IN VITRO INTERACTION OF THIELAVIOPSIS PARADOXA WITH MYCOFLORA OF COCONUT ROOT ZONE

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R. SANAL KUMAR

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
KASARAGOD - 671 124, KERALA, INDIA

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To

My Parents & Teachers

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R. Sanal Kumar

Dr. (Mrs.) ROHINI IYER
Scientist Selection Grade

Division of Plant Pathology,
C.P.C.R.I. (ICAR), KASARAGOD
KERALA - 671 124.

CERTIFICATE

This is to certify that the dissertation entitled ***"In vitro interaction of Thielaviopsis paradoxa with mycoflora of coconut root zone"*** *submitted in partial fulfilment for the award of the degree of* ***Master of Philosophy*** *in Bioscience (Plant Protection) of Mangalore University is a record of* ***bona fide*** *research carried out by* ***Mr. R. Sanal Kumar*** *under my guidance and supervision. No part of the dissertation has been submitted for any other degree or diploma so far.*

Dated 21st December 1990.

(Rohini Iyer)

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INTRODUCTION

1. INTRODUCTION

The coconut palm, 'Kalpa Vriksha', though hardy, is affected by a number of diseases. Of these, stem bleeding - ('Kara Olikkal' or 'Chennir Olikkal' in Malayalam; 'Raktha kariroga' in Kannada; Saru ozhugal noi in Tamil and 'Lakkaragadum' in Telugu) - is an important debilitating disease. It causes considerable economic loss and has been reported from almost all coconut growing countries (Menon and Pandalai, 1958; Ohler, 1984). All varieties of coconut are susceptible to this disease.

The disease was earlier considered to be one of uncertain etiology. However, recently, involvement of Thielaviopsis paradoxa (de Seynes) von Hohnel as the primary causative agent of this disease has been established (Nambiar et al., 1986). T. paradoxa, a soil borne organism, is a weak pathogen. This organism infects the coconut intially through growth cracks or wounds that occur at the ground level. A brownish liquid oozes from the cracks/lesions. Slowly the infection spreads upwards and laterally. In due course, these lesions coalesce and form extensive patches of discoloured tissues. The palm does not die suddenly; even though a gradual decline in the vigour causes reduction in nut yield. Later on, the stem apex gradually tapers and crown size gets reduced. Under neglected conditions the palm may succumb to the disease.

Present strategy for control of the disease includes management of the disease through phytosanitation, provision of proper drainage, alleviation of soil moisture stress and supply of adequate balanced fertilizers and organics along with 5 kg Neem cake per palm annually. Root feeding, stem injection, drenching and band application with systemic fungicides like Calixin and Bavistin tested in affected gardens at Central Plantation Crops Research Institute, Kasaragod and nearby areas have given encouraging results. However, a schedule for chemical control is yet to be formulated.

Disease development mainly depends on environment, extent of susceptability of host plant and virulence of pathogen. Out of these, environment, especially soil environment offers scope for manipulation, which can be made use of in controlling the disease. Many workers earlier (Anon, 1976) reported the presence of many other fungi along with _T. paradoxa_ from bark and soil samples of stem bleeding affected palms. Very few reports on the interaction of *T. paradoxa* with other fungi are available (Gowda, 1987; Usman, 1988). So the present study had been taken up to fill up this lacuna. *In vitro* interaction of *T. paradoxa* with other soil mycoflora of coconut root zone would help to identify the potential biocontrol agents against this fungus, which when manipulated properly might offer an efficient and environmentally safe method of control of *T. paradoxa* either singly or in combination with other measures of control. With a view to achieving the above objectives, the following programmes of work have been taken up in this study.

1. Periodic soil sampling (once in two months) from the root zone of stem bleeding affected coconut palms belonging to an ongoing stem bleeding field control trial of the following treatments.

    1.1. NPK
    1.2. NPK + Dolomite
    1.3. NPK + Dolomite + Neem cake
    1.4. Control

2. Isolation of fungi and bacteria
3. Purification of fungi
4. *In vitro* interaction of selected fungi against *Thielaviopsis paradoxa* isolate by

    4.1. Simulataneous inoculation
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REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Disease, origin and geographic distribution

Report on the origin of stem bleeding disease of coconut first came from Srilanka (Petch, 1906). Later on it was observed to occur in India (Sundararaman, 1922), Philippines (Lee, 1922), Malaysia (Sharples, 1923) Andaman Islands (Mitra, 1929), Trinidad (Briton Jones, 1940), Papua New Guinea (Dwyer, 1940), Fiji (Mc. Paul, 1962) and Indonesia (Renard *et al.*, 1984).

2.2. Symptoms

Exudation of reddish brown gummy fluid from the growth cracks in the trunk, which later turns to black on drying is the first symptom. (Petch, 1906; Sundararaman, 1922; Lee, 1922; Sharples, 1923; Mitra, 1929; Briton Jones, 1940; Salgado, 1942; John, 1952; Menon and Pandalai, 1958; Goberdhan, 1961; Radha, 1962; Mc. Paul, 1962; Ceeviyar, 1974; Thampan, 1984; Ohler, 1984; Renard *et al.*, 1984; Nambiar and Rohini Iyer, 1989; Njanadevan, 1990). These symptoms are first noticed at the base of the palm. The small irregular lesions formed traverse upwards and sometimes many lesions coalesce together forming larger patches. The external symptoms do not always reflect the extent of internal damages (Nambiar et al., 1986). The decay leaves behind only the fibrous tissue and this in later stages form cavities (Radha, 1962). However, under cooler conditions and in young palms the decay can reach deeper layers of the tissue (Nambiar et al., 1986). Attack of scoletid beetles like Diocalandra sp. and Xyleborus sp. has been found to accentuate the damage to vasculature, causing rapid deterioration of the palm (Radha, 1962).

In severe cronic cases of the disease, symptoms are also expressed on the crown. The outer whorl of leaves turn yellow and the yellowing gradually spreads to the inner whorl. The leaves droop one by one and fall prematurely. Production of nuts dwindles, followed by gradual tapering of stem apex, resulting in reduction of size of the crown, leading finally to the death of the palm (Ohler, 1984). The crown symptoms are more pronounced during summer season due to moisture stress. However, in rainy season and in well managed irrigated gardens, they are less conspicuous (Nambiar et al., 1986).

2.3. **Stem bleeding - Types**

Stem bleeding disease caused by T. paradoxa, bleeding on the base of the stem caused by *Ganoderma lucidum*, bleeding due to physiological disorders like poor drainage and prolonged drought, mild lightning attack, injury of stem due to bush fire, infestation of stem borers and damage to the stem caused by form implements during tillage etc. leads to stem bleeding (Nambiar and Kalpana Sastry, 1988). However, in this dissertation the word stem bleeding denotes the disease caused by *T. paradoxa*.

2.4. Etiology

Thielaviopsis paradoxa (de Seynes) von Hohnel - a weak pathogen - was reported to be associated with stem bleeding disease by Petch (1908). The perfect stage of the fungus is *Ceratostomella paradoxa* (de Seynes) Dade (Menon and Pandalai, 1958). The fungus was suspected to be the pathogen by many workers (Menon and Pandalai, 1958 and Ohler, 1984). However, recently involvement of *T. paradoxa* (de Seynes) von Hohnel as a primary causative agent of the disease had been established by Nambiar *et al*. (1986). Different types of stem bleeding were reported by Potty and Radhakrishnan (1978); Renard *et al*. (1984); Von Uexkull (1985); Nagarajan (1985) and Nambiar and Kalpana Sastry (1988). However, common stem bleeding disease is caused due to *T. paradoxa* infection and is widely prevalent in most of the coconut growing regions. (Nambiar *et al*., 1986).

Thielaviopsis paradoxa produces two types of spores viz : endoconidia (Phialospores) and chlamydospores. Endoconidia are initially hyaline, thin walled and rectangular later turn to oval, light brown and thick walled spores (Anil Kumar and Nambiar, 1988). T. paradoxa is pathogenic on a number of other plants like Palmyra, Arecanut, Cocoa, Sugarcane and Pineapple.

2.5. Variability in T. Paradoxa

Variability of some of the isolates of T. Paradoxa has been worked out by Gowda (1987) and Naik (1990). According to them the fungus exhibited wide variability with regard to a number of characters. Gowda (1987) noticed that the pattern of growth and rate of growth was different for different isolates of T. paradoxa in a given medium (Table - I). Variability was also noticed in the cultural and morphological characters of T. paradoxa isolates. None of the isolates grew well on Oak wilt fungus agar-c medium, while all the isolates of T.paradoxa were found to grow luxuriantly on sterile coconut rachillae. However, a few of the isolates did not show good growth on coconut tissue extract agar CTEA medium.

Some isolates of T. paradoxa produced fluffy colonies, grew faster and produced more mycelial growth. As against this another isolate of T. paradoxa had a shy

Table - 1. Growth of different isolates of *T. paradoxa* on eight culture media on the fourth day after inoculation (Gowda, 1987)

Culture medium used	Colony diameter of *T. paradoxa* isolates (Mean of four replications in cm.)					
	1	2	3	4	5	6
PDA	0.7	9.0	8.0	1.3	5.1	9.0
CMA	0.7	9.0	7.5	1.3	4.5	8.0
OMA	0.6	9.0	7.9	1.1	5.0	9.0
CTEA	0.6	9.0	9.0	1.2	4.6	5.5
OWA-A	0.6	9.0	8.0	1.1	5.5	7.0
OWA-C	0.5	0.9	0.5	0.5	0.6	0.5
DAPA	0.7	9.0	9.0	1.2	9.0	7.5
KA	0.7	9.0	9.0	1.2	7.1	7.5

PDA - Potato Dextrose Agar
CMA - Corn Meal Agar
OMA - Oat Meal Agar
CTEA - Coconut Tissue Extract Agar
OWA - A - Oak Wilt Agar - A
OWA - C - Oak Wilt Fungus Agar - C
DAPA - Dextrose Asparagine phosphate Agar
KA - Kirchoff's Agar

vegetative growth with flat colonies and produced abundant conidia and chlamydospores shortly after inoculation (Gowda, 1987).

Naik (1990) studied the effect of natural and synthetic media on the growth of different isolates of T. paradoxa isolated from coconut. Maximum growth of all isolates of T. paradoxa was obtained on almost all natural media while the growth on synthetic media was comparatively less. (Table 2 & 3). Sugarcane juice agar medium (SJA), Pineapple juice agar medium (PJA), Coconut tissue extract agar medium (CTEA) supported maximum gorwth. Among the natural media, minimum growth was observed on Oak wilt agar medium. On synthetic media, maximum growth of T. paradoxa was observed on Dextrose asparagine phosphate agar (DAPA) (Naik, 1990).

2.6. Disease indexing

For the sake of quantifying the extent of severity of the disease, and index has been developed by Jacob Mathew et al (1989) taking into account all visible symptoms of the disease. Height at which the lesion developed, lesion size, total number of leaves, number of leaves which showed yellowing and drooping, presence of tapering at trunk apex and number of bunches and nuts. According to this, the severity of stem bleeding can be quantified using the formula, 1.7 1 + 4.0 t, where '1' is

Table - 2. **Growth of _T. paradoxa_ (in cm.) on different natural media recorded after 2 days of incubation (Mean of four replicates) (Naik, 1990)**

S. No.	Description of Isolate	Different isolates of _T. paradoxa_														
		Kudlu(5)		Shiriya(6)		Adoor(7)		Vittal(8)		Kayankulam (9)		Kadoor(11)		Sagar		(12)
	Natural Media	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	Mean
1.	Sugar cane juice agar (SJA)	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0
2.	Pineapple juice agar (PJA)	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0
3.	Potato dextrose agar (PDA)	8.35	++++	8.26	++++	9.0	++++	8.5	++++	8.65	++++	8.71	++++	8.17	++++	8.61
4.	Oak wilt agar (OWA)	9.0	++++	7.81	++++	9.0	++++	9.0	++++	7.78	++++	7.62	++++	6.95	++++	7.81
5.	Coconut tissue extract agar (CTEA)	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0	++++	9.0
6.	Arecanut tissue extract agar (ATEA)	9.0	+++	9.0	+++	9.0	+++	9.0	+++	9.0	+++	9.0	+++	9.0	+++	9.0
	Mean	8.89		8.67		8.99		8.62		8.73		8.72		8.52		

Note: SG = Colony growth in cm. (average of four replicates
SP = Sporulation

		Spore count
+ :	Poor	: 1-10
++ :	Fair	: 10-50
+++ :	Good	: 50-100
++++ :	Excellent	: 100 and above

C.D. at	5% level
Isolates	: 0.299
Media	: 0.277
Isolates X Media	: 0.733

Table - 3. Growth of *T. paradoxa* (in cm.) on different synthetic media recorded after 2 days of incubation (mean of four replicates) (Naik, 1990)

S. No.	Description of Isolate	Different isolates of *T. paradoxa*														
		Kudlu(5)		Shiriya(6)		Adoor(7)		Vittal(8)		Kayamkulam (9)		Kadoor(11)		Sagar(12)		
	Natural media	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	CG	SP	Mean
1.	Dextrose asparagine phosphate agar media (DAPA)	9.0	+++	7.85	+++	9.0	+++	7.0	+++	9.0	+++	8.62	+++	8.0	+++	8.35
2.	Coon's agar media (SA)	9.0	++	7.13	++	9.0	++	6.80	++	7.35	++	7.12	++	7.02	++	7.62
3.	Czapek's sucrose nitrate agar media (CSNA)	9.0	++	7.53	++	9.0	++	8.00	++	7.62	++	6.75	++	7.25	++	7.87
4.	Richard's agar media (RA)	9.0	++	7.75	++	9.0	++	7.00	++	8.00	++	7.25	++	6.87	++	7.98
5.	Asthana - Hawkers agar media (AHA)	9.0	++	6.50	++	9.0	++	7.00	++	7.50	++	7.00	++	9.0	++	7.85
	Mean	9.0		7.35		9.0		7.36		7.89		7.34		7.62		

Note : CG = Colony growth in cm. (average of four replicates)
SP = Sporulation

		Spore count
+ :	Poor	: 1 - 10
++ :	Fair	: 10 - 50
+++ :	Good	: 50 -100
++++ :	Excellent	: 100 and above

C.D. at	5% level
Isolates	: 0.339
Synthetic Media	: 0.286
Isolated X Media	: 0.758

the lesion size in 1000 cm^2 and 't' is the score for stem tapering, ranging from 0 to 4.

2.7. Predisposing factors

Development of growth cracks, poor drainage, soil moisture stress, hard pan formation in soil, imbalanced nutrition, excessive salinity of soil, stem injury etc. are some of the various factors reported as predisposing factors responsible for the stem bleeding disease incidence. The fungus enters the stem tissues through growth cracks or wounds occurring naturally on the palms (Petch, 1908). Sudden heavy manuring (Briton Jones, 1940) or heavy rains followed by extensive dry period (Ohler, 1984) were reported be the cause of development of more growth cracks. Trash burning at the palm basin damaged the stem, opening the way for infection (Goberdhan, 1961; La Broma, 1973). Radha (1962) reported that undue fluctuations in soil reaction and moisture or ill drained soil conditions could cause severe stem bleeding and death of palms. Nambiar et al. (1989) opined that enhanced infection and decay in palms was favoured by high humidity and moderate temperature. Poor drainage and hard soil pan formation led to crippled root growth, imbalanced mineral nutrition or other physiological causes in palms (Salgado, 1942). Potty and Radhakrishnan (1978) noticed that increased levels of P, tended to increase the disease incidence. However, the application of N at fixed rate of 0.35 kg/palm reduced the disease. No significant difference was found in major nutrient content, in soil pH and electrical conductivity between healthy and diseased palms (Mathew and Ramanandan, 1980). Renard *et al.* (1984) and Von Uexkull (1985) believed that chlorine deficiency played a role in the disease incidence. Nagarajan (1985) found that excessive salinity due to high sodium content in water caused stem bleeding during summer.

2.8. Disease Cycle

2.8.1. Survival of *T. paradoxa*

Usman (1988) studied the survival of *T. paradoxa* in three different types of soil. Direct radiation, higher temperature and low moisture content reduced germination of chlamydospores in top soil upto a depth of 5 cm. Chlamydospore survival and germination was found to be low in cowdung and Neem cake ammended soil due to the enhanced growth of antagonistic mycoflora. Gowda (1987) and Usman (1988) reported that *Trichoderma species* affected the survival of *T. paradoxa* drastically.

2.9. Management of the disease

Since the pathogen is soil borne and can survive under a wide variety of conditions it becomes very difficult to treat large volumes of soil chemically to eliminate or reduce the pathogens population for than one reason. Firstly, the volume itself is too large to be treated economically and effectively. Secondly, such chemical treatments can drastically affect the non-target organisms and thus can be environmentally very unsafe. Therefore a multiproged approach to manage the disease has been evolved, phytosanitation, balanced nutrient supply, maintainence of soil moisture status, fungicide treatments in the early stages of the disease, removal of affected bark, protection of wound by the application of hot coaltar are the various components of the management practices. The chisseled tissues should be destroyed by burning to prevent the spread of the disease to other healthy palms (Radha, 1962). In areas where moisture stress is felt, irrigation should be given to alleviate the same. Proper drainage should be provided in water logged areas, where rainfall is poorly distributed. Application of recommended fertilizers namely NPK (N - 500 g/palm; P - 320g/palm and K - 1200g/palm) in two split doses along with 5kg Neem cake and control of stem borers by swabbing with BHC (0.2%). Application of systemic fungicides like Bavistin, Calixin through stem injection, root feeding, band application and drenching has been found to give encouraging results (Nambiar and Kalpana Sastry, 1988; Nambiar and Rohini Iyer 1988; Anon 1989; Radhakrishnan 1990). The systemic fungicides viz. Calixin and Bavistin at 10 to 10,000 ppm concentrations tested against *T. paradoxa* *invitro* inhibited the growth of *T. paradoxa* completely (Naik, 1990).

2.10. Effect of fertilizer and season on soil microflora

Several factors such as soil type, soil moisture, soil **pH,** temperature, fertilizer application, mode of cultivation, environmental factors, age and condition of the plants are known to influence the rhizosphere micro organisms (Rovira, 1965b; Katznelson and Rouatt, 1957; Rodica *et al*., 1969; Shetty and Rangaswami, 1970; Emmimath and Rangaswami, 1971; Subba Rao, 1977). Subba Rao (1977) opined that many experiments done to find out the effects of N, P and K additions on rhizosphere microflora did not lend themselves to any generalization since increase as well as decrease in R:S ratios had been reported as a sequel to fertilizer applications.

Nesbitt *et al*. (1979) studied the effect of organic matter in soil on soil microflora and observed that increase of organic matter led to rise in microbial population. Bopaiah and Bhat (1981) reported that the application of organic manure increased microbial population. The soil ammended with Neem cake and cowdung enhanced the population of antagonistic mycoflora (Usman, 1988). Bopaiah (1990) reported that fungi and bacteria were more in the rootzone soil as compared to the interspace soil in both coconut and arecanut. The population dynamics of bacteria, fungi and actinomycets in the rootzone soil of Areca indicated that bacterial and fungal numbers were less from March to July.

2.11. Biological control of plant pathogens

Biological control is the reduction of inoculum or disease producing activities of a pathogen in its active or dormant state, accomplished naturally or through manipulation of the environment and or host by one or more antagonists (Baker and Cook 1979). Hartley (1921) was the first person to start biological control through introduction of antagonists to the soil against damping off of coniferous seedlings. The technique was used against common scab of potato (Millard and Taylor, 1927) and against take all of wheat (Sanford and Broad foot, 1931). Komada (1988) reviewed the present status of the use of cross protection involving non-pathogenic form of a fungus in biological control programmes. Baker (1980) stated that occurence of a plant disease indicated that biological balance was not in equilibrium and so that many types of microbial interactions were involved in maintaining the biological balance.

2.11.1 Types of interactions and thier mechanisms.

Bull and Slater (1982) reported the different types of interactions that can occur between two membered populations, viz. Neutralism, Mutualism, Commensalism, Competition, Parasitism and Amensalism.

Neutralism :- The co-existence of two populations that have no effect at all on each other.

Mutualism :- Mutual benefit to the two interacting population. Synergism, Protocooperation and symbiosis are types of mutualistic interations.

Commensalism :- Benifit to one population from another that itself is unaffected.

Competition :- Competition between two populations or organisms for nutrients, space or other factors.

Parasitism :- Individual benefit to one population by harming another or feeding of one population on another.

Amensalism :- The inhibition or restriction of one population by the presence of a second population but the latter is not affected. Amensal effects occur when microorganisms produce major metabolites or antibiotics.

Competition, Amensalism and Parasitism all represent domains within a continuous spectrum of antagonistic interactions (Bull and Slater, 1982). Baker (1980) reported that biological balance of microorganisms is achieved through antagonism, 'the balance wheel of nature'. An antagonist is a mocroorganism that adversely affects the growth of another organism when they grow in mutual association (Mukhopadhyay, 1987).

2.11.2 Mechanisms involved in antagonistic interaction

Baker (1980) stated that antagonism includes competition for nutrients, favourable sites and oxygen; antibiosis by metabolites of other microorganisms; hyphal interation and parasitism or other forms of direct exploitation of a pathogen by other organisms.

2.11.2.1. Competition

Clark (1968) defined competition as the injurious effect of one organism on another because of the utilization or removal of some resources of the environment. Antagonistic ability of Trichoderma spp. may be the result of competitive action aroused by the concurrent consumption of the same energy sources in a given medium was reported by Dubos (1986). Mukhopadhyay (1987) supported this view. Blair (1943) reported the depressed growth of Rhizoctonia sp. due to nitrogen starvation of the mycelium caused by the rapid utilization of available nitrogen by cellulose - decomposing microorganisms. Marshall and Alexander (1960) noted two species of bacteria that suppressed the growth of *Fusarium oxysporum*. *f*. *cubense* with their ability to develop in the absence of aminoacids and growth factors. Park *et al*. (1988) indicated that the competitive interaction between *Pseudomonas putida* and non pathogenic isolates of *Fusarium oxysporum* resulted in the biocontrol of *Fusarium* wilt of cucumber.

2.11.2.2. **Antibiosis**

Mukhopadhyay (1987) stated that the liberation of an antibiotic substance or other chemicals formed by an antagonist may be harmful to the pathogen and results in antibiosis. Lee and Wu (1986) opined that certain *Penicillium* *sp*. exhibiting antagonistic interaction with *Sclerotina sclerotiorum* produce antibiotic substances which help in inhibiting the growth of the pathogen. Brian and Mc Gowan (1945) reported that *Trichoderma viride* produced a highly fungistatic antibiotic, 'Viridin'. Bastos *et al*. (1986) opined that an antifungal antibiotic was isolated from the culture filtrate of *cladobotryum amazonense*. The purified antibiotic inhibited the mycelial growth of *Crinipellis perniciosa* and *Phytophthora palmivora* *in vitro* and was also toxic to *P*. *palmivora* sporangia and *C*. *perniciosa* basidiospores.

2.11.2.3. Parasitism

This had been described by Boosalis (1964) as interaction between microbial populations that result in a negetive effect on the growth and survival of one. Parasitism includes hyperparasitism, mycoparsitism and direct parasitism.

2.11.2.3.1. Hyperparasitism

Microbial populations which over grow another population by absorbing nutrients and other materials from it. Boosalis (1956) reported that *Penicillium Vermiculatum* parasitising on *Rhizoctonia solani* (causing damping off disease) showed penetration pegs developed either from hyphae in direct contact with host mycelium or from mycelium coiling around the host hyphae.

2.11.2.3.2. Hyphal interaction

Henis *et al*. (1983) studied the penetration of hyphae of the *Trichoderma spp*. in sclerotia of *sclerotium rolfsii*. The hyphae of *Trichoderma* penetrated the rind and cortex, lysed the medullar tissue, produced chlamydospores inside and outside the sclerotia and finally underwent autolysis. Elad *et al*. (1987) observed the hyphal interaction of *Trichoderma harzianum* and the pathogen *Rhizoctonia solani* under scanning electron microscope (SEM) and found that the hyphae of the antagonist approach those of pathogen forming branches which grow directly towards the host. Production of hyphal coils by Trichoderma harzianum over the interaction zone was also noticed.

2.11.2.3.3. **Mycoparasitism**

Microorganisms derive nutrients from dead cells of the host, usually killed by the parasite before it invades the host. (Boosalis, 1964). Biological control experiments with the antagonistic Trichoderma, found to be a mycoparasite (Boosalis, 1964; Chet and Elad, 1982; Hadar et al., 1979; Elad et al., 1983).

Elad et al. (1981a) reported that the initial detectable interactions showed that the hyphae of the mycoparasite grew directly towards its host. The phenomenon appears to be due to the chemotrophic growth of Trichoderma towards the host (Chet and Elad, 1983).

2.11.2.3.4. **Lytic enzyme production**

Trichoderma viride produced enzymes viz. P - 1 - 3 gluconase and chitinase and was able to lyse hyphae of sclerotina sclerotiorum. This was reported by Jones and Watson (1969). Hadar et al. (1979) suggested that T. harzianum also produced the same enzymes while attacking Rhizoctonia solani cell walls and claimed that the main mechanism in the antagonism of T. harzianum against R. solani was the release of these lytic enzymes. Thin walled endoconidia produced by Thielaviopsis basicola were more subject to lysis by mycelial action than chlamydospores. (Tsao and Bricker, 1966).

2.11.2.3.5. Direct parasitism

Microorganisms directly attack other living organisms without killing them suddenly (Boosalis,1964). Gliocladium roseum exhibits direct parasitism on immature conidia and conidiophore of Helminthosporium sativum. This was reported by Barnett and Lilly (1962). Blakeman and Fokkema (1982) reported that Verticillium lecanii is a direct parasite of both insects and rusts including coffee rust Hemeleia vastatrix. Campbell (1956) found that on synthetic media, the conidia of Helminthosporium sativum were invaded and killed by Myrothecium verrucaria.

2.11.3. Interaction of antagonistic fungi with plant pathogens

2.11.3.1. Interaction of Trichoderma spp. and Gliocladium spp. with plant pathogens

Mukhopadhyay (1987) stated that the most frequently studied fungus in relation to biological control are species of *Trichoderma*. Soil borne pathogens like *Rhizoctonia solani*, *pythium spp*. and *Sclerotium rolfsii* etc. are effectively controlled by *Trichoderma spp*. (Comporota, 1985; Teyes and Dirks, 1985; Wells *et al*., 1972). Dennis and Webster (1971) described the antagonistic properties of *Trichoderma* in terms of antibiotic production. Many isolates of *Trichoderma* produce volatile and non volatile antibiotics (Bruce *et al*., 1984 and Camporota, 1985).

Nelson and Powelson (1988) reported that *Trichoderma hamatum* exhibited antagonistic interaction with *Botrytis cinerea*, causative of graymold snapbeans. Volatile compounds produced by *T. hamatum* reduced the mean radial growth of *B. cinerea* to 0.6 mm. *T. harzianum* produced volatile metabolites identified as alkyl pyrones, when added to a peat - soilmixture and these compounds suppressed *R. solani* induced damping off of lettuce was reported by Claydon *et al*. (1987). Dennis and Webster (1971) studied the interaction of *Trichoderma viride* with other fungi and found that *T. viride* produced antibiotics viz. gliotoxin and viridin while parasiting on other fungi. These antibiotics are stable only in acid solution. Acidification of citrus seed beds favoured the inhibition of Rhizoctonia solani by Trichoderma viride. Nikitina et al. (1977) investigated the effect of culture liquids of T. viride on the growth, development and infection of maize by Ustilago maydis. Antibiotics from the fungus caused degeneration and disintegration of the pathogen. T. viride and T. polysporum produced a chloroform soluble antibiotic such as trichodermin and another peptide antibiotic by T. hamatum (Dennis and Webster, 1971). Godtfredsen and Vangedal (1965) described trichodemin as least structurally complicated sequiterpenoid metabolite.

Howell and Stipanovic (1983) reported on the isolation and structure of a new toxic metabolite from Gliocladium virens, a diketopiperazine namely gliovirin, that is active against Pythium spp. Jones and Hancock (1988) found that Gliotoxin produced by Gliocladium virens inhibited the growth of Rhizoctonia solani and Pythium ultimum. Papavizas (1985) opined that Trichoderma and Gliocladium were not only good sources of various toxic metabolites and antibiotics but also various enzymes such as exo - and endoglucanases, cellobiase and chitinase. Chet and Baker (1980, 1981) reported that Trichoderma produced cellulase, β-(1-3)- glucanase and chitinase and degraded the glucans in the walls of Pythium spp. and the chitin in the walls of Rhizoctonia solani. The importance of β - (1-3) glucanase and chitinase as key enzymes responsible for fungal cell and sclerotial wall lysis and degradation was also stressed by Jones *et al.* (1974) and Elad *et al.* (1982, 1983).

Chet and Elad (1982) and Elad *et al.* (1983b) reported that biocontrol experiments with the antagonistic *Trichoderma* proved it to be a mycoparasite. Howell (1982) showed that cotton seed treatment with *Gliocladium virens* protected seedlings from damping off caused by *Rhizoctonia solani*. The antagonist parasitised hyphae of *Rhizoctonia solani in vitro* and reduced the number of viable sclerotia after three weeks in natural soil. Weindling (1934) noticed *Trichoderma lignorum*, having great potential for biocontrol, interact antagonistically by parasitizing the hyphae of other fungi *in vitro* and bringing about several morphological changes like coiling, production of haustoria, disorganisation of host cell contents and penetration of the host. Mutto *et al.* (1986) reported parasitic interaction of *Trichoderma harzianum* on the sclerotia of *sclerotium rolfsii*. *T. harzianum* grow inside the sclerotia cell walls and in the cell lumen. The cytoplasm of the host cells rapidly degenerated. The hyperparasite passed from cell to cell by lytic perforation of the walls of the pathogen. Germ tubes of medullar cells were also parasitised with wall lysis and digestion of cell cytoplasm of the pathogen by *Trichoderma harzianum*. Biles and Hill (1988) reported that a conidial suspension of *Trichoderma harzianum* reduced the

Table - 4. Other examples of successful biocontrol of plant pathoges by *Gliocladium spp.* and *Trichoderma spp.*

1	2	3	4	5	6
S. No.	*Gliocladium spp.* & *Trichoderma spp.*	Pathogens	Source of Pathogen	Reference	
1.	*Gliocladium catenulatum*	*Sclerotina sclerotiorum*	Sunflower	Karutova	(1987)
2.	*Gliocladium roseum*	*Pythium debaryanum* *Rhizoctonia solani*	Sunflower	Seasan *et al.*	(1986)
		Fusarium moniliforme & *Verticillium dahliae*	Cotton	"	
3.	*Gliocladium virens*	*Rhizoctonia solani*	Beetseed	Lewis and Papavizas	(1987)
4.	*Gliocladium virens*	Wood decomposing fungi	Trees	Bettucci *et al.*	(1988)
5.	*Gliocladium virens*	*Sclerotium rolfsii*	Grapes	Keyser and Ferreira	(1988)

	[illegible]	*Sclerotium rolfsii*	Snapbean	Papavizas and Lewis (1988)
7.	*Trichoderma aureoviride*	*Rhizoctonia solani*	Rice	Manian and Paulsamy (1987)
8.	*Trichoderma hamatum*	*Pythium aphanidermatum*	Peas Cucumber Pepper and Gysophilla	Sivan *et al*. (1984)
9.	*T. hamatum*	*Pythium ultimum* & *Fusarium solani*	Pea	Teyes and Dirks (1985)
10.	*T. hamatum*	*Rhizoctonia solani*	Beet	Lewis and Papavizas (1985)
11.	*T. hamatum*	*Corticium rolfsii*	Tomato	Wokochen *et al*. (1987)
12.	*T. hamatum*	*Sclerotina sclerotiorum*	Sunflower	Karutova (1987)

13.	*T. hamatum*	*Botrytis cinerea*	Grapevine leaves	Jailloux and Froidefond	(1987)
14.	*Trichoderma harzianum*	Hymenomycetes	Acer rubrum	Pottle *et al.*	(1977)
15.	*T. harzianum*	*Sclerotium rolfsii*	Soil	Elad *et al.*	(1980)
16.	*T. harzianum*	*Pythium aphanidermatum*	Peas, cucumber, Pepper and Gysophilla	Sivan *et al.*	(1984)
17.	*T. harzianum*	*Rhizoctonia solani*	Beet	Lewis and Papavizas	(1985)
18.	*T. harzianum*	*Fusarium oxysporum*	Cotton	Sivan and Chet	(1986)
		F. oxysporum	Melon		
		F. roseum	Wheat		
19.	*T. harzianum*	*Phythium debaryanum*	Sunflower	Sesan	(1986)
20.	*T. harzianum*	*Rosellinia neccatrix*	Apple	Freeman *et al.*	(1986)

1	2	3	4	5	6
21.	*T. harzianum*	*Corticium rolfsii*	Tomato	Wokochen *et al.*	(1986)
22.	*T. harzianum*	*Fusarium oxysporum* f.sp. *radicislycopersici*	Tomato Seeds	Sivan *et al.*	(1987)
23.	*T. harzianum*	*Gauemannomyces graminis* var. *tritici*	Wheat	Maas and Kotze	(1987)
24.	*T. harzianum*	*Rhizoctonia solani*	Cotton	Alagarsamy *et al.*	(1987)
25.	*T. harzianum*	*Botrytis cinerea*	Grape vine leaves	Jailloux and Froidefond	(1987)
26.	*T. harzianum*	*Cochliobolus sativus*	Wheat seedlings	Biles and Hill	(1988)
27.	*T.harzianum*	*Antrodia carbonica* *Lentinus lepidens* *Poria placenta* *Gleophyllum trabeum* *Coriolus versiocolar*	Trees	Murmanis *et al.*	(1988)

1	2	3	4	5	6
28.	*T. harzianum*	*Fusarium oxysporum* & *F. f.sp. melonis*	Rhizosphere soil "	Sivan and Chet	(1989)
29.	*T. harzianum*	*Sclerotium rolfsii*	Snapbean	Papavizas and Lewis	(1989)
30.	*T. harzianum*	*Phellinus weirii*	Conifers	Goldfarb *et al.*	(1989)
31.	*Trichoderma koningii*	*Nectria gallinena*	Apple	Meier	(1985)
32.	*T. koningii*	*Botrytis cinerea*	Grapevine leaves	Jailloux and Froidefond	(1987)
33.	*T. koningii*	*Botrytis cinerea*	*---	Kohl and Schlosser	(1988)
34.	*T. koningii*	*Gauemannomyces* var. *tritici*	Wheat	Simon	(1989)
35.	*Trichoderma longibrachiatum*	*Botrytis cinerea*	Grape vine leaves	Jailloux and Froide fond	(1987)

1	2	3	4	5	6
36.	*Trichoderma polysporum*	*Ceratocystis ulmi*	Elm	Webber and Hedger	(1986)
37.	*T. polysporum*	*Laccaria bicolar*	Black spruce	Summerbell	(1987)
38.	*T. polysporum*	*Gauemannomyces graminis* var. *tritici*	Wheat	Maas and Kotze	(1987)
39.	*T. polysporum*	*Antrodia carbonica* *Lentinus lepidens* *Poria placenta* *Gleophyllum trabeum* & *Corriolus versicolor*	Trees	Murmanis *et al*.	(1988)
40.	*T. polysporum*	*Phellinus weirii*	Conifers	Goldfarb *et al*.	(1989)
41.	*Trichoderma spp*.	*Fusarium oxysporum*	Watermelon	Dula and Aponyigaramvology	(1987)
42.	*Trichoderma spp*.	*Rhizoctonia solani*	Beet seed	Lewis and Papavizas	(1987)

43.	*Trichoderma spp.*	*Botrytis cinerea*	Grapes	Shimshoni *et al.* (1988)
44.	*Trichoderma sp.*	Wood decomposing fungi	Trees	Bettucci *et al.* (1988)
45.	*Trichoderma viride*	*Ceratocystis ulmi*	Elm	Ricard (1983) and Zimmerman (1985)
46.	*T. viride*	*Rhizoctonia solani*	Beet	Lewis and Papavizas (1985)
47.	*T. viride*	*Sclerotina sclerotiorum* *Pythium debaryanum* *Rhizoctonia solani*	Sunflower	Sesan *et al.* (1986)
		Fusarium moniliforme & *Verticillium dahliae*	Cotton	"
48.	*T. viride*	*Corticium rolfsii*	Tomato	Wokochen *et al.* (1986)

49.	*T. viride*	*Ceratocystis ulmi*	Elm	Webber and Hedger	(1986)
50.	*T. viride*	*Fusarium oxysporum* *Macrophomina phaseolina* *Rhizoctonia solani*	Eucalyptus	Taha *et al*.	(1987)
51.	*T. viride*	*Botrytis cinerea*	Grapevine leaves	Jailloux and Froidefond	(1987)
52.	*T. viride*	*Rhizoctonia solani*	Cotton	Algarsamy *et al*.	(1987)
53.	*T. viride*	*Laccaria bicolar*	Black Spruce	Summerbell	(1987)
54.	*T.viride*	*Rhizoctonia solani* & *Fusarium oxysporum*	Sesame seedling	Shing *et al*.	(1987)

1	2	3	4	5	6
55.	*T. viride*	*Botrytis allii* *Botrytis cinerea*	*---	Roulston and Lane	(1988)
56.	*T. viride*	*Botrytis cinerea*	*---	Kohl and Schlosser	(1988)
57.	*T. viride*	*Phellinus weirii*	Conifers	Goldfarb *et al.*	(1989)

*Crops not known

sporulation of cochliobolus sativus on excised winter wheat cultivars. Chung and Hoitink (1990) reported that the interaction of Trichoderma hamatum and thermophilic fungi in suppression of Rhizoctonia damping off in a bark compost - ammended container medium varied significantly. Efficacy of the biocontrol agent in medium ammended with compost from a 40 - 50 C zone was reduced significantly as compared with that of medium containing higher temperature composts. Damping off was suppressed by Trichoderma hamatum when the medium containing 40 - 50 C compost or compost with Humicola spp. was incubated for three or more weeks at 25° C.

2.11.3.2. Interaction of Aspergillus spp. with plant pathogens

Bailey and Garcia (1978) reported that the sporulation of Phytophtora palmivora from Cacao pods was inhibited by Aspergillus flavipes, A. flavus. oryzae and A. terreus. Frias and Garcia (1981) opined that the application of conidial suspension of A. terreus around the tree base and on the stump upto 1.8 m delayed the inhibition of Phytophthora causing black rot of cacao pods by 30 days. Gokulapalan and Nair (1984) reported that Aspergillus niger exhibits antagonistic interaction with Rhizoctonia solani (causing rice sheath blight) resulting in the inhibition of the pathogen in vitro.

Venkatasubbaiah and Safeeulla (1984) studied the interaction of Aspergillus niger with Rhizoctonia solani, the incitant of collar rot of coffee seedlings. A. niger hyperparasitised on R. solani completely in dual cultures. Seed treatment with A. niger significantly reduced the disease. Melgarejo et al. (1985) found Aspergillus flavus inhibiting the growth of Monilinia laxa on peach twings and flowers. Carkaci and Maden (1986) reported Aspergillus sp. along with other fungi to be parasitic on Sclerotinia sclerotiorum (Lib) de Bary, inhibiting the growth of the pathogen and also parasitising its sclerotia. Shukla and Kamala Uniyal (1989) studied the colony interaction between Ganoderma lucidum (Leyss). Karst and other soil fungi viz. Aspergillus niger van Tieghem, Aspergillus terreus Thom and Aspergillus fumigatus Fresenius. Of these three fungi, only Aspergillus terreus exhibited parasitic activity and penetrated into the hyphae of the pathogen and induced disintegration of clamp connections.

2.11.3.3. **Interaction of Penicillium spp. with plant pathogens.**

Boosalis (1954) reported that Rhizoctonia solani was severely parasitized by a Penicillium sp. grown on sand corn meal medium or PDA, medium containing 20g of dextrose/l. Yellow perithecia of the hyperparasite covered the surface of most isolates of *R. solani* cultures within 21 days, however the hyperparasite was not pathogenic to the host. Boosalis (1956) studied the interaction of *Penicillium vermiculatum* and *Trichoderma sp.* with the pathogenic fungus *Rhizoctonia solani* and found that the pathogen was parasitised by the two fungi. Kharchenko *et al*. (1977) studied the effect of culture liquids of *Penicillium implicatum* and other species of *Penicillium* on infection of barley by *Erysiphae graminis*. Powdery mildew of barley by *E. graminis* was reduced by treatment of barely plants with culture liquids of *Penicillium sp*. without affecting the plant growth. Frias and Garcia (1981) reported a *Penicillium sp*. as antagonistic to *Phytophthora palmivora* (Butl.) causing black rot of Cacao pods. Melgarejo and Sagasta (1986) reported that *Penicillium purpurogenum* interacted with *Monilinia laxa* in dual cultures and this resulted in the degeneration of the hyphae of *Monilinia laxa*. Utkhede and Rahe (1980) reported the production of antibiotics by *Penicillium nigricans* in culture, antagonistic to the growth of *sclerotium cepivorum* causing white rot of Onion. Decal *et al*. (1988) studied the antagonistic interaction of *Penicillium frequentans* with *Monilinia laxa* on peach twigs and found that the antagonist significantly inhibited the pathogen by the production of antibiotic substances.

2.11.3.4. Interaction of other fungi and bacteria with plant pathogens.

Mc Millan (1986) reported that foliar sprays of *verticillium lecanii* conidia at 100, 1000 or 10,000/ml effectively controlled rust (*colesporium domingense*) and associated defoliation of frangiapani (*Plumeria rubra*) in green house and field trials by their antagonistic interaction. *Verticillium dahliae* causing diseases in Artichoke plants and Olive trees was inhibited by antagonist *Talaromyces flavus* and the antagonist population increased after soil solarization (Tjamos and Paplomatas, 1987). Kim *et al*. (1988) reported that glucose oxidase, a metabolite produced in liquid culture by *Talaromyces flavus* mediated the *in vitro* inhibition of radial growth and germination of microsclerotia of *verticillium dahliae*. Spink and Rowe (1989) reported that pyrophyllite (pyrax) base pellets containing ascospores of *Talaromyces flavus*, used as ammendment in field soil acted as a potential biological control agent of *verticillium dahliae* in potato fields. Seed treatment with ascospores of *chaetomium globosum* reduced damping off of sugar beet caused by seed borne Phoma (*Pleospora*) *betae* and soil borne *Pythium ultimum* and *Rhizoctonia solani* (Walther and Gindrat, 1988).

Fedorov and Bobko (1988) studied the interaction of *Peniophora* (*phlebia*) *gigantea* and *Pleurotus ostreatus* with

Armillaria mellea, the honey fungus causing root rot of pines and found that the two interacting fungi were most promising fungi for the control of honey fungus. Jagar and Velvis (1988) reported that spraying of conidia and hyphal fragments of *verticillium biguttatum* killed sclerotia of *Rhizoctonia solani* on freshly harvested potato tubers by the mycoparasitic activity. Thompson and Wildermuth (1989) reported that VAM fungi *Glomus mosseae* exhibited antagonistic interaction with *Bipolaris sorkiniana* causing rootinfection of various crops and resulted in the reduction of disease intensity and inhibition of the pathogen. Repeated treatment of cucumber leaves with culture homogenates of the antagonist *Tilletiopsis sp*. resulted in 78-100% reduction in the production of conidia by the pathogen, *Erysiphae cichoracearum* (Knudsen and Skou, 1989)

Shin *et al*. (1987) reported that Bacillus *polymyxa* and *Streptomyces spp*. from rhizosphere soils exhibit antagonism to *Fusarium oxysporum*, causative organism of Sesame soil borne disease. Here they lysed the cell walls of hyphae and conidia of the pathogen and reduced the formation of macroconidia and chlamydospores. Jee and Kim (1987) studied the antagonistic interaction of certain bacteria and fungi against *Fusarium oxysporum* f. sp. *cucumerinum* causing Cucumber Wilt and found that *Pseudomonas fluorescens* strongly inhibited the germination of microconidia and germ tube of the pathogen on water agar.

Gordon - Lennox et al. (1987) studied the interaction of Pseudomonas sp., Pythium oligandrum and chaetomium globosum with Pythium ultimum, causative of sugarbeet diseases and found that the activity of the pathogen was controlled by all these antagonistic microorganisms. They also reported that Pseudomonas sp. and P. oligandrum protected sugarbeet against P. ultimum and Phoma (Pleospora) betae, but not against Rhizoctonia solani. However, Chaetomium globosum controlled all the three pathogens viz. Pythium ultimum, Phoma (Pleospora) betae and Rhizoctonia solani on cotton. In vitro studies revealed that Pythium oligandrum was mycoparasitic and Chaetomium globosum and Pseudomonas sp. had antibiotic activity and caused damage to the hyphae of pathogens. Joyce.E.Loper (1988) reported that Pseudomonas fluorescens from cotton rhizosphere soil protected cotton from seed colonization and pre-emergence damping off caused by Pythium ultimum by its antagonistic interaction. Becker and Cook (1988) reported that fluorescent Pseudomonads exhibit antagonistic interaction with Pythium ultimum var. sporangiferum, pathogenic to wheat cultivars and suppress the pathogen by the production of siderophores. The antagonist also exhibited plant growth promoting activity. Cho (1987) opined that antagonists like Pseudomonas cepacia, Bacillus polymyxa and another Bacillus sp. interact with the pathogen Phytophthora capsici on Capsicum annum and inhibit its growth. Brooks et al. (1988) studied the in vitro interaction of endophytic bacteria isolated from live Oaks with Oak Wilt pathogen, *Ceratocystis fagacearum* and found that the antagonist inhibited the growth of the pathogen by the production of siderophores and antibiotics. The antagonist also exhibited chitinase activity. *Pseudomonas cepacia* inhibited *Botrytis cinerea* causing gray mold and *Penicillium expansum* causing blue mold and protected Golden delicious apples and Bosc pears (Janisiewicz and Roitman, 1988). Keck (1988) reported that K - 84 strain of *Agrobacterium radiobacter* exhibited antagonism to *Agrobacterium tumefaciens* the Crown gall pathogen of Chrysanthemum plants resulting in the inhibition of the pathogen by the production of antibiotic, Agrocin - 84.

MATERIALS AND METHODS

3 . MATERIALS AND METHODS

3.1. Collection of soil samples

Soil samples were collected at sixty days intervals; during the first weeks of February, April and June from the rootzone of stem bleeding affected WCT coconut palms. They were over twenty years of age and belonged to an ongoing stem bleeding field control trial laid out at Uduma in cultivators' fields. The treatment imposed were T_1 - NPK; T_2 - NPK + Dolomite; T_3 - NPK + Dolomite + Neem cake and T_4 - Control. N was supplied as urea (500g/palm); P as Super phosphate (320g/palm) and K as muriate of Potash (1200g/palm). They were given in two split doses, one third at the onset of monsoon during May - June and two thirds during September - October every year. Dolomite at the rate of 1kg/palm/year was applied fifteen days prior to the application of first split dose. Neem cake @ 5kg/palm was applied during September along with the application of second split dose. In addition, 50kg green manure/palm was applied every year. Samples were collected from rootzones of three palms each, from T_1, T_2, T_3 and control - T_4 .

3.1.1. Method of soil sample collection

Soil samples were taken after scrapping off the surface layer of undecomposed organic matter and debris from each palm basin. Composite samples were collected by pooling four samples collected around the bole of each palm at a distance of 1 meter and from a depth of 0-25 cm and mixing them thoroughly. From this about 200g soil was drawn as sample. Clean well labelled bags were used for collecting these.

3.2. Isolation of fungi and bacteria

Whenever soil was wet it was air dried before plating. About half a gram soil measured out by a spatula prepared for the purpose was plated by 'soil plate method' (Warcup, 1950) on soil extract agar. As and when colonies appeared on the plate, they were transfered to fresh potato dextrose agar slants. These were examined later. Mixtures of fungal cultures were purified by single hyphal tip/single spore isolation and individual cultures were maintained on PDA slants. Bacterial cultures were purified by streak plate method on nutrient agar.

3.3. Identification of fungi

Some of the fungal cultures were identified based on morphological characters following Barnett (1960);

Gilman (1967) and Ainsworth, Sparrow and Sussman (1973) etc. And rest of the cultures were identified by Dr. (Mrs) Shashi Prabha Lal, scientist selection grade, Indian type culture collection, Indian Agricultural Research Institute, New Delhi - 12.

3.4. *In vitro* interaction studies of *Thielaviopsis paradoxa* with mycoflora of coconut rootzone

A fast growing isolate of *Thielaviopsis paradoxa* isolate No: 7 (Naik, 1990) was used for *in vitro* interaction studies with other soil fungi. Discs of 5 mm diameter cut from the periphery of a 48 hr growth on PDA were used. Other candidate fungi were also multiplied on PDA and discs of 5 mm diameter were cut from the periphery of a 48 hr growth and used for tests. 'Direct opposition method' as described by Webber and Hedger (1986) was used in the *in vitro* interaction studies. Two types of inoculation were followed, namely, 'simultaneous' and 'sequential (A & B)'. Sequence - A (*T. paradoxa* challenged by candidate fungus); sequence - B (candidate fungus challenged by *T. paradoxa*).

In the case of simultaneous inoculation the two discs were placed 3.5 cm apart, the fungal growth touching the medium. PDA was used for these tests and the petridishes were incubated at $27 \pm 2^{\circ}$ C in an incubator and were examined after 24 hrs, 48 hrs, 72 hrs, 168 hrs (7 days) and 360 hrs (15 days). In the case of sequential inoculation one of the organisms (*T. paradoxa* or the candidate fungus) was inoculated first and allowed to grow for 12 hrs. The challenging organism was then inoculated at a distance of 3.5 cm on Petri Plates of 8 cm diameter which were then icubated at 27 ± 2 °C. Observations were recorded as in the previous case. This included the growth of *T. paradoxa* towards the test fungus *vis a vis* in the opposite direction, i.e. Control. These measurments were used to find the percentage of inhibition of *T. paradoxa* by each candidate fungus.

3.5. Study of population dynamics of *T. paradoxa*

It was considered necessary to have an idea of the population dynamics of *T.paradoxa* also under the same set of conditions. For this, 20g of air dried sieved soil (through a sieve of mesh size 710 µm) was taken in a sterile petridish of 100mm dia. It was wetted with 2ml sterile water. Coconut rachillae bits of 1cm size were sterilized under 6kg (15 lb) pressure for 15 minutes. Each plate of soil was seeded with 25 bits of rachillae. Petridishes were incubated at room temperature for 15 days. Each bit was crushed, stained with trypan blue (0.5%) and examined for the presence of *T. paradoxa* conidia, chlamydospores and mycelium. Results were expressed as percentage of colonization of rachillae bits by *T.paradoxa.*

RESULTS

4. RESULTS

4.1. Isolation of fungi and bacteria from stem bleeding affected coconut palm root zone.

Soil samples from the basins of stem bleeding affected palms were plated seperately in soil extract agar as detailed in 'Materials and Methods'. The data is presented in Tables - 5 & 6.

In Table - 5a, the data revelas that the fungal population varied significantly depending on the month (period) of sampling. Among the three periods, namely February, April and June, maximum fungal population was in February and least in June. However, among treatments namely, NPK; NPK + Dolomite; NPK + Dolomite + Neem cake, there was no significant difference with regard to the total fungal population. A significant difference existed among the three periods and treatments with regard to the bacterial population (Table - 5b). When the month of sampling were considered, the maximum bacterial population was observed during February. Bacterial population was

Table - 5. Effect of seasons and fertilizer treatments on fungal and bacterial population in stem bleeding affected coconut soils.

Treatment	Palm No:	FEBRUARY		APRIL		JUNE	
		Total fungi/g soil	Total bacteria /g soil	Total fungi/g soil	Total bacteria /g soil	Total fungi/g soil	Total bacteria /g soil
Control	114	77	276	33	323	30	249
	115	67	301	58	332	33	198
	121	76	315	50	313	35	256
Mean		73	297	47	323	33	234
NPK	42	111	337	61	355	36	72
	44	73	406	57	351	53	107
	58	66	283	71	342	29	178
Mean		83	342	63	349	39	119
NPK+Dolomite	33	82	393	76	297	35	164
	34	60	279	57	305	57	175
	35	50	394	48	276	31	171
Mean		64	355	60	293	41	170
NPK+Dolomite + Neem cake	97	50	369	56	330	51	251
	98	79	461	63	332	52	228
	99	89	495	55	316	65	195
Mean		73	442	58	326	56	225

Table -5a Table of means; Effect of seasons and fertilizer treatments on fungal population in stem bleeding affected coconut soils.

Treatment	Time of sampling			Mean
	FEBRUARY	APRIL	JUNE	
	Total fungi/g soil	Total fungi/g soil	Total fungi/g soil	
Control	73.33	47.00	32.67	51.00
NPK	83.33	63.00	39.33	61.89
NPK + Dolomite	64.00	60.33	41.00	55.11
NPK + Dolomite+ Neem cake	72.67	58.00	56.00	62.22
Mean	73.33	57.08	42.25	

S.E./plot - 13.38 Gen. Mean - 57.56 C.V.(%) - 23.25

C.D. (P = 0.05) for period - 11.276

Table -5b Table of means; Effect of seasons and fertilizer treatments on bacterial population in stem bleeding affected coconut soils.

Treatment	Time of sampling			Mean
	FEBRUARY	APRIL	JUNE	
	Total bacteria /g soil	Total bacteria /g soil	Total bacteria /g soil	
Control	297.33	322.67	234.33	284.78
NPK	342.00	349.33	119.00	270.11
NPK + Dolomite	355.33	292.67	170.00	272.67
NPK + Dolomite + Neem Cake	441.67	326.00	224.67	330.78
Mean	359.08	322.67	187.00	

S.E/plot - 38.72 Gen. Mean - 289.58 C.V. (%)-13.37

C.D. (P = 0.05) for treatments - 37.672, C.D. (P = 0.05) for period - 32.625

C.D. (P = 0.05) for T X P - 65.249

Table - 6 Effect of seasons and fertilizer treatments on antagonistic fungi population in stem bleeding affected coconut soils.

Treatment	Palm No.	FEBRUARY	APRIL	JUNE
		Antagonistic fungi/g soil	Antagonistic fungi/g soil	Antagonistic fungi/g soil
Control	114	8	6	4
	115	6	6	6
	121	8	8	8
Mean		7	7	6
NPK	42	8	6	2
	44	4	4	4
	58	4	4	4
Mean		5	5	3
NPK + Dolomite	33	8	6	6
	34	10	14	4
	35	8	4	10
Mean		9	8	7
NPK + Dolomite Neem cake	97	8	12	12
	98	10	8	6
	99	12	6	6
Mean		10	9	8

significantly high in NPK + Dolomite + Neem cake treatment compared to that of others viz. NPK and NPK + Dolomite.

The data presented in table - 6a showed that a significant difference existed between treatments with regard to the antagonistic fungal population. The antagonistic fungal population was found to be maximum in NPK + Dolomite + Neem cake treatment; whereas, the minimum population was seen in NPK treatment. Attempts were made to understand the population dynamics of _T_. _paradoxa_ in these soils by using a bait technique. Percent colonization of bits by _T_. _paradoxa_ from the various treatments is given in Table - 7. Results indicate that throughout the period under study NPK + Dolomite + Neem cake registered the least amount of colonization (3.33%) which was followed by NPK + Dolomite (20%). There was no difference between the control and NPK which registered the highest degree of colonization (28 and 31 percent respectively).

4.2. Identification of fungi

The fungi isolated from palm basin soil were identified by observing specimens stained in cotton blue - lactophenol under the microscope. For identification standard text books and keys mentioned in 'Materials and Methods' were used. Some of the cultures were identified through the courtesy of Indian type culture collection

Table - 6a Table of means; Effect of seasons and fertilizer treatments on antagonistic fungal population in stem bleeding affected coconut soils.

Treatment	Time of sampling			
	FEBRUARY	APRIL	JUNE	Mean
	Antagonistic fungi/g soil	Antagonistic fungi/g soil	Antagonistic fungi/g soil	
Control	7.33	6.67	6.00	6.67
NPK	5.33	4.67	3.33	4.44
NPK + Dolomite	8.67	8.00	6.67	7.78
NPK + Dolomite+ Neem cake	10.00	8.67	8.00	8.89
Mean	7.83	7.00	6.00	

S.E./Plot - 2.56　　Gen. Mean - 6.94　　C.V.(%) - 36.87

C.D. (P = 0.05) for Treatments - 2.491

Table - 7 Table of means; Effect of seasons and fertilizer treatments on percentage colonization of baits by *T. paradoxa* in stem bleeding affected coconut soils.

Treatments	Time of sampling			Mean
	FEBRUARY	APRIL	JUNE	
	% colonization of baits by *T.paradoxa*	% colonization of baits by *T.paradoxa*	% colonization of baits by *T.paradoxa*	
Control	26.00	28.00	31.00	28.33
NPK	29.00	35.00	30.00	31.33
NPK + Dolomite	19.00	20.00	21.00	20.00
NPK + Dolomite + Neem cake	4.00	3.00	3.00	3.33
Mean	19.50	21.50	21.25	

S.E./plot - 5.13 Gen. Mean - 20.75 C.V. (%) - 24.73

C.D. (P = 0·05) for treatments - 4.251

Indian Agricultural Research Institute, New Delhi - 12. Fungi identified are presented in Table - 8.

4.3. *In vitro* interaction of *T. paradoxa* with thirteen fungi isolated from coconut root zone.

Thirteen fungi isolated from the root zone of stem bleeding affected palms viz. *Trichoderma koningii* (A1); *Paecilomyces sp.* (A2); *Trichoderma viride* - 1 (A3); *Trichoderma viride* - 2 (A4); *Rhizopus stolonifer* (A5); *Absidia sp*. (A6); *Thielavia terricola* (A7); *Penicillium pinophilum* (A8); *Aspergillus fumigatus* (A9); *Aspergillus terreus* (A10); *Gongronella butleri* (A11); *Fusarium solani* (A12); *Cunninghamella elegans* (A13) were used in the *in vitro* interaction with *T. paradoxa* by direct opposition method. Two methods of inoculation, as described in 'Materials and Methods' were followed. Reactions of the fungi showing antagonism towards *T. paradoxa* are summarised in Tables.

Of the three methods of inoculation tested, maximum percentage of inhibition was observed in sequential inoculation; sequence - B (candidate fungi challenged by *T. paradoxa* later) technique compared to the other two methods (Table - 9). Based on the results recorded after fifteen days of inoculation of the thirteen fungi tested (Table - 15), five fungi viz. *Trichoderma koningii* (A1);

Table - 8 List of fungi isolated from the rootzone soils of stem bleeding affected palms at Uduma.

1	2	3	4	5	6
S.NO.	Treatment	Seasons	Fungus	No.	Remarks
1.	Control	FEBRUARY	*Absidia sp.*	7	I (intermingling)
			Aspergillus fumigatus	4	MC (mutual inhibition)
			Aspergillus niger	5	not tested
			Curvularia sp.	4	not tested
			Gongronella butleri	9	I
			Paecilomyces sp.	8	I
			Penicillium pinophilum	8	I
			Penicillium verruculosum	7	not tested
			Pythium sp.	6	not tested
			Rhizopus stolonifer	5	O (overgrowth)
			Thielavia terricola	4	O
			Trichoderma viride	4	O

"	Control	APRIL	*Absidia sp.*	10	I
			Aspergillus fumigatus	6	MC
			Cunninghamella elegans	9	I
			Paecilomyces sp.	11	I
			Penicillium verruculosum	5	not tested
			Penicillium pinophilum	4	I
			Pythium sp.	10	not tested
			Rhizopus stolonifer	5	O
			Thielavia terricola	5	O
			Trichoderma viride	4	O
"	"	JUNE	*Absidia sp.*	8	I
			Aspergillus fumigatus	4	MC
			Cunninghamella elegans	5	I
			Paecilomyces sp.	2	I
			Penicillium verruculosum	4	not tested
			Penicillium pinophilum	2	I
			Pythium sp.	10	not tested
			Rhizopus stolonifer	5	O
			Thielavia terricola	5	O
			Trichoderma viride	4	O

2	NPK	FEBRUARY	Absidia sp.		
			Aspergillus fumigatus	4	MC
			Cunninghamella elegans	11	I
			Gongronella butleri	8	I
			Penicillium pinophilum	8	I
			Penicillium verruculosum	9	not tested
			Pythium sp.	6	not tested
			Rhizopus stolonifer	4	O
			Thielavia terricola	3	O
			Trichoderma viride	5	O
"	"	APRIL	Absidia sp.	9	I
			Aspergillus fumigatus	3	MC
			Aspergillus niger	2	not tested
			Aspergillus terreus	1	MC
			Cunninghamella elegans	7	I
			Fusarium solani	2	MC
			Gongronella butleri	8	I
			Penicillium pinophilum	5	I
			Penicillium verruculosum	3	not tested
			Pythium sp.	7	not tested
			Rhizopus stolonifer	3	O
			Syncephalastrum racemosum	1	not tested
			Trichoderma viride	3	O

"	NPK	JUNE	Absidia sp.	4	I
			Circinella sp.	7	not tested
			Cunninghamella elegans	8	I
			Gongronella butleri	5	I
			Paecilomyces sp.	4	I
			Penicillium pinophilum	4	I
			Penicillium verruculosum	2	not tested
			Pythium sp.	8	not tested
			Rhizopus stolonifer	5	O
			Thielavia terricola	3	O
			Trichoderma viride	2	O
3	NPK + Dolomite	FEBRUARY	Absidia sp.	8	I
			Aspergillus fumigatus	6	MC
			Aspergillus niger	5	not tested
			Circinella sp.	6	not tested
			Cunninghamella elegans	9	I
			Gongronella butleri	8	I
			Penicillium pinophilum	7	I
			Pythium sp.	6	not tested
			Rhizopus stolonifer	5	O
			Thielavia terricola	3	O
			Trichoderma viride	7	O

3	NPK + Dolomite	APRIL	*Absidia sp.*	6	I
			Aspergillus flavipes	3	not tested
			Aspergillus fumigatus	6	MC
			Aspergillus terreus	3	MC
			Cunninghamella elegans	5	I
			Gongronella butleri	9	I
			Paecilomyces sp.	6	I
			Penicillium pinophilum	8	I
			Pythium sp.	6	not tested
			Rhizopus stolonifer	3	O
			Thielavia terricola	5	O
			Trichoderma viride	4	O
"	"	JUNE	*Absidia sp.*	8	I
			Aspergillus fumigatus	3	MC
			Cunninghamella elegans	5	I
			Curvularia sp.	4	not tested
			Fusarium solani	4	MC
			Paecilomyces sp.	6	I
			Penicillium pinophilum	7	I
			Pythium sp.	9	I
			Rhizopus stolonifer	5	O
			Thielavia terricola	5	O
			Trichoderma viride	3	O

1	2	3	4	5	6
4	NPK + Dolomite + Neem cake	FEBRUARY	Absidia sp.	9	I
			Aspergillus fumigatus	8	MC
			Aspergillus niger	5	not tested
			Circinella sp.	3	not tested
			Cunninghamella elegans	7	I
			Curvularia sp.	4	not tested
			Gongronella butleri	8	I
			Paecilomyces sp.	2	I
			Penicillium pinophilum	3	I
			Penicillium verruculosum	3	not tested
			Pythium sp.	4	not tested
			Rhizopus stolonifer	6	O
			Thielaviopsis paradoxa	1	pathogen of stem bleeding disease of coconut
			Thielavia terricola	3	O
			Trichoderma koningii	3	O
			Trichoderma viride	5	O
"	"	APRIL	Absidia sp.	8	I
			Aspergillus fumigatus	4	MC
			Aspergillus niger	2	not tested

1	2	3	4	5	6
4	NPK + Dolomite + Neem cake	APRIL	*Circinella sp.*	4	not tested
			Cunninghamella elegans	6	I
			Curvularia sp.	5	not tested
			Paecilomyces sp.	3	I
			Pythium sp.	8	not tested
			Rhizopus stolonifer	6	O
			Thielavia terricola	5	O
			Trichoderma koningii	2	O
			Trichoderma viride	7	O
"	"	JUNE	*Absidia sp.*	7	I
			Aspergillus fumigatus	3	MC
			Aspergillus niger	2	not tested
			Circinella sp.	7	not tested
			Cunninghamella elegans	6	I
			Curvularia sp.	3	not tested
			Fusarium solani	4	MC
			Gongronella butleri	9	I
			Pythium sp.	8	not tested
			Rhizopus stolonifer	2	O
			Thielavia terricola	6	O
			Trichoderma koningii	3	O
			Trichoderma viride	4	O

Table - 9 Percentage of inhibition of *T. paradoxa* (24 hrs after inoculation)

fungi tested	Sequence - A inoculation	Simultaneous inoculation	Sequence - B inoculation
Trichoderma koningii (A1)	4.7	9.0	20.0
Trichoderma viride - 1 (A3)	10.0	19.0	33.3
Trichoderma viride - 2 (A4)	10.0	20.0	42.8
Rhizopus stolonifer (A5)	9.0	14.2	28.5

Trichoderma viride - 1 (A3); Trichoderma viride - 2 (A4); Rhizopus stolonifer (A5); Thielavia terricola (A7) had showed inhibitory effect on T. paradoxa. Mutual inhibition was observed in the case of Aspergillus fumigatus (A9); Aspergillus terreus (A10) and Fusarium solani (A12). The remaining five fungi viz. Paecilomyces sp. (A2); Absidia sp. (A6); Penicillium pinophilum (A8); Gongronella butleri (A11) and Cunninghamella elegans (A13) did not show any inhibitory effect on T. paradoxa. They intermingled freely with T. paradoxa.

Maximum inhibition of T. paradoxa was observed with Trichoderma viride - 2 (A4) (10.0%, 20.0% and 42.8%). Whereas the least inhibition of T. paradoxa was observed with Trichoderma koningii (A1) (4.7%, 9% and 20%), (Table - 9). Thielavia terricola (A7) showed inhibitory effect only after 48 hrs; unlike Trichodermas and Rhizopus who exhibited in 24 hrs. Details of interaction of the five fungi showing inhibitory effect on T. paradoxa and three fungi exhibiting mutual inhibition with the pathogen are given below.

4.3.1. In vitro interaction of T. paradoxa with Trichoderma koningii (A1).

Based on observation taken at 24 hrs after inoculation, the percentage inhibition on T. paradoxa obtained in the three methods of inoculation was 4.7% in sequence - A, 9% in simultaneous and 20% in sequence - B.

(Table - 10) (Plates - 1,2, & 3; Fig. - TP x A1). A feeble band formation (2mm width) was seen at the line of contact of both the organisms in simultaneous and sequence - B inoculation, whereas no band formation was observed in sequence - A inoculation after 48 hrs (Table - 11) (Plates - 5,6 & 7; Fig. - TP x A1). *Trichoderma koningii* was completely surrounded by *T. paradoxa* in sequence - A inoculation. However, the band width increased in simultaneous and sequence - B inoculation after 72 hrs (Table - 12) (Plates - 9,10 & 11; Fig. - TP x A1). After 168 hrs (7 days), radial growth towards the two interacting fungi remained the same as that of at 72 hrs. However, the control side of *Trichoderma koningii* (A1) increased in simultaneous and sequence - B inoculation (Table - 13). *Trichoderma koningii* also started to produce scattered spore masses (after 168 hrs) here and there on *T. paradoxa*, whereas no fructification was seen in sequence - A inoculation. Spore masses of *Trichoderma koningii* (A1) spread over *T. paradoxa* like a mat in simultaneous inoculation and fructification of A1 began to spread over *T. paradoxa* in sequence - B after 360 hrs (15 days) (Plates - 13,14 & 15; Fig. - TP x A1). In simultaneous and sequence - B inoculations, growth of *Trichoderma koningii* (A1) increased, whereas it remained the same as that of 168 hrs (7 days) in sequence - A inoculation (Table - 14). Therefore this type of interaction was included in the 'O' type interaction category (Table - 15).

Table 10 In vitro interaction of T paradoxa with fungi of coconut root zone (Observations taken 24 hrs. after inoculation).

Fungi tested	Sequence - A inoculation						Simultaneous inoculation						Sequence - B inoculation					
	X Colony radius of T.paradoxa		% of inhibition of T. paradoxa	X Colony radius of candidate fungus		% of inhibition of Candidate fungus	X Colony radius of T.paradoxa		% of inhibition of T. paradoxa	X Colony radius of Candidate fungus		% of inhibition of candidate fungus	X Colony radius of T.paradoxa		% of inhibition of T. paradoxa	X Colony radius of candidate fungus		% of inhibition of candidate fungus
	Radius towards candidate fungus	Control		Radius towards T.paradoxa	Control		Radius towards candidate fungus	Control		Radius towards T.paradoxa	Control		Radius towards candidate fungus	Control		Radius towards T.paradoxa	Control	
	(mm)	(mm)		(mm)	(mm)		(mm)	(mm)		(mm)	(mm)		(mm)	(mm)		(mm)	(mm)	
Trichoderma koningii A1	20	21	4.7	5	5	0	20	22	9.0	8	8	0	4	5	20.0	9	9	0
Trichoderma viride - 1 A3	19	21	10.0	6	6	0	17	21	19.0	9	9	0	4	6	33.3	11	11	0
Trichoderma viride - 2 A4	19	21	10.0	6	6	0	16	20	20.0	10	10	0	4	7	42.8	12	12	0
Rhizopus Stolonifer A5	20	22	9.0	6	6	0	18	21	14.2	9	9	0	5	7	28.5	10	10	0
Thielavia terricola A7	21	21	0.0	4	4	0	21	21	0.0	7	7	0	6	6	0.0	9	9	0
Aspergillus fumigatus A9	21	21	0.0	4	4	0	20	20	0.0	5	5	0	6	6	0.0	5	5	0
Aspergillus terreus A10	21	21	0.0	3	3	0	20	20	0.0	4	4	0	6	6	0.0	4	4	0
Fusarium Solani A12	22	22	0.0	3	3	0	21	21	0.0	4	4	0	6	6	0.0	4	4	0

Sequence - A inoculation : T. paradoxa inoculated 12 hrs before the inoculation of candidate fungus
Simultaneous inoculation :: T. paradoxa and candidate fungus were simultaneously inoculated
Sequence - B inoculation : T. paradoxa was inoculated after 12 hrs growth of the candidate fungus.
X : Mean of three replicates
Control : Radius vertically away from the opposing colony towards the side of the petridish.

Table - II In vitro interaction of T. paradoxa with fungi of coconut root zone. (Observations taken 48 hrs after inoculation)

Fungi tested	Sequence - A inoculation				Simultaneous inoculation				Sequence - B inoculation			
	Colony radius of *T. paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T. paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T. paradoxa*		X Colony radius of candidate fungus	
	Radius towards candidate fungus	Control	Radius towards *T. paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T. paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T. paradoxa*	Control
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
Trichoderma koningii A1	28	>23	7	7	25	>23	10	10	17	21	18	18
Trichoderma viride - 1 A3	23	>23	12	12	16	>23	18	18	12	21	23	23
Trichoderma viride 2 A3	23	>23	12	12	15	>23	19	19	11	21	24	>23
Rhizopus Stolonifer A5	25	>23	9	9	18	>23	15	15	15	21	20	20
Thielavia terricola A7	30	>23	5	5	25	>23	9	9	22	23	12	12
Aspergillus fumigatus A9	29	>23	5	5	28	>23	7	7	22	23	9	9
Aspergillus terreus A10	30	>23	5	5	27	>23	5	5	26	>23	7	7
Fusarium solani A12	30	>23	4	4	29	>23	6	6	26	23	7	7

Sequence A inoculation : *T paradoxa* inoculated 12 hrs before the inoculation of the candidate fungus
Simultaneous inoculation : *T paradoxa* and candidate fungus were simultaneously inoculated
Sequence B inoculation : *T paradoxa* was inoculated after 12 hrs growth of the candidate fungus
X : Mean of three replicates
Control : Radius vertically away from the opposing colony towards the side of the petridish.

In vitro interaction of *T.paradoxa* with fungi of coconut root zone. (Observations taken 72 hrs after inoculation)

Fungi tested	Sequence - A inoculation				Simultaneous inoculation				Sequence - B inoculation			
	X Colony radius of *T.paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T.paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T.paradoxa*		X Colony radius of candiate fungus	
	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
Trichoderma koningii A1	28	>23	7	9	25	>23	10	15	17	>23	18	20
Trichoderma viride - 1 A3	22	>23	13	13	16	>23	18	18	12	>23	23	23
Trichoderma viride - 2 A4	22	>23	13	13	15	>23	19	19	11	>23	24	>23
Rhizopus stolonifer A5	25	>23	10	10	18	>23	15	15	15	>23	20	23
Thielavia terricola A7	30	>23	5	7	25	>23	10	10	22	>23	12	14
Aspergillus fumigatus A9	30	>23	6	6	27	>23	8	8	22	>23	10	10
Aspergillus terreus A10	30	>23	5	8	28	>23	6	6	26	>23	7	9
Fusarium solani A12	30	>23	4	7	29	>23	6	8	26	>23	8	8

Sequence A inoculation : *T.paradoxa* inoculated 12 hrs before the inoculation of candidate fungus

Simulataneous inoculation : *T. paradoxa* and candidate fungus were simultaneously inoculated

Sequence - B inoculation : *T. paradoxa* was inoculated after 12 hrs growth of candidate fungus.

X : Mean of three replicates

Control : Radius vertically away from the opposing colony towards the side of the petridish.

Table - 13. *In vitro* interaction of *T. paradoxa* with Fungi of Coconut root zone. (Observation taken 168 hrs.(7 days) after inoculation)

Fungi tested	Sequence - A inoculation				Simultaneous inoculation				Sequence - B inoculation			
	X Colony radius of *T.paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T.paradoxa*		X Colony radius of candidate fungus		X Colony radius of *T.paradoxa*		X Colony radius of candiate fungus	
	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control	Radius towards candidate fungus	Control	Radius towards *T.paradoxa*	Control
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)
Trichoderma koningii A1	28	>23	7	9	25	>23	10	23	17	>23	18	23
Trichoderma viride - 1 A3	22	>23	13	21	15	>23	19	23	12	23	23	>23
Trichoderma viride - 2 A4	22	>23	13	20	14	>23	20	>23	10	>23	25	>23
Rhizopus stolonifer A5	25	>23	10	20	17	>23	16	20	15	>23	20	>23
Thielavia terricola A7	30	>23	5	7	25	23	10	12	22	23	13	15
Aspergillus fumigatus A9	30	>23	6	6	27	>23	8	10	22	>23	12	12
Aspergillus terreus A10	30	>23	5	8	28	>23	6	8	27	>23	7	9
Fusarium solani A12	30	>23	4	12	29	>23	6	9	26	>23	8	10

Sequence - A inoculation : *T.paradoxa* inoculated 12 hrs before the inoculation of candidate fungus
Simulataneous inoculation : *T. paradoxa* and candidate fungus were simultaneously inoculated
Sequence - B inoculation : *T. paradoxa* was inoculated after 12 hrs growth of candidate fungus.
X : Mean of three replicates
Control : Radius vertically away from the opposing colony towards the side of the petridish.

Table - 14. *In vitro* interaction of *T. paradoxa* with Fungi of Coconut root zone. (Observations taken 15 days after inoculation)

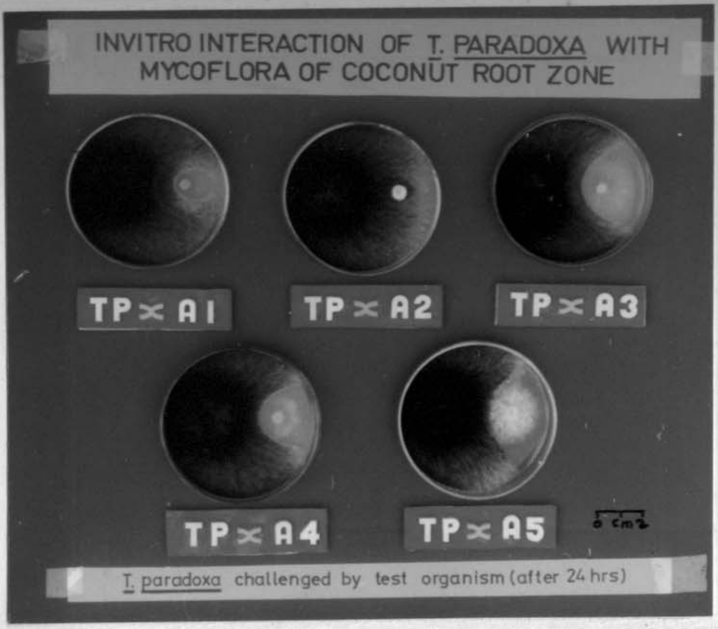
Fungi tested	Sequence - A inoculation				Simultaneous inoculation				Sequence - B inoculation				Interaction Category
	ˣColony radius of T. paradoxa		ˣColony radius of candidate fungus		ˣColony radius of T. paradoxa		ˣColony radius of candidate fungus		ˣColony radius of T. paradoxa		ˣColony radius of candidate fungus		
	Radius towards candidate fungus	Control	Radius towards T. paradoxa	Control	Radius towards candidate fungus	Control	Radius towards T. paradoxa	Control	Radius towards candidate fungus	Control	Radius towards T. paradoxa	Control	
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	
Trichoderma koningii A1	28	>23	7	9	25	>23	10	>23	17	>23	19	>23	O
Trichoderma viride - 1 A3	22	>23	13	21	15	>23	19	>23	12	>23	23	>23	O
Trichoderma viride - 2 A4	22	>23	13	20	14	>23	20	>23	10	>23	25	>23	O
Rhizopus stolonifer A5	25	>23	10	22	17	>23	16	20	15	>23	20	>23	O
Thielavia terricola A7	30	>23	5	7	25	>23	10	14	22	>23	13	16	O
Aspergillus fumigatus A9	30	>23	6	6	27	>23	8	10	22	>23	12	15	MC
Aspergillus terreus A10	30	>23	5	8	28	>23	6	9	27	>23	7	11	MC
Fusarium solani A12	30	>23	4	12	29	>23	6	9	26	>23	9	11	MC

Sequence - A inoculation	:	*T. paradoxa* inoculated 12 hrs before the inoculation of candidate fungus
Simultaneous inoculation	:	*T. paradoxa* and candidate fungus were simultaneously inoculated
Sequence - B inoculation	:	*T. paradoxa* was inoculated after 12 hrs growth of candidate fungus.
x	:	Mean of three replicates
Control	:	Radius vertically away from the opposing colony towards the side of the petridish.

Table - 15 In vitro interaction of T. paradoxa with fungi of coconut root zone (Observations taken 15 days after inoculation; Interaction category).

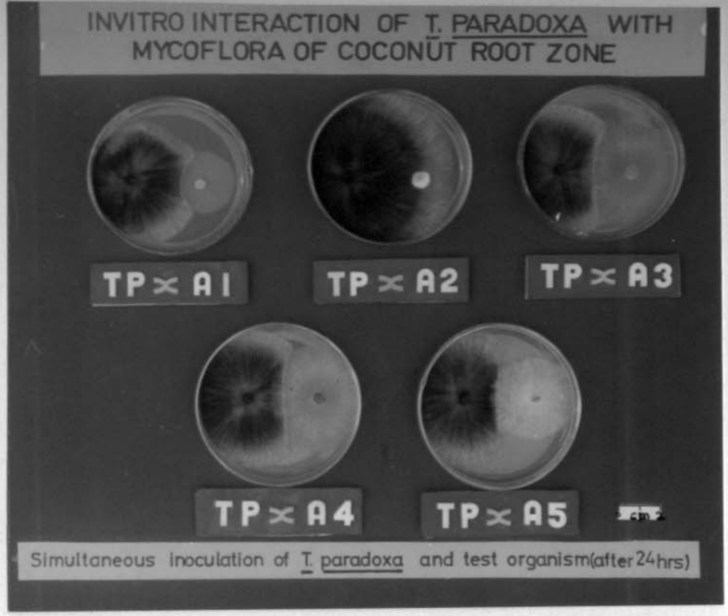
S.NO.	Category	Interaction	Fungi showing interaction with T.paradoxa.
1.	O	Overgrowth of T.paradoxa colony by candidate fungi usually accompanied by inhibition of T. paradoxa on or shortly after contact.	Rhizopus stolonifer, Thielavia terricola, Trichoderma koningii, Trichoderma viride (1&2).
2.	MC	Mutual inhibition of both the colonies with no apparent intermingling, a clearzone remaining between the colonies.	Aspergillus fumigatus, Aspergillus terreus, Fusariaum solani.
3.	I	Intermingling of colonies with no microscopic or macroscopic signs of interaction.	Absidia sp., Cunninghamella elegans, Gongronella butleri Paecilomyces sp., Penicillium pinophilum.

Plates - 1. Sequence - A inoculation

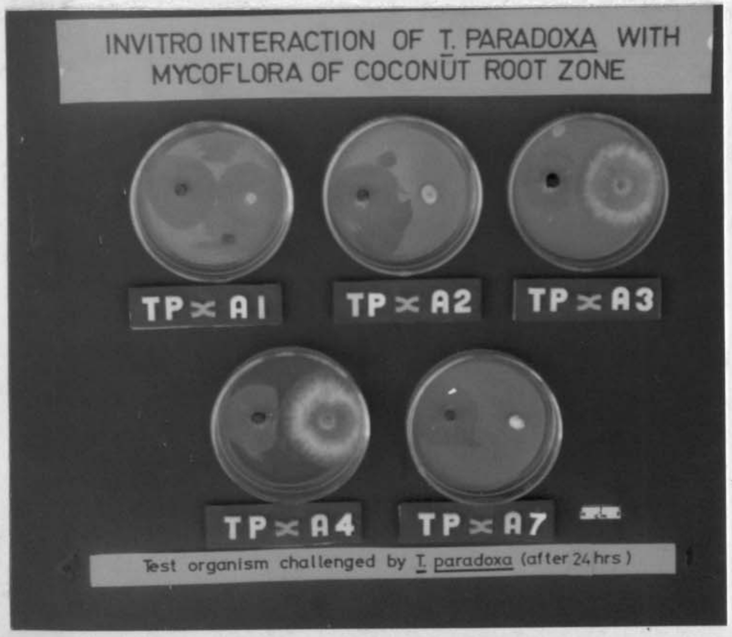


T. paradoxa challenged by test organism (after 24 hrs)

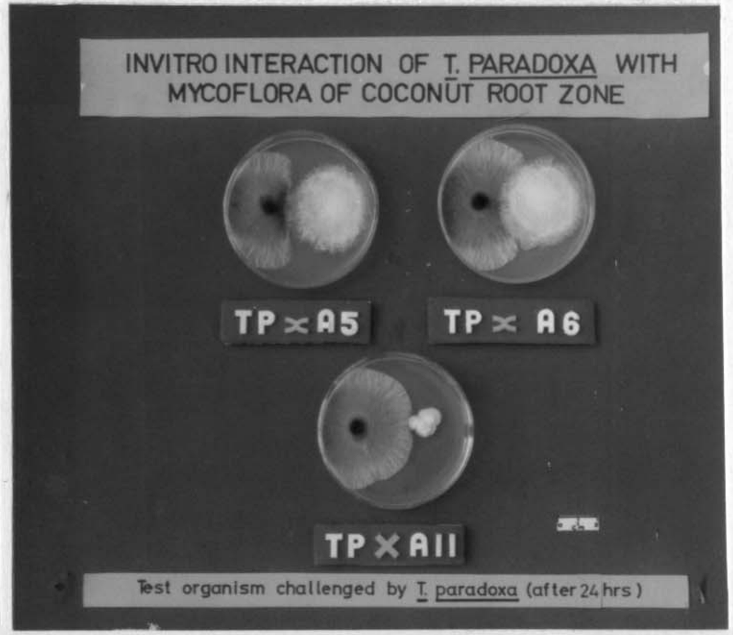
2. Simultaneous inoculation



Simultaneous inoculation of T. paradoxa and test organism(after 24hrs)

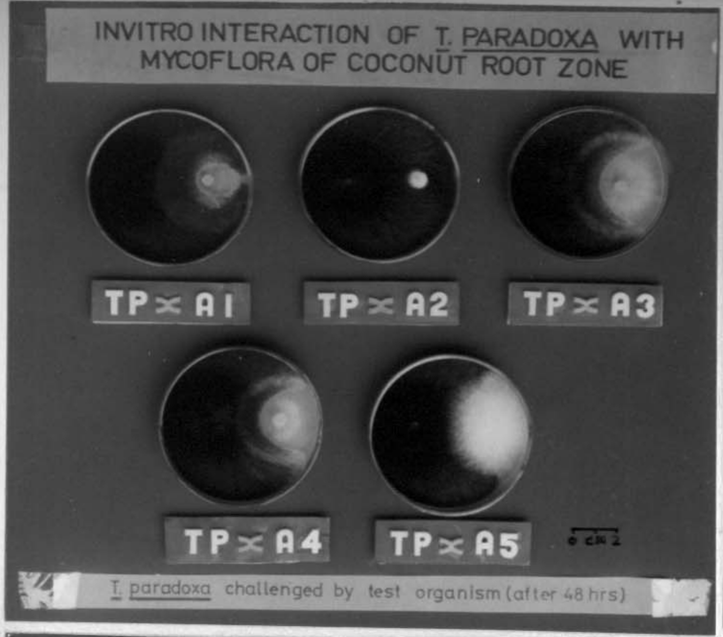


Test organism challenged by T. paradoxa (after 24 hrs)

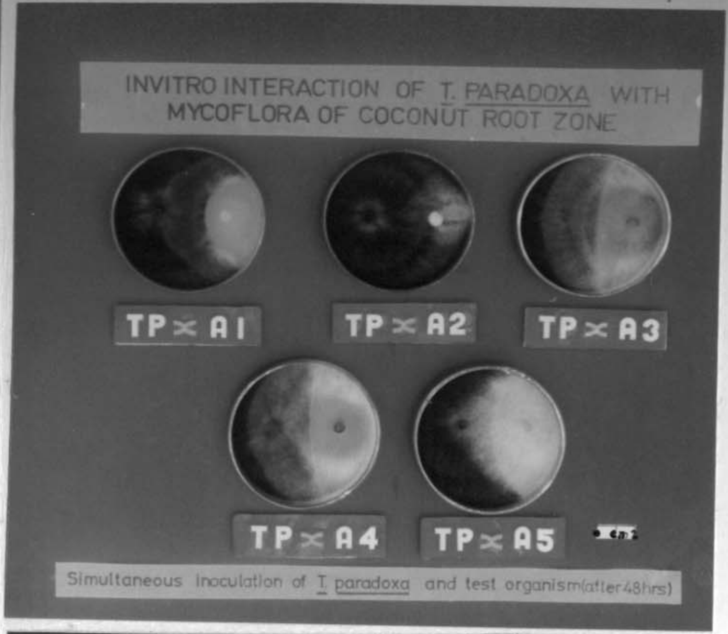


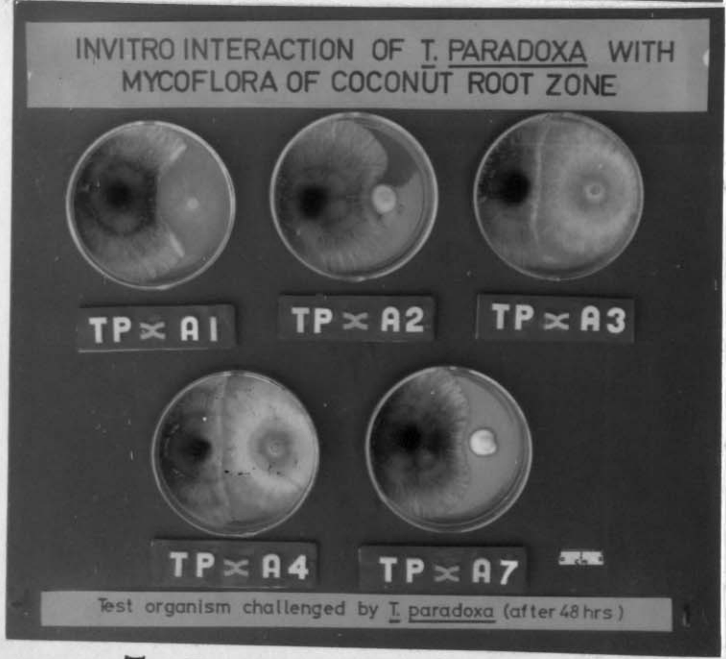
Test organism challenged by T. paradoxa (after 24 hrs)

Plates - 5. Sequence - A inoculation

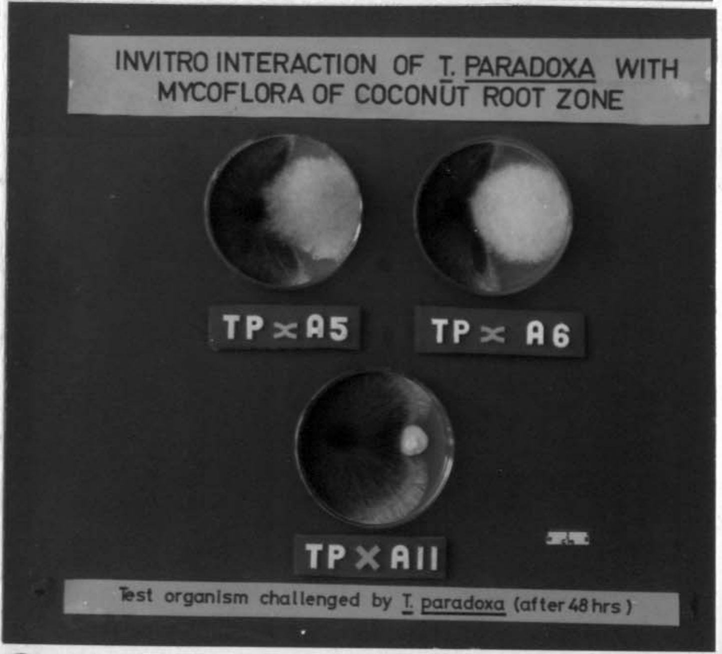


6. Simultaneous inoculation



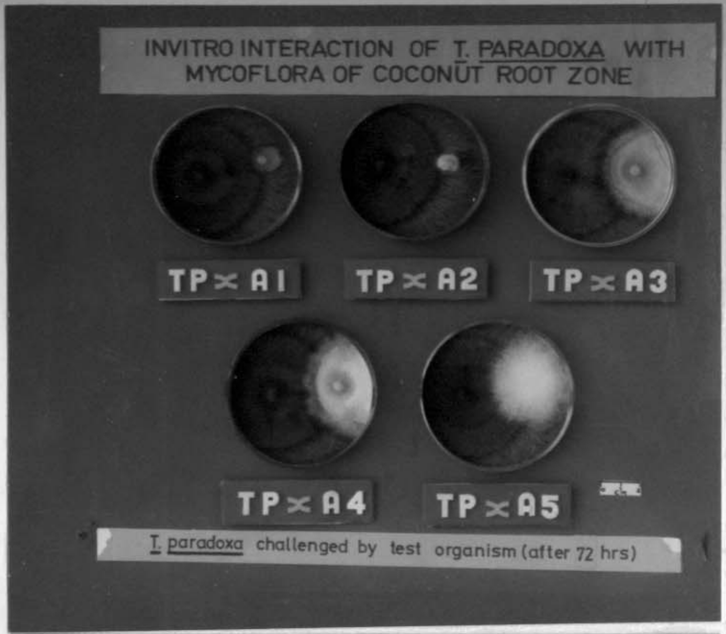


7. Sequence - B inoculation

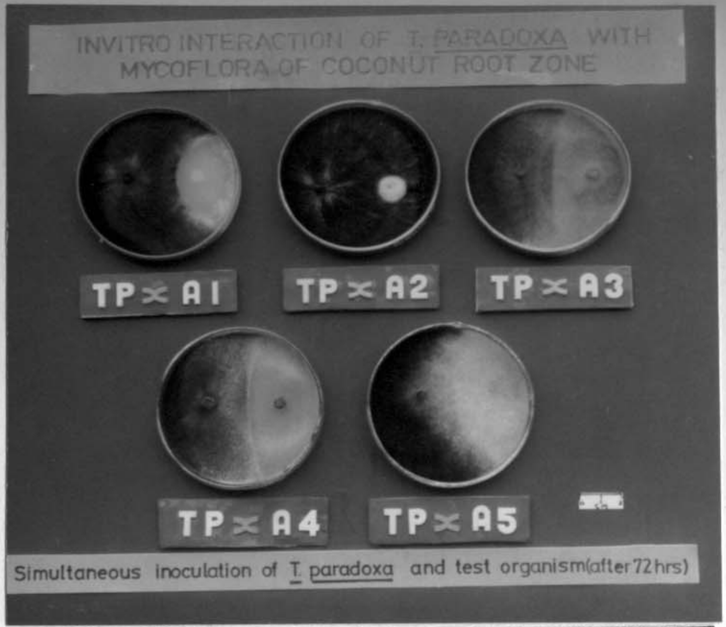


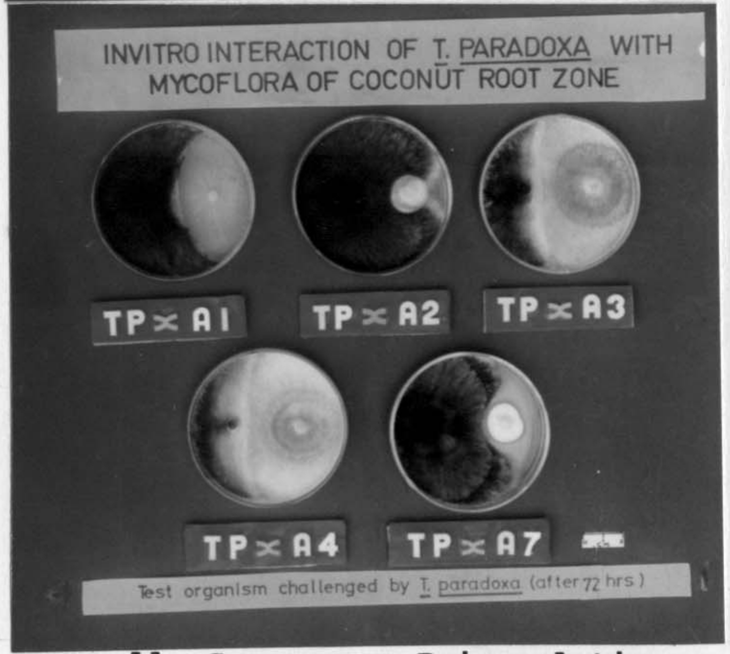
8. Sequence - B inoculation

Plates - 9. Sequence - A inoculation

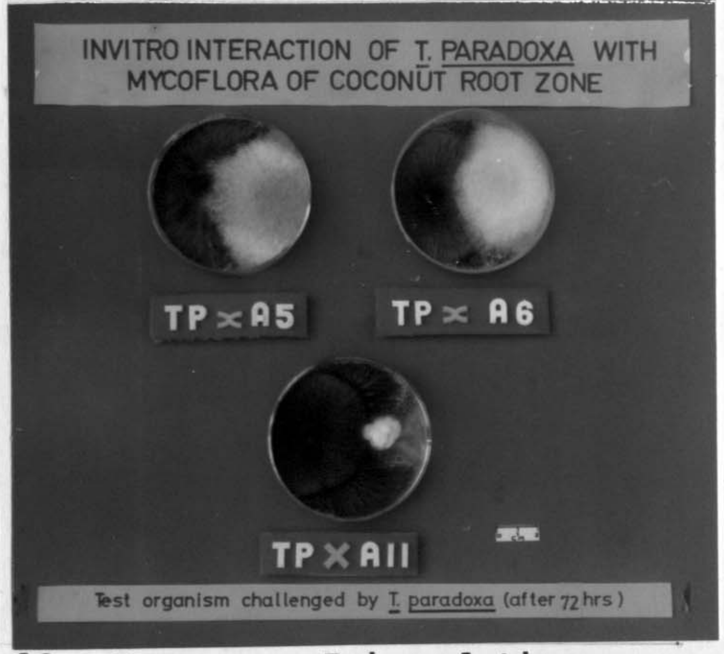


10. Simultaneous inoculation



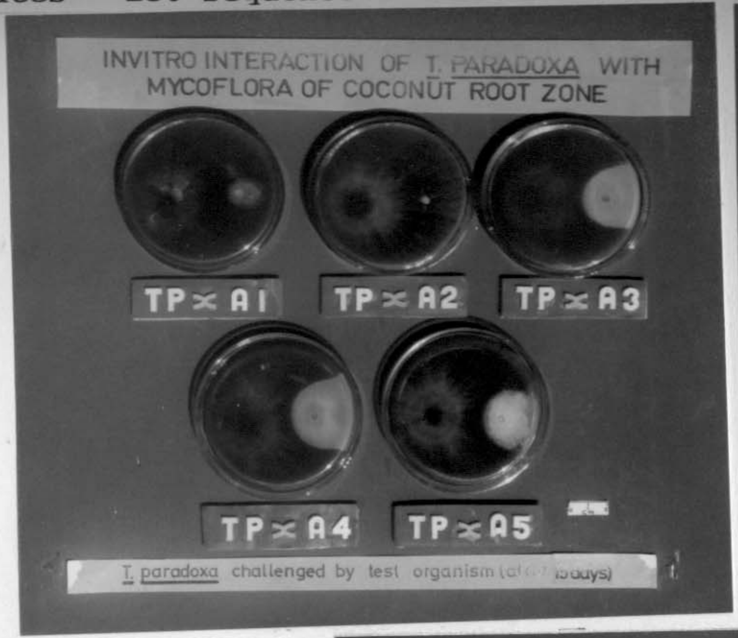


11. Sequence - B inoculation

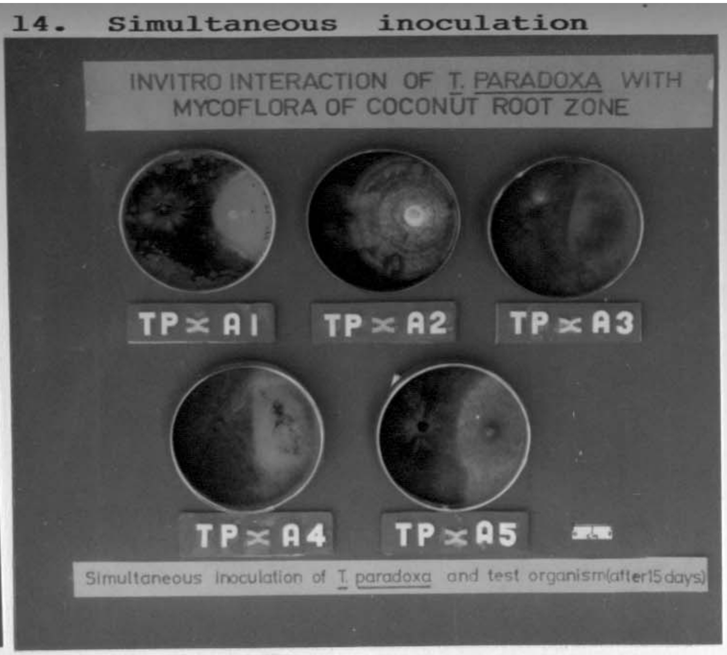


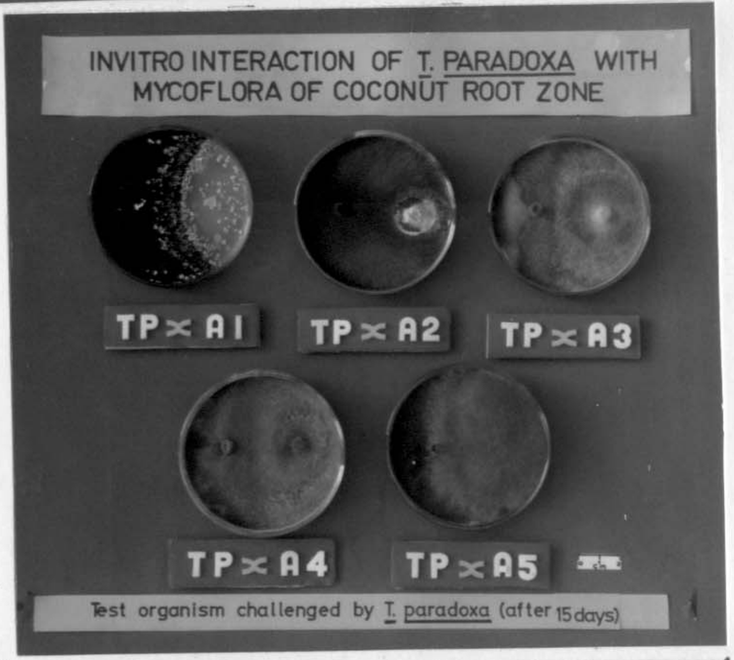
12. Sequence - B inoculation

Plates - 13. Sequence - A inoculation



14. Simultaneous inoculation





15. Sequence - B inoculation

4.3.2. *In vitro* interaction of *T. paradoxa* with *Trichoderma viride* - 1 (A3)

Based on 24 hrs of observation, the percentage of inhibition of *T. paradoxa* obtained in the three methods of inoculation were 10% in sequence - A, 19% in simultaneous and 33.3% in sequence - B (Table - 10) (Plates - 1,2, & 3; Fig. - TP x A3). A thin band formation were seen at the line of contact in all three types of inoculation after 48 hrs (Table - 11) (Plates - 5,6, & 7; Fig.- TP x A3). After 72 hrs *T. paradoxa* was slightly inhibited by *Trichoderma viride* - 1 (A3) in sequence - A inoculation whereas growth of both the organisms remained the same as that of at 48 hrs in simultaneous and sequence - B inoculation (Table - 12) (Plates 9,10 & 11; Fig.- TP x A3) Growth of both organisms remained almost the same as that of at 72 hrs in all three inoculation after 168 hrs (7 days) (Table - 13). After 360 hrs (15 days), A3 had completely overgrown on *T. paradoxa* in all three types of inoculations (Table - 14), (Plates - 13,14 & 15; Fig. TP x A3). This type of interaction come under 'O' type interaction category (Table - 15).

4.3.3. *In vitro* interaction of *T. paradoxa* with *Trichoderma viride* - 2 (A4)

Based on observation taken at 24 hrs after inoculation, the percentage of inhibition of T. paradoxa obtained in three methods of inoculation were 10% in sequence - A, 20% in simultaneous and 42.8% in sequence-B (Table - 10) (Plates - 1,2 & 3; Fig.- TP x A4). Band formation and overgrowth of Trichoderma viride - 2 (A4) at the line of contact was noticed in all three types of inoculations after 48 hrs (Table - 11) (Plates - 5,6 &7; Fig.- TP x A4). Observations taken at 72 hrs revealed that T. paradoxa was slightly inhibited by Trichoderma viride - 2 (A4) in sequence - A inoculation whereas no further addition to growth after 48 hrs in the other two types of inoculations. (Table - 12) (Plates - 9,10 & 11; Fig. - TP x A4). After 168 hrs (7 days), T. paradoxa was inhibited in simultaneous and sequence - B inoculation, however, there was no further addition to growth after 72 hrs in sequence - A inoculation. (Table - 13) Trichoderma viride - 2 (A4) had completely over grown on T. paradoxa in simultaneous and sequence - B inoculation. Growth of both the interacting fungi remained same as that of at 168 hrs (7 days) in all three types of inoculation after 360 hrs (15 days) (Table - 14) (Plates - 13,14 & 15; Figure - TP x A4) Trichoderma viride - 2 (A4) was seen to be more aggressive than A3. This interaction come under 'O' type interaction category (Table - 15).

4.3.4. In vitro interaction of T. paradoxa with Rhizopus stolonifer (A5).

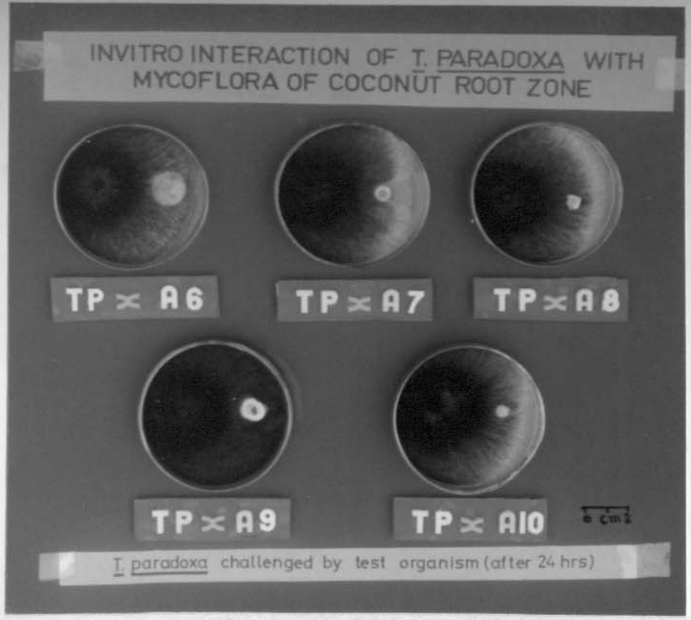
Based on observations taken at 24 hrs after inoculation, the percentage inhibition of T. paradoxa obtained were 9% in sequence - A, 14.2% in simultaneous and 28.5% in sequence - B (Table - 10) (Plates - 1,2, & 4; Figure - TP x A5). After 48 hrs, Rhizopus stolonifer (A5) started overgrowing on T. paradoxa at the point of contact (Table - 11) (Plates - 5,6 & 8; Figure - TP x A5). T. paradoxa was inhibited in sequence - A inoculation. However, growth of both organisms remained the same as that of at 48 hrs in all three types of inoculation after 72 hrs (Table - 12) (Plates - 9,10 & 12; Figure-TP x A5). After 168 hrs (7 days) inhibition of T. paradoxa by A4 was observed in simulataneous inoculation and the other two types of inoculation remained the same as that of at 72 hrs (Table - 13). The growth of both interacting fungi remained same as that of at 168 hrs (7 days) in sequence - A, simultaneous and sequence - B inoculation after 360 hrs (15 days) (Table - 14) (Plates - 13,14 & 15; Figure - TP x A5). This can, thus be included in 'O' type interaction category (Table - 15).

4.3.5. In vitro interaction of T. paradoxa with Thielavia terricola (A7).

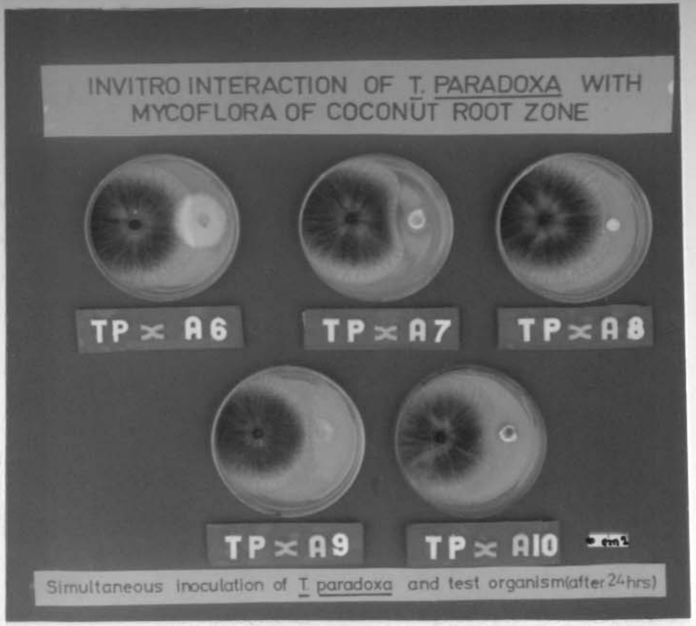
Based on 24 hrs of observation, no inhibition of

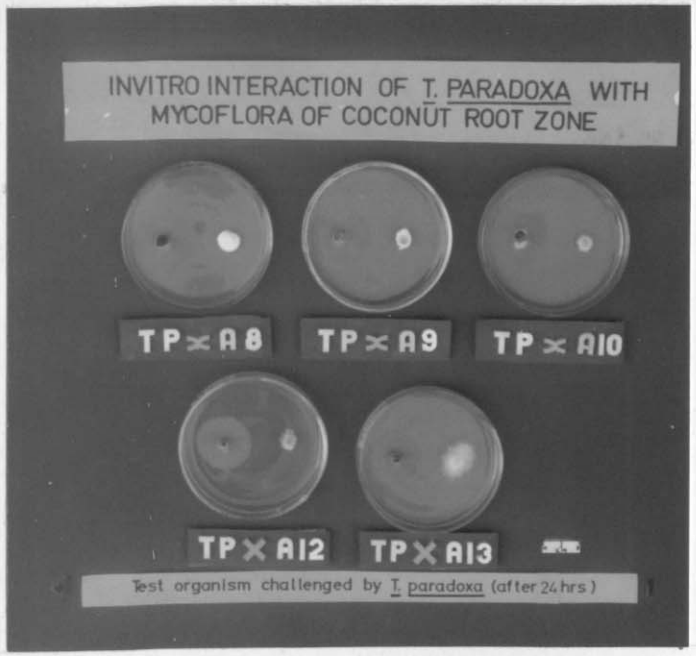
T. *paradoxa* was obtained in all three types of inoculation (Table - 10) (Plates - 16,17 & 3; Fig.- TP x A7). After 48 hrs, slight inhibition of *T. paradoxa* was observed in simultaneous and sequence - B inoculation whereas no inhibition of *T. paradoxa* was observed in sequence - A inoculation (Table - 11) (Plates - 19,20 & 7; Figure - TP x A7). *Thielavia terricola* (A7) started overgrowing by producing fructification on *T. paradoxa* in simultaneous and sequence - B inoculation after 72 hrs (Table 12) (Plates - 22, 23 & 11; Figure - TP x A7). The fructification of A7 spread throughout the plate in simultaneous and sequence - B inoculation after 168 hrs (7 days) whereas no change occured in sequence - A inoculation. The growth of the antagonist A7 remained the same as that at 72 hrs in sequence A and simultaneous inoculation and it had increased in sequence - B inoculation (Table - 13). After 360 hrs (15 days) growth of both interacting fungi remained the same as that of at 168 hrs in simultaneous and sequence - B inoculation whereas *Thielavia terricola* (A7) started overgrowing by producing fructifications on *T. paradoxa* in sequence - A inoculation (Table 14), (Plates - 25,26 & 27; Figure - TP x A7). This may be included in 'O' type interaction category.

Plates - 16. Sequence - A inoculation

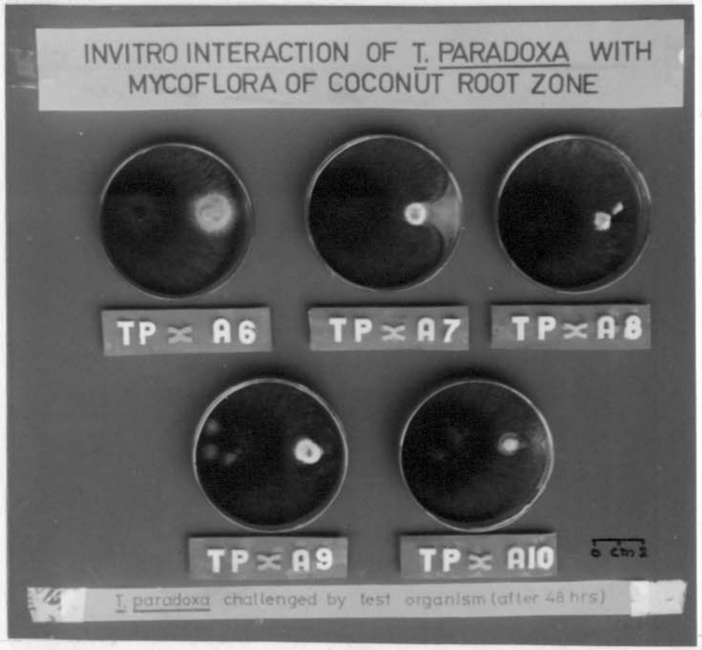


17. Simultaneous inoculation



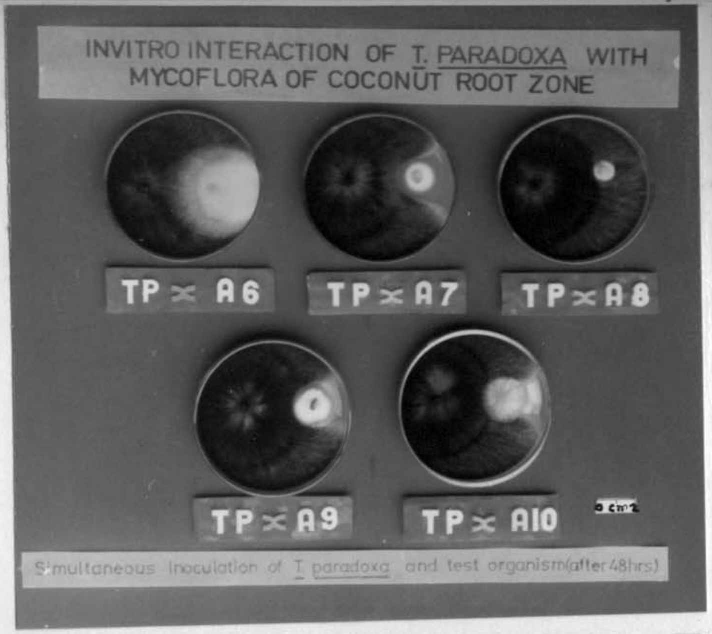


18. Sequence - B inoculation

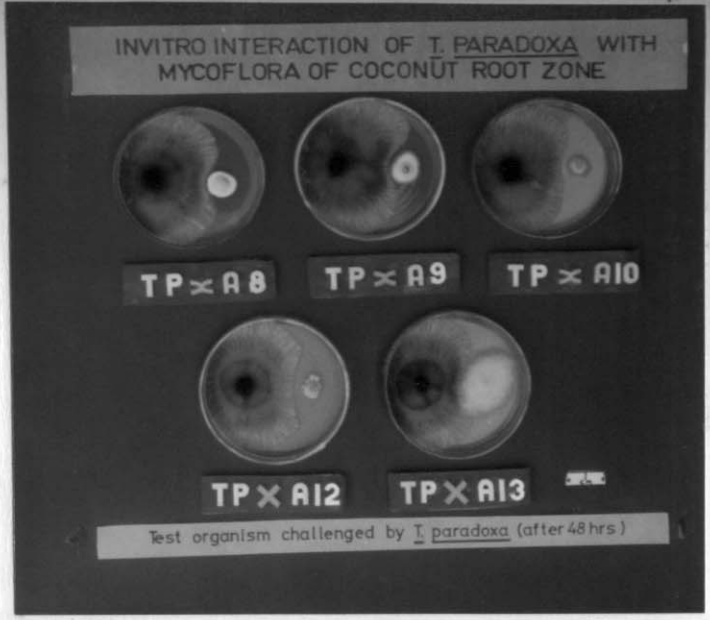


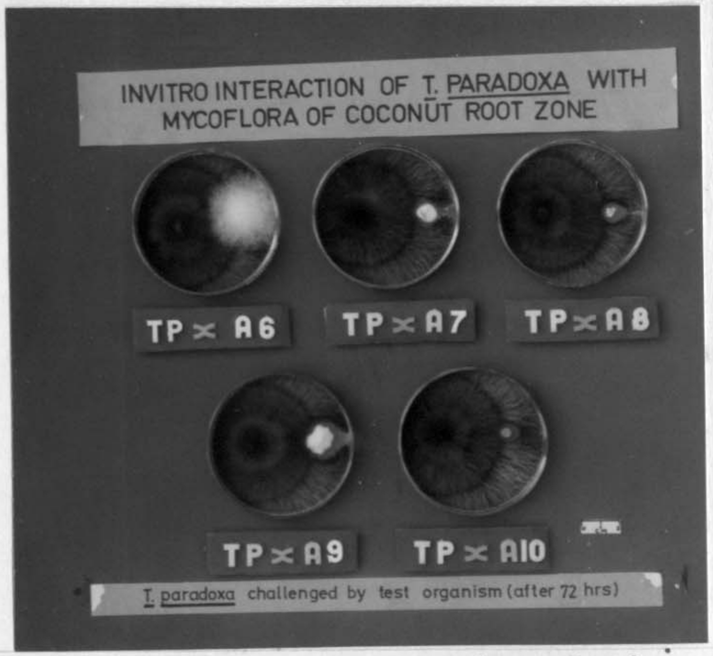
19. Sequence - A inoculation

Plates - 20. Simultaneous inoculation

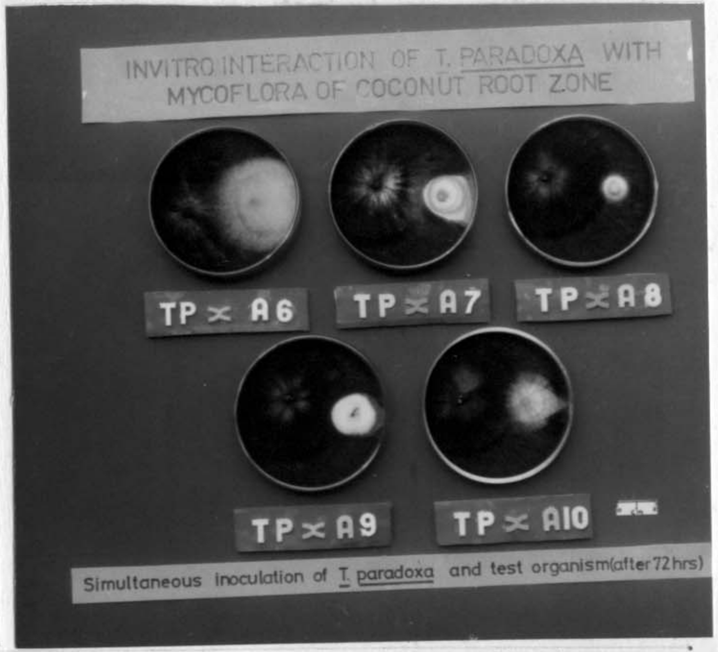


21. Sequence - B inoculation



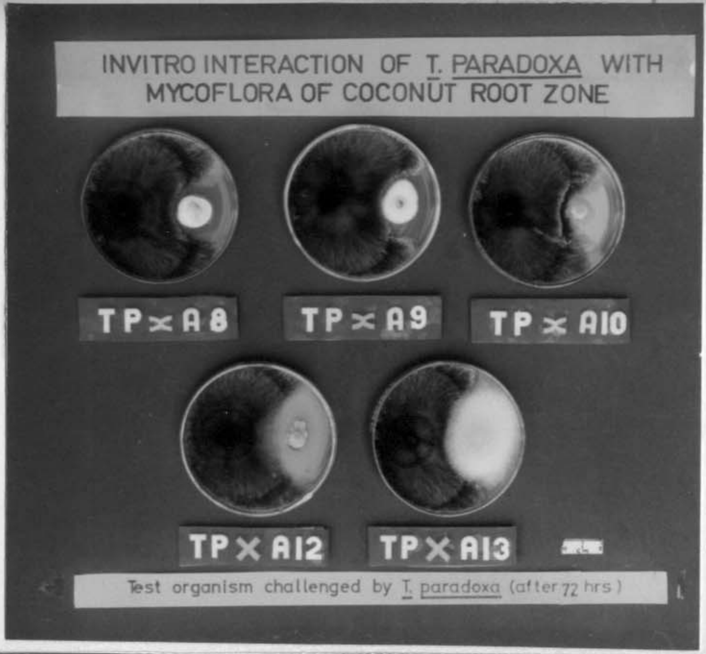


22. Sequence - A inoculation

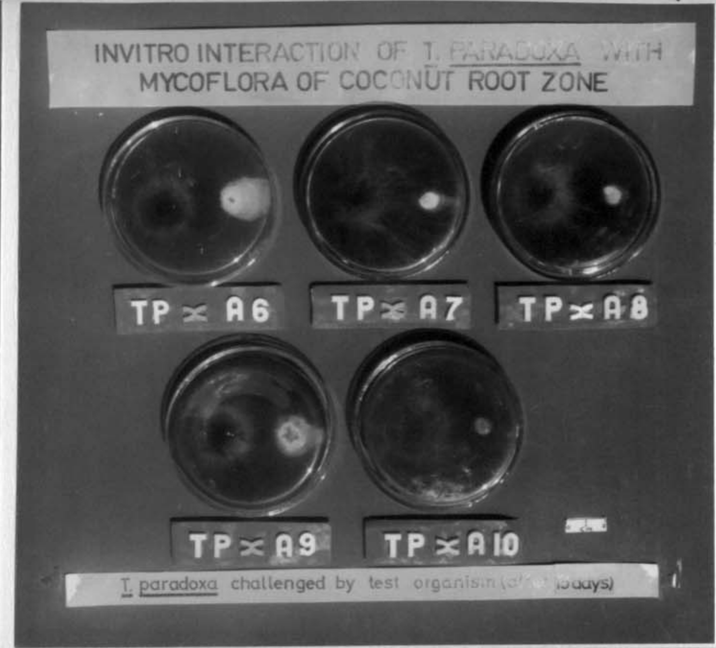


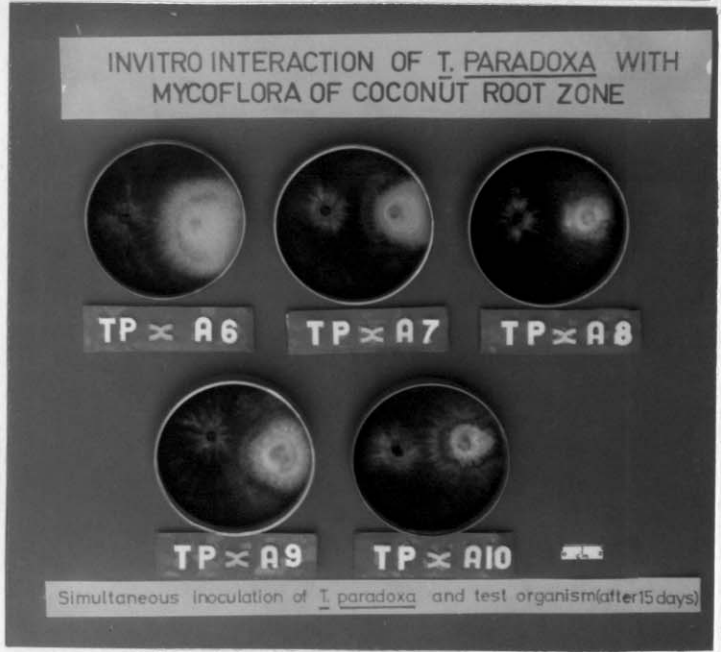
23. Simultaneous inoculation

Plates - 24. Sequence - B inoculation

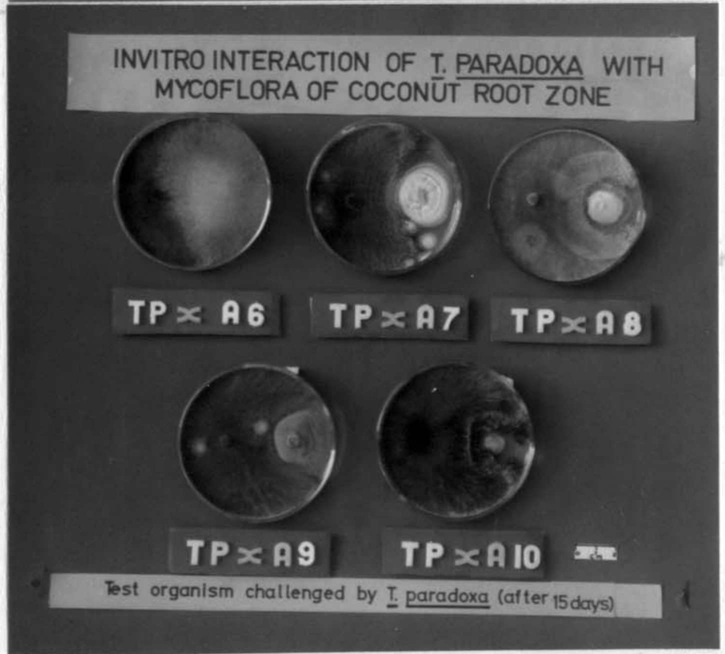


25. Sequence - A inoculation





26. Simultaneous inoculation



27. Sequence - B inoculation

4.3.6. In vitro interaction of T. paradoxa with Aspergillus fumigatus (A9)

After 24 hrs, no inhibition of T. paradoxa was observed in all three types of inoculation (Table - 10) (Plates 16,17 & 18; Fig.- TP x A9). An observation after 48 hrs were taken on the basis of the colony characters of both the interacting fungi T. paradoxa had surrounded Aspergillus fumigatus (A9) leaving little space for it in sequence - A inoculation (Plate - 19; Fig. TP x A9). However, the pathogen left a clearzone (width<2 mm) around the antagonist A9 showing a feeble band formation and a light yellow pigmentation was seen on the reverse of Aspergillus fumigatus (A9) in simultaneous inoculation (Plate - 20; Fig. TP x A9). Slight inhibition of T. paradoxa occured in sequence - B inoculation (Plate - 21; Fig. TP x A9). The growth of both the interacting fungi increased in all three types of inoculation (Table - 11). After 72 hrs, Aspergillus fumigatus (A9) increased it's growth in all three types of inoculation whereas no change in the growth of T. paradoxa was observed in sequence - A and sequence - B inoculation. However, a slight inhibition of the T. paradoxa was seen in simultaneous inoculation (Table - 12) (Plates - 22, 23 & 24; Fig. TP x A9). The growth of both the interacting fungi remained as that of at 72 hrs in sequence - A and simultaneous inoculation. However, a slight increase in the growth of Aspergillus fumigatus (A9) was observed in sequence - B inoculation after 168 hrs (Table - 13). After 360 hrs (15 days) no further increase in the growth of both the interacting fungi was observed (Table - 14). A gap was left between the two interacting fungi and the mycelium of either fungus was not observed in that area showing mutual inhibitory effect on each other. Therefore this type of interaction is included in the 'MC' type interaction category (Table-15).

4.3.7. In vitro interaction of T. paradoxa with Aspergillus terreus (A10)

In the three types of inoculation tested, no inhibition of T. paradoxa was observed in all three types of inoculation after 24 hrs (Table - 10) (Plates - 16,17 & 18; Fig. - TP x A10). After 48 hrs, T. paradoxa covered the entire plate excluding a small space (<2 mm) around the inoculation site of Aspergillus terreus (A10) in sequence - A inoculation. However, this inhibition zone was larger in simultaneous inoculation when compared to sequence - A. T. paradoxa exhibited a slight inhibition towards the antagonist (A10) in sequence - B inoculation (Plates - 19,20 & 21; Fig. - TP x A10). Both the interacting fungi increased their growth in all three types of inoculation upto 48 hrs (Table - 11). After 72 hrs, there was no further increase in the growth of the two interacting fungi in sequence - A and B except in simultaneous inoculation (Table - 12) (Plates - 22,23 & 24; Fig. - TP x A 10). Observation taken after 168 hrs (7 days) and 360 hrs (15 day) revealed that there was no further increase in the growth of the interacting fungi (Tables - 13 & 14) (Plates - 25,26 & 27; Fig. TP x A10) Mutual inhibition between the two interacting fungi was observed in simultaneous and sequence - B inoculation after 360 hrs (15 days), thus coming under the 'MC' type interaction category (Table - 15).

4.3.8. *In vitro* interaction of *T. paradoxa* with *Fusarium solani* (A 12).

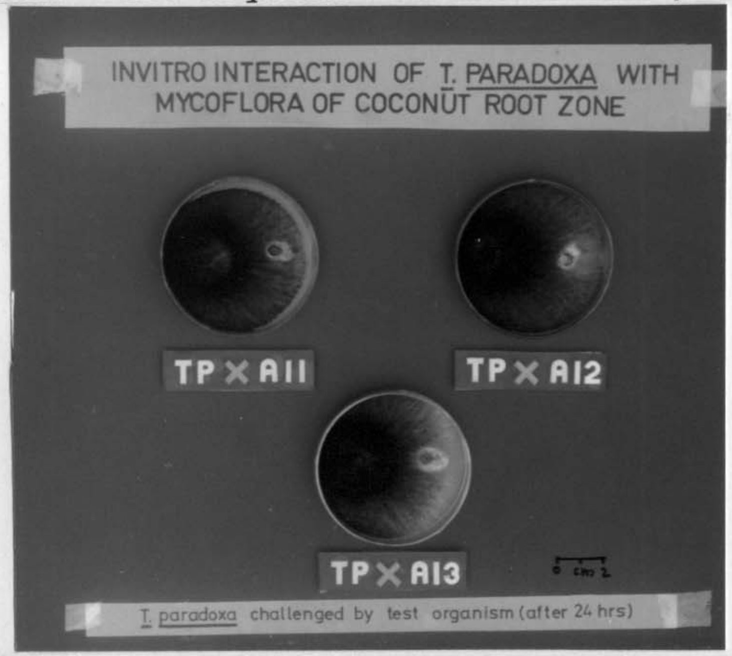
In all three types of inoculations tested, no inhibition of *T. paradoxa* was observed after 24 hrs (Table - 10) (Plates. - 28,29 & 18; Fig. TP x A12). After 48 hrs, *T. paradoxa* covered the entire plate leaving a 2 mm margin around the *Fusarium solani* (A12) colony of 7 mm dia in sequence - A and a larger margin (<2 mm) around the antagonist (A12) in simultaneous inoculation (Plates - 28 & 29; Fig. TP x A12). Slight inhibition of *T. paradoxa* was observed in sequence - B inoculation (Plate - 18; Fig. TP x A12). The growth of both the fungi increased in all three types of inoculation (Table - 11) upto 48 hrs. There was no further growth of the two interacting organisms as revealed by observations taken at

72 hrs (Table - 12). In the two types of inoculation, simultaneous and sequence - B a distinct band (2 mm) of T. paradoxa characterised by poor or no sporulation was observed around the antagonist A12 colony (Plates - 33 & 24; Fig. TP x A12). However, there was no such band of T. paradoxa in sequence - A inoculation (Plate - 32; Fig. TP x A12). Even after 168 hrs (7 days) and at 360 hrs (15 days) no change was observed in sequence - A and simultaneous inoculations whereas F. solani (A12) colony size increased in the sequence - B inoculation (Tables - 13 & 14) (Plates - 34,35 & 36 ; Fig. TP x A12). Mutual inhibition of both the fungi were observed at their line of contact in all three types of inoculation after fifteen days. This type of interaction, thus can be included in 'MC' type interaction category (Table - 15).

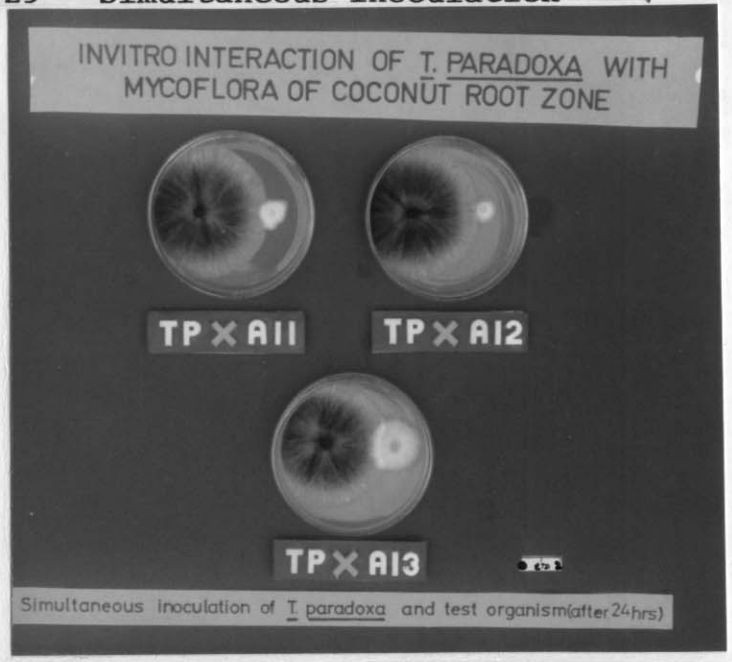
4.3.9. In vitro interaction of T. paradoxa with fungi showing intermingling colonies.

The fungi showing intermingling characters with T. paradoxa were Paecilomyces sp., (A2); Absidia sp. (A6); Penicillium pinophilum (A8); Gongronella butleri (A11); Cunninghamella elegans (A13). After 24 hrs, no inhibition of T. paradoxa by these fungi were observed (Plates - 1,2 & 3; 16,17 & 4; 16,17 & 18; 28,29 & 4; 28,29 & 18. Fig. TP x A2; TP x A6; TP x A8; TP x A11 and TP x A13).

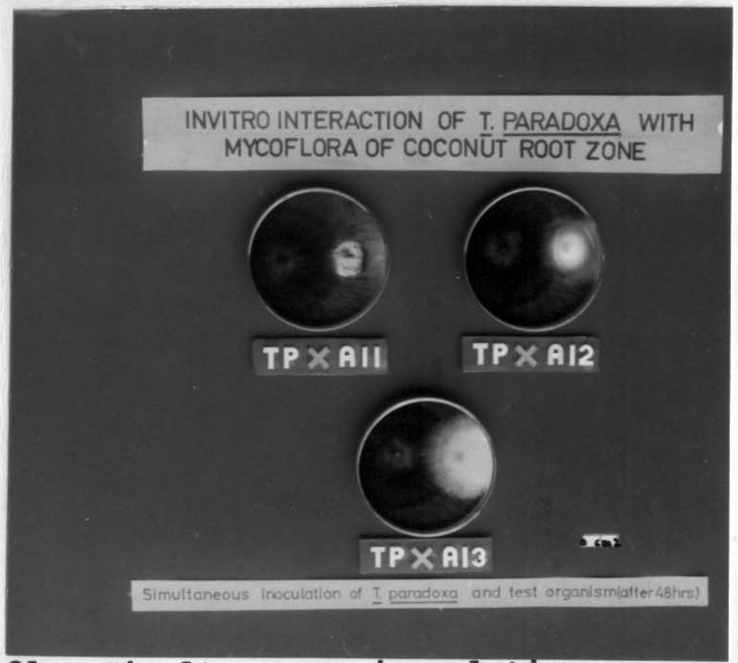
Plates - 28. Sequence A inoculation.



29 Simultaneous inoculation.

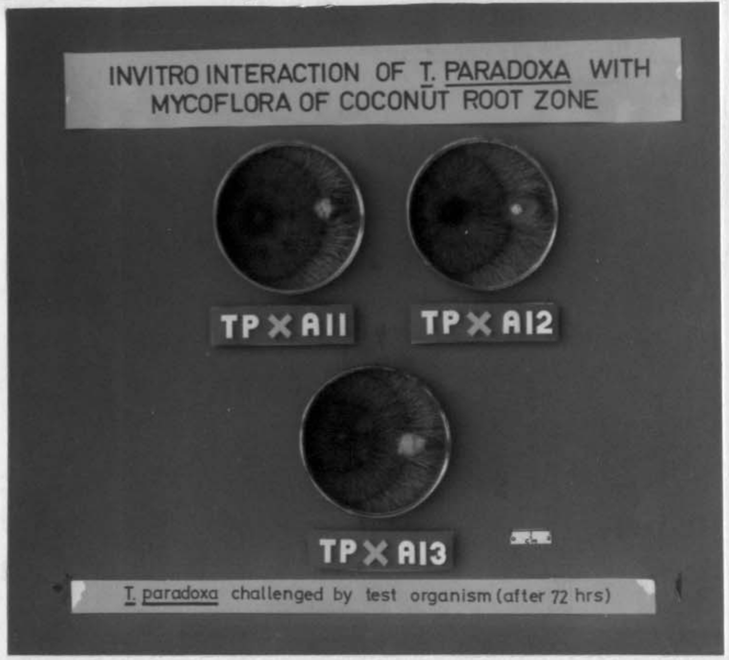


30 Sequence A inoculation

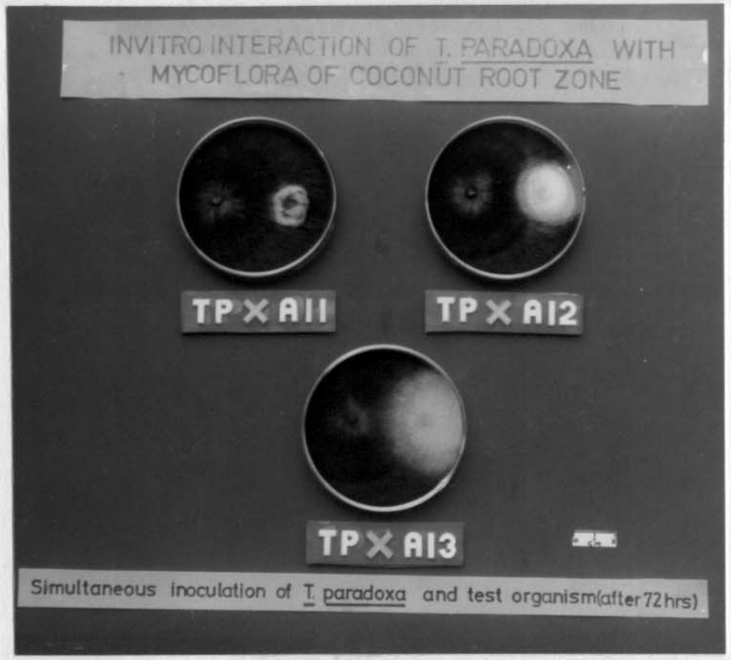


31 Simultaneous inoculation

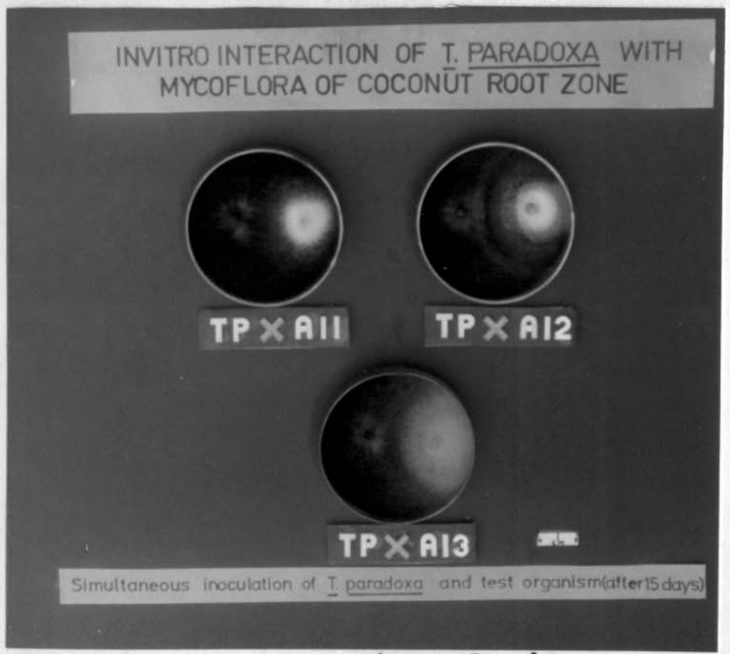
Plates - 32. Sequence - A inoculation.



33. Simulataneous inoculation



34. Sequence - A inoculation



35. Simultaneous inoculation

Plate - 36. Sequence - B inoculation

Among these fungi viz. Absidia sp. (A6), Penicillium pinophilum, Gongronella butleri (A11) and Cunninghamella elegans (A13) exhibited slight inhibition of T. paradoxa when inoculated simultaneously or under sequence - B. However, no inhibition of T. paradoxa was observed in Paecilomyces sp. (A2) after 48 hrs (Plates - 19,20 & 8; 19,20 & 21; 30,31 & 8; 30,31 & 21; 5,6 & 7. Fig. TP x A6; TP x A8; TP x A11 and TP x A13). T. paradoxa overcame the inhibition later on and intermingled freely with the candidate fungi after 72 hrs. However, Absidia sp. (A6) and Cunninghamella elegans (A13) restricted T. paradoxa at the boundary of the colony. In all the three types of inoculation aerial mycelium of these fungi traversed T. paradoxa colony. After 168 hrs (7 days) and 360 hrs (15 days) also no further change was observed and these five fungi intermingled with T. paradoxa without showing any microscopic and macroscopic sign of interaction (Plates - 13,14 & 15; 25,26 &27; 25,26 &27; 34,35 & 36; 34,35 & 36. Fig. TP x A2; TP x A6; TP x A8; TP x A11; TP x A13). Therefore these fungi could be grouped under the 'I' type interaction category (Table - 15).

DISCUSSION

5. DISCUSSION

Stem bleeding disease in coconut is caused by a soil borne fungus Thielaviopsis paradoxa. Inspite of being a weak wound - inhabiting fungus, it causes considerable damage to coconut. By virtue of being a soil dweller, the fungus is not easily amenable to control by conventional methods. Therefore it is necessary to adopt a multipronged approach to manage the disease so as to reduce the crop loss and to protect the plant's health. This obviously directs our attention to the manipulation of the soil, the very environ which supports and protects the pathogen.

While physical and chemical properties of the soil are important, the biological properties assume significance in understanding the various microflora, the influence of physical and chemical properties on them, their inter relations etc. Therefore a study of soil microflora and their interaction with T. paradoxa in a stem bleeding affected area subjected to various fertilizer regimes was taken up. An added reason for king up the study was the reports that application of eem cake has an ameliorative effect on stem bleeding isease.

.1. Effect of seasons and fertilizer treatments on microbial population in stem bleeding affected coconut soils.

Soil samples were collected from the following reatments, viz. NPK; NPK + Dolomite; NPK + Dolomite + Neem cake and control (Where only farmer's practices were adopted) The samples were collected during the first week of February, April and June. Samples could not be collected beyond this due to the limited time available for investigations under the M. Phil programme. Results indicated that fungal and bacterial population varied significantly depending on the month of sampling. Maximum population was in February and minimum population in June (Fig. 1 & 2, Table - 5 a & b). The reduction in fungal and bacterial population from February to May is mainly due to the continuous drought. By the time, the June samplings were taken, there were heavy down pores and the resultant flooding and anaerobiosis would have been responsible for the drastic reduction in fungal and bacterial population in June. Radha and Rawther (1959) reported that moderate rainfall was found to be favourable for the microflora, while heavy rains adversely affected

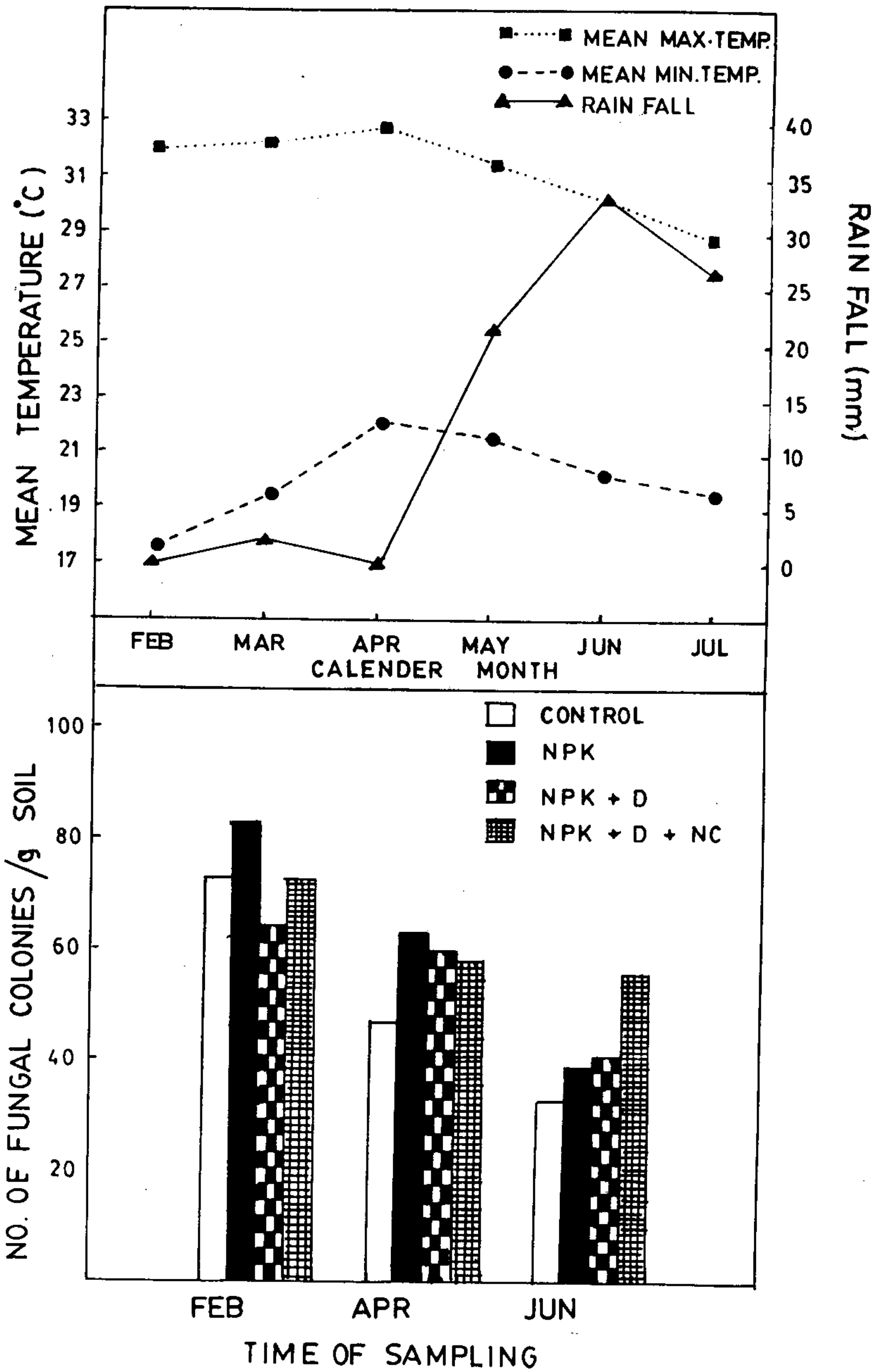


Fig. 1. Effect of seasons and fertilizer treatments on fungal population in stem bleeding affected coconut soils.

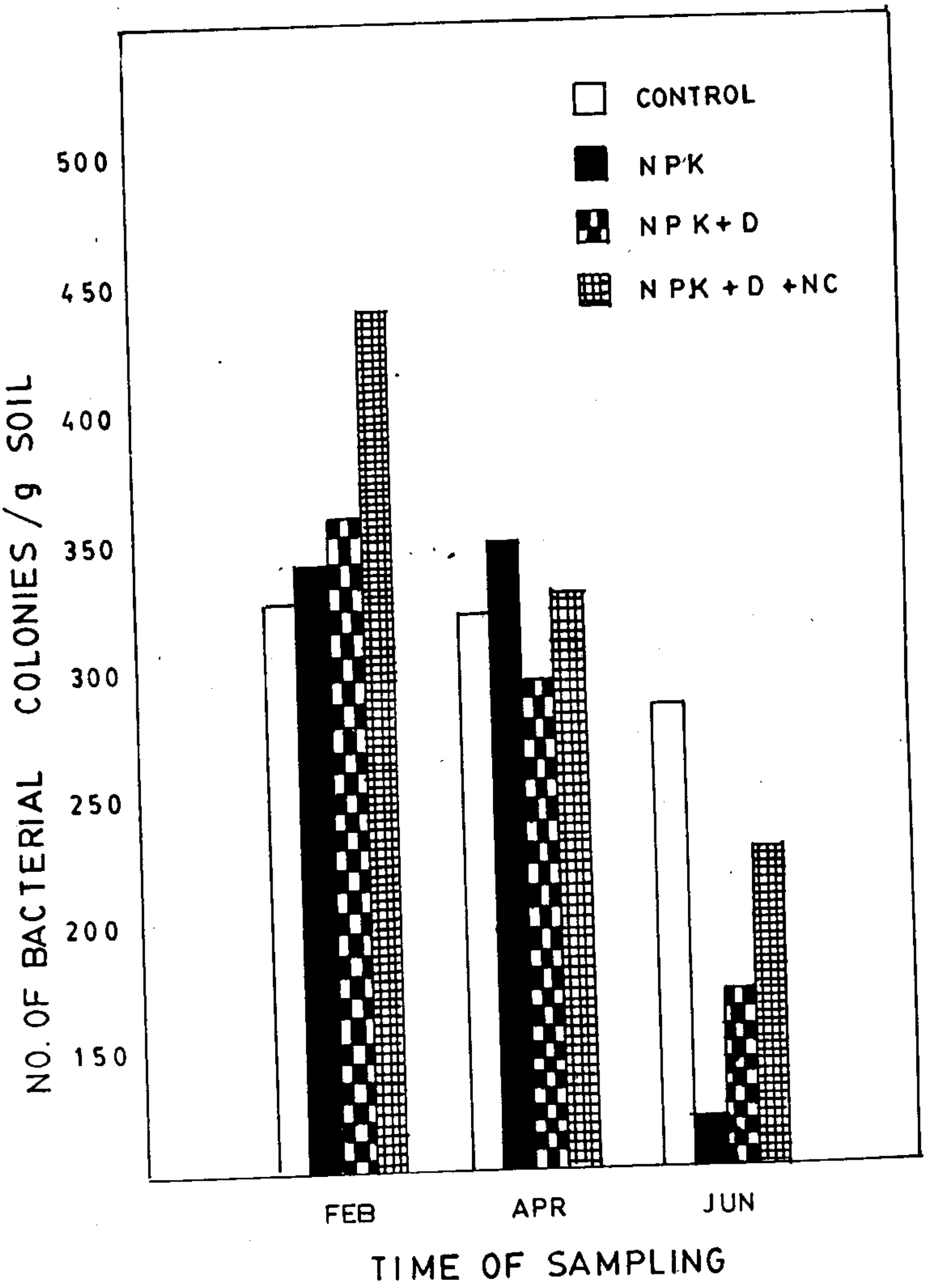


Fig. 2 Effect of seasons and fertilizer treatments on bacterial population in stem bleeding affected coconut soils.

in coconut rhizosphere. Similar observations were ecorded by Bopaiah (1990) in Arecanut. Same was the inding in other crops also (Timonian, 1940; Clark, 1949; huvaneshwari, 1958; Lakshmi - Kumari, 1964).

In this study, there was no significant difference mong the various treatments viz. control; NPK; NPK + olomite and NPK + Dolomite + Neem cake with regard to total fungal flora (Fig. 1 & Table - 5a). However, results indicated that the bacterial population was significantly enhanced in the NPK + Dolomite + Neem cake treatment compared to the others (Fig. 2 & Table - 5b). This suggests the beneficial effect of Neem cake on the population build up of bacteria in Neem cake ammended soils. Beneficial effects of organic matter in general has been recorded in arecanut soil by Bopaiah and Bhat (1981). However, this author is not aware of any literature pertaining to the influence of organic ammendments or Dolomite application or even fertilizer application in coconut in relation to the microbial population. The present investigations pertain only to one season's data, from one locality. Even though these observations are quite informative, they are of a preliminary nature. Therefore it would be worthwhile to investigate this in more detail at more localities for longer periods of time.

5.1.1. Effect of seasons and fertilizer treatments on antagonistic fungal population in stem bleeding affected coconut soils.

In the present investigation a large number of bacterial and fungal cultures were established for studying their interaction with T. paradoxa. Due to the pausity of time only fungal cultures were subjected to interaction studies. In the present investigation, the term antagonist refers to those fungi which exhibit antagonism or mutual inhibition towards T. paradoxa.

In Table - 6a, the effect of seasons and the various fertilizer treatments namely, Control; NPK; NPK + Dolomite and NPK + Dolomite + Neem cake on the population of antagonistic mycoflora is described. It could be observed that the total population gradually decreased from February to June (Fig. 3), following the same trend as the general population. This has already been discussed at an earlier occassion. However, with regard to the influence of various fertilizer regimes on these fungi, it was observed that their population was minimum in NPK alone treatment and the situation improved with the addition of Dolomite and the antagonistic population was maximum in NPK + Dolomite + Neem cake (Fig. 3). However, the difference between control, NPK + Dolomite and NPK + Dolomite + Neem cake was not statistically significant.

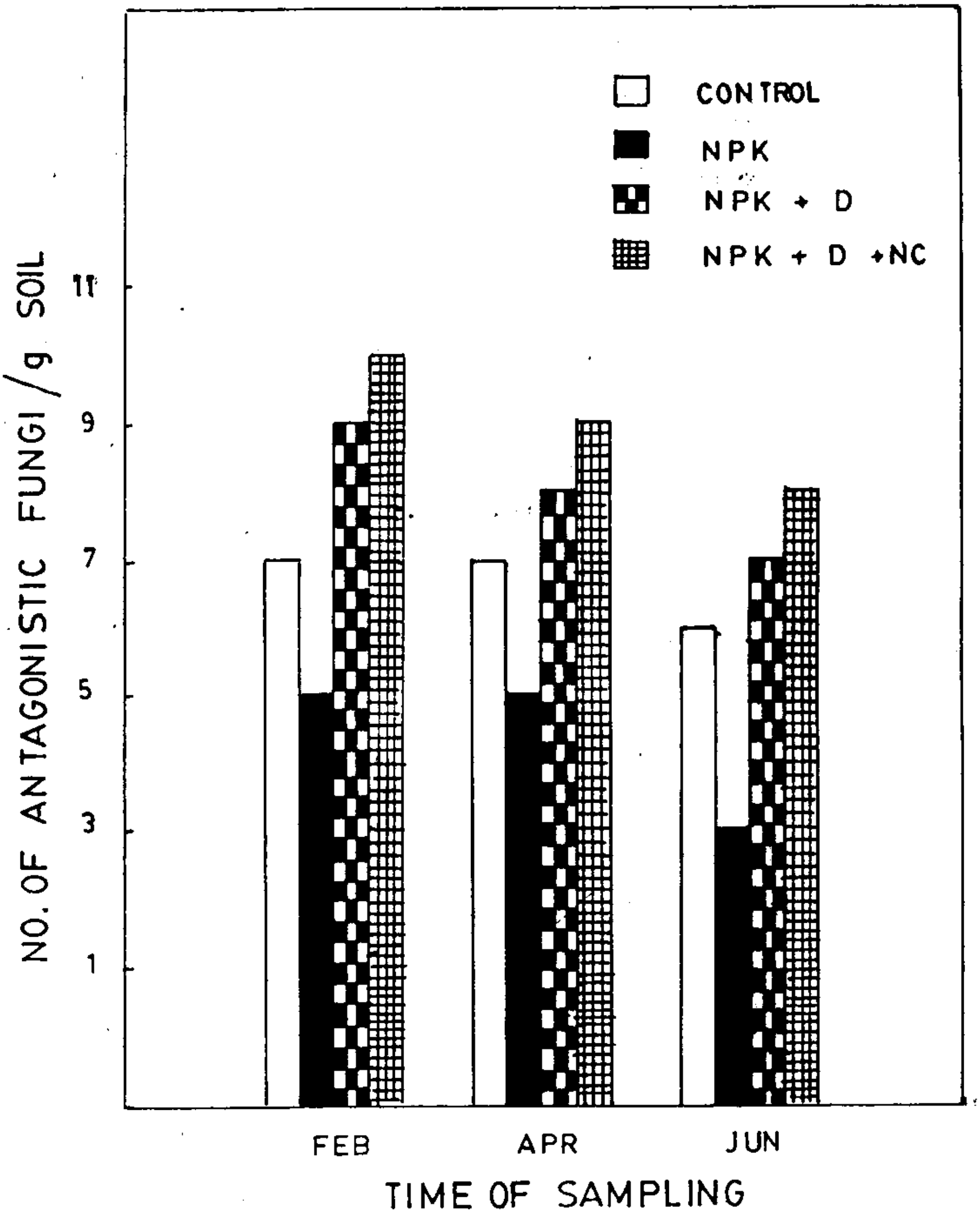


Fig.3. Effect of seasons and fertilizer treatments on antagonistic fungal population in stem bleeding affected coconut soils.

These results indicate that there is a likelyhood in the reduction of antagonistic mycoflora when the crop is supplied only with chemical fertilizers. The addition of Dolomite however, has an ameleorating effect, probably by altering the soil reaction to a favourable range and also by supplying trace elements. The situation is further improved with the addition of Neem cake which helps to retain moisture, improve the soil texture and also help in inhibiting *T. paradoxa.*

A study of the Table - 7, corraborates the observations discussed in the previous paragraph. It could be observed that the population of *T. paradoxa* was highest in control and NPK, less abundant in NPK + Dolomite and least in NPK + Dolomite + Neem cake (Fig. 4). The population trend of *T. paradoxa* is the reverse of that of the population of antagonist. Even though, there is no literature available on the influence of fertilizers of *T. paradoxa* population, Usman (1988) had reported the reduction of *T. paradoxa* in Neem cake ammended soils due to the adverse effect of the antagonists. Results obtained in the present investigation are in confirmation with these earlier evidences.

5.2. In vitro interaction of T.paradoxa with thirteen fungi isolated from coconut root zone.

The very objective of this investigation was to

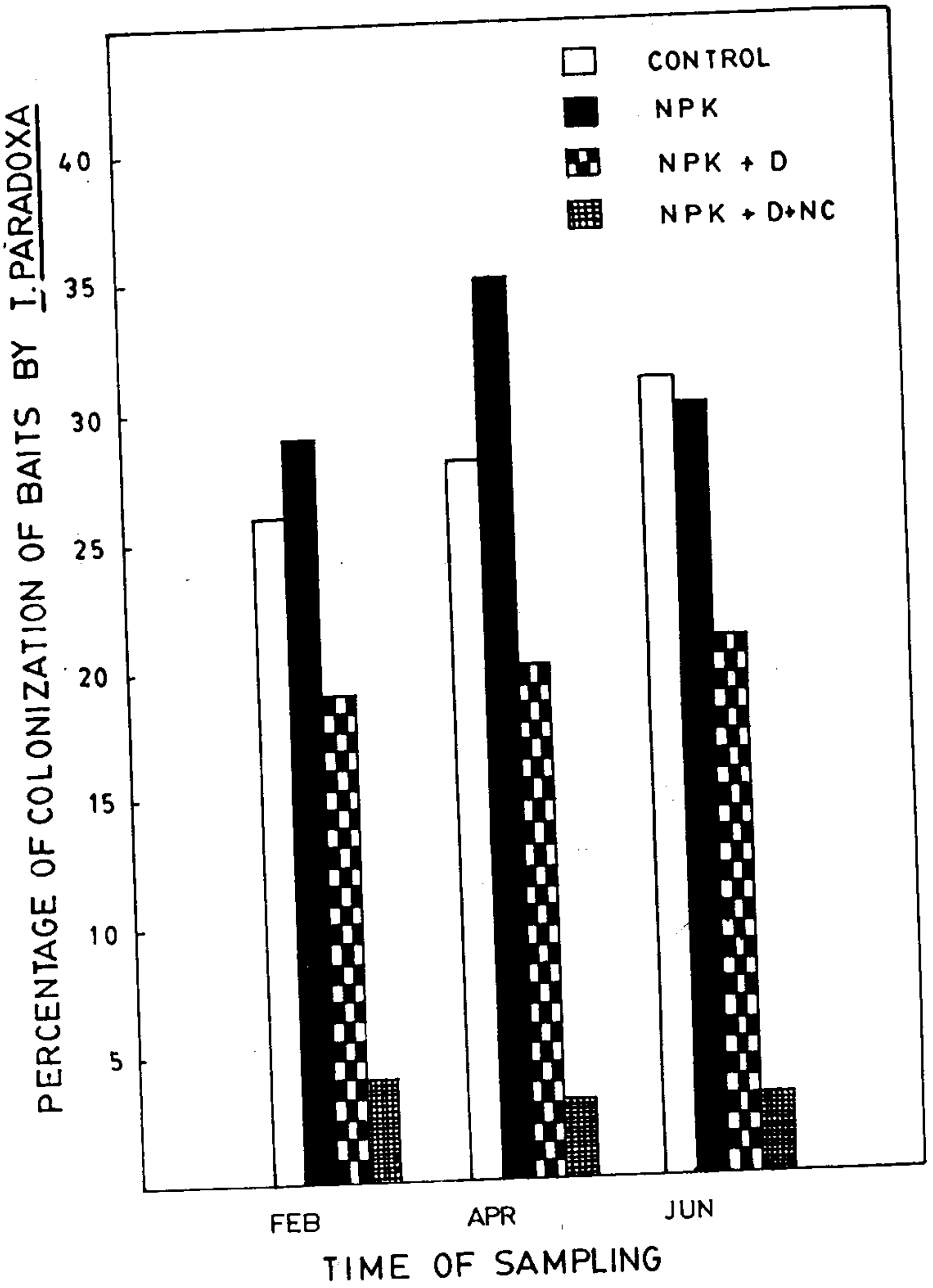


Fig. 4. Effect of seasons and fertilizer treatments on percentage colonization of baits by *T. paradoxa* in stem bleeding affected coconut soils.

identify the potential antagonists against T. paradoxa in the native soils. Antagonists present in the very locality are better adapted to the climate, soil and pathogen. Individual and cumilative effects of antagonists can considerably reduce the population of pathogen in the soil. Spatial distribution, seasonal abundance and antagonistic properties of the candidate fungi are all factors that affect the inoculum potential of the pathogen. For all these studies, the preliminary step involves collection of these fungi and information on their individual interactive effect on the pathogen.

For this, two types of inoculation of the pathogen verses candidate fungus has been tried in vitro. For assessing the percentage of inhibition of T. paradoxa, observations were taken after 24 hrs of inoculation. In general, sequence - B inoculation (candidate fungi challenged by T. paradoxa later) showed comparatively greater inhibition than sequence - A (T. paradoxa challenged by candidate fungus later) and simultaneous inoculation (Table - 9). This could be due to the fact that candidate fungus got an additional time to establish itself and be ready in defence to face the pathogen. These results would give us a clue in choosing those organisms that could be easily multiplied and applied to the soil or base of the trunk where they can establish in advance and lie in readines for the pathogen. Even though sequence - B inoculation were more effective in controlling T. paradoxa, situations in nature may not make available 'the antagonists in advance. Quite often a simultaneous infection of the pathogen and antagonists may take place as and when a growth crack appears on the tree trunk or it is also possible, that the pathogen may get a precedence in occupying the wound-site. Hence the results obtained from these tests are of great practical value. The useful organisms can be multiplied and used even after the disease symptoms appear.

The results indicated that maximum inhibition of T. paradoxa after 24 hrs of inoculation (Table - 9) was seen in

1. Trichoderma viride - 2 (A4) - Sequence - A, 10%; Simultaneous, 20% and Sequence - B inoculation, 42.8%.
2. Trichoderma viride - 1 (A3) - Sequence - A, 10%; simultaneous, 19% and Sequence - B inoculation, 33.3%.
3. Rhizopus stolonifer (A5) - Sequence - A, 9%; simultaneous, 14.2% and sequence - B inoculation 28.5%.
4. Trichoderma koningii (A1) - Sequence - A, 4.7%; simultaneous, 9% and Sequence - B inoculation 20%.

This gives a list of the candidate fungi which act fast in opposing T. paradoxa. However, Thielavia terricola (A7), an Ascomycete showing only perfect stage exhibited inhibitory reaction only at 48 hrs (Table - 11). A scan of the literature indicated that the inhibitory property of T. terricola and R. stolonifer against T. paradoxa were not hither to reported. Thus results indicate that these two organisms merit greater attention.

Number of species of Trichoderma have been recognised as antagonist against T. paradoxa and other species of ceratocystis. Trichoderma viride being the best. The observation of this author is also in confirmation with the literature (Gowda, 1987; Ricard, 1983; Zimmerman, 1985; Webber and Hedger, 1986). However, isolates have differed in the extend of inhibition and similar observation were recorded by this author.

All the results here pertain to isolate number - 7 of T. paradoxa maintained in C.P.C.R.I. It is quite likely that there could be variation in the reaction of these fungi to other isolates of T. paradoxa. Therefore it will be worthwhile to investigate the reaction of many isolates of antagonist against many isolates of T. paradoxa to get a more comprehensive picture.

Interactions between the opposing colony were assessed visually and microscopically after fifteen days (360 hrs) of incubation so as to facilitate stabilization of the interaction. For categorising the interactions, the terminology followed by Webber and Hedger, (1986) has been adapted. However, Webber and Hedger had recorded observations after one week of incubation. From the results recorded in Table - 15, it could be seen that the interaction fell into three distinct categories. Reaction 'O' characterised by over growth of the T. paradoxa colony by opposing candidate fungi, usually accompanied by inhibition of T. paradoxa on or shortly after contact was expressed by Rhizopus stolonifer, Thielavia terricola, Trichoderma koningii and Trichoderma viride 1 & 2. On microscopic examination, hyphal disintegration was seen virtually leaving only chlamydospores in the substratum. Similar observations with regard to T. viride has been well documented by Usman (1988). Information in respect to Rhizopus stolonifer, Thielavia terricola and Trichoderma koningii are been recorded for the first time in these dissertation.

Mutual inhibition of both the colonies with no apparent intermingling leaving a clear zone (< 2 mm) between the colonies, designated as 'MC' type of interaction was observed in Aspergillus fumigatus Aspergillus terreus and Fusarium solani (Table - 15). The inhibitory ('MC' category) properties of A. terreus on six isolates of *T. paradoxa* has been reported by Gowda, (1987). However the 'MC' type of reaction of *A. fumigatus* towards *T. paradoxa* is being reported for the first time. Webber and Hedger, (1986) has recorded 'MP' type (mutual inhibition with the production of pigmentation at the point of contact) reaction of *F. solani* towards *Ceratocystis ulmi*. Thus this observation of the 'MC' type of interaction of *F. solani* on *T. paradoxa* is a first report.

Interaction 'I' type characterized by intermingling of colonies with no microscopic or macroscopic signs of interaction, in otherwords 'Commenalism' was exhibited by *Absidia sp.*, *Cunninghamella elegans*, *Gongronella butleri*, *Paecilomyces sp*. and *Penicillium pinophilum* (Table - 15).

The results discussed here confine to the interaction of one isolate each (exception of *T. viride*) of the candidate fungi towards one isolate of *T. paradoxa*. This even though help us to get a very general idea of their interaction. It would be worthwhile to screen more number of isolates of organisms exhibiting both 'O' and 'MC' type of ineraction towards more number of isolates of *T. paradoxa*. In addition it would also be worthwhile to study the interaction between various isolates of *T. paradoxa* to each other so as to explore the possibiltiy of the existence of cross protection phenomenon. One merit of the present investigation is that it has brought out the antagonistic potential of *Rhizopus stolonifer* and *Thielavia terricola*, hitherto not exploited for plant disease control. However, the present investigation have given us a general idea under *in vitro* condition. Further investigation under *in vivo* condition would be worthwhile.

SUMMARY AND CONCLUSIONS

6 . SUMMARY AND CONCLUSIONS

1. The present investigation deals with the *in vitro* interaction of *Thielaviopsis paradoxa* (causative organism of stem bleeding disease of coconut) with other soil mycoflora of coconut root zone.

2. For this studies, fungi and bacteria were isolated from the soil samples collected once in two months for three periods (February, April and June) from the basins of stem bleeding affected coconut palms of an ongoing field control trial laid out at Uduma from the following treatments viz. control; NPK; NPK+Dolomite; NPK + Dolomite + Neem cake.

3. Total fungal and bacterial population was found maximum in February and least in June. Similar trend was also exhibited in antagonistic fungal population.

4. When various fertilizer treatments were considered there was no significant difference among treatments with regard to the fungal population.

5. However, the bacterial population was found high in NPK + Dolomite + Neem cake treatment.

6. Antagonistic fungal population was also found high in the treatment, NPK + Dolomite + Neem cake.

7. An assessment of *T. paradoxa* population using coconut rachis baits indicated that *T. paradoxa* populations were most adversely affected by NPK + Dolomite + Neem cake followed by NPK + Dolomite. Control and NPK had similar population levels. This observation complements the finding that antagonists population exhibited a reverse trend.

8. *In vitro* interaction of *T. paradoxa* with thirteen different fungi isolated as mentioned above were studied.

9. Three types of inoculation were followed: Sequence - A (*T. paradoxa* challenged by candidate fungus later), simultaneous (both *T. paradoxa* and candidate fungus inoculated at the same time) and Sequence - B (candidate fungus challenged by *T. paradoxa* later).

10. *T. viride* - 2 exhibited maximum percentage of inhibition of *T. paradoxa* in all three types of

inoculation (10%, 20% and 42.8%) followed by *Trichoderma viride* - 1 (10%, 19% and 33.3 %); *Rhizopus stolonifer* (9%, 14.2% and 28.5%); *Trichoderma koningii* (4.7%, 9% and 20%) respectively, 24 hrs after inoculation. *Thielavia terricola* exhibited inhibition of *T. paradoxa* only at 48 hrs. All these fungi came under 'O' type interaction category (over growth of the *T. paradoxa* colony by opposing candidate fungi, usually accompanied by inhibition of *T. paradoxa* on or shortly aftercontact)

11. Three fungi namely, *Aspergillus fumigatus*, *Aspergillus terreus* and *Fusarium solani* exhibited mutual inhibition with *T. paradoxa* after fifteen days and could be designated as 'MC' (mutual inhibition of both the colonies with no apparent intermingling leaving a clear zone (< 2mm) between the colonies).

12. Five fungi exhibited intermingling characters with *T. paradoxa*. They were *Absidia sp.*, *Cunninghamella elegans*, *Gongronella butleri*, *Paecilomyces sp.* and *Penicillium pinophilum*. So this were included in 'I' type interaction category. (intermingling of colonies with no microscopic or macroscopic signs of interaction).

The above mentioned studies were conducted for only one season and the antagonists were tested against only one isolate, i.e. isolate No: 7, *T. paradoxa*. Eventhough indications towards the choice of the antagonists and their method of inhibition of *T. paradoxa* are available, study involving large number of isolates of antagonists and *T. paradoxa* would help in confirming the observations and generalizing the conclusions.

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*Original not seen.

APPENDIX - 1

Effect of Seasons and Fertilizer treatments on fungal population in Stem bleeding affected Coconut soils

SUMMARY OF ANOVA:

Character: Fungi

SOURCE	D.F	S.S	M.S.S	F
Treat(T)	3	805.554688	268.518219	1.50
Period(P)	2	5801.054688	2900.527344	16.20 **
TxP	6	1074.281250	179.046875	1.00
ERROR	24	4298.000000	179.083328	
TOTAL	35	11978.890625		

TABLE OF MEANS

Treatments	Time of sampling			
	FEB	APR	JUN	Mean
Control	73.33	47.00	32.67	51.00
NPK	83.33	63.00	39.33	61.89
NPK+D	64.00	60.33	41.00	55.11
NPK+D+NC	72.67	58.00	56.00	62.22
Mean	73.33	57.08	42.25	

S.E/plot 13.38 Gen. Mean 57.56 C.V(%) 23.25

C.D. (P=0.05) for Period 11.276

APPENDIX - 2

Effect of Seasons and Fertilizer treatments on bacterial population in Stem bleeding affected Coconut soils

SUMMARY OF ANOVA:

Character: Bacteria

SOURCE	D.F	S.S	M.S.S	F
Treat(T)	3	21469.750000	7156.583496	4.77**
Period(P)	2	197377.500000	98688.750000	65.83**
TxP	6	41930.000000	6988.333496	4.66**
ERROR	24	35977.500000	1499.062500	
TOTAL	35	296754.750000		

TABLE OF MEANS

Treatments	Time of sampling			
	FEB	APR	JUN	Mean
Control	297.33	322.67	234.33	284.78
NPK	342.00	349.33	119.00	270.11
NPK+D	355.33	292.67	170.00	272.67
NPK+D+NC	441.67	326.00	224.67	330.78
Mean	359.08	322.67	187.00	

S.E/plot 38.72 Gen. Mean 289.58 C.V(%) 13.37

C.D. for Treatments 37.672 C.D. for Period 32.625

C.D. for TxP 65.249

APPENDIX - 3

Effect of Seasons and Fertilizer treatments on antagonistic fungal population in Stem bleeding affected Coconut soils

SUMMARY OF ANOVA:

Character: Antagonists

SOURCE	D.F	S.S	M.S.S	F
Treat(T)	3	97.222168	32.407387	4.94**
Period(P)	2	20.222168	10.111084	1.54
TxP	6	1.111206	0.185201	0.03
ERROR	24	157.333374	6.555557	
TOTAL	35	275.888916		

TABLE OF MEANS

Treatments	Time of sampling			
	FEB	APR	JUN	Mean
Control	7.33	6.67	6.00	6.67
NPK	5.33	4.67	3.33	4.44
NPK+D	8.67	8.00	6.67	7.78
NPK+D+NC	10.00	8.67	8.00	8.89
Mean	7.83	7.00	6.00	

S.E/plot 2.56 Gen.Mean 6.94 C.V(%) 36.87
C.D. for Treat 2.491

APPENDIX - 4

Effect of Seasons and Fertilizer treatments on percentage colonization of baits by I. Paradoxa in Stem bleeding affected Coconut soils

SUMMARY OF ANOVA:

SOURCE	D.F	S.S	M.S.S	F
Treat (T)	3	5681.000000	1893.666626	71.91**
Period (P)	2	38.000000	19.000000	0.72
TxP	6	106.000000	17.666666	0.67
ERROR	36	948.000000	26.333332	
TOTAL	47	6773.000000		

TABLE OF MEANS

Treatments	Time of sampling			
	FEB	APR	JUN	Mean
Control	26.00	28.00	31.00	28.33
NPK	29.00	35.00	30.00	31.33
NPK+D	19.00	20.00	21.00	20.00
NPK+D+NC	4.00	3.00	3.00	3.33
Mean	19.50	21.50	21.25	

S.E/plot 5.13 Gen. Mean 20.75 C.V(%) 24.73

C.D. for Treat 4.251