STUDIES ON SOME ASPECTS OF SCLEROTIAL WILT AND FUSARIUM DECLINE DISEASE OF BETELVINE IN PONNUR AREA OF GUNTUR DISTRICT OF ANDHRA PRADESH

THESIS SUBMITTED TO THE
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BY

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CERTIFICATE

Kum. G.Parvathi Devi has satisfactorily prosecuted the course of research work and that the thesis entitled "**STUDIES ON SOME ASPECTS OF SCLEROTIAL WILT AND FUSARIUM DECLINE DISEASE OF BETELVINE IN PONNUR AREA OF GUNTUR DISTRICT OF ANDHRA PRADESH**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON SOME ASPECTS OF SCLEROTIAL WILT AND FUSARIUM DECLINE DISEASE OF BETELVINE IN PONNUR AREA OF GUNTUR DISTRICT OF ANDHRA PRADESH**" submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture in the major subject of Plant Pathology of the Andhra Pradesh Agricultural University, Hyderabad is a record of bonafied research work carried out by Kum. G. Parvathi Devi under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of investigation have been duly acknowledged by her.

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Date: 29-12-89 **(G.PARVATHI DEVI)**

DECLARATION

I, Parvathi Devi. G, hereby declare that the thesis entitled "**STUDIES ON SOME ASPECTS OF SCLEROTIAL WILT AND FUSARIUM DECLINE DISEASE OF BETELVINE IN PONNUR AREA OF GUNTUR DISTRICT OF ANDHRA PRADESH**" submitted to the Andhra Pradesh Agricultural University is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier.

Date: 29-12-89

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ABSTRACT

Student	: G. Parvathi Devi
Title	: Studies on some aspects of Sclerotial wilt and Fusarium decline disease of betelvine in Ponnur area of Guntur district of Andhara Pradesh
Submitted for the Award of	: Master of Science in Agriculture
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Sclerotial wilt fungus Sclerotium rolfsii Sacc. and Fusarium decline disease fungus Fusarium solani (Mart) Sacc. were isolated in pure culture from diseased betelvine plants in Ponnur betelvine growing gardens. Pathogenicity tests with S.rolfsii had revealed that soil substitution method was effective in proving pathogenicity. However, introduction of inoculum through plastic tubes inserted at the time of planting was also effective, but the manifestation of wilt symptoms was delayed. Pathogenicity tests conducted with F.solani indicated that immersing the clipped roots in F.solani inoculum for 24 h was very effective in producing typical Fusarium wilt symptoms in soil and in hydroponics, similar to those observed under field conditions.

Field survey of second year betelvine gardens (both in apparently healthy as well as Fusarium decline diseased gardens) was conducted in Ponnur betelvine gardens, Guntur district (A.P) at 9 days interval, to correlate the per cent change in decline disease incidence with physical, bilogical and chemical properties of soil. The per cent change in decline disease incidence could be correlated with magnesium content of soil samples in the diseased gardens. As the magnesium content increased, the per cent change in decline disease incidence increased. Higher number of soil microflora (total fungi, total bacteria, total actinomycetes) are observed in diseased gardens and could be correlated with per cent change in decline disease incidence. As the soil microflora increased, the per cent change in decline disease incidence increased. The population of root-knot nematode <u>Meloidogyne incognita</u> was correlated with per cent change in decline disease incidence in diseased gardens. As the <u>M.incognita</u> population increased, the per cent change in decline disease incidence increased.

Interaction studies in pot culture experiments with <u>M.incognita</u> alone and in combination with <u>S.rolfsii</u> at different inoculum levels, indicated that maximum reduction in fresh shoot and root weight, dry shoot and root weight, shoot and root length was observed when the plants were inoculated with 4000 2nd stage juveniles of <u>M.incognita</u> & <u>S.rolfsii</u> simultaneously.

Trichoderma harzianum was a potent antagonistic fungus against *S.rolfsii* and *F.solani*. This antagonistic fungus can be used in field plots to control *S.rolfsii* or *F.solani*.

Thirteen betelvine cultivars collected from different states were tested for their resistance against *S.rolfsii* in pot culture. Cultivar "Karapaku" from Andhra Pradesh recorded 80% sclerotial infection. The cultivars Bangla Ponna patna (Orissa), Tellaku (Ponnur-R) (Andhra Pradesh), Tellaku (Ponnur-S) (Andhra Pradesh), Gachipan (Assam), Maghi (Bihar) and Kapoori (Bihar) gave 90% infection. However the remaining cultivars; Meetha cum Bangla (Uttar Pradesh), Bangla Desi (Uttar Pradesh), Bangla Nagaram (Uttar Pradesh), Kakair (Bihar), Godibangla (Orissa) and Bangla (Madhya Pradesh) recorded 100% infection.

INTRODUCTION

Betelvine or pan (*Piper betle* L.) is a perennial, dioecious, evergreen creeper cultivated in India for its leaf ever since the time of Mahabharatha. It occupies a significant place in every day life of the Indian people. Chewing of the leaf is considered to be an ancient habit among all classes of people. It is considered auspicious to offer betel leaf and arecanut in Hindu religious ceremonies and other occasions such as weddings and worships. The crop is grown throughout India in 50,000 hectares. It is largely grown in Andhra Pradesh, Assam, Orissa, Madhya Pradesh, Maharashtra, Karnataka, TamilNadu, Uttar Pradesh and West Bengal. Andhra Pradesh occupies about 3,000 hectares. It is chiefly cultivated in Cuddapah, Guntur, Chittoor, Nellore, Ananthapur, Visakhapatnam, Kurnool, Krishna, East Godavari, West Godavari and Medak districts. Ponnur area of Guntur district is famous for its quality betel leaf which is exported mainly to Maharashtra, Uttar Pradesh and West Bengal.

Moist humid shaded conditions favourable for crop growth also favour a variety of soil borne as well as foliar diseases like fungal and bacterial diseases and also several plant parasitic nematodes (Maiti, 1989).

Plant pathogenic fungi such as *Sclerotium rolfsii*, *Rhizoctonia solani*, *Phytophthora parasitica* var. *piperina* and *Colletotrichum capsici* were isolated from diseased betelvine plants (Dastur, 1927; Chowdary, 1945; Asthana and Mahmud 1944; Mahmud 1949, 1952; Mehrotra and Tiwari, 1967; Tiwari and Mehrotra 1974; Maiti and Sen, 1977, 1979 and 1982). A new decline disease of betelvine had been observed in 1977 in Ponnur area of Guntur district of Andhra Pradesh. Detailed information is available only on stem rot or foot rot of betelvine caused by Phytophthora parasitica var. piperina. Regarding the other diseases however, no information is available. Sclerotial wilt and decline disease are the two important diseases of betelvine in Ponnur area of Guntur district. The present investigation was undertaken with the following main objectives.

1) Isolation, identificaion of fungal pathogens from diseased beltelvine plants and testing their pathogenicity on commercially grown cultivar 'Tellaku'.

2) Survey of betelvine gardens suffering from Fusarium decline disease in Ponnur area to collect information on the prevalence of decline disease in relation to soil physical, biological and chemical characters.

3) Study of the interaction of root-knot nematode Meloidogyne incognita and S. rolfsii.

4) Study of the possible existence of antagonism between Trichoderma harzianum ans S. rolfsii; T. harzianum and F. solani in vitro.

5) Screening of different betelvine cultivars for their resistance against S. rolfsii.

REVIEW OF LITERATURE

Betelvine (*Piper betle* L.) is infected with several soil borne as well as foliar plant pathogens (fungal and bacterial) and also with different kinds of plant parasitic nematodes (Maiti, 1989).

Dastur (1927) first described the foot rot and wilt disease in betelvine caused by *Phytophthora parasitica* var. *piperina*. Later, Asthana and Mahmud (1944) described the symptoms like leaf tip burning, browning and necrosis of infected leaves. Foot rot and leaf rot of betelvine due to *P. parasitica* var. *piperina* was also reported by many investigators (Mahmud 1949, 1952; Mehrotra and Tiwari, 1967; Tiwari and Mehrotra, 1974) from different parts of Madhya Pradesh. Maiti and Sen (1977, 1979 and 1982) also described this important disease from West Bengal. Narasimhan and Ramakrishnan (1969) isolated *Phytophthora parasitica* from wilted betelvine plants from Madras. Singh and Chand (1973) reported that 44-86 per cent of losses were due to *P. parasitica* in diseased betelvine plantations around Jabalpur. Diwakar and Kulshrestha (1986) described the symptoms like drooping of affected plants leading to complete death of plants. Rotting of basal portion of the vine is a common symptom observed in this disease.

Fusarium spp. infects a wide variety of crop plants including many vegetables, ornamentals and plantation crops. Singh and Joshi (1972) isolated four species of *Fusarium* from diseased betelvine plants in Jabalpur. The species isolated were : *Fusarium equiseti* (corda) Sacc, *F. oxysporum*

Schlecht, F.moniliforme sheld and F. solani (Mart) Sacc. They observed symptoms like drooping, wilting and death of plants.

Ramalingam et al. (1985) reported Fusarium wilt of betelvine from Mysore. They isolated six species of Fusarium viz., F. coeruleum, F. merismiodes, F. moniliforme, F. maydis F. solani and F. udum from infected plants as well as from soil samples. They identified F. moniliforme as the causal agent of betelvine wilt. They noticed highest incidence of the disease in the months of December and January. Sulladmath et al. (1977) reported wide spread occurrence of betelvine decline disease due to F. solani from Karnataka State. Fusarium moniliforme was reported as a potential pathogen of betelvine wilt by Raut and Shukla (1973) from Maharashtra State.

Fusarium solani has been reported to infect a number of other cultivated crops. Killebrew et al. (1987) isolated F. solani from cortical lesions of primary and secondary roots of soybean plants. Lucas et al. (1988) isolated F. solani from lateral and tap roots of soybean plants. They noticed extensive root rot symptoms leading to sudden death of plants.

Gotlieb and Doriski (1983) isolated F. oxysporum from brown discoloured region of roots of birdsfoot-trefoil. They found hypertrophy, hyperplasia of parenchyma and presence of fungus mycelium in roots and stem tissues. Charcahr and

Kraft (1987) noticed extensive mycelial invasion without formation of vascular plugs in susceptible pea cultivars infected with F. oxysporum f. sp. pisi . They observed more conidial germination and germ tube growth in xylem fluids of susceptible pea cultivars.

Tessier and Mueller (1988) showed the response of vascular bundles in pea infected with F. oxysporum f. sp. pisi. They found more cytoplasmic activity in susceptible and resistant cultivars. They also noticed complete colonisation of the pathogen in susceptible cultivars. Martyn et al. (1988) reported F.oxysporum as the causal agent of root rot of sugarbeet. They noticed interveinal chlorosis, wilt and eventually collapse of leaves.

The mechanism by which wilting of Fusarium infected plants has been studied extensively by several investigators and several important features of wilt syndrome have been elucidated. Fusaric acid - a toxin produced by Fusarium spp. has been identified as one of the causes of wilting in infected plants. The toxin caused growth inhibition, respiratory inhibition, changes in cell wall permeability, cell injury and gel induction in diseased plants. The toxin also has been found to cause necrosis of cortical tissues overlying the vascular bundles of infected plants. (Beckman, 1964).

Pennypacker and Nelson (1972) observed the effects of different isolates of Fusarium spp. on their hosts. They found that F. oxysporum f. sp. dianthi caused vascular plugging, hypertrophy, hyperplasia and disintegration of xylem parenchyma cells, leading to formation of cavities in xylem vessels of carnations. An isolate of F. oxysporum f. sp. dianthi from Denmark, caused cell proliferation in xylem parenchyma with less vascular cavity formation.

Emberger and Nelson (1981) found plugging of xylem vessels with gum and pectinaceous materials, hypertrophy, hyperplasia of vascular cambium and formation of cavities within xylem vessels in susceptible chrysanthemum plants infected with F. oxysporum f. sp. chrysanthemi

Stuehling and Nelson (1981) observed breakdown of xylem parenchyma and vessel elements, occlusion of vessel elements by gums, accumulation of pectic substances and hypertrophy of cells in primary xylem. They also found tyloses in chrysanthemum plants infected with F. oxysporum f. sp. chrysanthemi.

Beckman and Mueller (1987) noticed the response of xylem parenchyma cells in tomato to vascular infecton by F. oxysporum f. sp. lycopersici. They observed upward movement of spores from vascular elements, penetration, colonisation and degradation of parenchyma cells.

A combination of these processes could be responsible for the breakdown of water economy of Fusarium infected plants and when the amount of water available to the leaves is below the minimum required for their functions, the stomata close and the leaves wilt and finally die followed in death by the rest of the plant.

Selerotial wilt is a destructive soil borne disease. In India, the disease was reported to cause severe losses in betelvine from different states. Under favourable conditions if the inoculum level is very high more than 50 per cent of plants in a particular garden will be affected by this disease. Chowdary (1945) reported some diseases of betelvine in Sylhet, Assam with emphasis on sclerotial wilt. He also described the sclerotial wilt disease symptoms. Decay of stem at soil level, growth of dense white cottony mycelial mass at the collar region leading to wilting of plants are the common symptoms observed in infected plants. Development of sclerotia was observed on stems and also on soil near the plant base (Maiti, 1989).

In a survey of betelvine gardens in Jabalpur (M.P.), Singh and Chand (1972) observed losses upto 42 - 62 per cent due to S. rolfsii infection. Maiti and Sen (1982) in a survey of betelvine gardens in three districts of West Bengal recorded losses from 25 to 90 per cent due to S. rolfsii. They found that the incidence of disease was more related to mean temperature > 22^{o}C.

Betelvine isolate of S. rolfsii could also infect potato, tomato and Chillies. However, brinjal plants were not infected (Nayak , 1966). Sclerotium rolfsii can also cause seedling blight and root rot in barley (Mishra and Bais,

1987) ; and seedling blight in groundnut (Anandavally Amma and Shanmugum, 1974).

2.1 Influence of soil mineral nutrients on disease incidence

The complex problem of mineral nutrition of higher plants can no longer be considered in isolation from the teeming millions of microorganisms of the soil. This interaction between the microbes and higher plants poses three problems.; competition for available minerals, mobilization of unavailable complexes, and immobilization (Sadasivan, 1965).

2.1.1 Calcium:

Soil amendment with $CaHPO_4$ at 1.0 per cent level not only retarded microbial decomposition of vegetative mycelium of Fusarium vasinfectum on cholodny slides but also prolonged its survival by promoting chlamydospore and conidia formation (Subramanian, 1946).

The influence of calcium on F. oxysporum f. sp. lycopersici, the causal agent of wilt of tomato (cv. Bonny best) was investigated by Edington and Walker (1958). They found reduced wilt severity with an increase in calcium concentration from 5 - 500 ppm. They also observed low disease index when boron at 0.001 to 0.25 ppm. and calcium at 500 ppm were applied externally.

Blanc et al. (1983) reported the effect of various levels of calcium on two cultivars of carnation differing in reaction to F. solani f. sp. dianthi. They found that the higher the initial calcium content the lower was the injury and the resistant cultivars had higher calcium content.

2.1.2 Calcium and Magnesium

Speigel (1987) investigated the role of calcium and magnesium salts in uninoculated as well as plants inoculated with F. oxysporum f. sp. melonis. They noticed higher amounts of magnesium and potassium and lower content of calcium in diseased plants than the healthy plants. They noticed low disease severity in seedlings treated with calcium nitrate rather than with magnesium.

2.2 Influence of soil p^H on disease incidence

Papavizas et al. (1968) indicated that the Fusarium root rot of beans was more pronounced at soil p^H values of 5.0 and 6.8 than at 9.0.

El - Abyad and Saleh (1971) reported that growth, sporulation and germination of F. oxysporum f. sp. vasinfectum the incitant of cotton wilt were best at neutral p^H.

Jones and Overman (1971) observed low incidence of wilt in tomato caused by F. oxysporum f. sp. lycopersici race 2 by increasing soil p^H from 6 to 7 or 7.5.

Sarhan (1982) observed inverse relationship between tomato Fusarial wilt infection and soil p^H. He found low disease incidence with an increase in NPK or an imbalance of micronutrients caused by calcium or increased p^H.

Chandrasekharan and Shanmugum (1984) noticed high incidence of chickpea root rot caused by F. solani at p^H. 6.0 followed by p^H 5.0 and 7.0. Least infection was recorded at p^H 9.0.

Oristsejafor (1986) found that soil p^H of 5.7 was favourable for growth and survival of F. oxysporum f. sp. claeidis. The pathogen could not be isolated from infested soil of p^H 3.0 after 12 weeks.

2.3 Influence of plant parasitic nematodes on disease incidence

Betelvine crop is infested with several kinds of plant parasitic nematodes viz., Meloidogyne arenaria, M. incognita, M. incognita acrita and Radopholus similis (Sitaramaiah, 1984).

Dhande and Sulaiman (1961) reported the occurrence of root-knot nematode M. incognita acrita on betelvine roots in Maharashtra State. They noticed blackening and drooping of the growing tip of infected plant with pale yellow coloured leaves leading to wilting of entire vine.

Balasubramanyam (1981) recorded Helicotylenchus retusus, Rotylenchulus reniformis, M. incognita and Hoplolaimus spp. with a frequency of 75, 59, 19 and 7 per cent respectively from betelvine gardens in Salem, Madurai and Coimbattore districts of Tamil Nadu.

Sivakumar and Marimuthu (1984) in a survey of plant parasitic nematodes affecting betelvine in Tamil Nadu recorded *M. incognita*, *Helicotylenchus* sp. throughout Tamil Nadu and *R. reniformis* and *R. similis* in isolated areas and also other ectoparasitic nematodes like *Hoplolaimus seinhorsti*, *Tylenchorhynchus brassicae*.

The plant parasitic nematodes reported from rhizosphere soil of betelvine in Ponnur area of Guntur district were *M. incognita*, *R. reniformis*, *Helicotylenchus* sp. *Hoplolaimus* sp. *Tylenchorhychus* sp. *Xiphinema* sp. *Longidorus* sp. and *Pratylencus* sp. Root knot nematode *M. incognita* was in higher population than the reniform nematode *R. reniformis* (Anonymous, 1989).

2.3.1 Interaction of plant parasitic nematodes with soil borne fungi

A synergistic increase in disease severity due to nematode - fungus interaction was recorded as early as 1892 by Atkinson, who observed that Fusarium wilt of cotton was always more in the presence of root knot nematodes. There is an increasing amount of evidence that root-knot nematodes facilitate entry and establishment of plant pathogenic fungi (Powell, 1971; Bergeson, 1972). This type of synergistic increase in fungus - nematode complex soil borne diseases were also described in number of crops; banana (Loos, 1959 and Newhall, 1958), beans (Ribeiro and Ferraz, 1984; Singh *et al*., 1981) cotton (Cooper and Brodie, 1963, Minton and Minton 1966; Norton 1960; Smith and Dick, 1960), Cowpea (Thomason *et* *al*. 1959), peas (Davis and Jenkins, 1963) tobacco (Melendez and Powell, 1967; Porter and Powell,1967) and tomato (Abawi and Barker, 1984; Jenkins and Coursen, 1957; Jones *et al*. 1976; Sidhu and Webster, 1983).

Porter and Powell (1967) reported that tobacco wilt was much more severe when *F. oxysporum* f. sp. *nicotianae* was inoculated 2 or 4 weeks after root-knot nematode (*M. incognita*, *M. arenaria*, *M. javanica*) inoculation rather than in plants inoculated simultaneously with fungus and root - knot nematodes or when roots were mechanically wounded without introduction of root-knot nematodes.

Bergeson *et al.* (1970) showed that propagules of *F. oxysporum* f. sp. *lycopersici* were more numerous in the rhizosphere soil of tomato infected with root-knot nematode *M. javanica* than in rhizosphere soil of non-infected plants. The combined infection of fungus and nematode resulted in an increased colonisation of the fungus in roots.

Invasion, rapid colonisation and extensive development of *F. oxysporum* f. sp. *nicotianae* hyphae in giant cells caused by root-knot nematodes on tobacco roots was observed by Melendez and Powell (1967).

Profuse growth of Fusarium in the tissues of cotton roots infected by root-knot nematode was observed by Minton and Minton (1963).

Goswami et al. (1970) reported that M. incognita and S. rolfsii showed a degree of synergism in wilting of egg-plant.

Association of Corticium rolfsii and M. incognita in wilting of Solanum khasianum was observed by Krishnaprasad et al. (1980). Maximum wilt incidence occurred when both the organisms were artificially inoculated.

Acharya et al. (1987) observed maximum reduction in height, weight of shoot and root of betelvine plants cv. 'Godibangla' when Meloidogyne incognita, Sclerotium rolfsii and Xanthomonas betlicola were inoculated simultaneously. They noticed lower nematode population when the root-knot nematode was inoculated 3 weeks after fungus and the bacterium inoculations.

2.4 Use of biological agents

Wells et al. (1972) studied the efficacy of Trichoderma harzianum against several important soil borne plant pathogens Sclerotinia trifoliorum, Rhizoctonia solani, Pythium aphanidermatum, P. myriotylum and Sclerotium rolfsii in agar cultures. They obtained good control of S. rolfsii in lupins, tomatoes and groundnut both under green house conditions and natural field conditions. Similarly T. harzianum was used as a biological agent in controlling S. rolfsii in different crops; beans, cotton , tomato (Elad et al. 1980; Wokocha et al. 1986) groundnut (Backman and Rodriguez- Kabana, 1975; Maiti and Sen, 1985), Sugarbeet (Upadhyay and Mukhopadhyay, 1986).

Upadhyay and Mukhopadhyay (1986) observed lysis of the mycelium and sclerotia of S. rolfsii in dual culture directly by T. harzianum. They found hyphal coiling, entry through haustorial-like structures and direct entry into the hyphae and sclerotia of S. rolfsii in sugarbeet. They achieved good control of S. rolfsii in sugarbeet by application of T. harzianum as infested sorghum grains under green house conditions. They noticed combined application of T. harzianum and Brassicol (Pentachloronitrobenzene) significantly reduced the disease incidence under field conditions.

Backman and Rodriguez -Kabana (1975) demonstrated significant reduction in S. rolfsii and an increase in yield of groundnut by application of T.harzianum granules. They reported that the control achieved by the application of this biological agent granules at the rate of 140 kg/ha was equivalent to application of soil fungicide Pentachloronitrobenzene (PCNB) at the rate of 112 kg/ha.

The antagonism between T.harzianum and Fusarium spp was studied in different crops; cucumber (Kudryavtseva, 1980), carnation (Mirkova, 1983) cotton (Sivan and Chet, 1987).

MATERIALS AND METHODS

3.1 Isolation of fungal pathogens

3.1.1 From decline affected betelvine plants

Roots of healthy and decline affected betelvine plants were collected from farmers field and also from experimental garden, All India Co-ordinated Research Project on betelvine, Chintalapudi, Ponnur, Guntur district. These roots were washed in tap water followed by sterile distilled water. Transverse sections of roots from both healthy as well as decline affected plants were taken, stained in lactophenol-cotton blue and observed under microscope. The decline affected plant roots exhibited brown discolouration of vascular vessels, where as the roots from healthy plants did not show any brown discolouration.

The root sections showing brown discolouration of vascular vessels were selected for isolation purpose. The root sections were surface sterilized with mercuric chloride (1:1000) for one minute followed by three washings with sterilized distilled water. The surface sterilized root sections were transferred onto potato dextrose agar medium (PDA) in petriplates and incubated at 28 - 32^{o}C.

Three days after incubation the fungus developed from the root sections. Profuse sporulation of the fungus was observed after 14 days.

The microscopic observation of suspected pathogen revealed both micro-conidia as well as macro-conidia. The isolated fungus culture was compared with earlier betelvine isolate (Ponnur) identified from International Mycological Institute, Ferrylane, Kew, Surrey, England (No. 315672) Hymavathi, 1988) . The earlier identified culture of Fusarium solani (Mart) Sacc. was maintained in the Department of Plant Pathology, Agricultural College, Bapatla and also at AICRP on betelvine, Chintalapudi, Ponnur, Guntur district. The isolated fungus was identified and confirmed as Fusarium solani (Mart) Sacc. For all the experimental purposes 14 days old culture was used.

3.1.1.1 **Maintenance of the fungus culture**

The fungus was maintained on potato dextrose agar medium (PDA) by subculturing it at an interval of 14 days.

3.1.1.2 **Pathogenicity tests of Fusarium solani**

Pathogenicity tests were conducted on three months old rooted susceptible betelvine cultivar 'Tellaku'. Two methods of inoculation tests were used: 1) Root dip method 2) Root clipping method

1. Root dip method:

The root system was washed thoroughly with tap water and then in sterilized distilled water. The root system was dipped in fungal spore suspension for 24 h. The spore load (8.95×10^5 spores/ml) was determined with a haemocytometer. After 24 h, the plants were removed from spore suspension and planted in sterilized soil. Control plants were kept in sterilized distilled water for 24 h and planted in sterilized soil. The plants were maintained by supplying Hoagland solution once in three days.

2. Root clipping method:

In this method the root system was washed with tap water and then with sterilized distilled water. The terminal 1 cm root portion of betelvine plants were clipped under water to prevent transpiration losses, with a pair of sterilized scissors and dipped in spore suspension for 24 h. After 24 h, the basal 0.2 cm portion of clipped roots were removed to prevent physical blockage of xylem vessels by pathogen propagules and planted in sterilized soil. Control plants were maintained by dipping the clipped roots in distilled water for 24 h and planting them in sterilized soil. The plants were observed for sixty days for the development of symptoms. The plants were maintained by supplying Hoagland solution once in three days.

3.1.1.3. Pathogenicity tests of *Fusarium solani* grown in hydroponics:

Pathogenicity tests were conducted on betelvine plants grown in hydroponics by adopting two methods: Root dip method and Root clipping method. Betelvine cuttings rooted in sterilized soil for three months were used.

1. **Root dip method:**

The root system was washed in tap water followed by sterilized distilled water. The plants were maintained in Hoagland solution for three days and then dipped in spore suspension for 24 h, prepared from 14 days old culture. The spore load (8.95 x 10^5 spores/ml) was determined with haemocytometer. After 24 h, the plants were removed from spore suspension and transferred to Hoagland solution in plastic buckets. The plants were aerated for two seconds ten times each day. The depleted Hoagland solution was replaced with fresh solution once in three days. Control plants were immersed in sterile distilled water for 24 h and transferred to Hoagland solution contained in plastic buckets.

2. **Root clipping method:**

The betelvine plants grown in sterilized soil for three months were used . The roots were washed in tap water followed by sterile distilled water. The plants were maintained in Hoagland solution for about three days. The terminal 1 cm root portion was clipped under water (to prevent transpiration losses) with a pair of sterilized scissors and the roots were dipped in spore suspension for 24 h. After 24 h, the basal 0.2 cm root portion was removed and the plants were replaced in Hoagland solution contained in plastic buckets. The roots of control plants were clipped in a similar manner and kept in Hoagland solution. The plants were observed regularly for symptom development. The depleted Hoagland solution was replaced with fresh Hoagland solution once in three days and the plants were aerated for two seconds ten times each day.

3.1.2 From basal root rot affected betelvine plants

Collar root rot affected plants were collected from farmers field and also from experimental garden, All India Co-ordinated Research Project (AICRP) on betelvine, Chintalapudi, Ponnur, Guntur district. The collar portions were cut into small pieces. These pieces were surface sterilized with mercuric chloride (1:1000) for one minute followed by three washnigs with sterilized distilled water. The surface sterilized pieces were plated on corn meal agar medium and incubated at 28 - 30 ^{o}C. Four days after incubation, hyphal growth was observed from plated cut pieces. Complete formation of sclerotia was observed in about 15 days.

For experimental purpose, 13 days old culture was used. Large drops of liquid material was seen on the sclerotial bodies during the period of maturation. The size of the sclerotia was 0.913 to 1.000 mm in diameter. The shape of the sclerotia was irregular to round.

The isolated fungal pathogen was identified and confirmed as <u>Sclerotium rolfsii</u> Sacc. by comparing with earlier betelvine isolate maintained at AICRP on betelvine, Chintalapudi, and also with the isolate maintained at Agricultural College, Bapatla.

3.1.2.1 **Maintenance of the fungus culture**

The fungus was maintained by subculturing at an interval of 13 days on corn meal agar medium.

3.1.2.2 **Pathogenicity tests for Sclerotium rolfsii**

Pathogenicity tests were conducted with three months old rooted betelvine cuttings cv. 'Tellaku'. Two methods were followed.

1. **Soil substitution method**

The soil around the base of the plant was removed and the gap was filled up with fungus grown on corn meal agar medium. Fifteen grams of fungus mycelial mat and sclerotia was used as inoculum. Care was taken not to disturb or injure the roots. Control plants were maintained by removing the soil at the base of the plants and filling up with sterilized soil without the addition of inoculum. The plants were maintained by supplying Hoagland solution (diluted) once in three days.

2. **Inoculation through plastic tubes**

In this method four hollow plastic tubes were inserted in the root zone of the vines at the time of planting. The fungus inoculum was introduced through the plastic tubes.

The object of introducing the inoculum through the plastic tubes was to avoid direct root injury. Thirteen days old culture was used at the rate of 15 grams of mycelium and sclerotia per kg of soil. Control plants received only sterilized water without inoculum through plastic tubes. The plants were observed regularly for symptoms.

3.2 Survey of betelvine gardens for Fusarium decline disease

The decline disease symptoms generally appear in second year betelvine gardens. The symptoms appear initially in the month of December and increase during the month of January and February. Therefore, for the survey purpose the second year gardens were selected. The survey was conducted from 17 th December 1988. Soil samples were collected both from apparently healthy (looking at the beginning of observations) as well as decline diseased gardens to a depth of 30 cm, as per stratified random technique. Due to high disease incidence (90%), the farmers completly removed the second year diseased gardens at the end of February 1989. Due to change of diseased gardens for recording observations the healthy gardens were also changed for observations. Hence; for this reason, the gardens were designated as H_1, H_2 and H_3 for three separate healthy gardens and D_1,D_2 and D_3 for diseased gardens.

The soil samples were collected into clean polythene bags at 9 days interval, to collect information on the prevalence of decline disease in relation to soil pH, electrical conductivity , calcium content and magnesium content under natural conditions.

Two hundred fifty grams of soil was separated from the total sample collected. The soil samples were utilized for estimation of total microbial population (total fungi, total bacteria and total actinomycetes) and also for isolation of plant parasitic and saprozoic nematodes. The soil calcium, magnesium content, soil pH and electrical conductivity were also determined.

3.2.1 Isolation of nematodes from soil samples

Plant parasitic and saprozoic nematodes were isolated from 250 g soil samples by modified Cobb's sieving method. One ml of final suspension was counted five times for observing different plant parasitic and also saprozoic nematodes. The identification of different genera of plant parasitic nematodes was done by following pictorial key of Mai et al (1968).

3.2.2 Estimation of total microbial population from soil

Serial dilution method was employed for determining microbial population (Allen, 1957). One gram of soil was taken into 100 ml conical flask, containing 9 ml of sterile distilled water and mixed thoroughly to get 10^1 dilution. This was used as stock solution. Further dilutions were prepared upto 10^6 from the stock solution. Three replications of petriplates were maintained for each dilution.

3.2.2.1 Total fungal population

Total fungal population was determined using rose bengal agar medium. A dilution factor of 10^4 was used for isolating fungi. One ml of diluent was transferred to sterile petriplates aseptically and molten sterile agar medium was added. The petridishes were rotated in clockwise and anticlockwise directions to ensure uniform mixing of the diluent with the medium. The petridishes with solidified media were inverted and incubated at 30°C. The fungal colonies were counted four days after plating by using a colony counter.

Composition of Martins rose bengal agar medium. (Martin, 1950) g/lit.

Glucose	= 10 g
Peptone	= 5 g
KH_2PO_4	= 1 g
$MgSO_4 . 7H_2O$	= 0.5 g
Rose bengal	= 0.035 g
Streptomycin	= 0.030 g
Agar	= 20 g
Distilled Water	= 1000 ml
Medium was adjusted to pH	= 6.0

Sterptomycin sulphate was added at the rate of one ml/20 ml medium.

3.2.2.2 Total bacterial population

Soil extract agar medium was used for determining total bacterial population. (Allen, 1957). A 10^6 dilution was utilized for enumerating the bacterial population. One ml of 10^6 dilution was added to petriplates before pouring the medium into petridishes. Care was taken to rotate petriplates for uniform dispersion of soil suspension into the medium. The inverted petridishes were incubated at 30° C $\pm$ 1. The bacterial colonies were counted 3 days after plating by using a colony counter.

Preparation of soil extract

One kg of soil suspended in one litre of tap water was autoclaved at 121°C for 30 min and a pinch of $CaCo_3$ was added. The soil suspension was filtered through whatman No.I filter paper.

Composition of soil extract agar medium

Glucose	= 10 g
K_2HPO_4	= 0.5 g
Yeast extract	= 0.5 g
Soil extract	= 100 ml
Agar	= 15 ml
Distilled water	= 900 ml
Medium was adjusted to pH	= 7.0

3.2.2.3 Total actinomycetes population

The dilution plate method employing starch ammonium agar medium (Kuznetsev and Arjuna Rao, 1972) was used for enumeration of total actinomycetes population present in the soil. One ml of 10^5 dilution was transferred aseptically into each petriplate and 20 ml of molten cooled medium was added. The petridishes were rotated to get uniform distribution of soil suspension into the medium and were then incubated at $30^{o}C \pm 1$. The actinomycetes population was recorded 7 days after plating by using a colony counter.

Composition of starch ammonium agar medium

Agar	= 20 g
Starch	= 10 g
$(NH_4)_2\ SO_4$	= 1 g
K_2HPO_4	= 1 g
$MgSO_4 . 7H_2O$	= 10 g
Nacl	= 1 g
$CaCO_3$	= 3 g
Tap water	= 1000 ml
Medium was adjusted to pH	= 7.0

3.2.3 Determination of Soil pH and electrical conductivity

Soil pH was determined in 1:2 ratio of soil water suspension using pH meter. (Model LI-10T, Elico Private ltd. Hyderabad). The same soil water suspension was also used for determination of electrical conductivity using conductivity bridge (Jackson, 1973)./

3.2.4 Chemical analysis of soil samples

The calcium and magnesium contents of soil samples, collected from both apparently healthy as well as Fusarium decline diseased gardens were estimated by ethylene diamine tetra acetic acid method (Jackson, 1973). Calcium was calculated by using the formula:

Titre value X0.0004008 X 1000 X 1/10 X 50/Aliquot taken .
.. mg /g dryweight of soil

The amount of magnesium was calculated by using the formula:

Titre value X 0.0002432 X 1000 X 1/10 X 50 / Aliquot taken
.. mg/g dryweight of soil

3.2.4 Quantification of disease spread

The cumulative occurrence of disease both in apparently healthy looking (at the beginning of observations) as well as Fusarium decline diseased gardens was calculated by using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total number (healthy and infected) of plants}} \times 100$$

The rate of spread of Fusarium decline disease was calculated by using the following formula (Van Der Plank, 1963)

$r = 2.3/t \times \log_e 1/1-x$

Where r = rate of spread

t = time interval

x = amount of disease

3.3 Interaction of root-knot nematode *Meloidogyne incognita* and *Sclerotium rolfsii*

3.3.1 Isolation and maintenance of root-knot nematodes (*Meloidogyne incognita*)

Original root-knot nematode culture was obtained from heavily infested betelvine roots in and around betelvine gardens from AICRP on betelvine, Ponnur. The culture was maintained from single egg mass culture. The root-knot nematode which infests betelvine plants was identified as *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 by looking at the perineal patterns of adult egg laying females (Singh and Sitaramaiah, 1973).

The nematode culture was maintained and increased on betelvine (*P.betle* L.) cv. "Tellaku" in autoclaved sand. The second stage juveniles were obtained by picking up uniform sized egg masses from infested betelvine roots and incubated at room temperature. The second stage juveniles hatched from egg masses were surface strilized with 0.02% ethoxy methyl mercuric chloride (Aretan) and 0.1% dihydrostreptomycin sulphate and used for inoculation after rinsing them with sterilized distilled water. (Sitaramaiah and Sinha, 1984).

3.3.2 **Inoculation of root-knot nematode *M. incognita* and *Sclerotium rolfsii***

Three months old betelvine plants were inoculated with 1000, 2000 and 4000 second stage juveniles of *M. incognita* alone or in combination with *S. rolfsii* simultaneously. Plants not inoculated with root-knot nematode or fungus served as control.

At the time of termination of the experiment plant height, fresh weight of shoot and root, dry weight of shoot and root, root - knot index, root rot index and root - knot nematode population were recorded.

Root - knot index and root rot index was determined by following Acharya et al (1987) classification.

Index	Root rot percentage / no. of galls
0	Nil
1	1-5
2	6-10
3	11-25
4	26-50
5	> 50

3.3.3 **Estimation of root-knot nematode population from infested betelvine roots**

The nematode infested roots were washed gently with tap water, plunged into boiling lactophenol-cotton blue (containing 0.1% cotton blue stain). Boiling was done for three minutes. The excess stain was removed by transferring the stained roots to pure lactophenol and kept until the roots were clear. The endoparasitic nematodes were dissected out and counted. (Hooper, 1970).

3.4 Antagonism between Trichoderma harzianum and Sclerotium rolfsii (betelvine isolate) and T.harzianum and Fusarium solani (betelvine isolate)

The culture of T. harzianum was obtained from Prof. A.N. Mukhopadhyay, Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttar Pradesh and maintained on potato dextrose agar medium (PDA).

The antagonistic nature of T.harzianum against S. rolfsii and F. solani was studied by two methods. a) Seeded agar method b) Dual agar culture.

3.4.1 Seeded agar method

The spore suspension of T.harzianum was prepared by adding 15 ml of sterile distilled water to 7 day old culture of the fungus grown on PDA slant. Five ml of spore suspension was mixed with 95 ml of slightly warm molten PDA. The seeded agar was poured into petriplates at the rate of 20 ml per plate and was allowed to solidify. Then the seeded petriplates were inoculated with 5 mm mycelial disc of S.rolfsii or F.solani separately in the centre of petriplate cut from the periphery of 2 weeks old cultures. The plates were incubated at 28 $\pm$ 2^{o}C. Each treatment was replicated 3 times. The radial growth of test fungus *S.rolfsii* or *F.solani* was recorded at 24 h interval.

3.4.2 Dual culture of *T.harzianum* and *S.rolfsii*; *T.harzianum* and *F.solani*

Molten potato dextrose agar medium was poured into petriplates at the rate of 20 ml / each plate and was allowed to solidify. The plates were inoculated with *T.harzianum* and *S.rolfsii* or *T.harzianum* and *F.solani* at a distance of 20 mm from each other. Three replications were maintained for each treatment. Control plates of *S.rolfsii* or *F.solani* alone and *T.harzianum* alone were maintained for comparison of growth of test fungi. The radial growth of *S.rolfsii* or *F.solani* was recorded at 24 h interval.

3.5 Screening of betelvine cultivars for disease resistance against *Sclerotium rolfsii*

The pathogen *S.rolfsii* originally isolated from diseased betelvine plants was maintained on corn meal agar medium (CMA). For large scale inoculation purpose the fungus was increased on corn meal medium. Different betelvine cultivars collected from various betelvine growing regions in India were utilized for testing their reaction against *S.rolfsii*. The cultivars were planted in sterilized soil. Three months old rooted betelvine cultivars were inoculated with the test fungus. Ten grams of fungus inoculum (mycelium + sclerotia) grown on liquid medium was placed at the basal region of each test cultivar. Observations were recorded daily for a period of three weeks. The following cultivars were tested:

Pungent varieties

1) Bangla Desi (Uttar Pradesh)
2) Bangla-ponna Patna (Orissa)
3) Bangla Nagaram (Uttar Pradesh)
4) Kakair (Bihar)
5) Godi Bangla (Orissa)
6) Gachipan (Assam)
7) Bangla (Madhya Pradesh)
8) Karapaku (Andhra Pradesh)
9) Maghi (Bihar)

Non-pungent varieties

1) Tellaku (Ponnur-R) (Andhra Pradesh)
2) Tellaku (Ponnur-S) (Andhra Pradesh)
3) Kapoori (Bihar)

Meetha variety

1) Meetha cum Bangla (Uttar Pradesh)

3.6 Media used

Composition of potato dextrose agar

Peeled potatoes	=	200 g
Glucose	=	20 g
Agar	=	20 g
Distilled water	=	1 lit

Composition of corn meal agar

Maize = 30 g

Agar = 20 g

Distilled water = 1 lit

Composition of corn meal medium

Maize = 30 g

Distilled water = 1 lit

Composition of potato dextrose extract

Peeled potatoes = 200 g

Dextrose = 20 g

Distilled water = 1 lit

Composition of Hoagland solution

Macronutrients	and	Micronutrients	
KNO_3 = 6 ml		KCl	1 ml
$Ca(NO_3)_2$ $4H_2O$ = 4 ml		H_3BO_3	
NH_4 H_2 PO_4 = 2 ml		$MnSO_4$ H_2O	
$MgSO_4$ $7H_2O$ = 1 ml		$ZnSO_4$ $7H_2O$	
		$CuSO_4$ $5H_2O$	
		H_2MoO_4(85% HCO_3)	
		Fe-EDTA =	1 ml

3.7 Stain used

Composition of lactophenol - Cotton blue

Lactophenol = 67 ml

Distilled water = 20 ml

Cotton blue = 0.1 g

RESULTS

4.1 Symptomatology of Fusarium decline disease in betelvine

The decline disease affected plants initially showed bending of terminal leaf towards stem and blackening of terminal bud. The diseased plants were characterised by epinasty, stunted growth with pale yellow colour leaves and defoliation, finally leading to wilting and death of plants (Plates 1&2). Healthy plants do not show any of the above symptoms (Plate 3). The root system of diseased plants showed brown discolouration of vascular vessels, when sectioned with razor blade(Plate 4). However, healthy plant root system did not show any brown discolouration (Plate 5). Fungus mycelium was also seen in xylem vessels of diseased plant root sections(Plate 6).

4.2 Isolation

The root sections showing brown discolouration of vascular vessels were used for the isolation of pathogen. The root sections were surface disinfected with mercuric chloride (1:1000) for one minute, followed by three changes of sterilized distilled water and plated on potato dextrose agar medium (PDA). The mycelial growth of the fungus was obtained from the plated root sections after three days of incubation. The fungus was mainatained on PDA medium by periodical transfers.

The mycelial growth was cottony in culture and round. Microscopic examination showed that the conidiophores

Plate 1:Yellowing of the leaves of Fusarium decline affected betelvine plants under field conditions

Plate 2: Characteristic wilting and death of betelvine plants due to Fusarium decline disease under field conditions

Plate 3: Healthy betelvine plants without any disease symptoms under field conditions

Plate 4:Fusarium decline affected betelvine plant root section (518x)

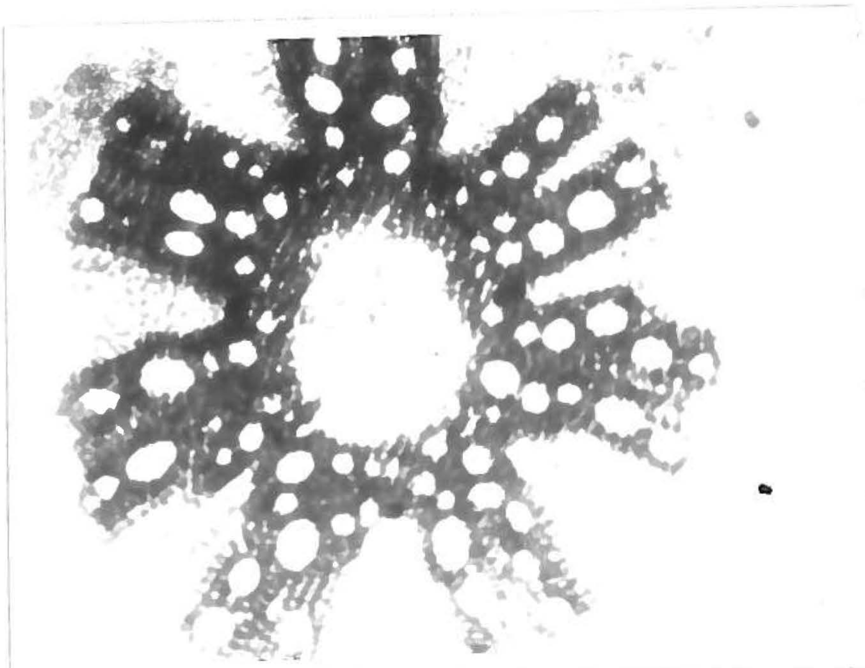

Plate 5:Healthy betelvine plant root section (466 x)

branched irregularly, bearing macro as well as micro conidia. The macro-conidia were several celled and typically canoe shaped. Micro-conidia were single celled and round. By microscopic observation and comparison with the original betelvine isolate identified from International Mycological Institute, Ferrylane, Kew, Surrey, England (Hymavathi, 1988), the isolated fungus was identified and confirmed as Fusarium solani (Mart) Sacc. (No: 315673)(Plate 7).

4.3 Pathogenicity tests

Pathogenicity studies were conducted by adopting two methods viz., root dip method and root clipping method.

1. Root dip method:

In this method, three months old previously rooted betelvine plants with intact roots were used. The root system was washed thoroughly with tap water and then with sterilized distilled water. The root system was dipped for 24 h , in fungal spore suspension prepared from 14 days old culture in sterilized distilled water. The spore load was determined with haemocytometer (8.95 x 10^5 spores/ml). After 24 h, the plants were removed from spore suspension and planted in sterilized soil. Control plants were dipped in sterilized water for 24 h and planted in sterilized soil.

2. **Root clipping method:**

Three months old rooted betelvine plants were used. The roots were washed initially with tap water and then with sterilized distilled water. The terminal one cm root portion was clipped with a pair of sterilized scissors and dipped in spore suspension for 24 h. After 24 h, the basal 0.2 cm portion of clipped roots were removed to prevent physical blockage of xylem vessels by pathogen propagules. The plants were repotted in sterilized soil. Control plants were maintained by dipping the clipped roots in sterilized distilled water only for 24 h and replanting them in sterilized soil.

Of the two methods used to prove the pathogenisity of Fusarium solani, inoculation of plants after clipping the roots, resulted in 100 per cent infection of plants (Table 1). The initial symptoms appeared in about 12 days after dipping the clipped roots in F. solani inoculum. The infected plants showed bending of the terminal leaf towards stem. The leaves turned to pale yellow in colour finally leading to complete wilting (Plate 8). When the infected plant root system was sectioned, the fungus mycelium was found in xylem vessels. The root system turned into black colour (Plate 9). However, the root dip method with intact roots did not give any infection and these plants remained healthy throughout the period of observation of 60 days (Table 1;Plate 10). From the

Table 1: Relative efficacy of method of inoculation of <u>Fusarium</u> <u>solani</u> on pathogenicity of betelvine cv. "Tellaku"

S.NO.	Method of inoculation	No.of plants inoculated	No. of plants infected	per cent infection
1	Roots dipped in spore suspension and planted in sterilized soil.	10	0	0
2	Roots dipped in distilled water and planted in sterilized soil. (control)	10	0	0
3	Clipped roots immersed in spore suspension and planted in sterilized soil.	10	10	100
4	Clipped roots immersed in distilled water and planted in sterilized soil.	10	0	0

Plate 6: Presence of fungus mycelium (F.solani) in xylem vessels of affected betelvine plant root section (1136x)

Plate 7: Growth of F.solani (betelvine isolate) on potato dextrose agar medium

Plate 8: Inoculated betelvine plants (clipped roots immersed in spore suspension of F. solani) showing symptoms of Fusarium decline disease in soil

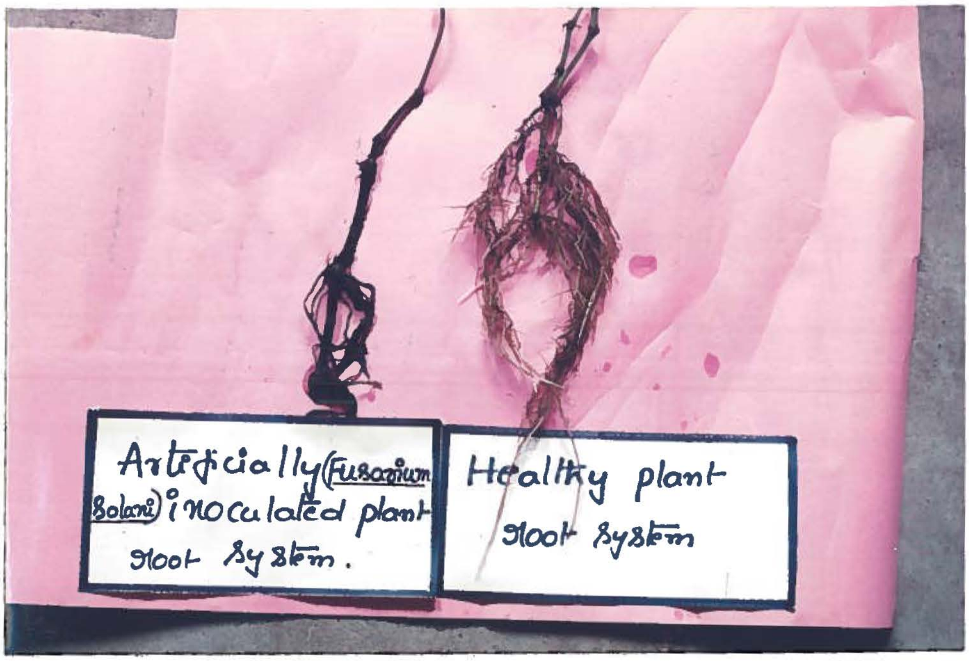

Plate 9: Inoculated betelvine plant roots showing black discolouration

Plate 10: Inoculated betelvine plants (roots dipped in spore suspension of F.solani) showing no symptoms of Fusarium decline disease in soil

treatment where the roots were clipped and then immersed in *F. solani* spore suspension betelvine plants showed typical decline disease symptoms which were identical to the symptoms observed in fields. From these plants, the fungul pathogen was re-isolated and was compared with the original isolate. Both of these isolates were found to be identical.

4.4 Pathogenicity of *Fusarium solani* on betelvine grown in hydroponics

Pathogenicity studies were conducted on betelvine plants grown in hydroponics by using the above two methods i.e root dip method and root clipping method. Betelvine cuttings rooted in sterilized soil for three months were used.

1. Root dip method:

The root system was washed in tap water followed by sterilized distilled water. The plants were maintained in Hoagland solution for three days and then dipped in spore suspension for 24 h. The plants were removed from spore suspension and transferred to Hoagland solution in plastic buckets. Check plants were immersed in sterilized distilled water for 24 h and transferred to Hoagland solution in plastic buckets.

2. Root clipping method:

The roots were washed in tap water followed by sterilized distilled water. The plants were maintained in

Hoagland solution for three days. The terminal one cm portion of roots was clipped under water. The clipped roots were immersed in fungal spore suspension for 24 h. After 24 h, the basal 0.2 cm was removed. The plants were transferred to Hoagland solution. The roots of control plants were clipped in a similar way and kept in sterilized distilled water for 24 h and trasferred to Hoagland solution.

Of the two methods used to prove the pathogenicity of Fusarium solani in hydroponics, immersion of clipped roots in fungal inoculum resulted in 100% infection of plants (Table 2, Plate 11). The symptoms appeared in about 12 days after the clipped roots were immersed in the fungal spore suspension for 24 h. The symptoms were mainly noticed in the terminal leaf. The terminal leaf was bent towards stem. The terminal bud also turned black. The leaves became pale yellow in colour and finally the entire plant wilted.

In the intact root dip method, the plants remained healthy through out the period of observation (Table 2;Plate 12). From the wilted betelvine plants roots the fungal pathogen F.solani was re-isolated and compared with the original isolate. Both these isolates were found to be indentical.

4.5 Symptomatology of Selerotial wilt (Selerotium rolfsii) in betelvine

The symptoms of diseased betelvine plants in field were observed mainly at the base of the plant. Decay of the stem

Table 2: Relative efficacy of method of inoculation of *Fusarium solani* on pathogenicity of betelvine cultivar "Tellaku" grown in hydroponics.

S. NO.	Method of inoculation	No. of plants inoculated	No.of plants infected	Per cent infection
1	Roots dipped in spore suspension and transferred to Hoagland solution.	10	0	0
2	Roots immersed in distilled water and placed in Hoagland solution.(control)	10	0	0
3	Roots clipped and immersed in spore suspension and transferred to Hoagland solution.	10	10	100
4	Roots clipped and then dipped in distilled water and placed in Hoagland solution.	10	0	0

Plate 11: Inoculated betelvine plants (clipped roots immersed in spore suspension of F solani) showing symptoms of Fusarium decline disease in hydroponics

Plate 12: Inoculated betelvine plants (roots dipped in spore suspension of F solani) showing no symptoms of Fusarium decline disease in hydroponics

at soil level was a common symptom observed where a white mycelial mass was found at the collar region and resulted in wilting. Numerous sclerotia also developed on infected stems and also on the surface of soil near the infected region of plants (Plate 13).

4.6 Isolation of Sclerotium rolfsii from diseased betelvine plants and its pathogenicity

The mycelial growth of the fungus was obtained in four days after incubation of surface sterilized collar portion of betelvine plants. The fungus was maintained by subculturing at an interval of 13 days on corn meal agar medium. The fungal mycelium was silky and when the fungus completely covered the surface of the medium in petriplates, the mycelial masses appeared as white tiny knots in 8 days of incubation. These knots gradually increased in size and the color changed to brownish black. Sclerotia were formed in about two weeks. The size of the Sclerotia was 0.913 to 1.000 mm in diameter (Plate 14).

4.7 Pathogenicity tests

Pathogenicity studies were carried out adopting two different methods with sterilized soil. a) Soil substitution method. b) Inoculation through plastic tubes : Three months old rooted betelvine plants were used for these studies.

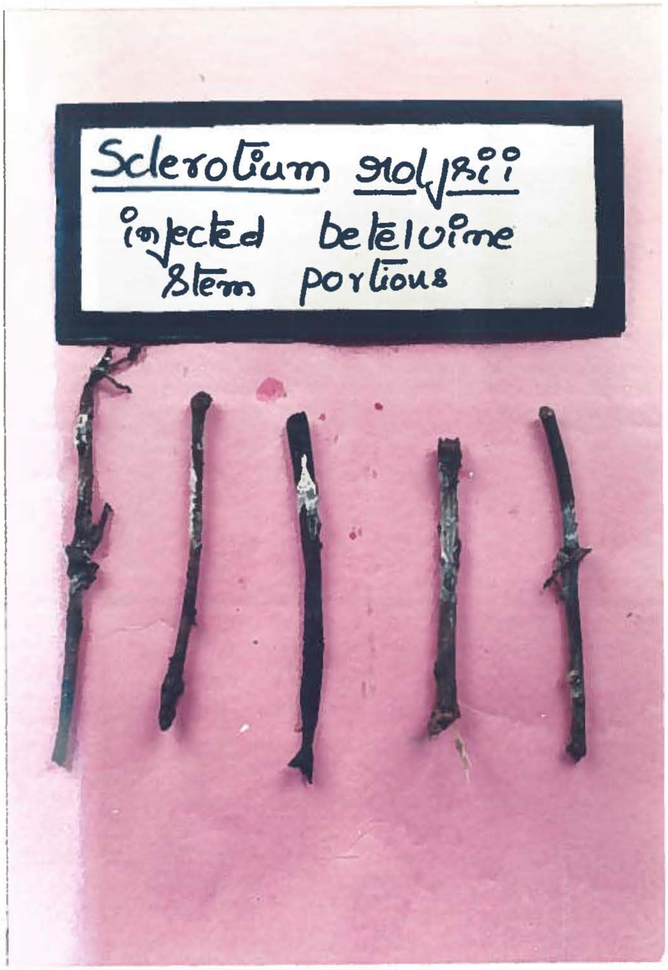

Plate 13: Presence of white mycelial mass of S.rolfsii on infected betelvine stems

Plate 14:Growth of S.rolfsii on corn meal agar medium

1. Soil substitution method:

The soil around the base of the plants was removed and the gap was filled up with fungal inoculum. Fifteen grams of inoculum (mycelium plus sclerotia) was used per kilogram of soil. Care was taken not to disturb or injure the roots. Control plants were maintained by removing the soil at the base of plants and filling up with sterilized soil only without the addition of inoculum. These plants were maintained by supplying Hoagland solution (diluted) once in three days.

2. Inoculation through plastic tubes:

Four plastic tubes (11 cm length) were inserted in the root zone at the time of planting betelvine cuttings. Sclerotium rolfsii grown on corn meal agar medium for 13 days was used at the rate of 15 g per kg of soil. The blended inoculum (fungus mycelium and sclerotia) was poured through the hollow plastic tubes. Control plants were maintained by pouring distilled water only through the plastic tubes. The plants were maintained by supplying Hoagland solution once in three days.

Of these two methods used to prove the pahogenicity of Sclerotium rolfsii soil subtitution method resulted in quick and maximum infection (100%) of plants (Table 3;Plate 15). The disease symptoms appeared in 10 to 12 days after inoculation with the fungus inoculum. In the second method of

Table 3: Relative efficacy of method of inoculation of <u>Sclerotium rolfsii</u> on pathogenicity of betelvine cv. "Tellaku".

S. NO.	Method of inoculation	No. of plants inoculated	No. of plants infected	Per cent infection
1	Soil substitution with *S. rolfsii* culture	10	10	100
2	Control (without fungus inoculum)	10	0	0
3	Inoculum (*S. rolfsii*) introduced through plastic tubes	10	10	100
4	Control (distilled water added through the plastic tubes)	10	0	0

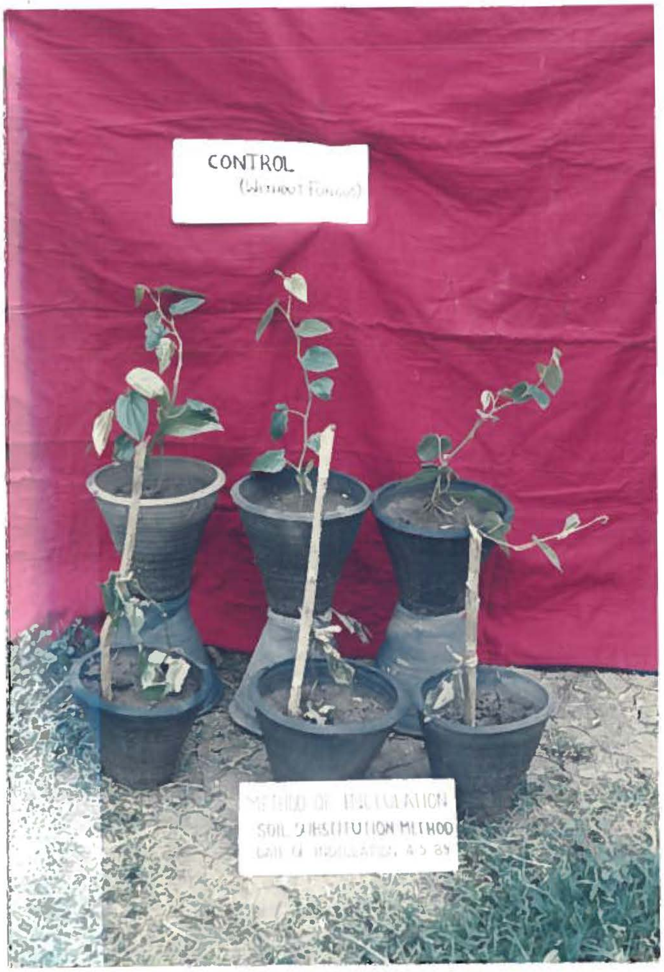

Plate 15: Inoculated betelvine plants showing symptoms of Scleotial wilt (soil substitution method)

Plate 16: Inoculated betelvine plants showing symptoms of Sclerotial wilt (inoculum of S.rolfsii introduced through plastic tubes)

inoculation (inoculation through plastic tubes), the disease symptoms appeared in about 20 days after inoculation. The basal portion of betelvine plant near the collar region was rotted. As a result, the plants wilted. However, at the end of 20 days the percentage of infected plants was 100 per cent (Table 3;Plate 16).

The fungal pathogen was re-isolated from the infected betelvine plants and it was compared with the original betelvine isolate and both of these isolates were found to be identical.

4.8 Survey of betelvine gardens for Fusarium decline disease

The decline disease caused by <u>Fusarium</u> <u>solani</u> was observed mainly in second year betelvine gardens in Guntur ditrict during winter months starting from December. Initial symptoms of the disease in fields appeared during the last week of December and increased during the month of January to February. The symptoms of this disease were noticed mainly at the terminal leaf, which showed bending towards stem and then blackening of the terminal bud. The infected plants exhibited considerable stunting, pale yellow coloured leaves and defoliation in course of infection. The fungus invaded cortical tissue and gained entry into vascular system resulting in discolouration of vascular system. Fungus mycelium could be seen in xylem vessels of infected plants.

A survey of betelvine gardens in Ponnur area was conducted during 1988-89 at 9 days interval both in apparently healthy gardens (at the beginning of observations) and also in Fusarium decline diseased gardens to collect information on the prevalence of Fusarium decline disease in relation to soil physical, biological and chemical characters.

Fusarium decline disease symptoms appear in second year betelvine gardens. Therefore, for survey purpose three second year diseased gardens (D_1, D_2 and D_3) and three second year healthy gardens (H_1, H_2 and H_3) were selected based on previous history of the gardens.

The per cent change in Fusarium decline disease incidence was correlated with soil physical properties (pH, electrical conductivity) chemical properties (calcium and maagnesium content), microbial population (fungi, bacteria and actinomycetes) and nematode population individually both in apparently healthy (H_1,H_2 and H_3) as well as Fusarium decline diseased gardens (D_1,D_2 and D_3).

4.8.1 **Healthy gardens :**

Soil pH of different soil samples collected from apparently healthy gardens (H_1, H_2 and H_3) was not correlated with per cent change in decline disease incidence at 9 days interval (Table 4).

The electrical conductivity (m.mhos/cm) of the soil samples could not be correlated with the per cent change in decline disease incidence in all the three (H_1, H_2 and H_3) healthy gardens (Table 4).

Calcium content (mg/g dry weight of soil) of soil samples collected from apparenty healthy gardens (H_1, H_2 and H_3) could not be correlated with per cent change in decline disease incidence (Table 4).

Magnesium content (mg/g dry weight of soil) of soil samples was not correlated with the per cent change in decline disease incidence in healthy gardens (H_1, H_2 and H_3) at 9 days interval (Table 4).

Total fungal population (10^4) per gram of soil was not correlated with the per cent change in decline disease incidence in all the three healthy gardens (H_1, H_2 and H_3) (Table 5). The total bacterial population/g soil (10^6) was found to be positively correlated with per cent change in decline H_1 healthy garden ($r = 0.968$). As the bacterial population increased, the per cent change in decline disease also increased at 9 days interval (Table 5;Fig. 1). However, the bacterial population could not be correlated with the per cent change in decline disease incidence in H_2 and H_3 healthy gardens.

Table 4: Correlation between soil physical, chemical properties and per cent change in Fusarium decline disease incidence in apparently healthy betelvine gardens.

Date of observation	Cumulative occurrence of decline disease incidence	Per cent change in decline disease incidence	Soil physical factors		Soil chemical factors		Table value
			p^H	Electrical conductivity (m.mhos/cm)	Calcium (mg/g soil)	Magnesium (mg/g soil)	
H_1 Garden							
17-12-88	0.89	0.89	8.48	0.46	4.96	3.02	
26-12-88	2.00	1.11	8.20	0.35	4.87	2.27	
04-01-89	4.89	2.89	8.33	0.33	6.66	2.28	
13-01-89	8.75	3.86	8.18	0.37	5.06	2.15	
22-01-89	13.52	4.77	8.38	0.38	6.30	2.32	0.666
31-01-89	20.75	7.23	8.31	0.36	5.97	3.06	
09-02-89	29.60	8.85	8.27	0.29	6.06	2.40	
18-02-89	37.50	7.90	8.61	0.31	4.56	2.18	
27-02-89	44.00	6.75	8.21	0.36	4.96	2.84	
calculated value			0.048 NS	-0.294 NS	0.116 NS	0.022 NS	
H_2 Garden							
08-03-89	5.62	5.62	8.27	0.38	5.39	1.65	
17-03-89	14.81	9.19	7.96	0.34	6.18	1.80	
26.03-89	27.50	12.69	8.02	0.62	5.12	2.48	
04-04-89	42.25	14.75	7.17	0.53	5.76	2.02	0.754
13-04-89	58.74	16.49	8.02	0.61	6.07	1.73	
22-04-89	70.75	12.01	7.21	0.56	6.06	1.97	
31-04-89	81.50	10.75	7.18	0.59	5.09	2.37	
calculated value			-0.342 NS	0.299 NS	0.273 NS	0.22 NS	
H_3 Garden							
09-05-89	11.75	11.75	7.09	0.73	4.98	3.08	
18-05-89	26.11	14.36	8.15	0.65	6.59	3.01	
27-05-89	44.18	18.07	8.14	0.64	6.70	1.87	0.811
05-06-89	55.00	10.82	8.07	0.78	6.68	2.27	
14-06-89	62.50	7.50	8.02	0.62	7.13	1.98	
23-06-89	67.75	5.25	8.40	0.46	6.67	2.20	
calculated value			-0.146 NS	0.456 NS	-0.148 NS	0.121 NS	

Note: Soil physical and chemical properties were individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from apendix 'H' from statistical procedures for Agricultural Research K.A. Gomez and A.A. Gomez.

* Significant at 5% level of probability.

Table 5: Correlation between soil microbial population and per cent change in Fusarium decline disease incidence in apparently healthy betelvine gardens

Date of observation	Cumulative Occurrence of decline disease incidence	Percent change in decline disease incidence	Soil microbial population/g soil: Total fungi (10^4)	Total bacteria (10^6)	Total actinomycetes (10^5)	Table value
H_1 Garden						
17-12-88	0.89	0.89	24.49	50.26	35.99	
26-12-88	2.00	1.11	42.86	51.25	25.42	
04-01-89	4.89	2.89	31.99	58.73	21.83	
13-01-89	8.75	3.86	32.16	60.09	32.79	
22-01-89	13.52	4.77	32.49	74.06	37.53	
31-01-89	20.75	7.23	37.83	75.66	37.53	0.666
09-02-89	29.60	8.85	39.86	85.89	41.43	
18-02-89	37.50	7.90	38.86	87.09	37.09	
27-02-89	44.00	6.75	43.03	82.53	31.73	
Calculated value			0.513 NS	0.968 *	0.622 NS	
H_2 Garden						
08-03-89	5.62	5.62	39.53	84.26	34.66	
17-03-89	14.81	9.19	44.39	80.19	39.22	
26-03-89	27.50	12.69	43.63	87.32	39.96	
04-04-89	42.25	14.75	42.83	87.46	40.23	
13-04-89	58.74	16.49	44.96	91.16	33.99	0.754
22-04-89	70.75	12.01	45.42	90.96	35.23	
31-04-89	81.50	10.75	43.49	89.72	31.92	
Calculated value			0.657 NS	0.634 NS	0.155 NS	
H_3 Garden						
09-05-89	11.75	11.75	48.76	92.06	35.29	
18-05-89	26.11	14.36	42.36	93.39	38.43	
27-05-89	44.18	18.07	42.13	91.56	36.56	
05-06-89	55.00	10.82	36.63	99.36	28.86	
14-06-89	62.50	7.50	35.06	100.96	28.69	0.811
23-06-89	67.75	5.25	29.01	95.82	29.65	
Calculated value			0.706 NS	-0.667 NS	0.801 NS	

Note: Soil microbial population was individually correlated with per cent change in Fusarium decline disease incidence

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

* Significant at 5 % level of probability

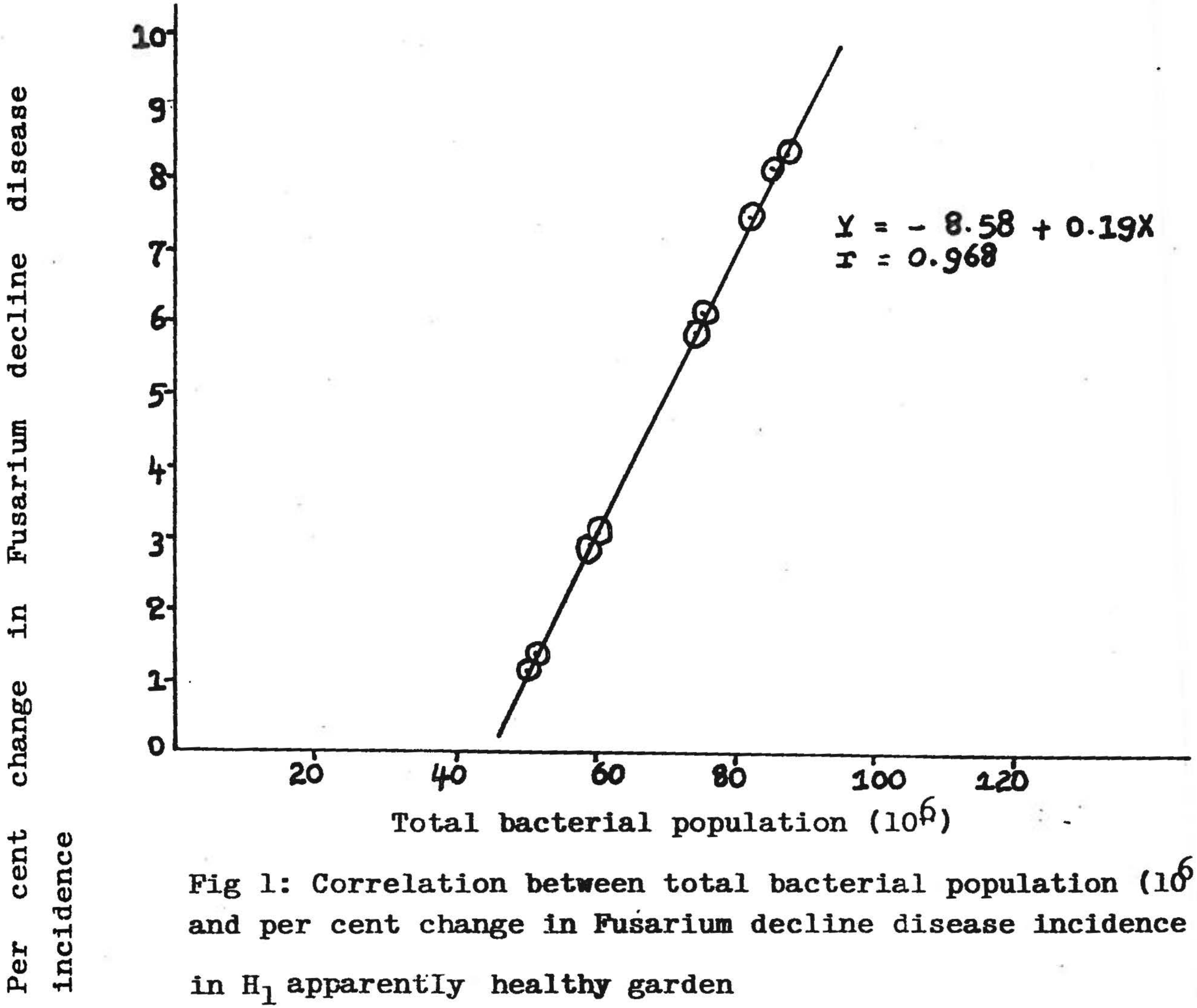

Fig 1: Correlation between total bacterial population (10^6) and per cent change in Fusarium decline disease incidence in H_1 apparently healthy garden

The total actinomycetes population/g of soil (10^5) was not correlated with the per cent change in decline disease incidence in healthy gardens (H_1, H_2 and H_3) (Table 5).

Plant parasitic nematodes (Rotylenchulus reniformis, Helicotylenchus spp, Hirschmanniella oryzae, Meloidogyne incognita, Pratylenchus spp and Tylenchorhycnhus spp) recoved from 250 g soil samples of apparently healthy gardens could not be correlated with per cent change in decline disease incidence at 9 days interval (Table 6).

Similarly the saprozoic nematodes (Aphelenchus avenae and Tylenchus spp were also not found to be significantly correlated with per cent change in decline disease incidence in all the three healthy gardens (H_1, H_2 and H_3). But , Tylenchus spp was found to be negatively correlated with per cent change in decline in H_1 healthy garden ($r = -0.683$). As the Tylenchus spp population decreased, the per cent change in decline disease incidence increased (Table 7;Fig 2).

4.8.2 **Decline diseased gardens:**

Soil pH of different soil samples collected from Fusarium decline diseased gardens (D_1,D_2 and D_3) could not be correlated with per cent change in decline disease incidence (Table 8).

Table 6: Correlation between plant parasitic nematodes and per cent change in *Fusarium* decline disease incidence in apparently healthy betelvine gardens

Date of observation	Cumulative Occurrence of decline disease incidence	Per cent change in decline disease incidence	Plant parasitic nematodes/250 g soil						Total Plant parasitic nematodes	Table value
			Rotylenchulus reniformis	*Helicotylenchus* spp.	*Hirschmanniella oryzae*	*Meloidogyne incognita*	*Pratylenchus* spp.	*Tylenchorhynchus* spp.		
H_1 Garden										
17-12-88	0.89	0.89	729	810	400	-	-	-	1939	
26-12-88	2.00	1.11	625	732	215	-	-	-	1572	
04-01-89	4.89	2.89	950	450	370	-	-	-	1770	
13-01-89	8.75	3.86	1080	600	300	90	-	-	2070	
22-01-89	13.52	4.77	740	490	540	-	10	-	1780	
31-01-89	20.75	7.23	1090	490	280	-	-	-	1860	0.666
09-02-89	29.60	8.85	850	620	200	120	-	-	1790	
18-02-89	37.50	7.90	980	830	140	-	-	10	1960	
27-02-89	44.00	6.75	725	130	160	-	-	-	1015	
Calculated value			0.42 NS	-0.267 NS	-0.468 NS	0.341 NS	-	-	-0.106 NS	
H_2 Garden										
08-03-89	5.62	5.62	850	610	590	10	-	10	2070	
17-03-89	14.81	9.19	850	600	600	10	-	-	2060	
26-03-89	27.50	12.69	1060	760	660	-	-	-	2480	
04-04-89	42.25	14.75	800	600	100	-	-	-	1500	0.754
13-04-89	58.74	16.49	1000	570	470	100	-	-	2140	
22-04-89	70.75	12.01	1140	800	730	-	-	-	2670	
31-04-89	81.50	10.75	670	840	200	130	-	-	2440	
Calculated value			0.300 NS	-0.073 NS	-0.441 NS	0.239 NS			-0.141 NS	
H_3 Garden										
09-05-89	11.75	11.75	625	840	610	120	-	-	2195	
18-05-89	26.11	14.36	770	750	580	100	-	10	2210	
27-05-89	44.18	18.07	940	570	560	70	-	-	2140	
05-06-89	55.00	10.82	950	940	970	-	10	-	2870	0.811
14-06-89	62.50	7.50	870	850	700	-	-	-	2440	
23-06-89	67.75	5.25	920	700	650	50	-	-	2320	
Calculated value			-0.097 NS	-0.031 NS	-0.338 NS	0.462 NS			-0.361 NS	

Note: Plant parasitic nematodes were individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

Table 7: Correlation between saprozoic nematodes and per cent change in Fusarium decline disease incidence in apparently healthy betelvine gardens.

Date of observation	Cumulative Occurrence of decline disease incidence	Per cent change in decline disease incidence	Saprozoic nematodes/250 g soil: *Aphelenchus avenae*	Saprozoic nematodes/250 g soil: *Tylenchus* spp	Total saprozoic nematodes	Table value
H_1 Garden						
17-12-88	0.89	0.89	60	80	140	
26-12-88	2.00	1.11	79	100	179	
04-01-89	4.89	2.89	85	90	175	
13-01-89	8.75	3.86	130	30	160	
22-01-89	13.52	4.77	40	70	110	0.666
31-01-89	20.75	7.23	100	80	180	
09-02-89	29.60	8.85	180	20	200	
18-02-89	37.50	7.90	170	40	210	
27-02-89	44.00	6.75	60	50	110	
Calculated value			0.599 NS	-0.683 *	0.290 NS	
H_2 Garden						
08-03-89	5.62	5.62	60	70	130	
17-03-89	14.81	9.19	40	65	105	
26-03-89	27.50	12.69	90	20	110	
04-04-89	42.25	14.75	80	40	120	0.754
13-04-89	58.74	16.49	70	52	122	
22-04-89	70.75	12.01	220	60	280	
31-04-89	81.50	10.75	170	70	240	
Calculated value			0.107 NS	-0.566 NS	-0.047 NS	
H_3 Garden						
09-05-89	11.75	11.75	230	140	370	
18-05-89	26.11	14.36	220	80	300	
27-05-89	44.18	18.07	340	280	620	0.811
05-06-89	55.00	10.82	320	380	700	
14-06-89	62.50	7.50	225	125	350	
23-06-89	67.75	5.25	150	100	250	
Calculated value			0.510 NS	0.345 NS	0.501 NS	

Note: Saprozoic nematodes were individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

* Significant at 5% level of probability.

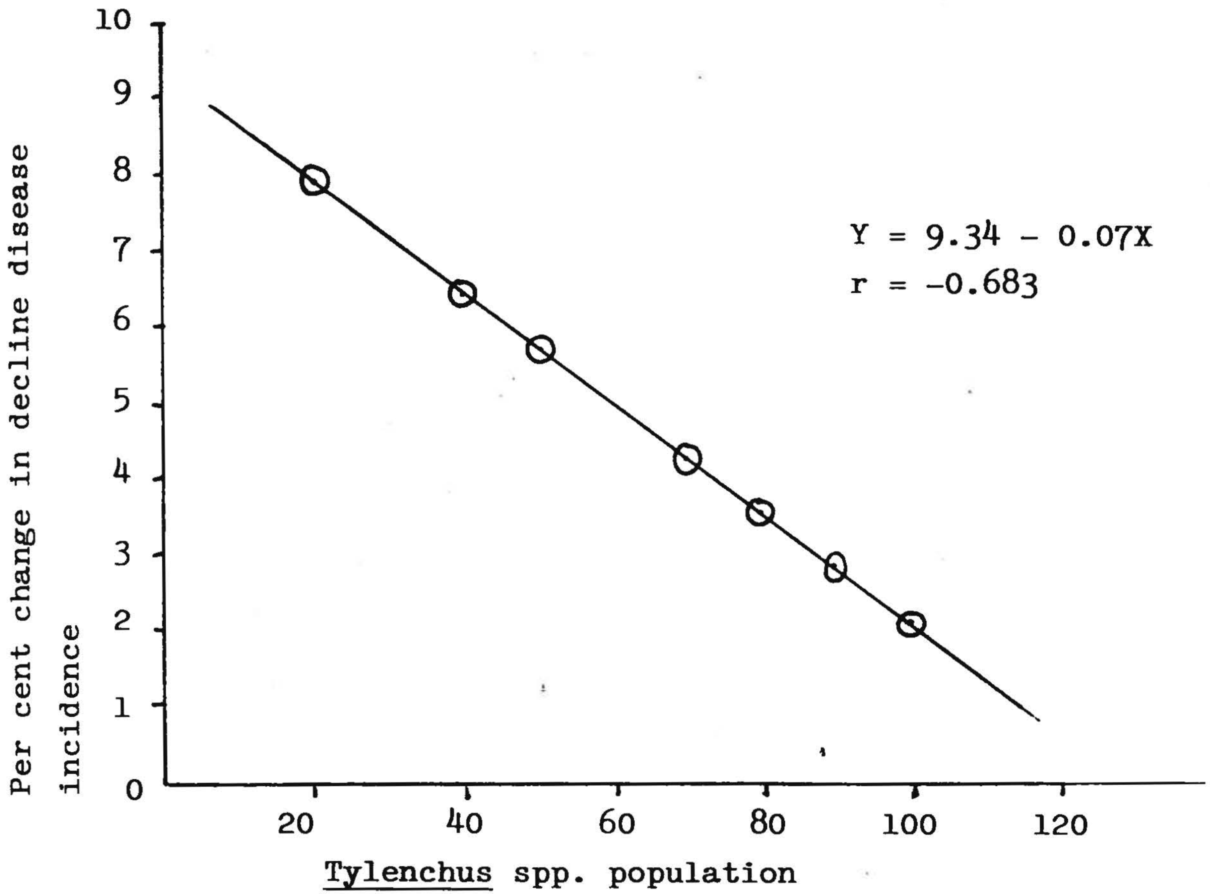

Fig 2:Correlation between *Tylenchus* spp. population and per cent change in Fusarium decline disease incidence in H_1 apparently healthy garden

The electrical conductivity (m.mhos/cm) of the soil samples was not found to be correlated with the per cent change in decline disease incidence in three diseased gardens (D_1,D_2 and D_3) (Table 8).

Calcium content (mg/g dry weight of soil) of the soil samples collected from three diseased gardens (D_1,D_2 and D_3) could not be correlated with the per cent change in decline disease incidence (Table 8).

Magnesium content (mg/g dry weight of soil) of soil samples was found to be positively correlated with the per cent change in Fusarium decline in all the three diseased gardens ($r = 0.824$, 0.826 and 0.909 for D_1, D_2 and D_3. respectively) (Table 8; Fig. 3,4 and 5). As the magnesium content of soil samples increased, the per cent change in Fusarium decline disease incidence also increased in all the three diseased (D_1,D_2 and D_3) gardens.

Rhizosphere microflora (total fungi, total bacteria and total actinomycetes) and fauna (total nematodes) of the soil samples collected from three diseased gardens were estimated at 9 days interval and results were correlated with per cent change in decline disease incidence individually.

The total fungal population (10^4) per gram of soil was not found to be correlated with per cent change in decline disease incidence in D_1 and D_2 diseased gardens. However,

Table 8: Correlation between soil physical, chemical properties and per cent change in Fusarium decline disease incidence in diseased betelvine gardens.

Date of observation	Cumulative Occurrence of decline disease incidence	Per cent change in decline disease incidence	Soil physical factors		Soil chemical factors		Table value
			pH	Electrical conductivity (m.mhos/cm)	Calcium (mg/g soil)	Magnesium (mg/g soil)	
D_1 Garden							
17-12-88	7.25	7.25	8.27	0.55	5.56	2.49	
26-12-88	16.72	9.47	8.12	0.52	4.70	3.00	
04-01-89	27.29	10.57	8.58	0.35	6.15	3.06	
13-01-89	39.00	11.71	8.20	0.38	4.70	3.25	
22-01-89	51.27	12.27	8.35	0.38	6.69	3.25	0.666
31-01-89	65.27	14.00	8.23	0.38	6.26	3.14	
09-02-89	76.92	11.65	8.17	0.32	4.37	3.24	
18-02-89	88.23	9.31	8.41	0.30	4.11	2.42	
27-02-89	92.75	6.52	8.43	0.32	5.08	2.57	
Calculated value			-0.266 NS	-0.242 NS	0.349 NS	0.824 *	
D_2 Garden							
08-03-89	6.25	6.25	8.36	0.52	4.75	2.39	
17-03-89	17.27	11.02	8.05	0.32	6.30	2.42	
26-03-89	31.00	13.73	7.89	0.49	6.57	3.06	
04-04-89	49.00	18.00	8.11	0.48	5.36	3.40	0.754
13-04-89	69.00	20.00	7.99	0.60	5.76	3.25	
22-04-89	79.25	10.25	8.01	0.54	6.13	3.00	
31-04-89	88.23	8.98	8.00	0.58	6.11	2.26	
Calculated value			0.678 NS	0.143 NS	0.117 NS	0.846 *	
D_3 Garden							
09-05-89	22.36	22.36	7.97	0.80	6.65	3.02	
18-05-89	50.50	28.14	8.07	0.77	6.50	3.25	
27-05-89	62.25	11.75	8.04	0.79	6.82	3.04	0.811
05-06-89	72.00	9.75	8.11	0.67	6.42	2.88	
14-06-89	80.00	8.00	7.96	0.56	7.12	2.89	
23-06-89	84.50	4.50	8.01	0.54	5.91	2.82	
Calculated value			0.106 NS	0.767 NS	0.145 NS	0.909 *	

Note: Soil physical and chemical properities were individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

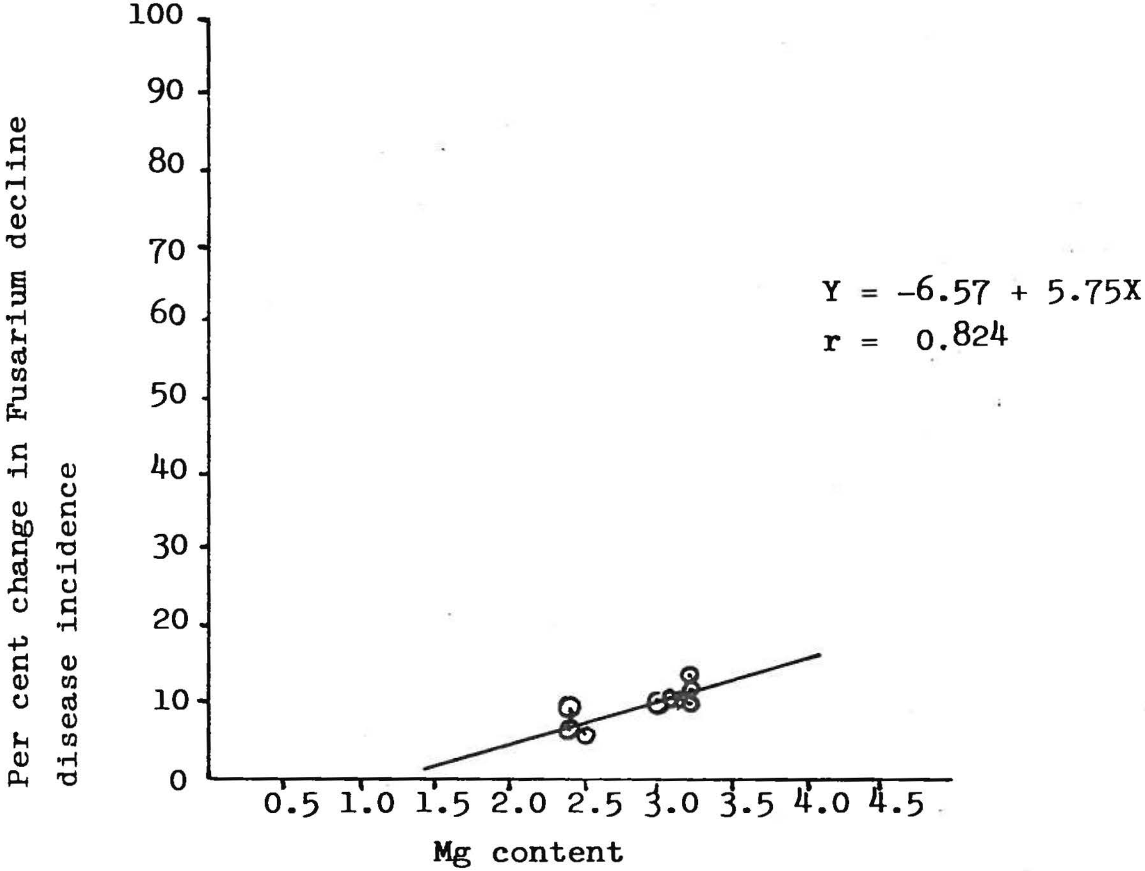

Fig 3:Correlation between magnesium content (mg/g soil) and per cent change in Fusarium decline disease incidence in D_1 diseased garden

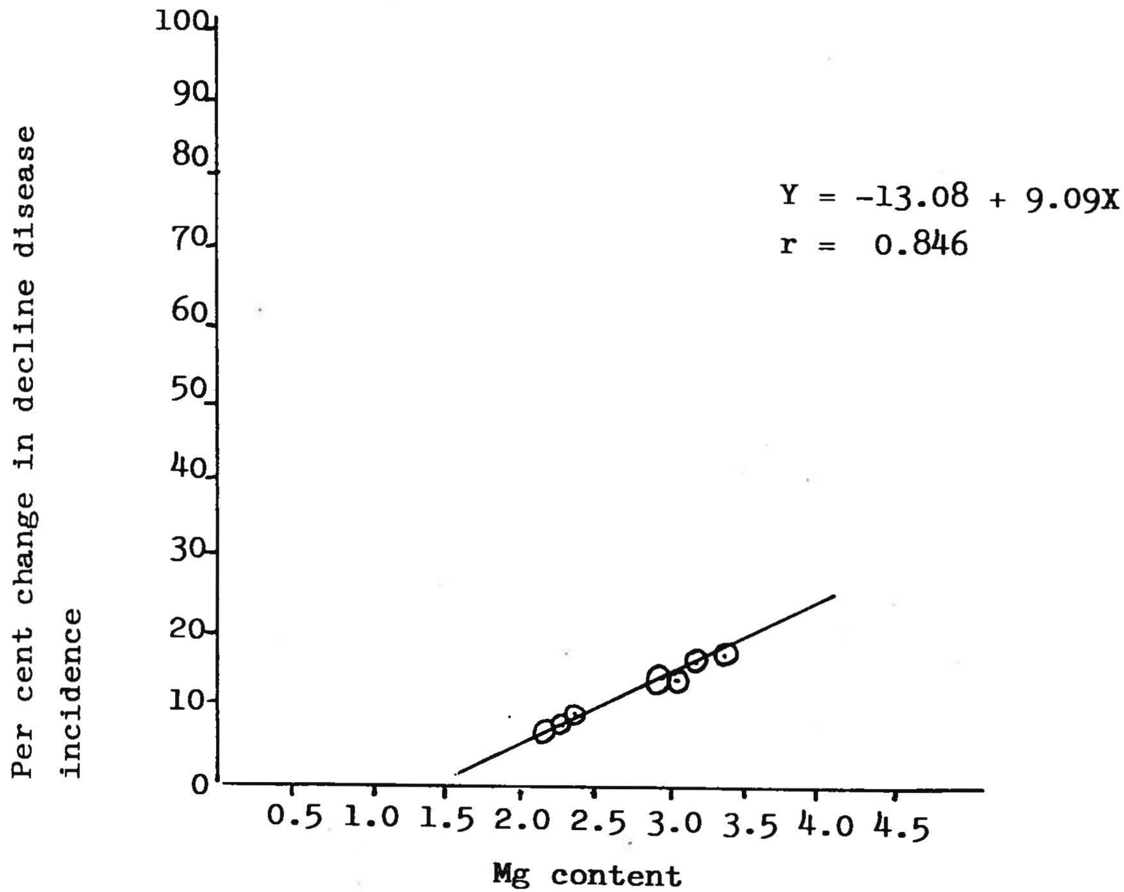

Fig 4: Correlation between magnesium content (mg/g soil) and per cent change in Fusarium decline disease incidence in D_2 diseased garden

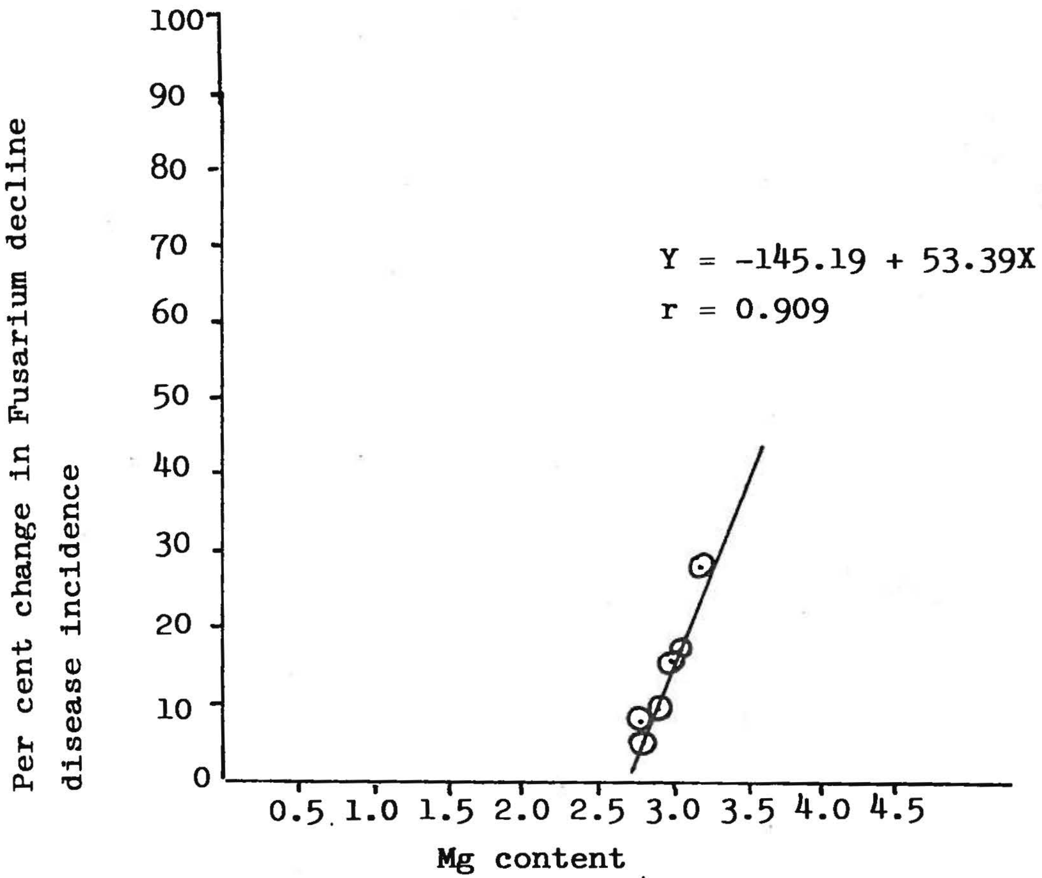

Fig 5: Correlation between magnesium content (mg/g soil) and per cent change in Fusarium decline disease incidence in D_3 diseased garden

total fungal population was found to be correlated with per cent change in decline disease incidence in D_3 garden (r = 0.924). As the total fungal population decreased, the per cent change in decline disease incidence also decreased (Table 9 and Fig 6).

The total bacterial population was found to be correlated with per cent change in decline disease incidence in D_2 and D_3 garden (r = 0.762 & 0.853), but not in D_1. garden. As the bacterial population increased the per cent change in decline disease incidence increased in D_2 garden. In D_3 garden, as the bacterial population decreased the per cent change in decline disease incidence decreased (Table 9; Fig 7 & 8).

The total actinomycetes population was not correlated with the per cent change in decline disease incidence in all three diseased gardens (D_1,D_2 and D_3) (Table 9).

Plant parasitic nematodes (Rotylenchulus reniformis, Helicotylenchus spp., Hirschmanniella oryzae, Meloidogyne incognita, Pratylenchus spp and Tylenchorhynchus spp.) recovered from 250 gms soil sample collected from the three diseased gardens could not be correlated with per cent change in decline disease incidence. However, Meloidogyne incognita was found to be correlated with per cent change in decline disease incidence in D_2 diseased garden (r = 0.768).

Table 9: Correlation between microbial population and per cent change in Fusarium decline disease incidence in diseased betelvine gardens.

Date of observation	Cumulative occurence of decline disease incidence	Per cent change in decline disease incidence	Soil microbial population/g soil: Total fungi (10^4)	Total bacteria (10^6)	Total actinomycetes (10^5)	Table value
D_1 Garden						
17-12-88	7.25	7.25	35.59	42.33	20.03	
26-12-88	16.72	9.47	32.48	63.35	28.14	
04-01-89	27.29	10.57	30.92	71.63	22.16	
13-01-89	39.00	11.71	31.16	61.97	28.66	
22-01-89	51.27	12.27	37.69	66.49	37.26	0.666
31-01-89	65.27	14.00	34.69	80.32	34.19	
09-02-89	76.92	11.65	38.36	78.22	38.03	
18-02-89	88.23	9.31	38.02	85.06	33.06	
27-02-89	92.75	6.52	39.09	83.09	35.79	
Calculated value			-0.260 NS	0.219 NS	0.351 NS	
D_2 Garden						
08-03-89	6.25	6.25	39.23	89.26	38.69	
17-03-89	17.27	11.02	41.82	95.27	36.09	
26-03-89	31.00	13.73	30.64	100.82	35.26	
04-04-89	49.00	18.00	42.23	100.00	39.23	
13-04-89	69.00	20.00	43.86	98.13	36.69	0.754
22-04-89	79.25	10.25	48.59	96.39	30.49	
31-04-89	88.23	8.98	45.59	95.79	35.29	
Calculated value			-0.049 NS	0.762 *	0.234 NS	
D_3 Garden						
09-05-89	22.36	22.36	45.13	97.32	41.99	
18-05-89	50.50	28.14	43.79	94.09	30.43	
27-05-89	62.25	11.75	37.06	92.43	35.19	0.811
05-06-89	72.00	9.75	38.23	90.23	29.32	
14-06-89	80.00	8.00	37.26	88.62	25.79	
23-06-89	84.50	4.50	32.60	85.56	23.79	
Calculated value			0.924 *	0.853 *	0.596 NS	

Note: Soil microbial population was individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

* Significant at 5% level of probability.

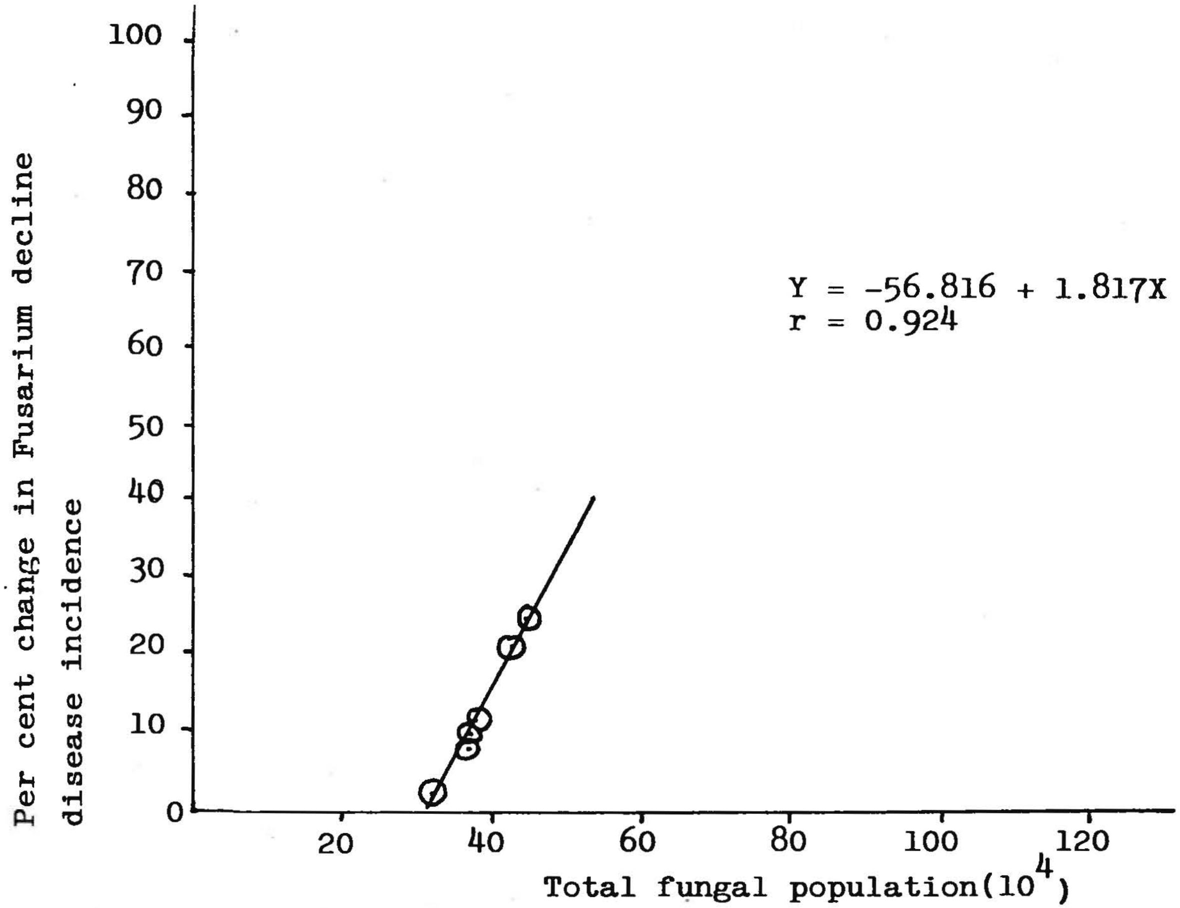

Fig 6: Correlation between total fungal population (10) and per cent change in Fusarium decline disease incidence in D_3 diseased garden

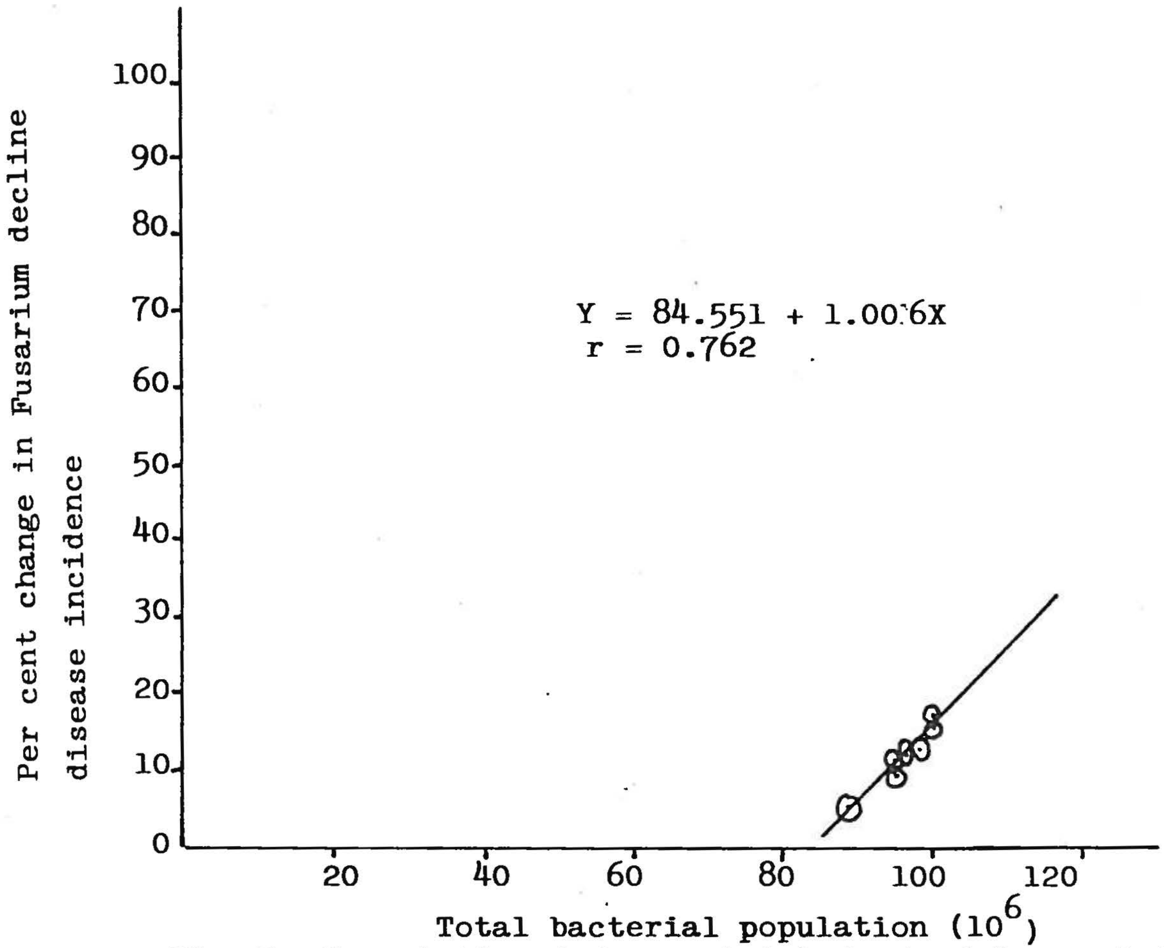

Fig 7: Correlation between total bacterial population and per cent change in Fusarium decline disease incidence in D_2 diseased garden

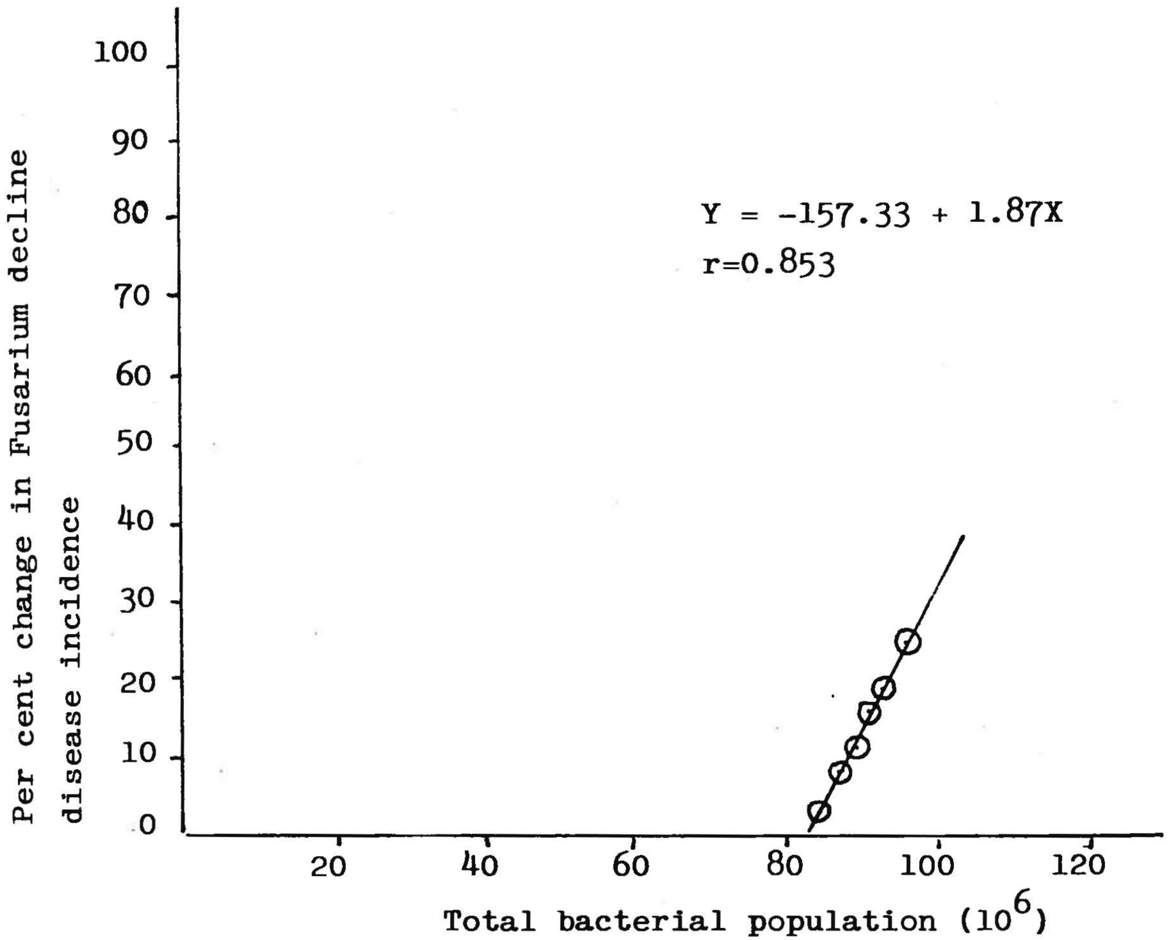

Fig 8: Correlation between total bacterial population (10^6) and per cent change in Fusarium decline disease incidence in D_3 diseased garden

Table 10: Correlation between plant parasitic nematodes and per cent change in Fusarium decline disease incidence in diseased betelvine gardens

Date of observation	Cumulative Occurrence of decline disease incidence	Percent change in decline disease incidence	Plant parasitic nematodes/250 g soil						Total Plant parasitic nematodes	Table value
			<u>Rotylenchulus reniformis</u>	<u>Helicotylenchus</u> spp.	<u>Hirschmanniella oryzae</u>	<u>Meloidogyne incognita</u>	<u>pratylenchus</u> spp.	<u>Tylenchorhynchus</u> spp.		
D_1 Garden										
17-12-88	7.25	7.25	1080	40	40	-	10	10	1200	
26-12-88	16.72	9.47	860	100	250	-	-	-	1210	
04-01-89	27.39	10.57	1090	470	100	-	-	-	1600	
13-01-89	39.00	11.71	1000	590	420	-	-	-	2010	
22-01-89	51.27	12.27	1040	620	300	100	10	10	2080	
31-01-89	65.27	14.00	1060	640	270	-	-	-	1970	0.666
09-02-89	76.92	11.65	1040	460	410	150	-	-	2060	
18-02-89	86.23	9.31	1080	830	490	200	-	-	2600	
27-02-89	92.75	6.52	1300	710	510	180	-	-	2700	
Calculated value			-0.441 NS	0.273 NS	0.0036 NS	-0.270 NS	-	-	-0.10 NS	
D_2 Garden										
08-03-89	6.25	6.25	760	700	550	50	-	10	2070	
17-03-89	17.27	11.02	970	600	550	70	-	-	2190	
26-03-89	31.00	13.73	960	600	450	80	-	-	2090	
04-04-89	49.00	18.00	820	640	700	100	10	-	2270	0.754
13-04-89	69.00	20.00	900	600	570	110	-	-	2180	
22-04-89	79.25	10.25	910	660	680	-	-	-	2250	
31-04-89	88.23	8.98	1140	680	700	-	-	-	2520	
Calculated value			-0.185 NS	-0.473 NS	0.00105 NS	0.768 *			-0.074 NS	
D_3 Garden										
09-05-89	22.36	22.36	670	840	800	-	10	-	2320	
18-05-89	50.50	28.14	750	840	530	200	-	-	2320	
27-05-89	62.25	11.75	870	770	610	-	-	-	2250	0.811
05-06-89	72.00	9.75	680	550	360	-	-	-	1590	
14-06-89	80.00	8.00	840	930	890	150	-	-	2810	
23-06-89	84.50	4.50	900	840	880	100	-	-	2720	
Calculated value			-0.579 NS	0.146 NS	0.282 NS	0.233 NS			-0.176 NS	

Note: Plant parasitic nematodes were individually correlated with per cent change in Fusarium decline disease incidence.

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

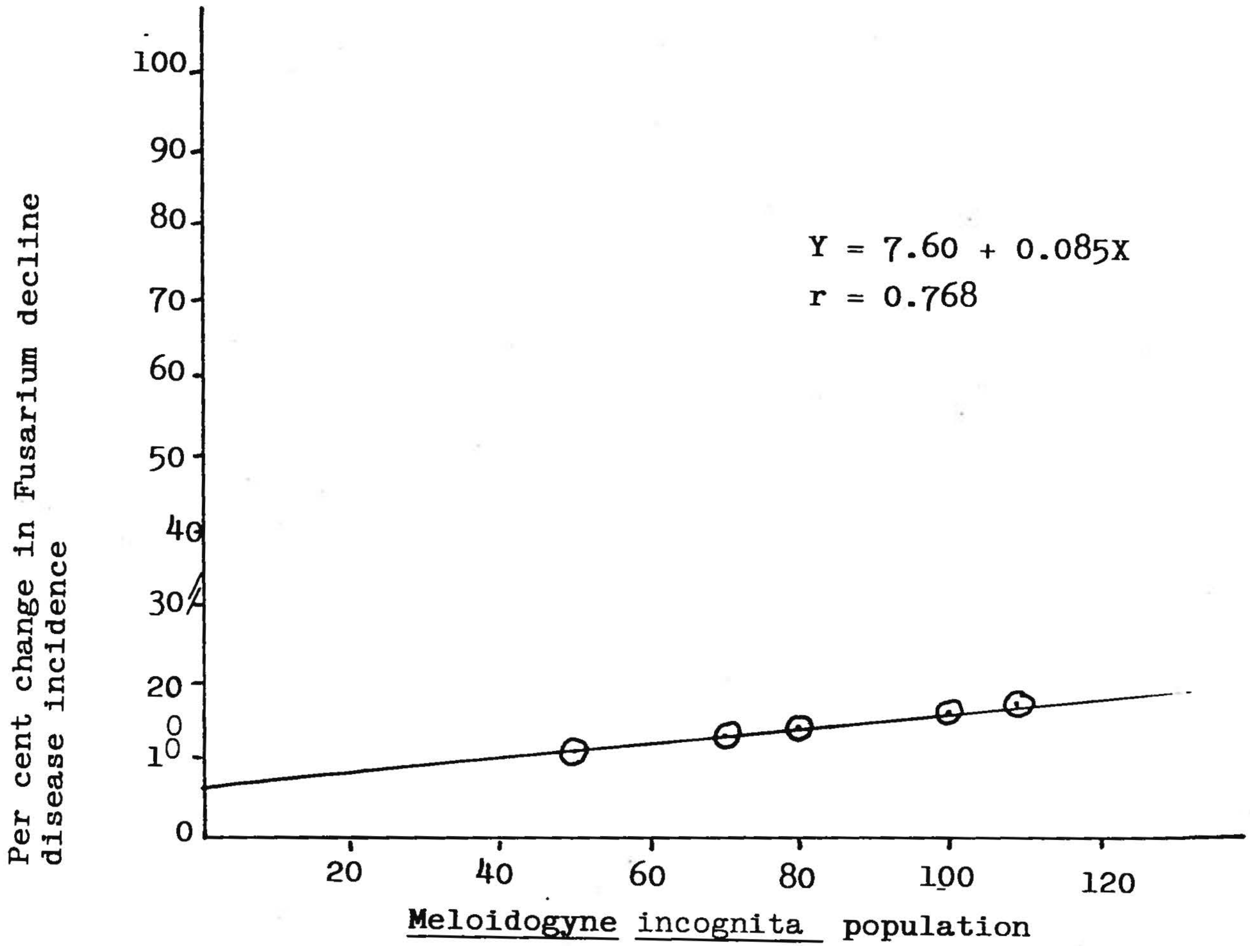

Fig 9:Correlation between *M.incognita* population and per cent change in Fusarium decline disease incidence in D_2 diseased garden

Table 11: Correlation between saprozoic nematodes and per cent change in decline disease incidence in diseased betelvine gardens

Date of observation	Cumulative occurrence of decline disease incidence	Per cent change in decline disease incidence	Saprozoic nematodes/ 250 g soil		Total saprozoic nematodes	Table value
			Aphelenchus avenae	*Tylenchus* spp		
D_1 Garden						
17-12-88	7.25	7.25	90	20	110	
26-12-88	16.72	9.47	60	80	140	
04-01-89	27.39	10.57	10	30	40	
13-01-89	39.00	11.71	100	10	110	
22-01-89	51.27	12.27	70	25	95	0.666
31-01-89	65.27	14.00	110	20	135	
09-02-89	76.92	11.65	40	40	80	
18-02-89	86.23	9.31	60	70	130	
27-02-89	92.75	6.52	40	60	100	
Calculated value			-0.169^{NS}	-0.477^{NS}	-0.036^{NS}	
D_2 Garden						
08-03-89	6.25	6.25	90	140	230	
17-03-89	17.27	11.02	140	70	210	
26-03-89	31.00	13.73	100	107	207	
04-04-89	49.00	18.00	120	100	220	0.754
13-04-89	69.00	20.00	100	48	148	
22-04-89	79.25	10.25	250	100	350	
31-04-89	88.23	8.98	100	180	380	
Calculated value			-0.271^{NS}	-0.676^{NS}	0.551^{NS}	
D_3 Garden						
09-05-89	22.36	22.36	90	110	200	
18-05-89	50.50	28.14	60	70	130	
27-05-89	62.25	11.75	120	80	200	0.811
05-06-89	72.00	9.75	140	130	270	
14-06-89	80.00	8.00	100	180	290	
23-06-89	84.50	4.50	110	100	210	
Calculated value			-0.768^{NS}	-0.500^{NS}	-0.737^{NS}	

Note: Saprozoic nematodes were individually correlated with per cent change in decline

Table 'r' value was taken from appendix 'H' from statistical procedures for Agricultural Research by K.A. Gomez and A.A. Gomez

As *M. incognita* population increased, the per cent change in decline disease incidence also increased (Table 10; Fig 9)

The saprozoic nematodes (*Aphelenchus avenae* and *Tylenchus* spp.) were not found to be correlated with per cent change in decline disease incidence in all the three diseased gardens (D_1,D_2 and D_3) (Table 11).

4.9 Quantification of rate of spread of decline disease incidence

4.9.1 Healthy gardens:

The rate of spread of decline disease was low in H_1 healthy garden i.e., 0.00156 per units per day in the initial stages. Later the rate of spread increased slowly from 0.00156 to 0.148 per units per day (Table 12;Fig 10).

In H_2 healthy garden the rate of spread was more than H_1 healthy garden and it increased to 0.431 per units per day from 0.040 per units per day (Table 12; Fig 10).

In H_3 garden also, the rate of spread of disease incidence was higher than H_1 garden and it increased from 0.077 to 0.289 per units per day (Table 12; Fig 10).

Out of the three gardens, the rate of spread of disease was maximum in H_2 healthy garden due to high incidence of disease (Table 12).

Table 12: Rate of spread of decline disease in second year apparently healthy and diseased betelvine gardens

Date of observation	Apparently healthy garden			Diseased garden		
	Cumulative occurrence of decline disease incidence	Rate of spread $r=2.3/t \times \log_e 1/1-x$	$\log_e 1/1-x$	Cumulative occurrence of decline disease incidence	Rate of spread $r=2.3/t \times \log_e 1/1-x$	$\log_e 1/1-x$
Garden No. 1						
17-12-88	0.89	-	0.0089	7.25	-	0.075
26-12-88	2.00	0.0052	0.0202	16.72	0.046	0.182
04-01-89	4.89	0.0128	0.0501	27.29	0.081	0.318
13-01-89	8.75	0.0230	0.0910	39.00	0.126	0.494
22-01-89	13.52	0.0370	0.1450	51.27	0.183	0.718
31-01-89	20.75	0.0590	0.2320	65.27	0.270	1.057
09-02-89	29.60	0.0890	0.3500	76.92	0.374	1.466
18-02-89	37.50	0.1200	0.4700	88.23	0.506	1.982
27-02-89	44.00	0.1480	0.5790	92.75	0.670	2.624
Garden No.2						
08-03-89	5.62	-	0.0570	6.25	-	0.064
17-03-89	14.81	0.0400	0.1600	17.27	0.048	0.189
26-03-89	27.50	0.0820	0.3210	31.00	0.094	0.371
04-04-89	42.25	0.1400	0.5490	49.00	0.172	0.673
13-04-89	58.74	0.2260	0.8850	69.00	0.299	1.171
22-04-89	70.75	0.3140	1.2290	79.25	0.401	1.572
31-04-89	81.85	0.4310	1.6870	88.23	0.546	2.139
Garden No.3						
09-05-89	11.75	-	0.1240	22.36	-	0.253
18-05-89	26.11	0.0770	0.3020	50.50	0.179	0.703
27-05-89	44.18	0.1480	0.5830	62.25	0.248	0.974
05-06-89	55.00	0.2040	0.7980	72.00	0.325	1.272
14-06-89	62.50	0.2500	0.9800	80.00	0.411	1.609
23-06-89	67.75	0.2890	1.1310	84.50	0.476	1.864

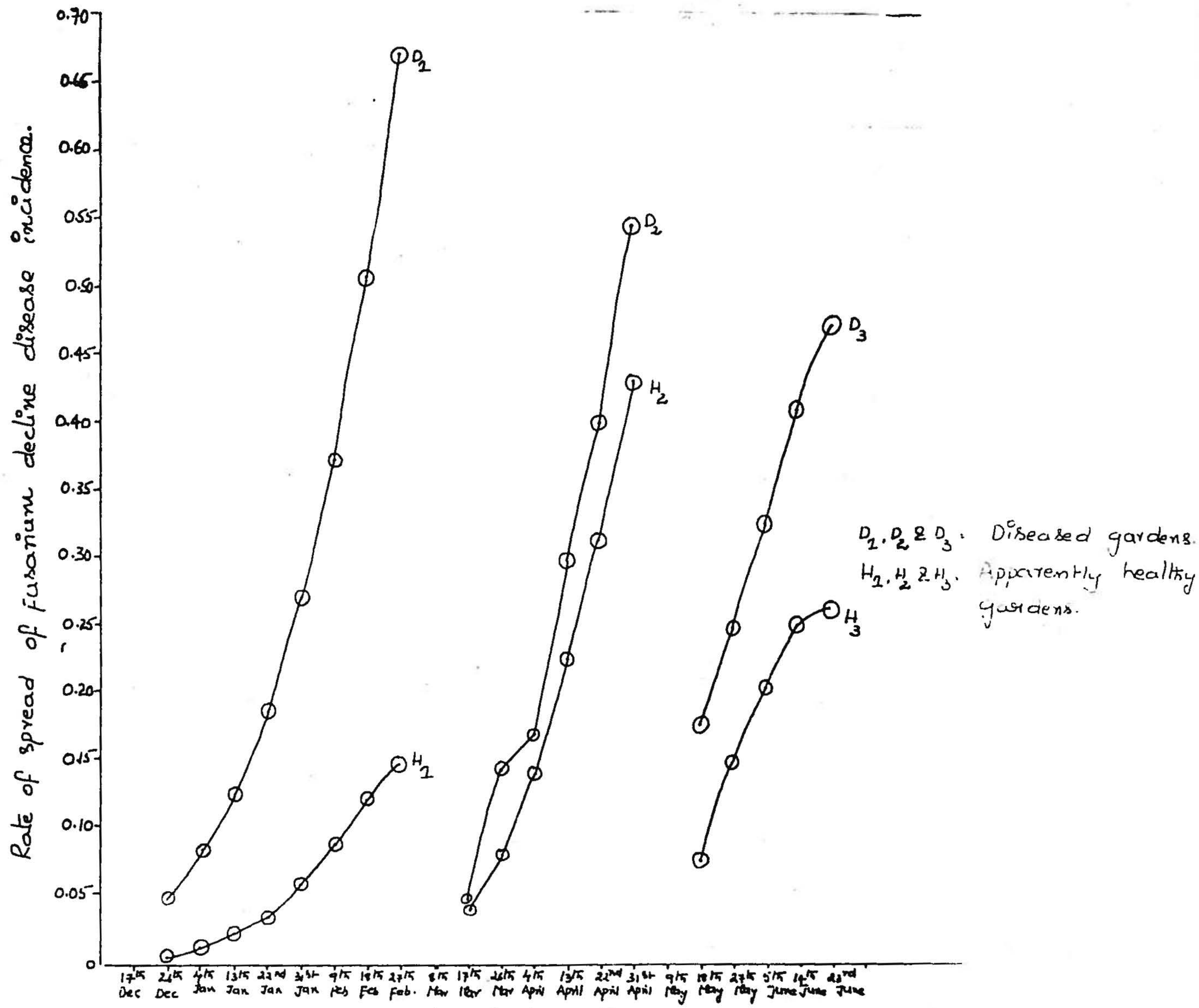

Fig 10: Rate of spread of Fusarium decline disease in apparently healthy and decline diseased betelvine gardens.

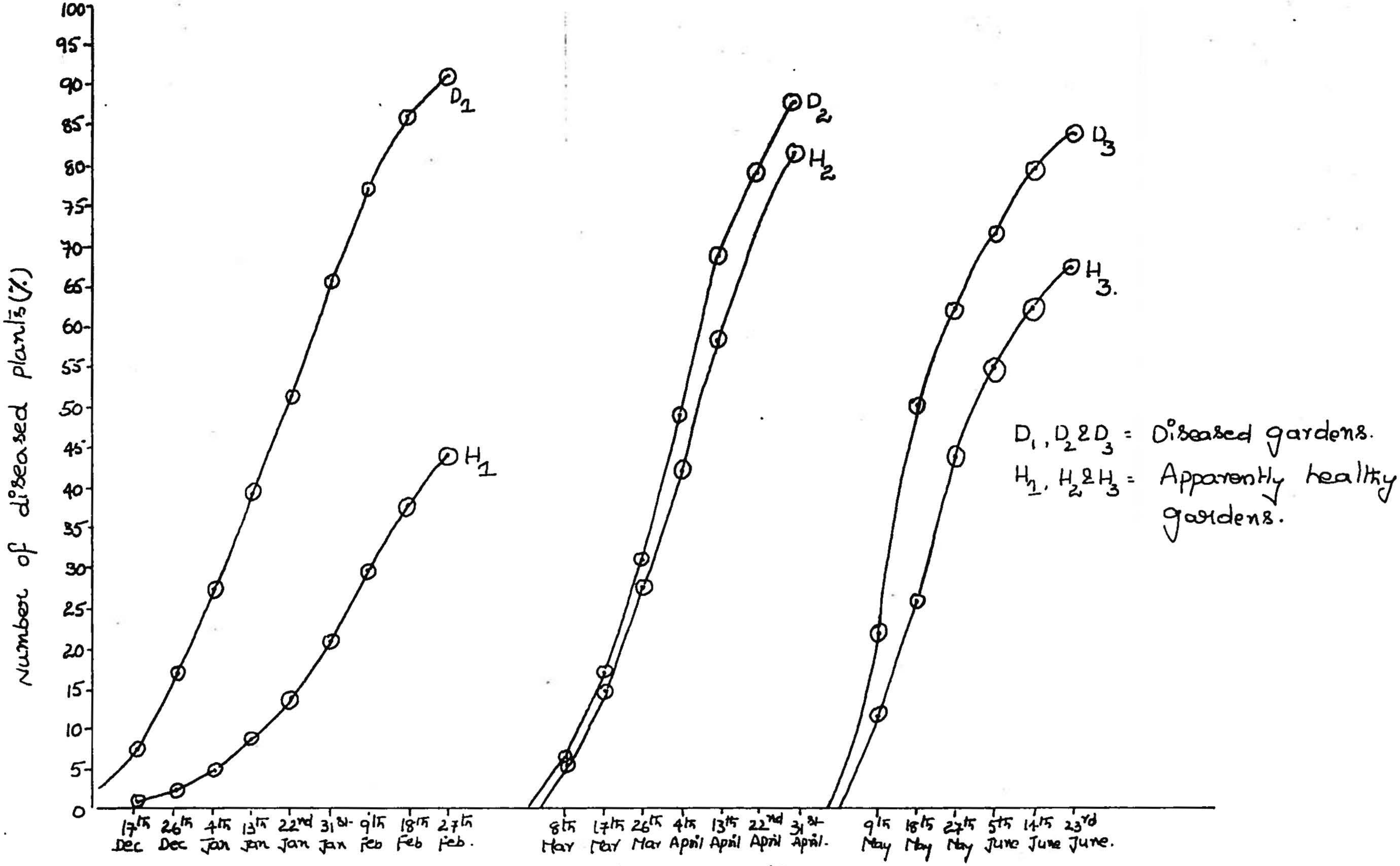

Fig 11: progress of Fusarium decline disease incidence in apparently healthy and decline diseased betel-vine gardens.

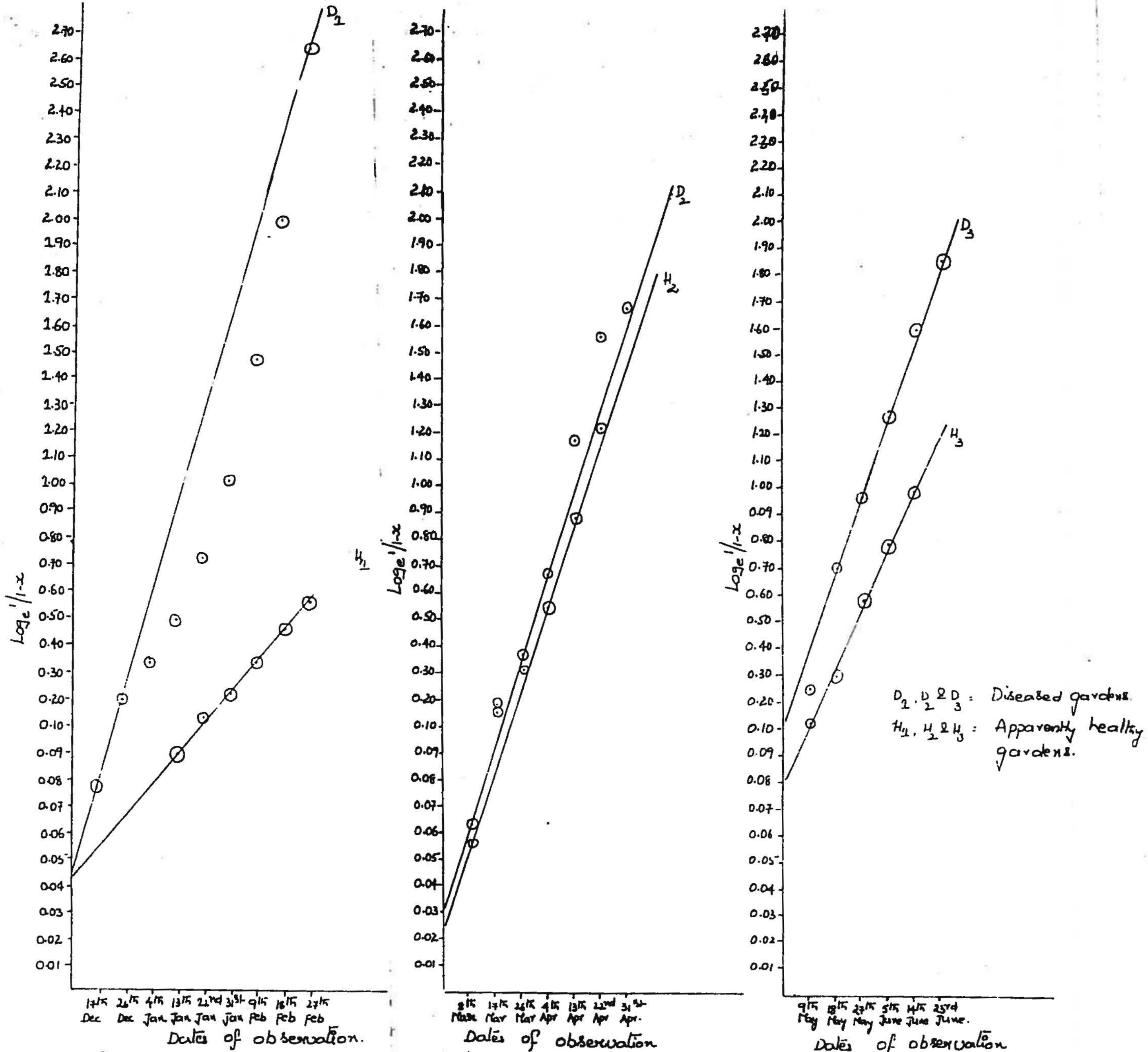

Fig: 12. Progress of Fusarium decline disease in apparently healthy and decline diseased betelvine gardens ($Log_e {}^1/_{1-x}$ plotted against date of observation)

4.9.2 **Diseased gardens**

The rate of spread of disease was gradually increased from 0.046 to 0.67 per units per day in D_1 diseased garden. Maximum incidence of disease was noticed in D_1 garden (92.75%) Table 12;Fig 10).

In D_2 diseased garden , the rate of spread of disease was found to be increased from 0.048 units to 0.546 units (Table 12;Fig. 10).

In D_3 garden, the rate of spread of disease was found to be 0.476 per units per day (Table 12;Fig 10).

When per cent diseased plants were plotted against different dates of observation (Time), a sigmoid curve of disease progression was obtained (Fig 11). The rate of spread gradually increased from the initial stages. The prediction curve was very steep in D_1 garden due to maximum disease incidence when compared to D_2 and D_3 garden and also H_1,H_2 and H_3 gardens (Fig 12), when $\log_e 1/1-x$ was plotted against time (dates of observation).

5.0 Influence of root-knot nemtode Meloidogyne incognita alone and in combination with sclerotium rolfsii at different nematode inoculum levels on sclerotium wilt of betelvine.

This experiment was conducted in earthen pots containing 2 kg of sterilized soil with three months old rooted betelvine plants cv "Tellaku". The soil in pots was inoculated with different M. incognita inoculum levels i.e., 1000, 2000 and 4000 2nd stage juveniles after surface sterilization with 0.02 per cent ethoxymethyl mercuric chloride (Aretan) and 0.1 per cent dihydrostreptomycin sulphate. Two weeks old fungus culture of S. rolfsii was used @ 30 g per pot. Control plants without inoculation of nematodes or fungus were maintained for comparison. The root-knot index, root rot index and vegetative growth parameters (fresh shoot and root weight, dry shoot and root weight, shoot length and root length) were recorded at the time of termination of experiment (60 days after inoculations).(plates 17 & 18).

5.1 **Fresh shoot weight**

significant differences in fresh shoot weight of betelvine plants inoculated with different inoculum levels of root-knot nematode M.incognita alone and in combination with S. rolfsii were recorded. The mean shoot weight of betelvine plants cv."Tellaku" inoculated with nematodes and S. rolfsii was significantly lower than the plants grown without M.incognita or S. rolfsii inocululations.

Among the treatments testd, plants inoculated with S. rolfsii and 4000 2nd stage juveniles of M.incognita simultaneously gave maximum decrease (39.11 %) in fresh shoot weight. This was followed by S. rolfsii and an inoculum level of 2000 2nd stage juveniles of *M.incognita* (33.29%); 1000 2nd stage juveniles of *M.incognita* and *S. rolfsii* inoculated simultaneously (30.97%). The inoculum levels of 1000, 2000 and 4000 second stage juveniles of *M.incognita* reduced the fresh shoot weight 23.47, 20.11 and 16.85 per cent respectively. The fungus *S. rolfsii* reduced the fresh shoot weight of betelvine plants to the extent of 9.73 per cent.(Table 13; Fig. 13).

5.2 Fresh root weight

Significant differences in mean fresh root weight were observed when betelvine plants were inoculated with different inoculum levels of *M.incognita* alone or *M.incognita* + *S. rolfsii* simultaneously or *S. rolfsii* alone.

Maximum reduction (52.62%) in fresh root weight was found in plants inoculated with *S. rolfsii* and 4000 2nd stage juveniles of *M.incognita* simultaneously. This was followed by *S. rolfsii* and an inoculum level of 2000 and 1000 2nd stage juveniles of *M.incognita* 49.20 and 47.55 per cent respectively. In the treatments, where betelvine plants were inoculated with 1000 2nd stage juveniles of *M.incognita* recorded 43.47 per cent reduction in fresh root weight. This was followed by an inoculum level of 2000 and 4000 2nd stage juveniles of *M.incognita* 30.96 and 29.15 per cent respectively. The fungus *S. rolfsii* alone reduced the fresh root weight of betelvine plants to the extent of 29.31 per cent (Table. 13, Fig. 13).

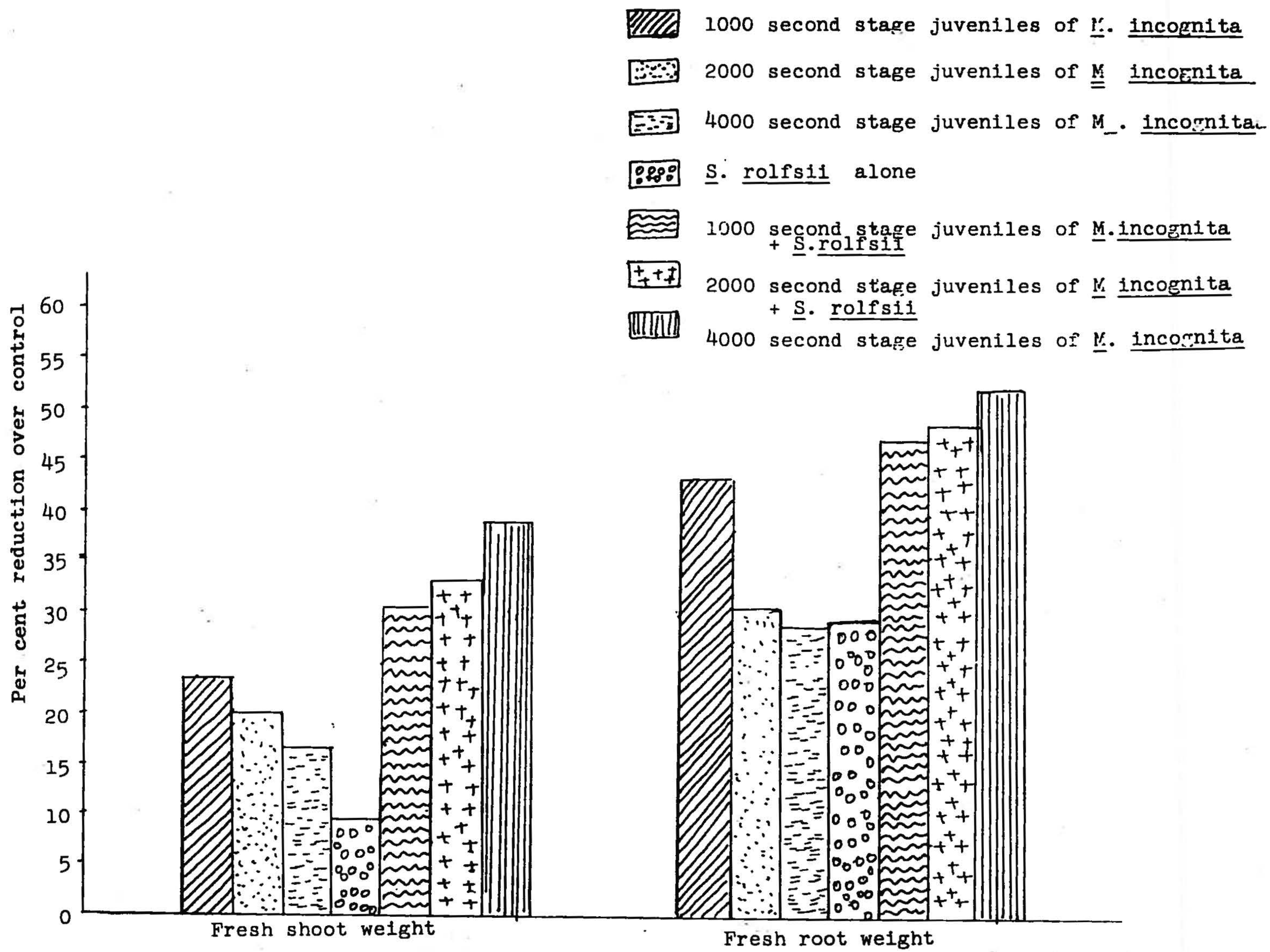

Fig.13 Influence of M.incognita alone and in combination with S.rolfsii at different inoculum levels on fresh shoot and root weight of betelvine cv. "Tellaku".

5.3 Dry shoot weight

Significant differences in mean shoot dry weight were recorded when plants were inoculated with *M. incognita* alone , *S. rolfsii* alone or *M. incognita* + *S. rolfsii* simultaneously at different nematode inoculum levels.

Maximum reduction (39.66%) in dry shoot weight was found in plants inoculated with *S. rolfsii* and an inoculum level of 4000 2nd stage juveniles of *M. incognita* simultaneously. This was followed by *S. rolfsii* and an inoculum level of 2000 and 1000 2nd stage juveniles of *M. incognita* 38.11 and 27.83 per cent respectively. Betelvine plants inoculated with 1000 2nd stage juveniles of *M. incognita* recorded 23.97 per cent reduction in dry shoot weight. This was followed by an inoculum level of 2000 and 4000 2nd stage juveniles of *M. incognita* 23.47 and 19.24 per cent respectively. Betelvine plants inoculated with fungus *S. rolfsii* alone recorded 14.38 per cent reduction in dry shoot weight (Table 13; Fig. 14).

5.4 Dry root weight

Maximum reduction (74.98%) in root dry weight was recorded in plants inoculated with *S. rolfsii* and 4000 2nd stage juveniles of *M. incognita* simultaneously. This was followed by *S. rolfsii* and an inoculum level of 2000 and 1000 2nd stage juveniles of *M. incognita* (69.14 and 67.65 per cent respectively). In the treatment where the plants were inoculated with 1000 2nd stage juveniles gave 49.17 per cent

Table 13: Influence of root knot nematode *Meloidogyne incognita* alone at different inoculum levels and in combination wtih *S. rolfsii* on betelvine vegetative growth, root knot index, root rot index and root population per one gram sample

Treatments	Mean fresh weight		Mean dry weight(g)		Mean length		Root-knot	Root rot	No.of larvae/ g root sample
	Shoot	Root	Shoot	Root	Shoot	Root	index	index	
Control (with out nematodes or fungus)	44.10 (-)	18.15 (-)	16.06 (-)	3.37 (-)	76.40 (-)	19.43 (-)	-	-	-
M. incognita 1000 second stage juveniles only	33.75 (-23.47)	10.26 (-43.47)	12.21 (-23.97)	1.713 (-49.17)	73.77 (-3.44)	13.70 (-29.49)	3.3	-	20
M. incognita 2000 second stage juveniles only	35.23 (-20.11)	12.53 (-30.96)	12.29 (-23.47)	1.92 (43.03)	74.63 (-2.32)	14.47 (-25.53)	3.7	-	25.33
M. incognita 4000 second stage juveniles	36.67 (-16.85)	12.86 (-29.15)	12.97 (-19.24)	2.05 (-39.17)	75.46 (-1.23)	15.57 (-19.87)	4.7	-	27.40
S. rolfsii alone	39.81 (-9.73)	12.83 (-29.13)	13.75 (-14.38)	2.07 (-38.58)	69.67 (-8.81)	13.23 (-31.91)	-	4.3	-
M. incognita 1000 second stage juveniles only + *S. rolfsii* (simultaneous inoculation	31.44 (-30.97)	9.52 (-47.55)	11.59 (-27.83)	1.09 (-67.65)	67.73 (-11.35)	11.67 (-39.94)	3.0	2.7	18.33
M. incognita 2000 second stage juveniles only + *S. rolfsii* (simultaneous inoculation	29.42 (-33.29)	9.22 (-49.20)	9.94 (-38.11)	1.04 (-69.14)	64.00 (-16.23)	11.63 (-40.14)	3.3	3.3	19.33
M. incognita 4000 second stage juveniles only + *S. rolfsii* (simultaneous inoculation	26.85 (-39.11)	8.60 (-52.62)	9.69 (-39.66)	0.843 (-74.98)	61.5 (-19.50)	10.64 (-45.24)	3.7	3.7	20.33
SEM	0.889	0.259	0.506	0.119	0.861	0.165	0.255	0.239	1.003
CD at 5% level	2.689	0.785	1.535	0.361	2.613	0.502	0.774	0.727	3.042
CD at 1% level	3.745	1.089	2.130	0.501	3.626	0.697	1.075	1.009	4.221

(-) Percent reduction over control

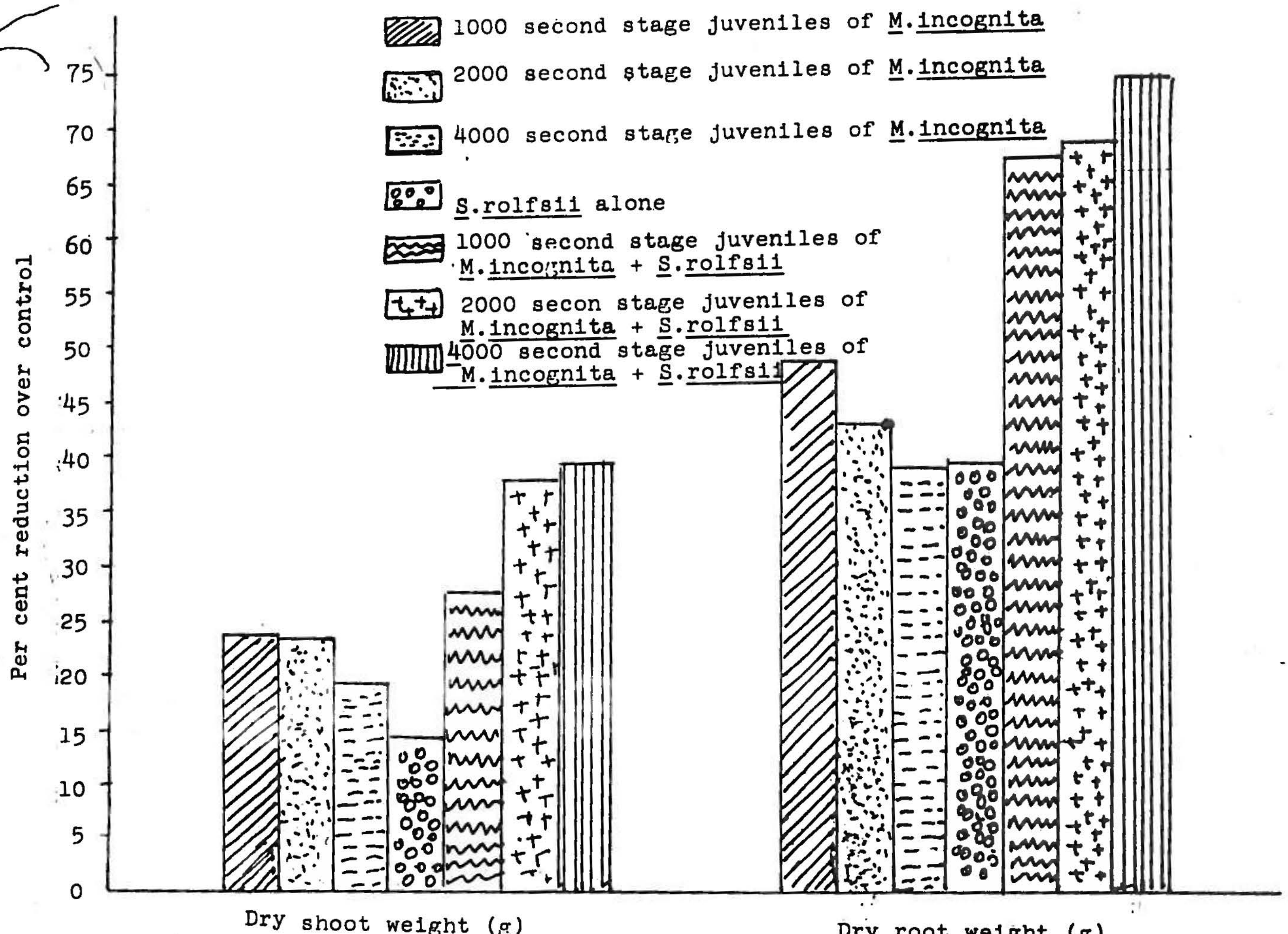

Fig.14 Influence of M.incognita alone and in combination with S.rolfsii at different inoculum on dry shoot and root weight of betelvine cv"Tellaku"

Plate 17: Influence of M.incognita in combination with S.rolfsii at different inoculum levels on Scelrotial wilt of betelvine

Plate 18: Influence of S.rolfsii alone on Sclerotial wilt of betelvine

reduction in dry root weight. This was followed by an inoculum level of 2000 and 4000 2nd stage juveniles of M. incognita (43.03 and 39.17 per cent respectively). The fungus S. rolfsii alone reduced the dry root weight to the extent of 38.58 per cent (Table 13; Fig.14).

5.5 **Shoot length**

Significant differences in shoot length of betelvine plants inoculated with different inoculum levels of M. incognita alone or in combination with S. rolfsii or S. rolfsii alone were recorded (table 13). The mean shoot length of betelvine cv. " Tellaku" plants inoculated with nematodes alone, S. rolfsii alone and M. incognita + S. rolfsii was significantly lower than plants maintained without M. incognita or S. rolfsii inoculations.

Maximum reduction (19.5%) in shoot length was observed in the treatment, where plants were inoculated with 4000 2nd stage juveniles of M. incognita and S. rolfsii simultaneously. This was followed by an inoculum level of 2000 and 1000 2nd stage juveniles of M. incognita and S. rolfsii inoculated simultaneously (16.23% and 11.35% respectively) and in the treatment, where plants were inoculated with S. rolfsii alone gave 8.81% reduction in shoot length. In the treatment where plants were inoculated with 1000 2nd stage juveniles of M. incognnita recorded 3.44 per cent reduction in shoot length. This was followed by

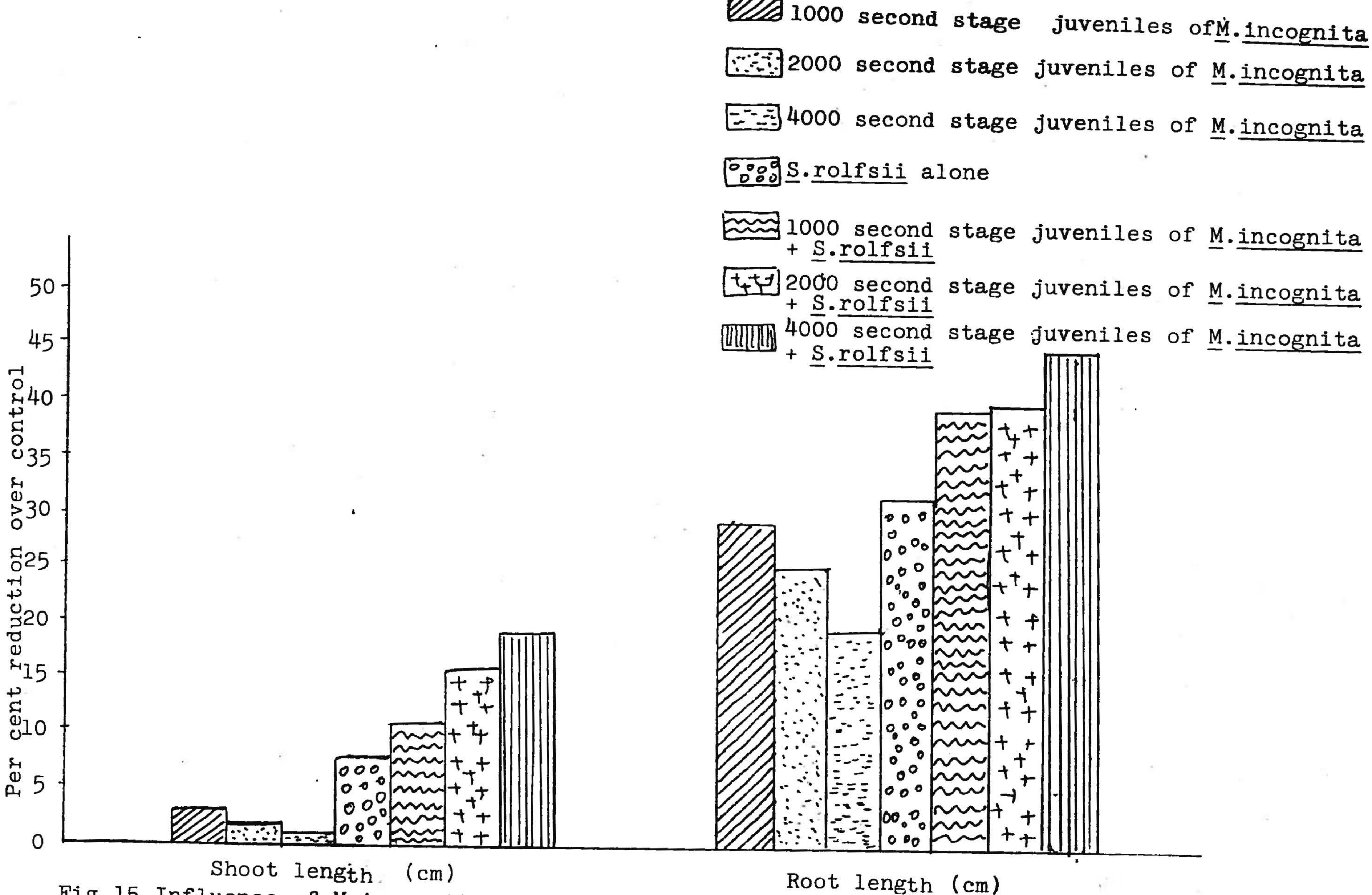

Fig.15 Influence of *M.incognita* alone and in combination with *S.rolfsii* at different inoculum levels on shoot length and root length of betelvine cv"Tellaku"

2000 and 1000 2nd stage juveniles of M. incognita 2.32 per cent and 1.23 percent respectively (Table 13;Fig 15).

5.6 **Root length**

Maximum decrease (45.24%) in root length was recorded in plants inoculated simultaneously with 4000 2nd stage juveniles of M. incognita and S. rolfsii. The reduction in root length was found to be on par in the treatments where plants were inoculated with 1000, 2000 and 4000 2nd stage juveniles of M. incognita and S. rolfsii simultaneously (39.94 and 40.14 per cent respectively). Plants inoculated with 1000, 2000 and 4000 2nd stage juveniles of M. incognita gave 29.49, 25.53 and 19.87 per cent reduction in root length. The fungus S. rolfsii alone gave 31.91 per cent reduction in root length of betelvine (Table 13;Fig 15).

5.7 **Root-knot index**

Maximum root-knot index (4.7) was recorded in plants inoculated with 4000 2nd stage juveniles of M. incognita. This was followed by 2000 2nd stage juveniles (3.7) and 1000 2nd stage juveniles of M. incognita (3.3). In the treatment where plants were inoculated with 1000 2nd stage juveniles of M. incognita and S. rolfsii simultaneously, recorded root-knot index of 3.0. This was followed by an inoculum level of 2000 and 4000 2nd stage juveniles of M. incognita and S. rolfsii inoculated simultaneously (3.3 and 3.7

respectively)(Table 13;Fig. 16).

5.8 Root rot index

Incidence of root rot was maximum (4.3) in the treatment where plants were inoculated with S. rolfsii alone. This was followed by simultaneous inoculation of 4000 2nd stage juveniles of M. incognita and S. rolfsii (3.7); 2000 2nd stage juveniles of M. incognita and S. rolfsii (3.3); 1000 2nd stage juveniles of M. incognita and S. rolfsii (2.7). No root rot index was noticed in the treatments where the plants were inoculated with different inoculum levels of M. incognita (Table 13;Fig 16).

5.9 Number of larvae per 1g root sample

Maximum root population (27.4) was found in the treatment where plants were inoculated with 4000 2nd stage juveniles of M. incognita. This was followed by 2000 2nd stage juveniles of M. incognita (25.33) and 1000 2nd stage juveniles of M. incognita (20). Significantly lower population was recorded in the treatment where S. rolfsii and 1000 2nd stage juveniles of M. incognita were inoculated simultaneously (18.33). This was followed by 2000 2nd stage juveniles + S. rolfsii; 1000 2nd stage juveniles of M. incognita +S. rolfsii (Table 13;Fig.17).

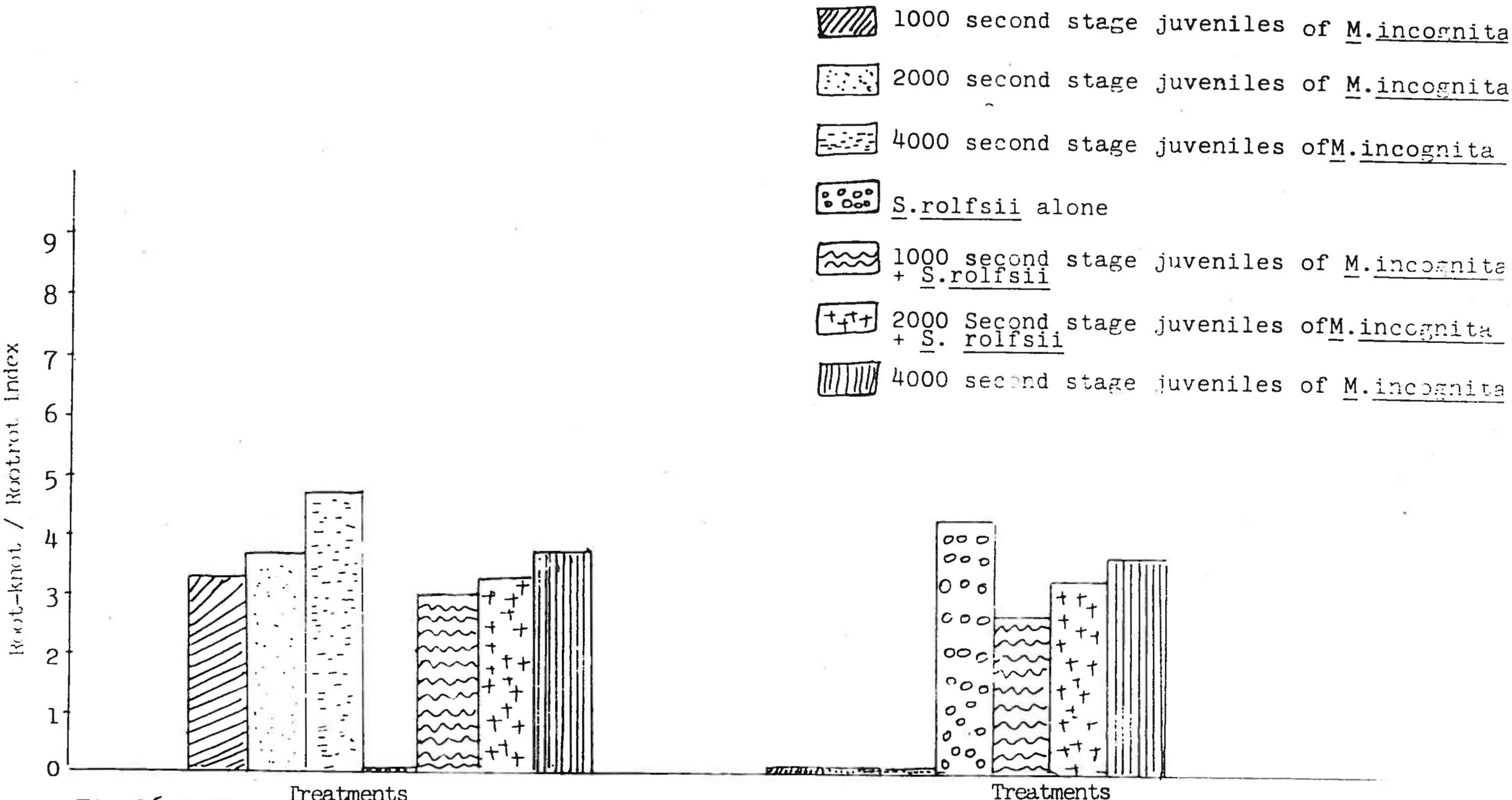

Fig.16 Influence of M.incognita alone and in combination with S.rolfsii at different inoculum levels on **betelvine** root knot index and root knot index

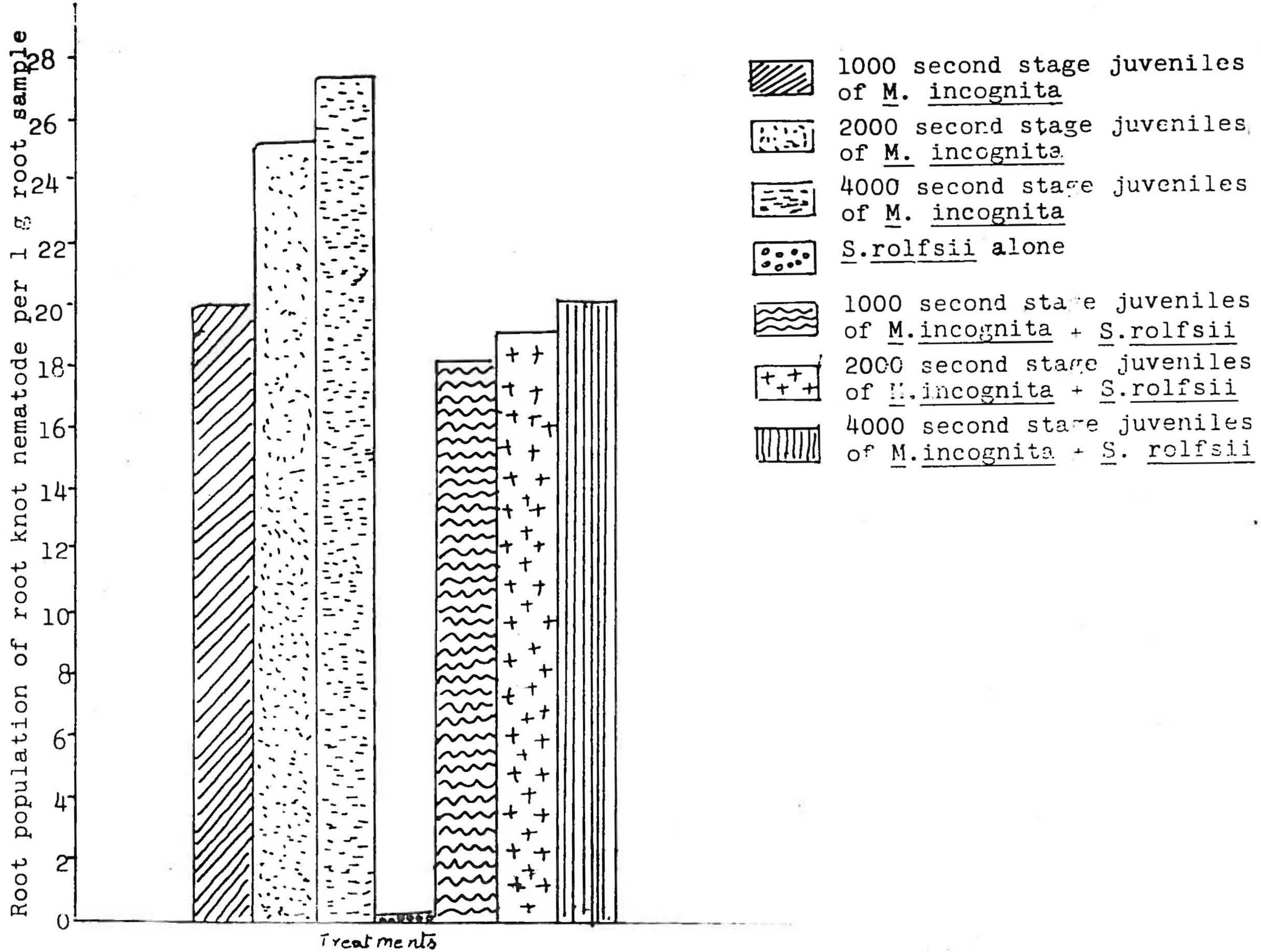

Fig.17 Influence of M.incognita alone and in combination with S.rolfsii at different inoculum levels on root population of root knot nematode.

6.D Studies on antagonism between Trichoderma harzianum and betelvine isolates of S. rolfsii and F. solani

The betel leaves are directly used for chewing purpose and biological control is right approach for controlling the soil borne plant diseases without any chemical residue problem. Keeping this in view an experiment was conducted to explore the possibility of controlling S. rolfsii and F.solani with the help of a potent antagonistic fungus T. harzianum..

The antagonistic effect of T. harzianum against s. rolfsii or F. solani (betelvine isolates) was studied by two methods i.e., seeded agar method and dual culture method. In both the methods T. harzianum showed its antagonistic effect against S. rolfsii or F. solani. By 24 h of incubation, T.harzianum covered the entire surface of medium (PDA) in the petriplate and it did not allow the development of S. rolfsii in seeded agar method. However, after 72 h of incubation, it allowed 18.5mm radial growth of the mycelial disc of S. rolfsii. By the end of 48 h of incubation, T. harzianum attained green colour (Plate 19).

When the fungal disc of T. harzianum was inoculated at a distance of 20mm from the mycelial disc of S. rolfsii, a black lytic zone was observed whenever the hyphae of T. harzianum and S. rolfsii came in contant with each other by 120 h of incubation. T. harzianum completely encircled the

Plate 19: Antagonism of T. harzianum against S. rolfsii in seeded agar method and dual agar culture method

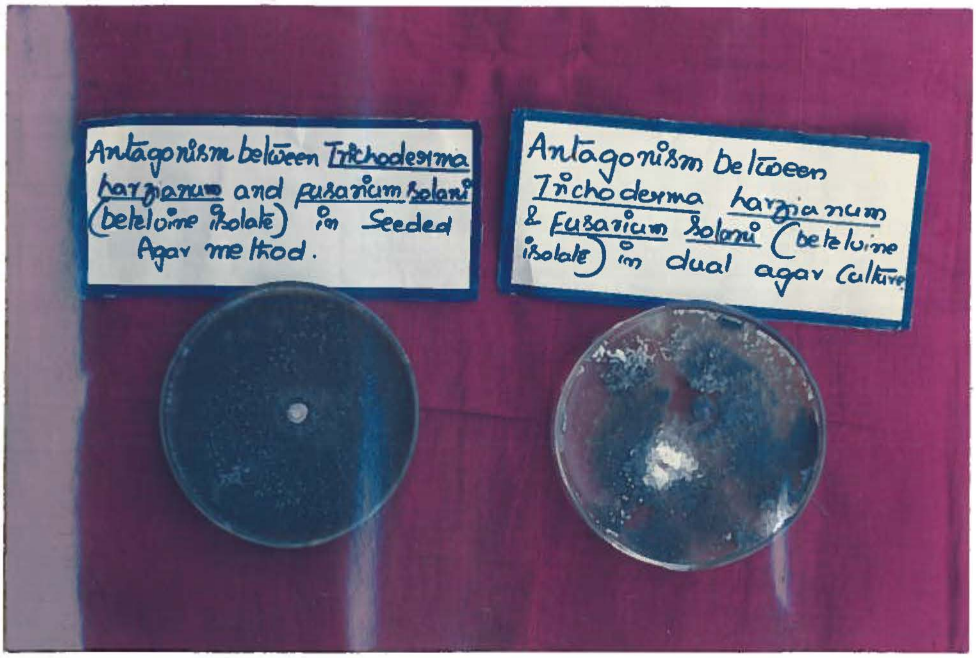

Plate 20: Antagonism of T. harzianum against F. solani in seeded agar method and dual agar culture method

mycelium of *S. rolfsii*.

Similarly *T. harzianum* showed its antagonistic effect against *F.solani* . *T. harzianum* allowed 6 mm radial growth of mycelial disc of *F.solani* at the end of 72 h . *T.harizianum* completely covered the entire surface of PDA in about 24 h of incubation and attained green colour at the end of 48 h. When *T.harzianum* was inoculated at a distance of 20 mm from the mycelial disc of *F.solani*, *T.harzianum* completely encircled the mycelium of *F.solani* by the end of 72 h of incubation (Plate 20).

7.0 Screening of different betelvine cultivars for their resistance against *S. rolfsii*

Different betelvine cultivars collected from different parts of India were planted in sterilized soil. Three months old rooted betelvine cultivars were inoculated with *S. rolfsii* (mycelium + sclerotia) grown on liquid corn meal medium @ 10 g and placed at the basal region of each cultivar. Observations were taken daily for a period of three weeks.

The results revealed that out of thirteen cultivars tested for their resistance against *S. rolfsii* all varieties were found to be susceptible to (100 % infection) *S. rolfsii*.

Plate 21:Different betelvine cultivars before inoculation withS.rolfsii

Plate 22:Different betelvine cultivars after inoculation with S.rolfsii

Table 14: Screening of different betelvine cultivars for their resistance against <u>Sclerotium rolfsii</u>

Varieties tested	No. of plants inoculated	No. of diseased plants at the end of 3 weeks	% disease incidence
1) Meethacumbangla	10	10	100
2) Bangla Desi	10	10	100
3) Bangla Ponnapatna	10	9	90
4) Bangla Nagaram	10	10	100
5) Tellaku (Ponnur-S)	10	9	90
6) Tellaku (Ponnur-R)	10	9	90
7) Kakair	10	10	100
8) Godibangla	10	10	100
9) Gachipan	10	9	90
10) Bangla	10	10	100
11) Karapaku	10	8	80
12) Maghi	10	9	90
13) Kapoori	10	9	90

Note: 1 - 9 Non-pungent varieties
10 - 12 Pungent varieties
13 - Meetha varietiy

However, the variety "Karapaku" showed 80 per cent infection to S. rolfsii (Table 14). Six cultivars viz., Bangla Ponnapatna (Orissa), Tellaku-Ponnur-R; Tellaku Ponnur-S (Andhra Pradesh); Gachipan (Assam); Maghi (Bihar) and Kapoori (Bihar) recorded 90 per cent infection of sclerotial wilt. The remaining cultivars viz., Meethacumbangla (Uttar Pradesh); Bangla Desi (Uttar Pradesh);Bangla Nagaram (Uttar Pradesh);Kakair (Bihar);Godibangla (Orissa) and Bangla (Madhya Pradesh) recorded 100 per cent infection of sclerotial wilt.

DISCUSSION AND CONCLUSIONS

Betelvine (Piper betle L.) is infected with several kinds of fungal plant pathogens and also with different plant parasitic nematodes. Fungal plant pathogens like Sclerotium rolfsii, Rhizoctonia solani, Phytophthora parasitica var. piperina are some of the pathogens isolated from diseased betelvine plants (Maiti, 1989). A new decline disease of betelvine due to Fusarium solani has been recently reported from Ponnur betelvine growing regions in Guntur District of Andhra Pradesh (Hymavathi, 1988).

Sclerotial wilt caused by Sclerotium rolfsii and Fusarium decline disease caused by Fusarium solani are very important and destructive soil borne diseases in Ponnur betelvine gardens. These two diseases can cause enormoøus losses (> 50-75% death of plants), if the inoculum level is very high (Anonymous, 1989). Sclerotial wilt was mainly observed during summer and rainy seasons starting from March to October months; where as Fusarium decline was observed during winter months (December to February). The earlier reports made by Singh and Joshi (1972); Raut and Shukla (1973) and Sulladmath et al (1977) on Fusarium wilt disease were mainly concerned with the occurrence of the disease in different betelvine growing regions of the country. Similarly the reports made by Chowdary (1945); Singh and Chand (1972) and Maiti (1989) on S.rolfsii were concerned with disease symptoms and losses caused by this soil borne pathogen.

In the present investigation, the Fusarial wilt and Sclerotial wilt causing fungi were isolated in pure culture from diseased betelvine plants collected from Ponnur betelvine gardens. Pathogenicity tests were conducted with isolated fungi. A survey of betelvine gardens in Ponnur area was conducted during the year 1988-89 to collect detailed information on the prevalence of decline disease in relation to soil physical, biological and chemical properties. Interaction studies with root-knot nematode Meloidogyne incognita at diffrent inoculum levels in combination with S.rolfsii were conducted to get information on the synergistic effect of sclerotial wilt pathogen and the root-knot nematode parasite. Experiments were conducted in vitro to test the antagonistic agent Trichoderma harzianum in controlling S.rolfsii or F.solani. Different betelvine cultivars collected from different states were screened for their resistance against S.rolfsii.

Pathogenicity tests with S.rolfsii had conclusively proved that soil substitution method (where the soil around the base of the plant was removed and the gap was filled up with fungus inoculum), gave quick and maximum incidence of wilt disease symptoms in about 10-12 days after inoculation. However, when the inoculum was applied through hollow plastic tubes inserted in the rootzone at the time of planting the cuttings, the appearance of sclerotial wilt was delayed. However, at the end of 20 days after inoculation with the fungus the percentage of wilted plants was 100. This delayed manifestation of wilt symptom was attributed to the delay of infection of roots and then basal stem portion.

Pathogenicity tests conducted with F.solani in the present investigation had clearly shown that clipping of roots of betelvine plants and dipping them in the fungal inoculum for 24 h and planting them in sterilized soil or in hydroponics gave 100% infection. This method was found to be most effective in reproducing Fusarium wilt symptoms similar to those symptoms observed in field. This could be attributed to direct penetration of the pathogen into the root system and also to availability of proper food base for root colonisation. However, dipping intact roots without clipping in the fungus inoculum for 24 h and planting them in sterilized soil or in hydroponics did not produce wilt disease sympotms and the plants remained healthy throughtout the period of investigation (Table .1). Similar observations were reported by Armstrong and Armstrong (1958) in pathogenicity studies with F.solani in cotton, tomato, cowpea. Clark (1979,1980) also observed that root wounding was a prerequisite for infection of F.solani in sweetpotato.

Observations of stained transverse sections of betelvine roots on proliferation of F.solani in naturally infected betelvine roots during disease development revealed the association fungal mycelium of F.solani in the xylem vessels. Emberger and Nelson (1981); and Stuehling and Nelson

(1981) also reported similar observations in chrysanthemum roots infected with F.oxysporum f.sp Chrysanthemi. Gotlieb and Doriski (1983) also noticed the presence of fungus mycelium in roots and stem tissues of birdsfoot-trefoil, infected with F. oxysporum. Charchar and Kraft (1987) found extensive mycelial invasion and colonisation of roots of pea cultivars infected with F. oxysporum f. sp. pisi.

Distortion and occlusion of xylem vessels with gums and tyloses and plugging of xylem vessels were observed in the present investigation. Similar observations were reported by Pennypacker and Nelson (1972) in carnations; Stuehling and Nelson (1981) in chrysanthemum and Gotlieb and Doriski (1980) in birdsfoot-trefoil. They noticed plugging of xylem vessels with gum and pectinaceous material, hypertrophy and hyperplasia of vascular parenchyma.

Browning of vascular bundles was consistently noticed in betelvine roots inoculated with F. solani. Similar reports were made by Baayen and Elgersma (1985). They observed vascular browning in carnation plants when inoculated with F. oxysporum f. sp. dianthi.

Observations on sclerotial wilt caused by S. rolfsii revealed that rotting of basal portion of plant near the collar region and development of fungus mycelium was seen on soil surface near the base of the plant. Complete decay of root system and development of sclerotia on stems and on soil surface near the plant base was noticed. Chowdary (1945) and Maiti, (1989) also made similar observations. They noticed decay of basal portion of stem at soil level, formation of dense white cottony mycelial mass at the collar region and also development of sclerotia at the infected region and also on soil surface near the plant base.

The field survey of healthy gardens looking apparently healthy (at the beginning of observations) conclusively revealed that soil pH, electrical conductivity, calcium content, magnesium content were not correlated with per cent change in decline disease incidence of betelvine in all the three gardens surveyed. Microbial population (total fungi, total actinomycetes) was not also correlated with per cent change in decline disease incidence. However, total bacteria were found to be positively correlated with per cent change in decline disease incidence in H_1 healthy garden (r = 0.968; Table 5). As the bacterial population increased, the per cent change in decline disease also increased.

Plant parasitic nematodes (Rotylenchulus reniformis, Helicotylenchus spp ,Hirschmanniella oryzae, Meloidogyne incognita, Pratylenchus spp. and Tylenchorhynchus spp.) recovered from soil samples of apparently healthy gardens could not be correlated with per cent change in decline disease at 9 days interval (Table 6). Similarly the saprozoic nematodes (Aphelenchus avenae and Tylenchus spp.) were also not found to be significantly correlated with per cent change in decline disease. However, Tylenchus spp. was found to be negatively correlated with per cent change decline disease in H_1 healthy garden (r = -0.683). As the Tylenchus spp. population decreased the per cent change in decline disease increased (Table 7). Mycophagous nematodes Tylenchus spp. are ubiquitous in their occurrence, normally present in the root zone of crops (Das, 1960; Sitaramaiah, 1984) and frequently observed in association with diseased roots feeding on mycelium. The negative correlation could be due to absence of Tylenchus spp. resulting in increased disease incidence. In general, it was observed that total nematode populations were inconsistent at different intervals of observation. Therefore it would be difficult to draw any definite conclusion with regard to correlation of nematode population and decline disease incidence.

Field survey of decline diseased betelvine gardens conclusively revealed that soil p^H, electrical conductivity and calcium content of soil samples collected at 9 days interval could not be correlated with per cent change in decline disease incidence (Table 8). However magnesium content of soil samples was found to be positively correlated with per cent change in decline disease incidence in all the three diseased gardens (r = 0.824,0.826 and 0.909). As the magnesium content of soil samples increased, the per cent change in decline disease incidence also increased in all the three diseased gardens (D_1,D_2 and D_3) (Table 8). The observations made by Speigel (1987) find support from the present investigation. He noticed higher amounts of magnesium and potassium and lower content of calcium in diseased plants inoculated with F. oxysporum f. sp. melonis .

The total fungal population was found to be positively correlated with per cent change in decline disease incidence in D_3 garden (r= 0.924) (Table 9) and the total bacterial population was found to be correlated with per cent change in decline disease incidence in D_2 and D_3 gardens (r= 0.762 and 0.853) (Table 9) in the present investigation. This was attributed to the production of antibiotics and toxins formed in soils, in situ change in the microecology i.e., in soil microflora (Waksman, 1922) . Rhizosphere, is a zone of intensified microbial activity around the growing roots of higher plants. Plants under diseased conditions produce various kinds of root exudates compared to those under healthy conditions (Rovira, 1965). These root exudates may stimulate or suppress the soil microflora present in the vicinity of root depending upon the type of reaction. The roots of diseased plants exhibit higher metabolic activity and there by secrete large amounts of organic acids which ultimately increase the microbial population in soil. Balasubramanian (1975) reported high concentration of exchangable cations in soil from downy mildew affected sorghum plants and these cations were presumed to increase the soil microflora. The total actinomycetes population, however, was not correlated with per cent change in decline disease incidence in all the three diseased gardens (D_1,D_2. and D_3) surveyed (Table 9).

Plant parasitic nematodes (Rotylenchulus reniformis, Helicotylenchus spp., Hirschmanniella oryzae, Meloidogyne incognita, Pratylenchus spp. Tylenchorhynchus spp.) recovered from soil samples could not be correlated with per cent change in decline disease incidence in all the three diseased gardens investigated. However, root-knot nematode M. incognita was found to be correlated with per cent change in decline disease incidence in D_2 garden (r = 0.768) (Table 10). As the root-knot nematode M. incognita population increased, the per cent change in decline disease incidence also increased. The root-knot nematode M. incognita feeds on roots and causes wounds. These wounded roots are predisposed to the infection of soil borne fungi, thereby increasing the decline disease incidence. Minton and Minton (1963) observed that Fusarium grew profusely in cotton root tissues damaged by root-knot nematode M. incognita acrita. Melendez and Powell (1967) found that Fusarium oxysporum f.sp nicotianae hyphae penetrated readily and developed extensively in giant cells caused by root-knot nematode M . incognita.

The rate of spread of decline disease incidence was maximum (0.67 per units per day) in diseased D_1 garden. This was attributed to the high incidence of decline disease in

D_1 garden (Table 12). A sigmoid curve of disease incidence progression was obtained when per cent diseased plants were plotted aganist time (Talbe 12; Fig. 11). The prediction curve was very steep in D_1 diseased garden due to maximum incidence of disease (Fig. 13) when $\log_e 1/1-x$ was plotted against time.

Influence of root knot nematode M. incognita alone and in combination with S. rolfsii at different inoculum levels on Sclerotial wilt of betelvine.

Combined inoculation of betelvine plants with root-knot nematode M. incognita and S. rolfsii significantly reduced the fresh shoot and root weight, dry shoot and root weight, shoot length and root length when compared to inoculation with the root-knot nematode alone at different inoculum levels and also inoculation with S. rolfsii alone (Table 13). Goswami et al.(1975) reported that root- knot nematode M. javanica, Rhizoctonia bataticola pathogen complex in tomato reduced vegetative growth of tomato. Chahal and Chhabra (1984) observed that inoculation of tomato plants with M. incognita and R. solani resulted in significant growth reduction, in comparison to single pathogen inoculation. Taylor and Wyllie (1958) recorded increased death of soybean plants due to Rhizoctonia solani in the presence of M. incognita or M. hapla. Bergeson et al. (1970) reported that combined infection F. oxysporum f. sp. lycopersici and M.incognita results in increased colonisation of the fungus in roots. Sharma et al. (1980) observed synergistic effect between M. incognita and R. bataticola on okra. Golden and Van Gundy (1972) showed root exudates from tomato roots infected with M. javanica favoured R. solani and Thielaviopsis basicola, even when the fungus was separated from galled roots by a semipermeable membrane.

Antagonism between Trichoderma harzianum and S. rolfsii; T. harzianum and F. solani

Microbial antagonism plays an important role in biocontrol of several soil borne plant pathogens. Trichoderma harzianum and T. viridae potent antagonistic fungi have been used for controlling several soil borne plant pathogens (Wells et al, 1972; Elad et al, 1980,83; Backman and Kabana, 1975; Maiti and Sen, 1985; Upadhyay and Mukhopadhyay, 1986; Kudryavtseva, 1980 ; Mirkova, 1981; and Sivan & Chet, 1987).

In the present investigation T. harzianum was used as an antogonistic agent to see its effect on S. rolfsii and F. solani. The results revealed that T. harzianum did not allow the development of S. rolfsii in seeded agar method after 24 h of incubation. However, at the end of 72 h of observation it allowed 18.5 mm radial growth of mycelial disc of S. rolfsii. Formation of black lytic zone was observed where ever the hyphae of T. harzianum and S. rolfsii were in contact with each other in dual agar culture method. This black lytic zone was clear only at the end of 120 h of incubation. This gives evidence of the lysis of S. rolfsii cells when they come in contact with the antagonistic fungus T. harzianum. Similar observations were made by Upadhyay and Mukhopadhyay (1986) ; Maiti & Sen (1985).

Elad et al. (1983) with the help of scanning electron microscopy, noticed hyphal coils, appressoria by which T. harzianum attached to the hyphae of S. rolfsii. They also observed lysed sites and penetration holes in S. rolfsii cells following the removal of the parasitc hyphae of the antagonistic fungus T. harzianum. Upadhyay and Mukhopadhyay (1985) observed lysis of the mycelium and sclerotia of S. rolfsii in dual culture directly by T. harzianum. They noticed hyphal coiling entry through houstoria like structures.

The antagonisitc fungus T. harzianum allowed 6 mm F.solani radial growth at the end of 72 h in seeded agar method. The antagonistic fungus completely encircled the mycelium of F. solani at the end of 72 h of incubation. These laboratory observations demonstrated for the first time that the antagonistic fungus could be used for controlling F. solani. However, further studies (pot culture and field) are required to recommend the use of this antagonistic fungus for large scale field application in betelvine gardens.

Screening of different betelvine cultivars for their resistance against _S. rolfsii_.

For the sclerotial wilt pathogen _S. rolfsii_, there is no effective fungicide which can be used for effective control in field. If a resistant variety is found, there will be no need for the chemical control of this soil borne plant pathogen. The resistant variety can be multiplied for large scale use. With this view, the thirteen betelvine cultivars comprising pungent, non-pungent and meetha cultivars were screened in a pot culture experiment. The results revealed that all the betelvine cultivars in pots were found to be suceptible to _S. rolfsii_ (Table 14). However, the cultivar "Karapaku" from Andhra Pradesh showed 80 per cent infection to _S. rolfsii_. This could be attributed to higher phenolic content of this variety (Venkateswara Rao, 1987).

SUMMARY

Sclerotial wilt causing fungus Sclerotium rolfsii and Fusarium decline causing fungus Fusarium solani were isolated in pure culture from diseased betelvine plants collected from ponnur betelvine gardens. Pathogenicity studies were conducted with the isolated fungi on betelvine cultivar "Tellaku". The results revealed that soil substitution method was found to be the best method for inducing sclerotial wilt symptoms. For proving pathogenicity of F. solani on betelvine, clipping of roots and immersing them in fungal inoculum was effective method in reproducing typical Fusarium wilt symptoms.

A field survey was conducted during 1988-89 at 9 days interval both in apparently healthy (at time of observation) as well as decline diseased betelvine gardens (second year). Maximum Fusarium decline disease incidence was noticed in D_1 diseased garden and H_2 healthy garden. Total bacterial population was positively correlated with per cent change in Fusarium decline disease incidence ($r = 0.968$) in H_1 garden. As the bacterial population increased, the per cent change in decline disease incidence also increased at 9 days interval. The saprozoic nematode Tylenchus spp was negatively correlated with per cent change in decline disease incidence in H_1 garden ($r = -0.683$). As the Tylenchus spp nematode population decreased, the per cent change in decline disease incidence increased. Magnesium content of soil was positively correlated with the per cent change in decline disease incidence in all the three diseased gardens (r = 0.824, 0.826 and 0.909). As the magnesium content of soil samples increased, the per cent change in decline disease incidence also increased in all the three diseased gardens (D_1,D_2 and D_3). The total fungal population was correlated with per cent change in decline disease incidence in D_3 garden (r = 0.924). As the total fungal population decreased, the per cent change in decline disease also decreased. The total bacterial population was correlated with per cent change in decline in D_2 and D_3 gardens (r = 0.762 and 0.853), but not in D_1 garden. As the bacterial population increased, the per cent change in decline disease incidence increased in D_2 garden. In D_3 garden, as the bacterial population decreased, the per cent change in decline disease incidence decreased. The population of root-knot nematode <u>Meloidogyne incognita</u> was positively correlated with per cent change in decline in D_3 diseased garden (r = 0.768) As <u>M. incognita</u> population increased the per cent change in decline disease incidence also increased.

The response of betelvine plants (cv. Tellaku) to inoculation with root-knot nematode <u>Meloidogyne incognita</u> alone and in combination with <u>Sclerotium rolfsii</u> at different inoculum levels revealed that significant decreases were recorded in fresh and dry weight of shoot and root system and also shoot and root length. Significant differences were also observed in the root-knot index, root rot index and in nematode population /g of root sample.

Maximum reduction in fresh and dry weight of shoot and root system, shoot and root length was observed when the plants were inoculated with 4000 second stage juveniles of M. incognita and S. rolfsii simultaneously, followed by inoculum levels 2000 and 1000 2nd stage juveniles of M . incognita and S. rolfsii. Incidence of root rot was maximum (4.3) when the plants were inoculated with S. rofsii alone. Significantly lower root-knot nematode population was recorded in the combined inoculation of plants with M . incognita and S. rolfsii at an inoculum level of 1000 2nd stage juveniles.

Trichoderma harzianum was a powerful antagonist aganist S. rolfsii and F. solani. This antagonistic fungus can be used for large scale field application in betelvine crop for controlling S. rolfsii and F. solani.

Screening of different betelvine cultivars collected from different states were tested for their resistance against S. rolfsii indicated that the cultivar "Karapaku" from Andhra Pradesh recorded 80 per cent infection. The cultivars viz., Bangla ponna patna(Orissa), Tellaku (Ponnur-R) (Andhra Pradesh), Tellaku (Ponnur-S) (Andhra Pradesh) , Gachipan (Assam), Maghi (Bihar) and Kapoori (Bihar) showed 90 per cent infection. However, 100 per cent infection was recorded in the remaining cultivars namely Meetha cum Bangla (Uttar

Pradesh), Bangla Desi (Uttar Pradesh), Bangla Nagaram (Uttar Pradesh), Bangla (Madhya Pradesh), Kakair (Bihar) and Godibangla (Orissa).

LITERATURE CITED

Abawi, G.S. and K.R.Barker. 1984. Effects of cultivar, soil temperature and population levels of Meloidogyne incognita on root necrosis and Fusarium wilt of tomatoes. Phytopathology. 74: 433-438.

Acharya, A.; S.C.Dash and N.N. Padhi. 1987. Pathogenic association of Meloidogyne incognita with Sclerotium rolfsii and Xanthomonas betlicola on betelvine. Indian Journal of Nematology. 17(2): 196-198.

Allen, M.F. 1957. Experiments in soil bacteriology. Burgess Publishing Co., Minneapolis, Minnesota. USA. 117 p.

Anandavally Amma, P and N. Shanmugam. 1974. Studies on root rot of groundnut caused by Sclerotium rolfsii Sacc. Madras Agricultural Journal. 61: 973-978.

Anonymous. 1989. All India Co-ordinated Research Project on Betelvine . Status report of Andhra Pradesh Agricultural University. Chintalapudi. Ponnur - 522 124.

Armstrong, G.M. and J. K. Armstrong. 1958. Effect of cutting roots on the incidence of Fusarium wilt of cotton, tomatoes, cowpeas and other plants. Phytopathology. 48: 341.

Asthana, R.P. and K. A. Mahmud. 1944. Tip burn of Piper betle in the central provinces. Current Science. 13(9): 234.

Atkinson, G.F. 1892. Some diseases of cotton. Albama Polytechnique Institute Agricultural Experimental Station Bulletin No. 41: 61-65.

Baayan, R.P. and D.M. Elgersma. 1985. Colonisation and histopathology of susceptible and resistant carnation cultivars infected with Fusarium oxysporum f. sp. dianthi. Netherland Journal of Plant Pathology. 91(3): 119-135.

Backamn, P.A. and R. Rodriguez-kabana. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology. 65(7): 819-821.

Balasubramaninan, K.A. 1975. Pattern of distribution of certain cations in downy mildew - Affected sorghum and soil. Plant and Soil. 43:621-626.

Balasubramanyam, P. 1981. Nematode problem in betelvine grown in Tamil Nadu. Proceedings Improvement of betelvine cultivation. NBRI, Lucknow. Feb. 27-28 1981(edit. S.D. Khanduja and V.R. Balasubramaniyam.). P. 87.

Beckman, Carl.H. 1964. Host responses to vascular infection. Annual Review of Phytopathology 2: 231-251.

Beckman,C.H. and W.C. Mueller. 1987. Response of xylem parenchyma cells in tomato to vascular infection by Fusarium oxysporum f. sp. lycopersici. Phytopathology. 77: 45 (Abstr.).

Bergeson, G.B. 1972. Concepts of nematode-fungus associations in plant disease complexes : A review . Experimental Parasitology. 32: 301-314.

Bergeson, G.B.; S.D. Van Gundy and I.J. Thomason. 1970. Effect of Meloidogyne Javanica on rhizosphere microflora and Fusarium wilt of tomato. Phytopathology 60; 1245-1249.

* Blanc, D; R. Tramier and C. Pallot. 1983. Calcium nutrition and its effect on the receptivity of carnation to Fusarium oxysporum f. sp. dianthi. Acta Horticulturae. ~~Volume number~~ 141: 115-123.

Chahal, P.P.K. and H.K. Chhabra. 1984. Interaction of Meloidogyne incognita with Rhizoctonia solani on tomato. Indian Journal of Nematology. 14(1):56-57.

Chandrasekharan, A. and N. Shanmugam. 1984. Effect of soil types on chickpea root rot caused by Fusarium solani (Mart) Sacc. Indian Journal of Mycology & Plant Pathology. 14: 92-94.

Charchar, M. J. A. and J.M. Kraft. 1987. Response of near isogenic pea cultivars to infection by *Fusarium oxysporum* f. sp. *pisi* race 1 & 5. Phytopathology. 77(12): 397 (Abstr.)

Chowdary, S. 1945. Diseases of pan (*Piper betle*) in Sylhet, Assam-V : Sclerotial wilt. Part VI. Gloeosporium leaf spot. Proceedings of Indian Academy of Science Section B. 22(3): 175-190.

Clark, C.A. 1979. Surface rot and proximal end rot of sweet potato caused by *Fusarium solani*. Phytopathology. 69: 1-A_3.

Clark, C.A. 1980. End rot, surface rot and stem lesions caused on sweet potato by *Fusarium solani*. Phytopathology. 70: 109-112.

Cooper, W.E. and B. B. Brodie. 1963. A comparison of *Fusarium* wilt indices of cotton varieties with root-knot and sting nematodes as predisposing agents. Phytopathology 53: 1077-1080.

Dastur, J.F. 1927. A short note on the foot rot diseases of pan in the central provinces. Agricultural Journal of India. 22(2): 105-108.

* Das, V.M. 1960. Studies on the nematode parasites of plants in Hyderabad (Andhra Pradesh). India. Zeitsehrift Für Parasiten Kunde. ~~Volume~~ 19: 553-605.

Davis, R.A. and W.R. Jenkins. 1963. Effects of *Meloidogyne* spp. and *Tylenchorhynchus claytoni* on pea wilt incited by *Fusarium oxysporum* f. sp. *pisi* race I. Phytopathology 53: 745 (Abstr.)

Dhande, G.W. and M. Sulaiman, 1961. Occurrence of root-knot nematodes on betelvine in Maharashtra. Current Science 30: 351-352.

Diwakar, M.C. and S.P. Kulshrestha. 1986. Common diseases of pan in India and their control. Pesticides. 20(9): 35-36.

Edington, L.V. and J.C. Walker. 1958. Influence of calcium and boron on development of Fusarium wilt of tomato. Phytopathology 48(6): 324-332.

El-Abyad, M.S. and Y. E. Saleh. 1971. Studies with Fusarium oxysporum f. sp. vasinfectum the cause of cotton wilt in Egypt. Germination, sporulation and growth. Transactions of the British Mycological Society. 57(3): 427-437.

Elad, Y.; I. Chet and J. Katan. 1980. Trichoderma harzianum ; A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia solani.. Phytopathology. 70:119-120.

Elad, Y.; I. Chet; P. Boyle and Y. Henis. 1983. Parasitism of Trichoderma spp. on Rhizoctonia solani and Sclerotium rolfsii - Scanning Electron Microscopy & Fluorescence Microscopy. Phytopathology 73: 85-88.

Emberger, G. and P. E. Nelson. 1981. Histopathology of a susceptible chrysanthemum cultivar infected with Fusarium oxysporum f. sp. chrysanthemi. Phytopathology 71: 1043-1050.

Golden, J.K. and S.D. Van Gundy. 1972. Influence of Meloidogyne incognita on root rot development by Rhizoctonia solani and Thielaviopsis basicola in tomato. Journal of Nematology. 4: 225 (Abstr.).

Gomez, K.A. and A.A. Gomez. 1983. Statistical procedures for agricultural research. A Wiley-Interscience Publication. John Wiley & Sons. pp. 641.

Goswami, B.K.; D.V. Singh ; M.L. Seth and G.L. Gupta. 1970. Studies on association of root-knot nematode Meloidogyne incognita and Sclerotium rolfsii Sacc. in brinjal (Solanum melongena) Indian Phytophatology. 23: 587-589.

Goswami, B.K.; M.L. Seth; G.L. Gupta and D.V. Singh . 1975. Inter relationship of Meloidogyne javanica and Rhizoctonia bataticola in tomato. Indian Phytopathology. 28: 387-388.

Gotlieb. A.R. and H. Doriski. 1983. Fusarium wilt of Birdsfoot-Trefoil in Vermont and Newyork. Phytopathology. 73:366.

Hooper, D.J. 1970. In Laboratory methods for work with plant and soil nematodes. Technical Bulletin number 2. (Edited by Southey, J.F.). Her Majesty's Stationary Office, London. pp. 55-56.

Hymavathi. M. 1988. Investigations into the betelvine (Piper betle) decline in Guntur distirct. M.Sc. (Ag.) thesis submitted to Andhra Pradesh Agricultural University, Rajendranagar, Hyderabad.

Jackson, M.L. 1973. Soil Chemical Analysis. Prentice-Hall of India Private Limited, New Delhi.

Jenkins, W.R. and B.W. Coursen. 1957. The effect of root-knot nematodes, Meloidogyne incognita acrita and M. hapla, on Fusarium wilt of tomato. Plant Disease Reporter. 4(1): 182-186.

Jones. J.P. & A.J. Ovrman. 1971. Control of Fusarium wilt of tomato with lime and soil fumigants. Phytopathology. 61(12): 1415-1417.

Jones, J.P. ; A.J. Overman and P. Crill. 1976. Failure of root-knot nematode to affect Fusarium wilt resistance of tomato. Phytopathology. 66: 1339-1341.

Killebrew, F.;K. Roy; G. Lawrence and K. Mclean . 1987. Occurrence and pathogenicity of Fusarium solani on soybean seedlings. Phytopathology. 77(12) : 122 (Abstr.).

Krishna Prasad, K.S.; A. L. Siddaramaiah and P.K. Hedge. 1980. Note on the association of Corticium rolfsii and Meloidogyne incognita in the wilt complex of Solanum khasianum. Indian Agricultural Journal of Science. 50: 454.

* Kudryavtseva, K.I. 1980. The possibility of using Trichoderma in the control of root rot of Cucumber in the glass house. Leningrad Academy of Agricultural Sciences. pp. 111-119.

Kuznetsov, V.D. and V. Arjuna Rao. 1972. Actinomycetes antagonistic to phytopathogenic fungi from some South Indian soils. Indian Phytopathology. 25: 307-309.

Loos, C.A. 1959. Symptom expression of Fusarium wilt disease of the Gros Michel banana in the presence of Radopholus similis (Cobb. 1893) Thorne, 1949 and Meloidogyne incognita acrita Chitwood, 1949. Proceedings of Helminthological Society, Washington. 26: 103-111.

Lucas, B.S.; T.D. Wyllie and E.W. Palm. 1988. The role of Fusarium solani in Sudden death syndrome of soybeans. Phytopathology 78(12):403.

Mahmud, K.A. 1949. Survey of foot rot of pan (Piper betle) in the central provincies. Magazine of Agricultural College, Nagpur. 24(3-4): 3-6.

Mahmud, K.A. 1952. Review of literature on Phytophthora foot rot of pan (Piper betle L.) Bulletin of Botanical Society, Bengal. 6(2): 79-88.

Mai, W.F.; H.H Lyon and T.H. Kruk. 1968. Pictorial key to genera of plant parasitic nematodes. Cornell University. Ithaca, New York.

Maiti, D. and C. Sen. 1985. Integrated biocontrol of Sclerotium rolfsii with nitrogenous fertilizers and Trichoderma harzianum. Indian Journal of Agricultural Science. 55(7): 464-468.

Maiti, S. 1989. The Betelvine. All India Co-ordinated Research project on Betelvine. Extension Bulletin. Indian Institute of Horticultural Research, Bangalore. pp 9-14.

Maiti, S. and C. Sen. 1977. Leaf-rot and foot rot of Piper betle caused by Phytophthora palmivora. Indian Phytopathology. 30(3): 438-439.

Maiti, S. and C. Sen. 1979. Fungal diseases of betelvine. Pest Articles and News Summaries. 25(2): 150-157.

Maiti, S. and C.Sen. 1982. Incidence of major diseases of betelvine in relation to weather. Indian Phytopathology. 35(1) 14-17.

Martin, J.P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Science. 69: 215-233.

Martyn, R.D.; C.M. Rush,; C.L. Biles and E.M. Barker. 1988. Fusarium oxysporum, causal agent of root rot of sugarbeets in the Texas panhandle. Phytopathology 78(12) : 15-44.

Mehrotra, R.S. and D.P. Tiwari, 1967. Studies on the foot rot and leaf rot of Piper betle L. Behaviour of Phytophthora parasitica var. piperina in soil. Indian Phytopathology. 20(2): 161-167.

Melendez, P.L. and N.T. Powell. 1967. Histological aspects of the Fusarium wilt - root knot complex in flue cured tobacco. Phytopathology 57: 286-292.

Minton, N.A. and E.B. Minton. 1963. Infection realtionship of Meloidogyne incognita and Fusarium oxysporum f. sp. vasinfectum in cotton. Phytopathology 53:6-24.

Minton, N.A. and E.B. Minton. 1966. Effect of root-knot and sting nematodes on expression of Fusarium wilt of cotton in three soils. Phytopathology 55: 319-322.

* Mirkova, E. 1983. Use of Trichoderma harzianum Rafai to control wilt of glass house carnation caused by Fusarium oxysporum Schl. f. dianthi (Prill & Del) Snyd. & Hans. Gradinarska i lozarskanauka. 20(1): 65-69.

Mishra, B.K. and B.S. Bais. 1987. Studies on Seedling blight and root rot of barley caused by Sclerotium rolfsii. Indian Phytopathology. 40: 161-167.

Narasimhan, V. and K. Ramakrishanan. 1969. Studies on wilt diseases of betelvine. Madras Agricultural Journal. 56(2):68-73.

Nayak, B.K. 1966. Studies on Sclerotium wilt of betelvine caused by Sclerotium rolfsii. M.Sc. (Ag.) thesis, Orissa University of Agriculture and Technology. Bhuvaneswar.

Newhall, A.G. 1958. The incidence of panama disease of banana in the presence of the root-knot and the burrowing nematodes (M. incognita and Radopholus similis). Plant Disease Reporter. 42:853-856.

Norton, D.C. 1960. Effect of combinations of pathogenic organisms at different temperatures on the cotton seedling diseases. Texas Agricultural Experimental Station Miscellaneous Publication pp. 412.

Oritsejafor, J.J. 1986. Influence of moisture and pH on growth and survival of F. oxysporum f. sp. claeidis in soil. Transactions of the British Mycological Society. 87: 511-517.

Papavizas, G.C.; J.A. Lewis and B. Adams. 1968. Survival of root infecting fungi in soil. II. Influence of amendment and soil C to N balance on Fusarium root rot of beans. Phytopathology 58: 365-372.

Pennypacker, B.W. and P.E. Nelson. 1972. Histopathology of carnation infected with Fusarium oxysporum f. sp. dianthi. Phytopathology. 62: 1318-1326.

Porter, D.M. and N.T. Powell. 1967. Influence of certain Meloidogyne species on Fusarium wilt development in fluecured tobacco. Phytopathlogy 57: 282-285.

Powell, N.T. 1971. Interaction of plant parasitic nematodes with other disease causing agents. In Plant Parasitic Nematodes volume 2. (Eds:Zuckerman, B.M.; W.F.Mai and R.A. Rhode) Academic Press, New york. pp. 119-136.

Ramalingam, A.; M. Anandaraj and K.V.Srinivasa Murthy. 1985. Fusarial wilt of Betelvine. Indian Journal of Botany. 8(1) : 1-8.

Raut, S.P. and V.N. Shukla. 1973. Fusarium moniliforme a potential pathogen of betelvine (Piper betle L.) wilt. Punjab Rao Krishi Vidhyanilaya Research Journal. 2(1): 59-60.

* Ribeiro, C.A. and S. Ferraz. 1984. Studies on the interaction between Meloidogyne javanica and Fusarium oxysporum f.sp phaseoli in Phasealus vulgaris. Fitopatologica Brasileria. 8: 439-446.

Rovira, A.D. 1965. Plant root exudates and their influence upon soil micro organisms. In Ecology of soil borne plant pathogens. University of California press. pp: 172-186.

Sadasivan, T.S. 1965. Effect of mineral nutrients on soil micro organisms and plant disease. In Ecology of soil borne plant pathogens. University of California press. pp. 462-470.

* Sarhan, A.R.T. 1982. The influence of soil P^H on the severity of tomato Fusarium wilt infection. Acta Phytopathologica Academae Scientiarum Hungaricae. 17: 291-294.

Sharma,. J.K.; Singh I., and Chhabra, H.K. 1980. Observations on the influence of Meloidogyne incognita and Rhizctonia bataticola on okra. Indian Journal of Nematology 10: 148-151.

Sidhu, G.S. and Webster, J.M. 1983. Horizantal resistance in tomato against the Meloidogyne-Fusarium complex : an artifact of parasitic epistasis. Crop protection. 2: 205-210.

Singh, B.P. and J.N. Chand. 1972. Assessment of losses of betelvine (Piper betle) caused by Sclerotium rolfsii in Jabalpur (M.P). (Corticium rolfsii). Science & Culture. 38(12): 526-527.

Singh, B.P. and J.N. Chand. 1973. A note on the estimation of losses of betelvine (Piper betle L.) due to Phytophthora Dastur in Jabalpur (M.P.). Science and Culture. 39(4):201-202.

Singh, B.P. and L.K. Joshi. 1972. Studies on the diseases of *Piper betle* in Jabalpur (Madhya Pradesh). Four new world records of Fusaria. Science and Culture. 38(6): 385.

Singh, D.B.; P.P. Reddy and S.R. Shrma. 1981. Effect of root-knot nematode *Meloidogyne incognita* on Fusarium wilt of French beans. Indian Journal of Nematology. 11:84-85.

Singh, R.S. and K.Sitaramiah. 1973. Control of plant parasitic neamtodes with organic amendments of soil. Experimental station Research Bulletin No.6. G.B Pant University of Agricultural and Technology. Pantnagar. pp 289.

Sitaramaiah, K. 1984. Plant Parasitc Nematodes of India. Today and Tomorrow's Publishers and Printers, New Delhi. pp. 292.

Sitaramaiah, K. and S.K. Sinha. 1984. Histological aspects of *Pseudomonas* and root-knot nematode wilt complex in brinjal. Indian Journal of Nematology. 14(2): 175-176.

Sivakumar, M. and T. Marimuthu. 1984. Parasitic nematodes associated with betelvine (*Piper betle*) in Tamil Nadu. Madras Agricultural Journal. 71(2): 108-110.

Sivan, A. and I. Chet. 1987. Possible mechanism for control of *Fusarium* species by *Trichoderma harzianum*. Pests and Diseases. 2:865-872.

Smith, A.L. and J.B. Dick. 1960. Inheritance of resistance to Fusarium wilt in upland and sea land cotton as complicated by nematodes under field conditions. Phytopathology. 50: 44-48.

Speigel, Y.; D. Netzer and V. Kafkafi. 1987. The role of calcium nutrition on Fusarium wilt syndrome in muskmelon. Journal of Phytopathology. 118(3):220-226.

Stuehling, B.A. and P.E. Nelson. 1981. Anatomy of a tolerant chrysanthemum cultivar infected with *Fusarium oxysporum* f. sp. *Chrysanthemi*. Phytopathology. 71:1162-1168.

Subramanian, C.V. 1946. Some factors affecting the growth and survival of Fusarium vasinfectum Alk. the cotton wilt pathogen in the soil, with special reference to microbiological antagonism. Journal of Indian Botanical Society. 25:89-101.

Sulladmath, V.V.; P.C. Hiremath,; K.M. Ponnapa and H.C. Govindu. 1977. Investigations on betelvine decline in Karnataka. Current Research. 6: 91-92.

Taylor, D.P. and T.D. Wyllie . 1958. Interrelationship of root-knot nematode and Rhizoctonia solani on soybean emergence. Phytopathology. 49: 552. (Abstr.).

Tessier, B.J. and W.C. Mueller. 1988. Histopathology and ultrastructure of vascular responses in peas infected with Fusarium oxysporum f. sp. pisi. Phytopathology. 78(12):130 (Abstr.).

Thomason, I.J. ;D.C. Erwin and M.J. Garber. 1959. The relationship of the root-knot nematode Meloidogyne javanica to Fusarium wilt of cowpea. Phytopathology. 49:602-606.

Tiwari, D.P. and R.S. Mehrotra 1974. Studies on foot-rot and leaf rot of Piper betle-saprophytic colonisation of dead organic substrate in soil by betelvine Phytophthora Jouranl of Indian Botanical Society. 53(1/2):119-123.

Upadhyay, J.P. and A.N. Mukhopadhyay.1986. Biological control of Sclerotium rolfsii by Trichoderma harzianum in sugarbeet. Tropical Pest Management. 32(3):215-220.

Van Der plank,J.E. 1963. In PLANT DISEASE: Epidemics and control. Academic Press. New York. pp. 124-125.

Venkateswara Rao, Z. 1987. Investigations into the mosaic disease of betelvine (Piper betle L.) M.Sc.(Ag.) thesis submitted to A.P. Agricultural University. Rajendranagar, Hyderabad.

Waksman, S.A. 1922. Microbiological analysis of soil as an index of soil fertility. 111. Influence of fertilization upon number of microorganisms in the soil. Soil Science. 14:321-346.

Wells, H[redacted]. D.;D[redacted], K. Bell and C[redacted], A.Jaworski. 1972. Efficacy of Trichoderma harzianum as a biocontrol for Sclerotium rolfsii. Phytopathology 62(4): 442-447.

Wokocha, R.C.; A.C. Ebenebe and I.D. Erinle. 1986. Biological control of basal stem rot disease of tomato caused by Sclerotium rolfsii Sacc. Curzi in Northern Nigeria. Tropical Pest Management. 32(1):55-59.

* Original not seen

APPENDICES

Anova for table 13: Fresh shoot weight

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Repli- cations	2	1.21206	0.606	0.255	3.74
Treat- ments	7	681.16450	97.3092	40.955*	2.77
Error	14	33.26	2.376	-	-
Total	23	715.633	31.114	-	-

Anova for table 13: Fresh root weight

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Repli- cations	2	0.512	0.256	1.274	3.74
Treatments	7	202.494	28.930	143.920*	2.77
Error	14	2.8163	0.201	-	-
Total	23	205.822	8.950	-	-

Anova for table 13: Dry shoot weight

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	4.1832	2.0916	2.723	3.74
Treatments	7	88.614	12.659	16.483*	2.77
Error	14	10.7528	0.768	-	-
Total	23	103.55	4.502	-	-

Anova for table 13: Dry root weight

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	0.09193	0.0459	1.078	3.74
Treatments	7	13.8023	1.9710	46.285*	2.77
Error	14	0.59687	0.0426	-	-
Total	23	14.4911	0.63004	-	-

Anova for table 13:Shoot length

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	6.0612	3.031	3.162	3.74
Treatments	7	656.24	93.748	42.134*	2.77
Error	14	31.149	2.225	-	-
Total	23	693.45	30.15	-	-

Anova for table 13:Root length

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	1.226	0.613	2.38	3.74
Treatments	7	164.522	23.503	91.097*	2.77
Error	14	3.6107	0.258	-	-
Total	23	169.3587	7.363	-	-

Anova for table 13:Root-knot index

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	0.583	0.2915	1.487	3.74
Treatments	7	63.628	9.089	46.376*	2.77
Error	14	2.747	0.196	-	-
Total	23	66.958	2.911	-	-

Anova for table 13:Root rot index

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	0.25	0.125	0.722	3.74
Treatments	7	77.83	11.120	64.280*	2.77
Error	14	2.42	0.173	-	-
Total	23	80.50	3.5	-	-

Anova for table 13:Number of larvae for one g root sample

Source	Degress of freedom	Sum of squares	Mean sum of squares	Fcalculated value	Ftable value
Replications	2	5.08	2.54	0.842	3.74
Treatments	7	2334.0	333.43	110.48 *	2.77
Error	14	42.25	3.018	-	-
Total	23	2381.33	103.54	-	-

V I T A

I, Parvathi Devi. Gopaluni, was born on 12th May, 1966 to Smt. Vasundhara Devi and Sri. Hanumantha Rao in Markapur, Prakasam district in Andhra Pradesh. I had my school education at Z.P.G. High School, Markapur and Intermediate education at S.V.K.P.College, Markapur.

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