato were reported in Florida on sandy soils (Jones *et al.,* 1972). In India Chand and Thakur (1969) reported that Fusarium wilt occurs in tomato in all types of soils but with more severity in sandy soils. Further, it was

observed that per cent infection appeared to increase with higher per ce..tage of sand in the soil. Kapoor (1988) also found the disease in different soil types from sandy loam to heavy clay, but it was most serious on warm sandy soils. In our studies also the highest per cent disease incidence (PDI) was observed in sandy soils with 84 followed by sandy loam with 80.50 and the lowest in clay soils (37.5). Propagules of the pathogen also were highest in sandy soils followed by sandy loam. These results were found to be in agreement with those of early reports (Table 18).

Pre-emergence and post - emergence seedling mortality and wilt occurred even at lower inoculum levels of 1:64 and 1:32 (inoculum : soil) ratios. However, more than 50 per cent seedling mortality and wilt incidence occurred at a higher inoculum level with a dilution of 1:16 ratio (Table 19). Similar observations were reported by Haymaker (1928); Padua and Quebral (1972) and Kapoor (1987) in wilt incidence in tomato caused by *F. oxysporum* f.sp. *lycopersici* and *Fusarium* spp. Inoculum densities in general are known to be directly proportional to the disease severity (Baker, 1968). In case of Fusarium this is upto certain levels only, since it survives in the form of micro, macro conidia and chlamydospores which may occur in soil aggregates (Alabouvette, 1984).

Seventeen varieties of tomato were evaluated under pot culture in glass house. The results indicated (Table 20) that the coefficient of infection varied from 28.92 to 67.42 among the varieties. By following CODEX all the varieties were grouped as susceptible, however Rupali with CODEX of 28.75 followed by Punjab Chuhhara with 28.92 were better than other varieties. Pusa Ruby (67.42), Pusa Early Dwarf (53.42) and Best of All (53.21) were found to be highly susceptible in our studies. Earlier several workers have evaluated tomato breeding lines cultivars and varieties by assessing wilt index (Kesavan and Chowdhury, 1977, Chauhan, 1988 and Banerjee *et. al.*, 1990). Our studies are in agreement with the earlier workers.

Seedlings varying in age (14 days to 42 days) were tested to ascertain whether age of the plant had any effect on final disease severity. Results given in Table 21 indicate that seedling susceptibility is indirectly related to the age of seedlings with aged seedlings more tolarent. although 50% seedlings were susceptible till they were 28 days old. Earlier Foster and Walker (1947); Retig *et al.*, (1967) and Kesavan and Chowdhury (1977) reported that neither size nor vigour of the host plant had any consistent correlation with disease development. Our studies also indicate similar situation where disease occur even on 42 day old seedlings.

The present results given in Table 22, indicated that the age of the fungus culture should be at least three weeks old to produce significantly higher wilt incidence, although young cultures of one or two weeks could produce wilt but significantly lower than three weeks old cultures and above. Earlier reports regarding the age of the fungal culture to be used unanimously state that culture must be sufficiently old, preferably 3 to 4 weeks, to produce wilt (Fisher, 1935; Wellman, 1939; Foster and Walker, 1947; Gerdemann and Finley, 1951; Kesavan and Chowdhury, 1977). The fact that fungus *F. oxysporum* f. sp. *lycopersici* produces significant quantities of pectolytic enzymes (Gothoskar *et al.*, 1958) and wilt toxins (Sanwal, 1956) at the end of the second week after seeding in liquid media may explain the above results. It is likely that the fungus could produce effective amount of propagules at this period so as to cause wilting. These factors coupled together, confirm the results of the present study.

An efficient and quick method of inoculation is a pre - requisite to get an optimum disease incidence for proving pathogenicity and also for testing the efficacy of different control practices. An experiment was conducted to assess the efficacy of seven methods of inoculation on disease development. The results of the experiment are given in Table 23. Among the methods studied severed root dip in fungul spore suspension, unsevered root dip in suspension, and planting in pre infested soil were at par with each other. However, progress of the disease was quicker in root dip method. Planting in infested soil was chosen for all the studies unless mentioned otherwise as it is at par with root dip method and closely resembles natural condition. Earlier reports by Walker (1965), Kesavan and Chowdhury (1977) also suggested soil inoculation method as a superior one, attributing that soil environment endowed with fungal propagules favoured the disease to develop at faster rate.

Seedlings of eleven popular tomato varieties were inoculated at different ages ranging from 5 days to 30 days at 5 day interval. The results showed that at young age of 5-10 days all the varieties showed complete mortality except Rupali. As the age of seedlings increased there was a gradual reduction in disease incidence. However the varieities Pusa early Dwarf and Pusa Ruby were found to be highly susceptible (vide Table 24.)

In the present studies addition of neem cake, pongamia cake, dry leaves of pongamia and eucalyptus, FYM, and fresh leaves of pongamia as soil amendments gave good protection against seedling disease and wilt (Table 25). Neem cake was found to be

significantly superior to all other amendments. The superiority of this amendment may be due to several factors. It appears that these amendments may release some inhibitory substances on decomposition affecting the population of *F. oxysporum* f. sp. *lycopersici.* Besides, the nutrient content of these amendments may have a possible role in enhancing the host growth and vigour enabling them to withstand or resist the attack by the pathogen. Several earlier workers reported that varied concentrations (0.5 to 5 per cent) of amendments effectively reduced soil - borne diseases (Papavizas, 1963; Singh and Pandey, 1968; Kotasthane and Om Gupta, 1986). The present study revealed that a minimum of concentrations of 0.1 per cent for oil cakes, 1.5 per cent for dry amendments and three per cent for green leaf amendments are required to obtain maximum protection against both the seedling disease and wilt. Regarding incubation period, two weeks time was found sufficient for decomposition and to exhibit inhibitory effects on the pathogen (Table 26 - 31). Papavizas and Davey (1960) reported that a minimum of 2-3 weeks time was necessary for decomposition of amendments of corn, oat and bean to give protection against *R. solani* in bean seedlings.

The amendments are also attributed in enhancing the population of selective antagonistic microflora and thereby reduce pathogen population as reported by several investigators who worked on different soil - borne diseases using amendments (Papavizas and Davey, 1960; Mixon and Curl, 1967; Gautam and Kolte, 1979; Zakaria and lockwood, 1980; Zakaria *et. al.*, 1980; and Bouhot, 1981).

In the present studies it was found that there was a gradual increase in rhizosphere microflora upto 30 days after planting and decreasing trend later on. Treatment FYM recorded the highest bacterial population, while actionomycetes and fungal population was the highest in neem cake treatment. The microflora population was significantly higher in all amended treatments than control (Table 33). The pathogen propagules were found reduced significantly by all the amendments compared to unamended control However neem cake recorded the lowest number of propagules (Table 32). Increased microflora and reduced pathogen population of the treatments featuring organic amendments was reported by several workers (Papavizas, 1963; Singh and Singh, 1970; Prakash *et. al.*, 1979; Prakash *et al.*, 1985; Khalis and Manoharachary, 1985; Jeyarajan *et. al.*, 1987; Goswami and Bhattacharya 1989; Chakrabarthi and Sen 1991 and Homma *et al.*, 1979).

In the earlier studies watery compost preparations were tested against plant pathogens attacking aerial plant parts causing diseases *viz.*, late blight of potato, downy mildew, powdery mildew and rusts. These studies revealed significant disease reduction when compost extracts were sprayed (Weltzein and Ketterer, 1986; Ketterer 1990, Stindt, 1990; Winterscheidt *et. al.*, 1990 and Weltzein, 1991). Compost extracts from cattle manure were reported to reduce late blight of potato and increase the yield by 168% even under severe incidence under natural conditions. It was indicated that extracts with high biological activity could be used successfully in plant disease control of certain pathogens which are both air - borne and soil - borne such as *Phytophthora infestans* causing late blight of potato and *Botrytis cinerea* causing grey mold of straw berries (Stindt and Weltzein, 1988 and Stindt, 1990). Compost extracts of cattle manure, horse manure, goat and pig manure composts were tried by several methods at different ageing (fermentation) periods for their efficacy against plant diseases. Ageing had a significant effect on the disease reduction by compost extracts (Ketterer, 1990 and Winterscheidt et al., 1990). The effect of heat on the efficacy of extracts had been worked out (Stindt, 1990) and heat treated extracts were reported to be ineffective. However, the extension of extraction time reduced heat susceptibility of compost extracts. Microbial composition of the extracts was studied and effectiveness of selected microbes on plant pathogens like *Phytophthora infestans* and *Botrytis cinerea* was reported (Ketterer, 1990 and Stindt 1990).

In the present studies three Modified *Panchagavya* preparations (MPG-1, MPG-2 and MPG-3) when assayed for their efficacy showed that MPG-3 was superior to the other two. Dilutions of MPG from 0 to 1000 times were tried with water and dilution upto 10 times was as effective as 0 dilution in reducing the seedling disease and wilt incidence significantly over control (Table 35). Ageing/fermentation of 10 - 14 days was found to increase the efficiency of formulation in protecting the plants both at seedling and adult plant stage from wilt. The reduction in inoculum density of pathogen was not higher either with carbendazim or amendments as compared to MPG - 3. However, the plant growth was at par with healthy plants. Heating of the extracts by autoclaving and also boiling reduced its efficacy indicating some thermolabile compounds present in MPG might be responsible for suppression of mycelial growth of the pathogen. *In vitro* studies indicated that when MPG-3 extract was added 1:1 v/v to PDB followed by inoculation with pathogen, mycelial dry weight was reduced significantly over control and was at par

with those of culture filtrates of some antagonists. Conidial germination of *F. oxysporum* f. sp. *lycopersici* was also observed to be reduced by 63 per cent with MPG-3 (Table 40).

The use of *panchagavya,* can be traced back to the times of great indian epic the *'Mahabharata'* as a purification act of persons, who committed misdeeds as per Hindu religion (Shastri, 1982). The term *panchagavya* according to the explanation of technical terms used in *'Ayurveda'* includes cow's ghee, juice of cowdung, curds prepared of cow's milk, cow's milk and cow's urine. For preparing *panchagavya ghritha* cow's ghee should be cooked by adding the juice of cowdung,sour curds, cow milk and cow urine each of them in equal quantities. This medicated ghee cures epilepsy, jaundice and fever. *Mahapanchagavya ghritha* contains many drugs besides *panchagavya* and is like ambrosia in the treatment of epilepsy, insanity, oedema, abstinate abdominal diseases, tumers, piles, anaemia, jaundice and irregular fevers (Sharma, 1992).

In the present study the components of *panchagavya* were not effective against the test pathogen when applied to sick soil individually. cow dung juice and urine had phytotoxic effect when sprayed without dilutions individually on tomato. But when all the components are combined and allowed to ferment for 10 to 14 days, no phytotoxicity was noticed when sprayed on to tomato foliage.

Munshi (1964) in his Monograph, 'Agriculture in Ancient India', has given an account of protection of crops from pests and diseases described originally in *vedic* literature. According to him, Surapala in his famous book *Vrksayurveda* described the "Science of Medicine for Plants". The cow products *viz.* milk, curds, ghee, dung and urine were extensively used both individually and in combination for curing several disorders, diseases, insect pests and animals. Brhtsamhita, Agnipurana also mentioned various instances where in cow products were used for seed treatment, and as a remedy for plant ailments caused by vermin, wind, heat, lightning, cold and others. The plant ailments were categorised into wind, bile and phlegm disorders. The wind disorders were reported to be removed by medicines of bitter pungent and astringent taste, bilious affections by bitter and hot, salt and acid medicines and the phlegmatic affections by oily, sweet acid and salt medicines. A tree which was affected by bilious disorder had yellow leaves, dry branches was lean and could not bear the heat of the Sun. Besides, ripening of fruits was also delayed. Such trees recovered quickly when oily, cool and sweet potions were administered and by nurturing them with cold and perfumed waters in which Sita

(*Annona squamosa*) and Vidanga (*Embelia ribes*) were dissolved. The treatment was also given with Usira (*Andropogon muricatus*), Musta (*Cyprus rotundus*), honey, milk and ghee followed by fumigation with the fumes of Sita, honey and ghee.

It was reported earlier that a colourless and odourless liquid at times called "living water", was derived from sea water, cow manure and just low cost simple ingredients that were transformed by fermentation process in to a substance with remarkable qualities in enhancing crop growth, was prepared by Jim Martin in as early as 1950s. He used a milking cow manure and sea water to which added simple ingredients and fermented before use. He mainly emphasized the use of this preparation as a fertilizer and a stimulant of plant growth but nothing was mentioned about control of plant diseases (Curtis, 1992)

Although chemical methods are spectacular, impressive, quick and convincing even to an illiterate farmer, developing countries like India with low gross national product (GNP) can not depend entirely on these capital intensive chemical controls without economic strain on national budget. Further, reports of environmental pollution due to escalated indiscriminate use of deleterious pesticides would restrict the use of chemicals and encourage sustainable agriculture which is largely based on low cost, low input and long lasting technology through biological control. However, available research on chemicals would enable us to choose and integrate them with other methods in disease management.

Studies on *Fusarium* suppressive soils on tomato wilt (Couteaudier *et al.*, 1985, Callegarin and Cugudda, 1986) show encouraging trends towards biological control which is practically exploited in Europe and USA to find out effective biocontrol agents against wilt of tomato and to develop management techniques as a feasible component in the overall Integrated Disease Management (IDM) strategy.

Several possibilities of existence of microbial interactions have been evidenced, of which stimulating, inhibiting, non - influencing, mutual intermingling, overgrowing of microflora isolate and overgrowing of test pathogen and inhibition at a distance have been enumerated (Porter, 1924; Ghaffar, 1969 and Sharma and Sen, 1991).

Rhizosphere of healthy plants have been proved to be an excellent source for antagonists (Bhelwa *et al.*, 1962 and Linderman *et al.*, 1983). Therefore, it was thought

relevant to utilize such soil for screening. A preliminary screening of the soil microflora for suitable antagonists against *F. oxysporum* f. sp. *lycopersici* resulted in detection of three types of inhibition.

1. Intermingling with test pathogen
2. Overgrowing the underlying test pathogen;
    a) fast growth;
    b) slow growth
3. Inhibition (antibiosis) slight inhibition

Among the twelve isolates of antagonists, 10 were *Trichoderma* spp., showing mycelial overgrowth on the underlying test pathogen or showing slight inhibition alongwith two bacterial isolates showing slight inhibition (Fluorescent Pseudomonad and *Bacillus subtilis)*

Strong antagonism by *Trichoderma* species against *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum.* f. sp. *radicis lycopersici* have been reported (Yehia *et al.*, 1981; Costache *et al.*, 1980; Marios and Mitchell, 1981; D'Ercole, 1983; Sivan and Chet, 1986 and 1987 and Monaco *et al.*, 1991). *Trichoderma* represents the most widely and thoroughly studied fungus exhibiting antagonistic activity against a range of soil - borne plant pathogens(Papavizas, 1985).

Among bacteria, two strains of *Pseudomonas* against *F. solani* (Welvaert, 1961) and *F. oxysporum* f. sp. *lycopersici* (Garcia, 1982) and *Bacillus subtilis* against *F. oxysporum* f. sp. *lycopersici* (Yehia, 1981; Podile et al., 1985; and Kapoor, 1988) were claimed as biocontrol agents. Several fungi and bacteria are major constituents among the soil microflora, identified and studied in detail as biocontrol agents. Fungi probably rank third as potential antagonists although under specific situations they are considered as the most important group. Bacteria are extremely important in biological control of plant pathogens and a few bacterial antagonists are extensively studied against soil borne plant pathogens (Kerr, 1980 and Kloepper *et al.*, 1980).

In our studies, *Trichoderma viride* (A_4)and florescent *Pseudomonas* (B_1) were adjudged as the best biocontrol agents against *F. oxysporum* f. sp. *lycopersici* selected among the 12 potential antagonists tested both *in vitro* and *in vivo*. A_4 and B_1 caused maximum inhibition of radial growth and lysis area of *F. oxysporum* f. sp. *lycopersici* (Table 37 and 38).

In vitro studies employing several isolates of *Trichoderma* showed the suppression of mycelial growth of *F. oxysporum* f. sp. *lycopersici* with more isolates of *Trichoderma* overgrowing the test pathogen. Reisolation of *F. oxysporum* f. sp. *lycopersici* from the zone of interaction between antagonist and test pathogen using *Fusarium* specific medium showed that with few isolates of *Trichoderma* (A_1, A_4, and A_9) there was total lysis of *F. oxysporum* f. sp. *lycopersici*. None of the isolates of *Trichoderma* employed in our study showed coiling of hyphae of *F. oxysporum* f. sp. *lycopersici* but resulted in bulging of hyphae, excessive vacuolation and chlamydospore formation.

Dennis and Webster (1971c) reported the coiling of isolates of *Trichoderma* immediately on contact with *Fusarium oxysporum* as well as delayed coiling with other isolates and no interaction with some other isolates of *Trichoderma*.

Sivan and Chet (1986) reported that *T. harzianum* did not parasitize colonies of *Fusarium* sp. when tested on agar, however secreted β - 1 - 3 gluconase and chitinase into medium when grown in liquid medium supplemented with laminarin or chitin as sole source of carbon.

In our studies also some of the *Trichoderma* isolates (A_1, A_4 and A_9) are highly inhibitory to *F. oxysporum* f. sp. *lycopersici*. The major advantage of antibiosis observed in our studies with volatile substances is that the inhibitory substances produced may diffuse through air filled pores in soil and thus actual physical contact between antagonist and pathogen may not be necessary for pathogen inhibition. Several workers have reported the inhibitory effect of volatile compounds produced by *Trichoderma* spp. on several soil-borne pathogens (Dennis and Webster, 1971 b; Hutchinson and Cowan, 1972 and Upadhyay and Mukhopadhyay, 1983)

A shift in pH of the synthetic liquid medium towards acidic range was observed (Table 39). The pH of crude culture filtrates of the isolates of *Trichoderma* ranged from 3.96 (A_7) to 5.10 (A_3). The reduction in radial growth of *F. oxysporum* f. sp. *lycopersici* was not observed to be significant when grown in PDA amended culture filtrates of *Trichoderma* wherein pH was unaltered or altered. However, *Fusarium* in control plates gave thick and profuse mycelial growth compared to other treatments. Dennis and Webster, 1971 b have reported the increased antagonistic activity when the pH of culture filtrate was 4.0 as compared to 6.5.

In our studies growth of *F. oxysporum* f. sp. *lycopersici* was found markedly reduced when grown in liquid medium, PDB supplemented with culture filtrates of antogonists both in heat sterilized and filter sterilized and unadjusted pH conditions. All the treatments were significantly superior to control (Table 40) and showed reduced mycelial dry weights. It can be seen from this study that measurement of growth parameters through mycelial dry weight is superior to measurement of linear growth in assessing antagonistic abilities of both the isolates of *Trichoderma* and Bacterial antagonists. Growth inhibition of several soil-borne pathogens such as *Pythium, Sclerotium rolfsii* and *Rhizoctonia solani* by culture filtrates has been reported (Chet and Baker, 1980 and 1981; Upadhyay and Mukhopadhyay, 1983; Martinez and Garcia, 1984; Naik, 1989 and Kapoor, 1988).

The culture filtrates of *Trichodema* spp. and bacterial antagonists suppressed the conidial germination in *F. oxysporum* f. sp. *lycopersici*. The highest suppression was with *T. viridae* (A_4) and fluorescent *Pseudomonas* (B_1), followed by *Trichoderma* sp. (A_9) and (A_6) (Table 40). The earlier reports of several workers also indicated the inhibition of conidial germination by culture filtrates of different fungal and bacterial antogonists (Rishbeth, 1950; Naik, 1989 and Somasekhara, 1992).

Biological seed treatments, to replace the more deleterious chemicals, must be consistently effective against a range of pathogens. In *in vivo* assay seedlings raised from antagonist coated seeds indicated a positive reaction with all the antagonists tested (Table 42) although they exhibited varying degrees of reduction in disease intensity. Highest number of healthy seedlings of 64.6% and 63.3% emerged when seeds were treated with in *T. viride* (A_4) and *Pseudomonas* (B_1). Similar trend was noticed when the antagonists were multiplied and applied to infested soil well before sowing of seeds. Wilt incidence was also markedly reduced by A_4 and B_1 when applied as root dip and soil application (Table 42). All the isolates of antagonists were found to be significantly superior to control, although with variations among them in giving protection against both seedling disease and wilt. Inconsistency in the *in vitro* and *in vivo* behaviour of few isolates of *Trichoderma* (A_1, A_2 and A_9) against *F. oxysporum* f. sp. *lycopersici* was also observed in our studies (Tables 37, 38 and 40). Stevenson, 1956 reported that inconsistency in the results obtained between *in vitro* and *in vivo* application of antagonists; rate of reduction in disease severity in fields and magnitude of inhibition in petridish cultures were sometimes contradictory. The percentage of isolates showing *in vivo* antagonisms as lower as compared to *in vitro* studies were reported (Broadbent *et. al.*, 1971).

Effective and economical methods of biological control lies in the suitable introduction of the antagonist into the soil through planting material. This approach proves to be ideal with the recent progress made in seed bacterization with *Rhizobium* spp. before sowing to have better germination and general growth of legumes (Brown, 1974). Importance of seed dressing with microbial agents have been emphasised (Cook and Baker, 1983). Seed pelleting with *B. subtilis* improved pigeon pea stand (Singh, *et. al.* 1965). Seed treatment with *Trichoderma* sp. against *F. oxysporum* in tomato resulted in better seedling stand (Monaco *et. al.*, 1991). Similarly, Turhan (1981) reported that either immersion of seeds or roots and lower parts of stems of tomato seedlings into the homogenate of $^{C}/_{2}$ - 9 a new race of *Streptomyces ochraceouschloroticus* before planting in infested soil with *F. oxysporum* protected nearly 73% tomato seedlings. Garcia (1982) observed similar effect in laboratory inoculation tests with *Pseudomonas pseudoalcaligenes* and the C - 3 strain of *Bacillus cereus* against *F. oxysporum* f. sp. *lycopersici.* Marshall (1982) observed that soaking snapbean seeds in conidial suspension of *Trichoderma harzianum* resulted in a 65% improvement in seed germination and seedling stand.

One of the most critical obstacles to biological control by direct massive soil augmentation has been the lack or scarcity of methods for mass multiplication and delivering antagonists to soil. The production and commercialization of biological products is a dynamic process. Successful introduction depends on a number of factors such as production, formulation, toxicity evaluation, quality assurance, efficacy of marketing, and governmental regulations. These factors are not totally different from those used to evaluate chemicals except that biological pesticides represent a living dynamic system. Despite the limited success, scientists are attempting to develop newer biological agents, methods and their delivery. Some of the growth media employed for commercial multiplication for fungi have been reported by several workers (Wells *et. al.*, 1972; Akhtar,1977; Henis *et. al.*, 1978; Elad *et. al.*, 1980; Mukhopadhyay 1983, 1986; Mukhopadhyay et al.,1986; Papavizas et al., 1984 and Jones et al.,1984).

In the present investigations an effort has been made to multiply antagonists *Trichoderma viride* (A_4) and *Pseudomonas* (B_1) on naturally available inexpensive materials like FYM, biogas slurry, MPG-3, and well decomposed cotton waste amended with jaggery and unamended. It was observed that both the antagonists multiplied readily on above sterilized material but colonised slowly with the above unsterilised material. Amending with jaggery had no significant effect on the multiplication of A_4 and B_1

antagonists. However, FYM amended with jaggery one per cent supported high population of both A_4 and B_1 while MPG - 3 was the least effective (Table 43). Several techniques have been tested for delivery of antagonists viz; direct application through liquid broths (Kerr, 1980, Marios *et al.*, 1982); seed soaking (Marshall, 1982 and Sivan and Chet, 1986); pelleting of seeds in methyl cellulose (Wu, 1982); root dippings (Yuen *et al.*, 1983); use of organic matter like saw dust (Duda and Sierota 1987); vermiculite or pyraz clays (Maul *et. al.*, 1980 and Fravel *et, al.*, 1983) and polymer gels or alginate (Bashan, 1986). Such publications in recent years about the delivery of antagonists indicate positive out look towards this approach.

In our studies antagonists were applied in five different ways with or without carriers against seedling disease and four methods against wilt in pot culture trials, for optimizing the delivery systems. Seed pelleting with CMC and seed soaking in conidial/ bacterial cell suspension of antagonists recorded higher per cent healthy seedlings (Table 44) compared to FYM, biogas slurry and direct application against seedling disease. Interestingly, it was observed that in all the methods wherever antagonist was used the per cent healthy seedling stand was superior to control. Costache et al., (1980) observed that seed treatment with *T. viride*. retarded symptom development by *F. oxysporum* f. sp. *lycopersici*. Similar reports were made by several scientists (Yehia, 1981, Marios and Mitchell, 1981). Such improvement in healthy seedling stand could be attributed to the protection offered by the antagonists against pathogen. The exact mechanism of action in reducing plant disease is unknown however, antibiosis and mycoparasitism may be involved simultaneously, at different times or act independently as indicated by various studies in culture *in vitro*.

Besides seedling disease, application methods of antagonists have a profound influence on wilt incidence. Cutting across the methods of application, antagonists reduced wilt incidence significantly compared to control (Table 45). Root dip method of delivery of antagonists to tomato gave better root protection followed by spore suspension or carriers such as FYM or biogas slurry. Several scientists have employed different techniques for delivering the antagonists (Marios and Mitchell, 1981; Callegarin and Cugudda, 1986; Sivan and Chet, 1987; D' Ercole 1988 and Hartman and Fletcher, 1991).

In the present studies, the efficient amendment neem cake (NC), antagonists *T. viride* (A_4), fluorescent *Pseudomonas* (B_1) and MPG-3 were tested both individually and in

various combinations among themselves in comparison with carbendazim (1000 ppm) and uninoculated control. Results indicated that MPG-3 + NC (T_5) and MPG-3 (T_3) were the best in controlling both the seedling disease and wilt by protecting the seedlings better than carbendazim (Table 46). All the treatments were found effective compared to inoculated control in reducing disease. Neem cake with its ability to supply nutrients as well as decomposed products inhibitory to pathogen might have given additional protection to seedlings when combined with MPG-3. Even MPG-3 (T_3) alone has protected the seedlings from attack by *F. oxysporum* f. sp. *lycopersici*.

The effect of compost extracts against certain foliar diseases has been well documented by several workers (Weltzein and Ketterer, 1986; Stindt and Weltzein, 1988; Stindt, 1990 and Ketterer, 1990). The role of neem products in suppressing soil borne diseases has received considerable attention world-wide (Parveen and Mashkoor Alam, 1993).

Success in pot culture studies employing neem cake and MPG-3 individually and in combination, in comparison with carbendazim led to field trials. This study revealed that both the neem cake and MPG-3 are superior to carbendazim in protecting tomato plants against Fusarium wilt (Table 47). Treatment 5 (NC + MPG-3) gave the highest protection under natural field conditions with all the treatments superior to control. When taken into consideration the Cost Benefit Ratio (Appendix III), MPG-3 proved the best treatment. It is quite obvious that the cost of neem cake and carbendazim are high and application of neem cake at the rate of 2.5 tons/ha is essential to obtain optimum disease control. Hence it can be suggested to employ neem cake in nurseries to obtain healthy seedlings. The microbial load of all the treatments increased significantly over control and might have a positive role in protecting plants against attack by pathogen. (Table 48).

The results obtained in the management of Fusarium wilt of tomato with living / biological water MPG are encouraging. However, this has to be further subjected to critical studies under varied environmental conditions as well as soils before large scale recommendations. The studies should be further extended to other serious root and foliar plant pathogens.

The results presented in Table 49 indicates that soil flooding drastically reduced pathogen population by the end of one week. No significant reduction in inoculum density of *F. oxysporum* f. sp. *lycopersici* was observed when flooding was continued for next two weeks. Soil augmentation with *T. viride* and *Pseudomonas* also considerably

reduced pathogen population Wherever it can be advocated, soil flooding especially during warm periods, when the oxygen demand of the soil microbiota is greatest, can provide highly effective and inexpensive biological control of soil borne plant pathogens. Flooding helps in biologically destroying the pathogen inoculum, which is probably accomplished by anaerobes that decay the metabolically inactive propagules and produce toxic materials inhibitory to the propagules. Flooding seems more effective if used in combination with crop rotation. Cotton rotated with paddy following the wet dry method is widely used to control Fusarium wilt of cotton in the People's Republic of China (Cook, 1981). In Florida, rotations of rice with vegetables provided economic control of pathogens of vegetables. Flooding the soil is an ancient but still important and widely used method of biological control (Cook and Baker, 1983). *Trichoderma* spp. (particularly *T. harizianum*) when applied to soil effectively reduced sclerotial population of *S. rolfsii* in tomato (Wells *et. al.*, 1972), and peanuts (Davet *et al.*, 1981). Carbendazim was found to be highly effective against fusaria when applied as dry mix. Carbendazim drenching resulted in suppression of fusari-al activity in soil and which is known to persist upto 30 days (Arora *et al.*, 1977; Solel *et al.*, 1979 and Sinha *et al.*, 1980). Addition of bacterial antagonists to infested soil reduced populations of *Fusarium* (Naik, 1989).

SUMMARY

VI. SUMMARY

Random Survey of tomato fields in Karnataka revealed the prevalence of seedling disease and wilt incidence in all the 13 districts being surveyed. Disease Incidence varied from two to eight per cent attacking seedlings right from nursery stage to maturity of crop. Isolations revealed the predominance of *F. oxysporum* f.sp. *lycopersici* as the principle pathogen followed by F. solani

Based on the pathogenicity and virulence in pot culture studies, 12 isolates of *F. oxysporum* f. sp. *lycopersici* and two isolates of *F. solani* were identified causing both seedling disease and wilt. F_1 isolate (Nelamangala) followed by isolate F_6 (Tumkur) and isolate F_2 (Hebbal) were found more virulent in inducing the disease.

Sandy soils followed by sandy loam soils were found to be more favourable for the disease incidence while clay soil was least favourable.

The pathogen inoculum was multiplied on sand maize meal medium and diluted with different proportions of sterilized soil. Seedling disease and wilt occurred even at very low ratio of inoculum 1:64 dilution but threshold level for getting above 50 per cent disease was found to be at dilution 1:16. Pathogen population also increased with increase in inoculum level and at 1:16 dilution it was found to be 42×10^2 g^{-1} soil.

Tomato variety Pusa Ruby was found susceptible with per cent disease incidence (PDI) of 90. Seedlings upto 28 days were found to be highly susceptible while five weeks and above aged seedlings showed reduced disease incidence.

An incubation period of a minimum of three weeks when the culture was grown in sand maize meal medium was essential to get a disease incidence of around 80 per cent whereas higher incubation periods of more than three weeks did not result in any significant increase in disease incidence.

Among the several host inoculation techniques employed, splitting the tap root of 4 weeks old seedlings and dipping them in pathogen spore suspension yielded the highest PDI of 93; followed by severing the roots and dipping (83) and transplanting into sick soil (82).

Eleven popular varieties of tomato were assayed in pot culture studies using different aged seedlings (5-30) days for their reaction to *F. oxysporum* f.sp. *lycopersici* by inoculating with five mm diameter mycelial discs at the collar region. Young seedlings of Five to Ten days old in all the varieties exhibited 100 per cent mortality except variety Rupali (80% at 10 days old). Aged seedlings showed lesser disease incidence although Pusa Ruby and PED showed disease upto 30 days

Wide range of organic amendments (oil cakes at 0.1%; dry amendments at 1.5% and green leaves at 3%) when tried against disease showed varying degrees of protection with neem cake (0.1%) and FYM (1.5%) being effective. A minimum of two weeks of incubation period after the incorporation of organic amendments was required to express the inhibitory action against *F. oxysporum* f.sp. *lycopersici*. Augmented total microbial population in the rhizosphere of tomato plants was noticed which might have lead to reduced pathogen population as well as disease.

Living / biological water comprising exclusively of cow products (ghee, curds made of cow's milk, milk, dung and urine) supplemented with two per cent common salt and 0.01% yeast was designated as modified *Panchagavya* (MPG). MPG had a considerable influence in suppressing both the seedling disease and wilt incidence. Pot culture studies with MPG-3 formulation at 10^1 dilution with water resulted in economical disease control which was on par with the chemical control (Carbendazim 0.05%). Both the seed treatment and seedling dip before sowing and planting followed by drenching the soil 3 times viz., at the time of planting, subsequently twice at an interval of 10 days was found to give the best reduction in disease incidence. The microbial population in the MPG-3 formulation was found predominated by bacteria upto 14 days while the fungal population gradually was observed to increase from 4th to 18th day. Ten to 14 days of fermentation period for MPG-3 was found optimum in achieving maximum disease control.

Trichoderma spp. (19 isolates) and bacteria (5 isolates) were tested for their antagonistic abilities using dual culture technique against *F. oxysporum* f.sp. *lycopersici*. Ten isolates of *Trichoderma* and two isolates of bacteria showed varying degrees of antagonism. The culture filtrates of the *Trichoderma*, bacterial isolates and MPG-3 were found inhibitory to both the conidial germination and mycelial growth in *in vitro* studies. The antagonists produced inhibitory substances that were thermo stable or thermo labile in culture filtrates.

The antagonists reduced both the seedling disease and wilt in tomato to varying degrees when seeds and seedlings were soaked or root dipped in spore suspensions. Similar trend was observed when isolates of *Trichoderma* were introduced into pathogen sick soil in the form of wheat bran cultures and bacterial antagonists were introduced by pouring 24-48 hr. old bacterial suspension into sick soil.

T. viride (Hebbal) designated as A_4 and fluorescent Pseudomonad (Doddaballapur) designated as B_1 were found superior to other antagonists in affording protection in pot culture studies. Both *T. viride* and fluorescent *Pseudomonas* multiplied quickly on the

serilized organic materials especially FYM and biogas slurry but failed to grow faster on the same unsterilized materials. Among the several methods assayed for application of antagonists, dipping roots in spore suspension, corboxy methyl cellulose (CMC) pelleting and seed soaking were found superior to other methods.

MPG-3 (10^1 dilution) alone and MPG-3 combined with neem cake (25 $q.ha^{-1}$) resulted in maximum disease reduction when tried both under pot culture and field conditions. Neem cake combined with MPG-3 gave maximum fruit yield as compared to treatment MPG-3 and Carbendazim (0.05%).Highest cost benefit ratio was found to be with MPG-3 treatment alone. MPG-3 alone, neem cake along with MPG-3 induced higher total microflora resulting in reduced inoculum density of *F. oxysporum* f.sp. *lycopersici* propagules g^{-1} soil.

Studies on soil survival of the pathogen showed that flooding the soil drastically reduced the propagules which was followed by introducing the antagonist *T. viride.* into sick soil.

FUTURE LINE OF WORK

Organic amendments in the form of green leaves dry leaves, and oil cakes have been found effective against both the seedling disease and wilt in our studies.

A combination of these amendments could be studied for their efficacy cost effectiveness and local availability.

Preliminary work on the age old living biological water Panchagavya when suitably modified has been found to be effective against the disease. This needs to be further critically studied under varying disease incidences, environmental and soil conditions.

Mass multiplying of the promising antagonists on sterilized, easily available and inexpensive organic wastes has been successful. Further attempts need to be worked out for largescale multiplication.

Intergration of various methods of disease control such as use of organic amendments, antagonists, MPG-3 along with fungicidal control need to be worked out for their compatability and cost effectiveness under field conditions.

F. oxysporum f.sp. *lycopersici* being soil-borne fungus various methods of reducing population density in soil need to be worked out combining methods like soil flooding, fallowing and solarization.

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* Originals not seen

** English translation of Sanskrit original

*** Telugu translation of the original

APPENDICES

Appendix I

Culture Media used

1. Isolation of Micro organisms from soil

1a. Fungi : Peptone Dextrose Rose - Bengal Agar (Martin, 1950)

Dextrose	10.0 g
Peptone	5.0 g
KH_2PO_4	1.0 g.
$MgSo_4.7H_2O$	0.5 g.
Agar	20.0 g.
Rose Bengal (1%)	3.3 ml.
Water	1000 ml.
Streptomycin	300 mg.
pH	6.5-7.0

1.b. Bacteria : Soil Extract agar (Allen, 1957)

Glucose	1.0g.
K_2HPO_4	0.5 g.
Yeast Extract	0.5 g.
Soil Extract	100 ml.
Agar	20.0 g.
Distrilled water	900 ml.
pH	6.8 - 7.2

steam 1000 g. garden soil in 1000 ml of water in an autoclave for 30 minutes at 15 lb PSI. One g of $CaCO_3$ was added and soil suspension filtered through Whatman No.1 filter paper. One hundred ml of the filtrate was added to other chemicals and autoclaved.

For fluorescent Pseudomonads : King's B Medium

Protease peptone	20.0
Glycerol	10.0 g
K_2HPO_4 (anhydrous)	1.5 g
$MgSO_4.7H_2O$	1.5 g
Agar	15.0 g
pH	7.2

1.c. Actinomycetes : Starch Casein Agar (Kuster and Williams, 1964)

Agar	18.0 g
Starch	10.0 g
Casein (vitamin free)	0.30 g
KNO_3	2.0 g
NaCl	2.0 g

	K_2HPO_4	2.0 g
	$MgSO_4.7H_2O$	0.5 g
	$CaCO_3$	20 mg
	$FeSO_4.7H_2O$	10 mg
	Water	1000 ml

2. Maintenance of Micro Organisms.

I. Fungi : Potato Dextrose Agar (Riker and Riker, 1936)

Agar	20.0 g
Potato (Peeled and Sliced)	200.0 g
Dextrose	20.0 g
Water	1000 ml
pH	6.0 - 6.5

Potato Sucrose Agar (PSA) medium

Potato (Peeled and Sliced)	200.0 g
Sucrose	20.0 g
Water	1000 ml
pH	6.4

For the study of morphological and cultural characters of isolates of *F. oxysporum* f.sp. *lycopersici* and *F. solani*

II. Maintenance of Bacteria : Nutrient Agar (Difco Manual, 1953)

Agar	15.0 g
Beef extract	3.0 g
Peptone	5.0 g
NaCl	8.0 g
Water	1000 ml
pH	7.3

3. Enumeration of Fusarium Population

Fusarium specific medium (FSM) (Nash and Snyder, 1962)

Medium modified **

Difco peptone	1.5 g
KH_2PO_4	0.1 g
$MgSO_4.7H_2O$	0.05 g
**Dicrysticin	500 ppm
*PCNB (75 wp)	1000 ppm
Agar	20.0 g
Distilled Water	1000 ml
pH	6.4

* Add after autoclaving

** Dicrysticin has been substituted for streptomycin in the original formula.

4. Trichoderma Specific Medium (Elad et. al., 1981)

$MgSO_4.7H_2O$	0.2 g
$K_2H\,PO_4$	0.9 g
KCl	0.15 g
$NH_3\,NO_3$	1.0 g
Glucose	3.0 g
Rose Bengal	0.15 g
Agar	20.0 g
Chloramphenicol	0.25 g
Dexon (60% W.P.)	0.30 g
PCNB (75 WP)	0.20 g
Captan	0.25 g
Distilled water	1000 ml

5. Extraction of Inhibitory Substances Produced by Trichoderma

Weindling Medium (Weindling, 1941)

Glucose	25.00 g
Peptone	2.50 g
$KH_2\,PO_4$	2.00 g
$MgSO_4.7H_2O$	1.00 g
$FeCl_3$	0.01 g
Distilled water	*1000 ml*
pH	6.50

APPENDIX - II

Survey of Fusarium wilt disease in tomato

DATA SHEET

1	Name of the reporter/ Cooperator	:	
2.	Sample Number	:	
3.	Date of Collection	:	
4.	Name of the Cultivator	:	
5.	Locality		Village Taluk District
6.	Name of the Variety	:	
7.	Irrigated / Rainfed	:	
8.	Total cropped area	:	
9.	Stage of the crop	:	Nursery Transplanted Flowering Fruiting
10.	Condition of the Crop	:	
	a) Symptoms aboveground Root systems:		Stunting of plants Yellowing of leaves wilting Total plant/partial
	b) Percentage of plants infected	:	Number of plants infected. Total number of plants counted.
	c) Total area affected.		
11.	Previous crop(s)	:	
12.	Soil type	:	Sandy loam, Red sandy loam, Red clay loam.
13.	Any other information	:	(Plant Protection Chemicals/ Fertilizers, Organic Manures etc. used).

Guidelines for Collection of Soil/Root Samples

1. Collect representative samples of about 250 grams of soil around rootzone and 100 g of roots. From this separate 50 g of Soil and 20 g of roots for despatching.
2. Soil should be sufficiently wet during sampling time.
3. Immediately after collection put the sample in a polythene bag, put a label inside and tie with a rubber band, label outside too.
4. When the plant showing wilt symptoms is cut open longitudinally at the base of the stem (ground level) along the roots brown vascular discoloration is seen.
5. Fill the details in the data sheet on the spot of sampling. Keep one copy with you and despatch the other to **Sri. B. Padmodaya, Ph. D. Scholar, Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore - 560 065.**

NOTE : Bacterial Wilt can be identified by sudden wilting, presence of adventitious roots at ground level and when up rooted roots will be healthy apparently.

Appendix III

Economic returns of different management practices

Treatment	Addl. yield Tons/ha	Addl. Income Rs.	Addl. Expenditure		Total Expenditure (Rs.)	C. B. Ratio
			Input Rs.	Labour Rs.		
Un inoculated Control	6.40	19,200	-	-	-	-
Carbendazim	2.54	7,620	1,800	108	1,908	1:40
Neem Cake	3.85	11,500	10,500	36	10,536	1:1.09
MPG-3	4.60	13,800	540	108	648	1:21.30
NC+MPG-3	8.99	26,970	11,040	108	11,148	1:2.42

Tomato fruits	:	Rs. 3,000 per ton
Neem Cake	:	Rs. 500 per quintal
Carbendazim (50% WP)	:	Rs. 50 per 100 g.
MPG-3	:	Rs.240 per 100 litres.
Labour	:	Rs. 36 per day of 8 hrs.

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