EFFECTS OF EXTRACTS OF *Clerodendron infortunatum* ON THE EPILACHNA BEETLE *Henosepilachna vigintioctopunctata* F WITH RELATION TO SAFETY OF ITS NATURAL ENEMIES

By

LILY B

THESIS

submitted in partial fulfilment of the requirement

for the degree

MASTER OF SCIENCE IN AGRICULTURE

Agricultural Entomology

Faculty of Agriculture

Kerala Agricultural University

Department of Agricultural Entomology

COLLEGE OF AGRICULTURE

Vellayani – Thiruvananthapuram

1995

DECLARATION

I hereby declare that the thesis entitled "**Biocontrol of rhizome rot of ginger (*Zingiber officinale*) by antagonistic microorganisms** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other University or Society

Vellanikkara
10 6 96

V SHANMUGHAM

Dr A SUKUMARA VARMA
Associate Professor
Department of Plant Pathology

College of Horticulture
Vellanikkara
Date 10 6 96

CERTIFICATE

Certified that this thesis entitled **Biocontrol of rhizome rot of ginger (*Zingiber officinale*) by antagonistic microorganisms"** is a record of research work done independently by **Mr V Shanmugham** under my guidance and super vision and that it has not previously formed the basis for the award of any degree diploma fellowship or associateship to him

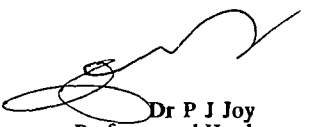
Dr A SUKUMARA VARMA
Chairman
Advisory Committee

CERTIFICATE

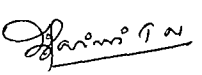
We the undersigned members of the Advisory Committee of **Mr V Shanmugham** a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Pathology** agree that this thesis entitled "**Biocontrol of rhizome rot of ginger (*Zingiber officinale*) by antagonistic microorganisms**" may be submitted by Mr V Shanmugham in partial fulfilment of the requirement for the degree

Dr A Sukumara Varma
Associate Professor
Department of Plant Pathology
College of Horticulture Vellanikkara
(Chairman)

Dr James Mathew
Professor and Head
Department of Plant Pathology
College of Horticulture
Vellanikkara
(Member)



Dr P J Joy
Professor and Head
Department of Entomology
College of Horticulture
Vellanikkara
(Member)



Smt T N Vilasini
Associate Professor
Department of Plant Pathology
College of Horticulture
Vellanikkara
(Member)

EXTERNAL EXAMINER

ACKNOWLEDGEMENT

I express my deep sense of gratitude and sincere thanks to **Dr A Sukumara Varma** Associate Professor of Plant Pathology and Chairman of my Advisory Committee for his personal attention keen interest constant inspiration and expert guidance during the course of this research work and preparation of the thesis

Let me place on record my sincere thanks to **Dr James Mathew** Professor and Head Department of Plant Pathology and member of my Advisory Committee for the help and co-operation received from him during the entire programme

I express my heartful thanks to **Dr P J Joy** Professor and Head Department of Agricultural Entomology and member of my Advisory Com mittee for the valuable suggestions rendered during the preparation of the thesis

My profound gratitude is due to **Smt T N Vilasini** Associate Professor Department of Plant Pathology member of my Advisory Committee for her constructive criticism and valuable advice during the course of investigation as well as in the preparation of the thesis

I extent my sincere thanks to Dr Koshy Abraham Associate Professor Department of Plant Pathology whose timely help has contributed much towards the completion of this thesis

My hearty thanks are expressed to Dr P S John Associate Professor Department of Agronomy for providing the required facilities for conducting the field work at the College of Horticulture Vellanikkara

My sincere thanks are due to Dr R Vikraman Nair Professor and Head Department of Agronomy and Dr Madhusuthanan Pillai Associate Professor Department of Microbiology College of Veterinary and Animal Sciences Mannuthy for extending helping hands during different periods of my work

I am extremely grateful to Dr K T Prasannakumari Assistant Professor AICRP on M & AP for her valuable help in taking photomicro graphs

With all regards I acknowledge the co-operation and help rendered by all the staff members of the Department of Plant Pathology during the period of the study

I am very much thankful to Prof P V Prabhakaran Professor and Head Department of Agricultural Statistics and Sri V K G Unnithan Associate Professor Department of Agricultural Statistics for their good help in the statistical analysis of the data

No word can truly express my deepest gratitude for the sincere help timely suggestions and encouragement provided by Angaiah Edwin Prem and Ajaykumar through out the study period

My sincere thanks are due to Rajesh and Ajithmohan and all other friends for their timely help in different stages of my research work

On a personal note I am grateful to my parents and brother Saravanan whose love and affection and constant help and encouragement which had always been a source of inspiration for me and which made me realise this dream

The grant of Junior Fellowship by Kerala Agricultural University is gratefully acknowledged

I thank Sri Joy for the neat and prompt typing of the thesis

Above all I bow my head before God Almighty who blessed me with health and confidence which stood me in good stead for the successful completion of the work

V SHANMUGHAM

Dedicated to
mother and father

CONTENTS

Chapters	Title	Page No
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	4
3	MATERIALS AND METHODS	19
4	RESULTS AND DISCUSSION	30
5	SUMMARY	69
	REFERENCES	i xv
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No	Title	Page No
1	Quantitative estimation of rhizosphere microflora	33
2	Growth of antagonists and pathogen	35
3	Growth of *Pythium aphanidermatum* and *Rhizopus* sp in dual culture	39
4	Growth of *P aphanidermatum* and *A carneus* in dual culture	39
5	Growth of *P aphanidermatum* and *A niger* in dual culture	42
6	Growth of *P aphanidermatum* and *A fumigatus* in dual culture	42
7	Growth of *P aphanidermatum* and *A flavus* in dual culture	46
8	Growth of *P aphanidermatum* and *T viride* in dual culture	46
9	Growth of *P aphanidermatum* and *A flavus* (sclerotial) in dual culture	51
10	Growth of *P aphanidermatum* and *E javanicum* ITCC No 4 595 in dual culture	51
11	Growth of *P aphanidermatum* and *E javanicum* ITCC No 4 596 in dual culture	54
12	Growth of *P aphanidermatum* and *Streptomyces* sp in dual culture	54
13	Reactions of the antagonists with the pathogen in dual culture	56
14	Effect of cell free culture filtrates of antagonists on the growth of pathogen (Poisoned food technique)	57
15	*In vitro* sensitivity of *P aphanidermatum* to selected fungicides	57

16	*In vitro* sensitivity of antagonists to selected fungicides	61
17a	Effect of antagonists and selected fungicides on rhizome rot of ginger in pot culture experiment percentage of rhizome rot incidence at monthly intervals from 120 DAP	63
17b	Effect of antagonists and selected fungicides on the rhizome rot of ginger in pot culture per cent efficiency over control	64

LIST OF PLATES

Plate No	Title
1	*Pythium aphanidermatum* showing single lobed sporangium (720x)
2	*P aphanidermatum* showing bi lobed sporangium (720x)
3	*P aphanidermatum* showing multilobed sporangia (720x)
4	*P aphanidermatum* showing sexual reproductive structures (720x)
5	*Aspergillus niger* x *P aphanidermatum* (inoculated on the same day) in dual culture on the third day
6	*A niger* x *P aphanidermatum* (inoculated on the same day) in dual culture on the ninth day
7	*A niger* x *P aphanidermatum* (inoculated one day after) in dual culture on the second day
8	*A niger* x *P aphanidermatum* (inoculated one day after) in dual culture on the fourth day
9	*A flavus* x *P aphanidermatum* (inoculated on the same day)in dual culture on the third day
10	*A flavus* x *P aphanidermatum* (inoculated on the same day) in dual culture on the tenth day
11	*A flavus* x *P aphanidermatum* (inoculated three days after) in dual culture on the second day
12	*A flavus* x *P aphanidermatum* (inoculated three days after) in dual culture on the tenth day
13	*Trichoderma viride* x *P aphanidermatum* (inoculated on the same day) in dual culture on the second day
14	*T viride* x *P aphanidermatum* (inoculated on the same day) in dual culture on the ninth day
15	*T viride* x *P aphanidermatum* (inoculated one day after) in dual culture on the second day
16	*T viride* x *P aphanidermatum* (inoculated one day after) in dual culture on the sixth day

Introduction

INTRODUCTION

Ginger (*Zingiber officinale* Rosc) belonging to the family Zingiberaceae is an important spice crop of the world grown and sold as fresh or dried rhizome in India and is believed to be originated in India/China (Purseglove 1972) It is used in medicine edible and soft drink products

In Kerala it is cultivated in an area of 14 040 ha with an annual production of 44 500 tonnes Kerala alone contributes for about 40 per cent of India s production Ginger contributes about 15 5 crores of the export earnings from spices to our country

Ginger is subject to a number of diseases leading to varying degrees of crop damage and yield reduction Well known among them are the rhizome rot disease (*Pythium* spp) bacterial wilt (*Pseudomonas solanacearum*) yellow disease (*Fusarium* spp) and leaf spot disease (*Phyllosticta zingiberi*) Among the diseases the rhizome rot caused by the fungus *Pythium* spp is the most serious threat to ginger cultivation due to its widespread occurrence and devastating nature Apart from the crop damage in the field the rhizomes stored for seed purpose are also affected by these pathogens Several species of *Pythium* are reported to cause rhizome rot of ginger resulting in the death of the plant in the field and shrinkage and rot of stored rhizomes Among them *Pythium aphanidermatum* (Edson) Fitz (Mitra and Subramanian 1928) and *P myriotylum* Drechsler (Uppal 1940 Park 1941 Bertus 1942) are known to be most common and potent pathogens

Mainly two sources of infection are identified viz infected seed materials and overwintering oospores or hybernating mycelium in the soil The

disease incidence is greater when infected seed materials are used for planting resulting in pre emergence rotting So also severity of the disease is more when there is heavy rainfall resulting in post emergence rotting

Management of the disease under field conditions using chemicals is very difficult because of cost of chemicals cost of labour and more over is posing problems of environmental hazards Application of chemicals in the soil adversely affect non target organisms including beneficial organisms and thereby causing derangement of the ecological balance For these reasons importance of biological control and their use as candidates for inclusion in the arsenal of weapons for disease management has increased during recent years Now biological control is regarded as one of the basic components of the integrated disease management system in modern agriculture

In recent years considerable success has been achieved by various workers in the control of *Pythium* by introducing antagonistic fungi like various species of *Trichoderma Chaetomium Penicillium Gliocladium Pythium* and also species of bacteria like *Pseudomonas Corynebacterium Bacillus* and *Enterobacter* If the native antagonists are found effective in checking the population of the pathogen *Pythium* they can best be utilised as biocontrol agents for controlling rhizome rot of ginger Such a practice of management if evolved will be more cheap long standing and not affecting the environment Considering these facts the present study was undertaken with the following objectives in view

(i) Isolation of the pathogen from the infected rhizomes of the host plant and testing its pathogenecity

(ii) Identification and characterization of the pathogen

(iii) Isolation of the soil microorganisms from the rhizosphere of healthy ginger plants among the rhizome rot affected plants in the diseased field

(iv) Identification and characterization of the isolated microorganisms

(v) Evaluation of the isolated microorganisms for their antagonistic properties against the pathogen under laboratory and pot culture conditions

(vi) Evaluation of the microorganisms showing antagonism for their antibiotic producing ability

(vi) Assessment of compatibility of the selected biocontrol agents with the commonly used fungicides for the management of rhizome rot

Review of Literature

REVIEW OF LITERATURE

The most serious disease of ginger is the rhizome rot caused by *Pythium* species Rhizome rot of ginger is of common occurrence in all ginger growing tracts in India Butler (1907) first recorded this serious disease of ginger in Gujarat caused by a species of *Pythium* which was later identified as *Pythium gracile* Schenk Sarma *et al* (1979) reported the prevalence of *P aphanidermatum* as the major pathogen causing rhizome rot of ginger in Kerala Kannan and Nair (1965) reported losses upto 80-90 per cent due to rhizome rot of ginger in Kerala

Pythium spp have been found to survive in soil for periods of 2 12 years (Hoppe 1966) but the survival structures in different species have not been adequately demonstrated The disease is carried over and propagated through diseased rhizomes and resting structures of the causal organism McRae (1911) considered the use of diseased rhizomes to be the principal factor in the dissemina tion He has also stated that the fungi responsible for rhizome rot are common soil dwellers and they have saprophytic ability to live in soil for a long time even in the absence of host Oospores and Chlamydospores reach the soil from infected rhizomes left to rot in the field or from the scales or the lower sheaths may also serve as important sources of infection High soil moisture is highly conducive to the disease development The disease becomes severe under poor drainage (Sarma *et al* 1979)

2 1 Screening for host resistance

Among the available varieties Maran Suas showed the field resistance against *P aphanidermatum* when tested by Indrasenan and Paily (1973) Nadia and

Narasapattam are also found as moderately resistant in Kerala (Balagopal *et al* 1974) Nybe and Nair (1979) reported rhizome rot incidence from 16-25 5 per cent in varieties Himachal Pradesh Taiwan Tafingiva Rio de Janiero and 3 2 7 5 per cent in varieties Maran Vengara Wynad local Wynad Manantody and Kuruppam pady

2 2 Quantitative estimation of soil microorganisms

Waksman and Curtis (1916 1918) and Jensen (1943) made attempts to estimate the microflora of soil and reported that the population varied from 300 50 00 000 per gram of soil depending on soil types Skinner *et al* (1952) reported several hundred thousands to hundred millions of bacteria per gram of dry soil Waksman (1952) observed that the fungi were lesser in number than the actinomy cetes and the population of fungi in soil ranged from few to as many as ten lakhs per gram

When the soil depth increased the microbial population was found to decrease A gradual reduction in microflora in deeper layers of soil was observed by different workers (Aristovskaya 1951 1957 Waksman 1952 Rose 1954 Milosevic 1958 Tsao *et al* 1959 Corke and Chase 1964 Venkatesan 1964 Vinod 1988)

The soil microbial population was found to decrease as the organic carbon availability of the soil decreased (Waksman and Curtis 1916 1918 Starc 1942 Laudelout *et al* 1949 Aristovskaya 1951 1957 Rose 1954 Blue *et al* 1955 Zhukova 1956 Jagnow 1958 Tsao *et al* 1959 Popova 1963 Vinod *et al* 1988)

2 3 Disease control with chemicals

Park (1935) observed that immersing seed ginger in 0 1 per cent mercuric chloride for two hours either just after harvesting or just before sowing yielded many more plants than untreated seed Thomas (1940) reported dipping of rhizomes in 0 25 per cent ceresan for 30 minutes to be effective in controlling rhizome rot in the field Bhagwat (1960) reported that rhizome rot of ginger caused by *P myriotylum* could be satisfactorily controlled by dipping the rhizomes in 2 2 50 Bordeaux mixture and by application of the fungicide to the soil eight days before sowing He also reported good control of the disease by soil drenching with cheshunt compound at the rate of 1 ounce/2 gallon of water Shahare and Asthana (1962) reported that seed treatment of ginger by different chemicals was not effective but soil treatment with Bordeaux mixture (4 4 50) 0 35 per cent perenox and 0 15 per cent dithane Z 78 suppressed the disease quite effectively

Kothari (1966) observed that treatment with mercuric chloride gave the highest germination of ginger No germination took place in the plots even after two months which were drenched with Bordeaux mixture Plots treated with thiram 0 2 per cent (soil drench) showed poor development of ginger High percentage of germination was obtained by drenching with dithane Z 78 and difolatan and highest yield with blitane (zinc + copper oxychloride) dithane Z 78 and difolatan when used as soil drench or drench plus seed treatments (Sarma *et al* 1979) Sharma and Dohroo (1982) observed that seed dip in 0 2 per cent solution of either dithane M 45 or daconil was effective in controlling the rhizome rot as well as increasing yield in the field

2 4 Disease control with biological agents

In a period when study of antibiotics amounts to a fad and the growing concern of both environmentalists and public health authorities on the increasing use of potentially hazardous fungicides in agriculture become the prime consideration the possibility of controlling plant pathogenic fungi by antagonistic microorganisms added either as a substitute or as an additive to fungicides has been the subject of extensive research The history of biological control dates back to 1908 when Potter showed that plant pathogens could be inhibited by their own metabolic products Sanford (1926) studying the potato scab caused by the actinomycete *Streptomyces scabies* brought the rather sudden realization that other soil microorganisms must exert a natural biological control even on root disease pathogens Garrett (1956) defined biological control of plant disease as any condition or practice whereby survival (or) activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of the disease caused by the pathogen

Antibiosis is a phenomenon employed in biological control and it means a relation between organisms in which one organism the antagonist creates adverse circumstances for the other for its growth According to Park (1961) categories of antagonism are antibiosis competition or exploitation

Baker and Cook (1974) stated "antagonistic potential resides in every soil microorganism and any random soil sample should yield antagonists to some microorganisms Metabolites are secreted and one of these would certainly prove inhibitory to some other microorganisms" Biological control by introduction of

antagonist into non treated soil is difficult to achieve because it attempts to establish an alien antagonist in a biologically buffered community (Baker and Cook 1974) Although this is difficult it can be done when the right organisms are obtained by screening the microorganisms isolated from soil for their antagonistic properties and properly used

2 4 1 Antagonistic microorganisms

A considerable amount of work has been carried out on the possibility of biological control of *Pythium* species using different antagonistic microorganisms which include fungi actinomycetes and bacteria

2 4 1 1 Antagonistic fungi

Weindling (1932 1934 1937) and Weindling and Emersen (1936) showed that *Trichoderma lignorum* (= *T viride*) and *Ghocladium fimbriatum* common inhabitants of wet soils produce a substance gliotoxin which inhibits the growth of *Pythium* Thomas (1940) suggested the use of biological control for *Pythium* spp by application of *T lignorum* Reduction of tobacco damping-off (*Pythium aphanider matum*) by *Trichoderma harzianum* was reported by Fajola and Alasoadura (1975) Among the fungi isolated from soil and inhibiting the development of *Pythium* spp *Penicillium cyclopium* and *T lignorum* were the most active antagonists (Shekunova and Volkova 1977) Dumitras and Fratilescusesan (1979) observed *T viride* as strongly antagonistic to *Pythium debaryanum* from sugarbeet pea and cotton seedlings In *in vivo* tests *T viride* protected the seedlings as effectively as fungicidal principles

In lab studies treatment of radish and pea seeds with conidia of *Trichoderma hamatum* in a methocel slurry protected seeds and seedlings from *Pythium* spp or *Rhizoctonia solani* nearly as effective as fungicide seed treatments (Harman *et al* 1980) Chet *et al* (1981) found in the attack by *T hamatum* on other fungi parasitism followed by lysis rather than the involvement of antibiotics

Yehia *et al* (1981) reported that *T viride Streptomyces griseus* and *Bacillus subtilis* reduced damping off of tomato caused by *P debaryanum Phytophthora (nicotianae)* var *parasitica* and *Fusarium oxysporum* f sp *lycopersici* in the glass house In dual culture *T hamatum* inhibited radial extension of *P ultimum* prior to hyphal contact Dohroo and Sharma (1984) obtained > 80 per cent control of rhizome rot of ginger in storage caused by *Pythium pleroticum* (wet rot) and *Fusarium equiseti* (dry rot) with *T viride* Seedling root rot caused by *Pythium graminicola* in canes did not occurred when *T viride* was incorporated in the soil (Padmanaban and Alexander 1984 1986 1987) Sivan *et al* (1984) obtained efficient control of damping off induced by *P aphanidermatum* in peas cucumbers tomatoes and peppers by application of wheat bran/peat preparation of *T harzianum* to soil

Mukhopadhyay and Chandra (1986) achieved control of damping off of sugarbeet and tobacco incited by *P aphanidermatum* by the application of wheat bran saw dust preparation of *T harzianum* at different layers to soil They also studied the mode of antagonism and found that it caused lysis and disintegration of protoplasm of the test fungi when grown on potato dextrose agar plates in dual culture They also found that *T harzianum* showed antibiotic activity towards *P aphanidermatum* Incorporation of the antagonists (*T harzianum* and *T viride*) to

the soil nine days before sowing protected tobacco seedlings from damping-off caused by *P aphanidermatum* upto 25 days (Nagarajan and Reddy 1986)

Bhardwaj *et al* (1988) obtained good control of rhizome rot of ginger caused by *P aphanidermatum* and *F equiseti* during storage by steeping inoculated rhizomes in a spore suspension of *T viride* (or) smearing with *T hamatum* Bhardwaj and Gupta (1991) reported that all the three species of *Trichoderma* viz *T viride T harzianum* and *T hamatum* inhibited the growth of *P aphanidermatum* and *F equiseti* causing rhizome rot of ginger when tested by dual culture method

Thomas *et al* (1991) observed that the rhizome rot disease of small cardamom (*Elettaria cardamomum* Maton) caused by *Pythium vexans* and *R solani* can be effectively controlled in *in vitro* by *T viride T harzianum Laetisaria arvalis Aspergillus* sp and *B subtilis* The antagonists interacted with the pathogens by growth inhibition anastomosis and hyphal lysis Integrated control of *Pythium* damping off of sugarbeet by metalaxyl and *T harzianum* was reported by Sawant and Mukhopadhyay (1991) Vinod *et al* (1991a) reported *T harzianum Trichoderma koningii* and *Trichoderma longibracheatum* showing dual culture reac tion of die back and disintegration of the test organism *P myriotylum* indicating mycoparasitism as their antagonistic property The *in vitro* inhibition of hyphal growth of *P aphanidermatum* by the isolates of *Trichoderma* viz *T harzianum* T_{82} and *Trichoderma* sp NF_9 was observed by Xu *et al* (1993)

The antimetabolite production by various species of *Trichoderma* was reported by several workers Brian and McGowan (1946) isolated a fusidic acid like antibiotic named viridin from *T viride* which possessed antifungal properties Dennis and Webster (1971) reported that *T hamatum* produced volatile and non

volatile metabolites having antifungal activity Papavizas (1984) reported production of trichodermin by *T lignorum* (*T viride*) and also opined that *Trichoderma* and *Gliocladium* produced various enzymes such as endo and exoglucanase cellobiase and chitinase When the isolates of different *Trichoderma* species were tested for the production of toxic metabolites using dual culture method and also agar layer technique many of them produced non volatile metabolites toxic to *P aphanider matum* the common mcitant of damping off disease in many crop nurseries including tobacco (Raju 1991) Devaki *et al* (199?) reported that *T harzianum* suppressed the growth of *P aphanidermatum* and *P myriotylum* infecting tobacco in dual culture by releasing a non volatile antibiotic ß (1 3) glucanase

The antagonistic properties of various species of *Aspergillus* were reported by several workers against other pathogens

Trevinoo and Espinosa (1981) applied conidial suspension of different species of *Aspergillus* with potato saccharose agar to cocoa against *Phytophthora palmivora* Only *Aspergillus terreus* was found to retard the start of disease by 30 days Bora (1977) observed that *Aspergillus niger* has shown greatest antagonism against *R solani* from egg plant when its antagonistic property was estimated among other soil fungi The inhibition of growth of *R solani* by parasitising the hyphae by *A niger* was reported by Gokulapalan and Nair (1984)

Mahadevamurthy *et al* (1988) noticed that germinatio of sclerotia of *Claviceps fusiformis* causing ergot of *Pennisetum americanum* was inhibited in soil amended with *A niger Fusarium oxysporum T harzianum T viride* and *B subtilis* Sclerotia colonized by the antagonists disintegrated and when fragments were incubated colonies of the antagonists were obtained Germination of the host

seed was not affected Gaur and Sharma (1991) observed that the microorganisms (34 fungi six bacteria and four actinomycetes) isolated from the rhizosphere soil of pigeon peas when tested for their antagonistic action towards *Fusarium udum* on Czapek s sucrose nitrate agar medium *T viride* followed by *A niger Streptomyces* sp *Penicillium* sp and *Bacillus* sp were the most effective in controlling the pathogen The antagonistic property of white sterile fungus *A niger Penicillium citrinum* and *B subtilis* against *Xanthomonas campestris* pv *cyamopsidis* causing bacterial blight of *Cyamopsis tetragonoloba* was reported by Sindhan *et al* (1991) Parashar *et al* (1992) reported that the antagonists *P citrinum* white sterile fungus *A niger* and *B subtilis* reduced the incidence of bacterial blight of *C tetragonoloba* caused by *X campestris* pv *cyamopsidis* compared with untreated controls when used as seed dressings or spray treatments and culture filtrate sprays of *P citrinum A niger* and the white sterile fungus were also effective in reducing the incidence

Dhedhi *et al* (1990) observed *Aspergillus fumigatus* as an effective antagonist to *F oxysporum* f sp *ciceris* causing vascular wilt of *Cicer arietinum* in *in vitro* studies

Masroor and Chandra (1987) reported that of 27 fungal isolates tested against *X campestris* pv *citri* the incitant of citrus canker the highest activity was shown by *Aspergillus flavus Aspergillus clavatus* and *A niger* The nonvolatile activity of *A flavus* against the growth of *Sclerotium rolfsii* was reported by Deo (1990) *A flavus* inhibited *F oxysporum* f sp *lini* from flax and *F oxysporum* f sp *lycopersici* from tomato by 28 2 and 26 per cent respectively (Dwivedi *et al* 1993)

Raistrick and Smith (1935) reported production of terrein by *A terreus* The production of geodin terricin and terric acid was also reported (Marcus 1947) Zaehner *et al* (1963) reported that the antibiotic properties of the metabolites of *A terreus* were due to flavipin eridin geodin patulin terric acids and sideramine ferrichysin The antibiotic production by *A niger* was reported by Broadbent (1966) The antibiotic jawaherene was detected in the study

Mukhopadhyay (1991) observed that species belonging to the genera *Gliocladium* and *Trichoderma* have proved highly effective in controlling plant pathogens especially soil borne ones like *Pyt m* spp

In the dual culture studies with *Penicillium citrinu* it has been reported to have strong antagonistic activities towards *Gaeumanomyces graminis* and *Pythium* sp (Domsch 1960) Seed treatment with comula of *Penicillium oxalicum* reduced seed rot and damping off of chickpea caused by *P ultimum* in naturally infested soils (Kaiser and Hannan 1984) Sharif (1988a) observed in plastic greenhouse treatment of cucumber plants with mycelial preparations of *Penicillium pinophilum P stiptatum T harzianum* effectively controlled *P aphanidermatum* Efficient control of damping off induced by *P aphanidermatum* in cucumber was obtained by *Penicillium stipitatum* and *T harzianum* (Sharif *et al* 1988b) Krishnamoorthy and Bhaskaran (1991) obtained an isolate of *Penicillium* from soil which was found to be most inhibitory to *Py hum indicum* in dual culture

The antibiotic production by *P citrinum* has been observed by many workers Hetherington and Raistrick (1931) found that citrinin an aromatic polycylic compound was produced by *P citrinum* Citrinin has been reported to have fungistatic and hyphae narrowing properties (Robinson and Park 1966) The

dual culture reaction of inhibition at a distance and disintegration of *P myriotylum* by *P citrinum* was reported by Vinod (1988) He also found that the cell free culture filtrate of *P citrinum* showing 100 per cent inhibition against *P myriotylum* indicating the production of antibiotics by the antagonist

2 4 1 2 Antagonistic actinomycetes

Streptomyces is the promising group of actinomycetes well known for the production of many different types of antibiotics Many species among these groups of organisms produce specific antibiotics under ideal conditions These group of actinomycetes have been well studied by research workers to reveal their antibiotic properties

Ainsworth (1977) stated that amphotericine was a polyene antibiotic obtained from *Streptomyces* sp and was found to be antifungal The degradation of hyphae of *P aphanidermatum* upon contact with soil particularly with some actinomycetes in *in vitro* was reported by Domsch *et al* (1980)

Yehia *et al* (1981) found that the antagonists *T viride Streptomyces griseus* and *B subtilis* effectively reduced damping off disease of tomato seedlings caused by *P debaryanum Streptomyces* spp isolated from peat effectively inhibited the growth of *P debaryanum* causing damping off of sugarbeet both *in vitro* and *in vivo* was reported by Tahvonen (1982) Tahvonen (1988) observed that spraying the peat substrate with a suspension of *Streptomyces* spp reduced root diseases of cucumbers caused by *Pythium* spp Rath and Wolf (1992) controlled seed rot and damping off of sugarbeet caused by *P ultimum* by combined application of *Pseudomonas fluorescens* B 5 and *Streptomyces* sp A 102

2 4 1 3 Antagonistic Bacteria

Tedla and Stanghellini (1992) observed that in tripartite rhizosphere (host fungus and bacteria) interactions bacterial antagonism was found to be responsible for the general suppression of *P aphanidermatum* activity at low soil temperature

Henis and Inbar (1968) observed that the metabolites from *B subtilis* inhibited the growth of *P ultimum* Vinod *et al* (1991b) reported that an isolate of *B subtilis* obtained from forest soils showed good antagonistic and antibiotic properties against the test fungi *P myriotylum P palmivora* and *R solani* In dual culture studies *B subtilis* parasitised *P myriotylum* and caused lysis and distintegra tion *P ultimum* causing damping off in *Pinus sylvestris* was inhibited by selected strains of *Pseudomonas Pseudomonas putida* and *B subtilis* in both *in vitro* and by seed and soil applications (Zaspel and Suss 1992) Smith *et al* (1993) reported that application of a suspension of *B cereus* strain UW 85 to cucumber fruits protected them against cottony leak disease caused by *P aphanidermatum*

2 5 System of growth and delivery of antagonists into soil

One of the most critical obstacles to biological control by direct massive soil augmentation has been the lack or scarcity of methods for mass culturing and delivering antagonists to soil Despite the limited progress scientists are attempt ing to develop effective experimental systems of growth and delivery of antagonists into soil For bacterial antagonists nutrient broth has been extensively used (Mitchell and Hurwitz 1965 Merriman *et al* 1974 Mangenot and Diem 1979) Broadbent *et al* (1971) grew potential bacterial antagonists in shake cultures of

yeast mannitol broth or nutrient broth *B subtilis* was cultured on potato dextrose broth for applying to kernels of corn (Kommedahl and Mew 1975)

Formulation of biocontrol agents facilitated better application Suslow *et al* (1979) suggested that formulation of *Pseudomonas* may be prepared by coating the cells with gums and polysaccharides that stabilize them so that they can be formulated as a dry powder

Development of growth media for large scale production of antagonists and of commercially acceptable carriers for their production into soils is brighter for fungi than for bacteria A diatomaceous earth granule impregnated with a molasses solution was found suitable for growth and delivery of *T harzianum* (Backman and Rodriguez Kabana 1975) Akhtar (1977) used wheat straw as the growth medium for *T viride* Many workers reported the use of wheat bran as a growth medium for *T harzianum* (Hems *et al* 1978 1979 Chet *et al* 1979 Maiti and Sen 1985 Mukharjee *et al* 1987) Elad *et al* (1982) reported high lytic activity of *T harzianum* in wheat bran culture of the fungus and in soil inoculated with the culture Lewis and Papavizas (1984) used a mixture of wheat bran sand and water to grow the antagonists viz *T viride T harzianum T hamatum Gliocladium roseum Gliocladium virens Gliocladium catenulatum Talaromyces flavus* and *Aspergillus ochraceous* Sivan *et al* (1984) studied the growth potential of *T harzianum* on organic food bases including several agricultural wastes like wheat bran wheat straw compost ground wheat straw ground cotton straw peat and a wheat bran/peat mixture (1 1 v/v) They found that wheat bran/peat was the best medium for the growth and survival of *T harzianum*

A medium consisting of equal volumes of wheat bran peat moss and water was used for culturing *T harzianum* (Chang *et al* 1986) Mukhopadhyay *et al* (1986) prepared *T harzianum* in a wheat bran sawdust tap water mixture Biological control of sugarbeet and tobacco damping off was achieved in glasshouse by the application of wheat bran sawdust preparation of *T harzianum* to *Pythuum* infested soils (Mukhopadyay and Chandra 1986) Mass multiplication of *T viride* in sand sorghum medium (sand 100 g and sorghum 20 g) was reported by Padmanaban and Alexander (1986)

Truong *et al* (1988) mass produced *T harzianum* in a mixture of rice bran rice hull water (3 1 1 2 v/v/v) and the culture was incorporated into baked and unbaked silt/loam soil at 50 cc per litre of soil effectively controlled *P aphanider matum* causing damping off on tobacco *T harzianum* when grown in a semi solid fermentation medium on wheat bran peat effectively controlled diseases of *Pythium* spp in green house tests (Ordentlich and Chet 1989)

Potato dextrose agar was used for culturing *Chaetomium globosum* (Kommedahl and Mew 1975) and *P oxalicum* (Kommedahl and Windels 1978) Modified Czapek liquid medium was used to obtain highly active culture filtrate of *A clavatus A flavus* and *A niger* which are active against X *campestris* pv *citri* causing citrus canker (Masroor and Chandra 1989)

G roseum was prepared in a mixture of peat soil and nutrients (Moody and Gindrat 1977) Lumsden *et al* (1992) noted that *G viren* produced detectable levels of gliotoxin when grown with an alginate wheat bran food base delivery system in peat moss vermiculate soil less medium (PV medium)

The antagonist *Corticium* first grown on corn leaf meal (CLM) and incorporated into *Pythium* infested field gave reduction in the incidence of damping off of table beet (Hoch and Abawi 1979) *L arvalis* was grown on wheat bran and used for the control of damping off of tomato by *Pythium* sp and root rot of black gram caused by *R batatıcola* (Martin *et al* 1984)

The foregoing amount of literature indicates that there is gre t potential to bring about reduction of the soil borne plant pathogen *Pythium* by selecting correct antagonists and applying them to the field at the correct time

Materials and Methods

MATERIALS AND METHODS

The laboratory and pot culture experiments were conducted at the College of Horticulture Vellanikkara during the year 1994 95 for the selection of promising antagonists both *in vitro* and *in vivo* against the pathogen inciting rhizome rot of ginger

3 1 Isolation and identification of the pathogen

The organism causing the rhizome rot disease was isolated from the infected rhizomes of diseased ginger plants by employing standard isolation methods (Riker and Riker 1936) The culture of the fungus was maintained in oatmeal agar medium For studying the morphological characters actively growing culture discs of the fungus were kept in sterile distilled water for 24 h and it was observed under the microscope

The measurements of the pathogen were carr out by using Olympus research microscope with maximum possible magnification The morphological characters like colour and thickness of mycelium size and shape of sporangia size and shape of oogonium and antheridium and their attachment were observed and recorded

3 2 Pathogenecity test

The pathogenecity tests were carried out in both *in vitro* and *in vivo* For artificial inoculation the detached ginger rhizomes were clearly washed in running water to remove the soil particles and it was then surface sterilized with absolute

alcohol Small holes were made on the surface of rhizomes ıth sterilized 5 mm cork borer and ɣoung mycelial discs (5 mm culture discs) were placed ın ıt under aseptic condition The ınoculated rhizomes were kept ın aseptic moist chambers and ıncubated at room temperature (24 to 30°C) tıll complete rottıng of the rhizome took place

One month old potted plants were used for provıng pathogenecıty test The mycelial mat of *P aphanidermatum* grown on oat meal agar medium was mixed with top 5 cm soil and wet cotton wool was placed over the ınoculated soil surface for 48 hours The development of symptoms were observed at weekly ıntervals

3 3 Collection of soil sample

The soil samples were collected during October 1994 from the rhizosphere of healthy ginger plants among the rhizome rot affected plants ın the diseased field The samples were pooled shade dried and were quant vely and qualitatively estimated

3 4 Quantitative estimation of microflora

The quantitative assay of microflora was carried out by serial dilution plate technique (Johnson and Curl 1972) The soil sample (10 0 g) was added to 100 ml sterile distilled water ın 250 ml conical flasks and shaken for 30 mm ın orbital shaker Ten ml of this soil dilution was then transferred to another flask containing 90 ml sterile d stilled water to get 10^{2} dilutions Later 10^{4} and 10^{6} dilutions were prepared from this by serial dilution

3 4 1 Estimation of fungal population

One ml of 10^{4} soil dilution was pipetted into sterile petri dishes to which 20 ml of melted and cooled Martin s rose bengal streptomycin agar media was poured Three petri dishes were kept as replications for the sample The petri dishes with the media were swirled thoroughly to get uniform distribution After solidification the dishes were incubated at room temperature for three days The fungal colonies developed at the end of three days were counted using dark field colony counter and expressed as number of colonies per gram of dry soil

3 4 2 Estimation of actinomycete population

The estimation of actinomycete population was done with a soil dilution of 10^{6} using Kenknight s agar medium and the method followed was as in the estimation of fungal population The dishes were incubated for seven days at room temperature and the actinomycete colonies were counted usmg dark field colony counter and expressed as number of colonies per gram of dry soil

3 4 3 Estimation of bacterial population

Bacterial population was estimated using 10^{6} soil dilution in nutrient agar medium The method employed for the estimation of fungal population was followed The dishes were incubated for 48 h at room temperature The bacterial colonies developed were counted with the help of dark field colony counter and expressed as number of colonies per gram of dry soil

3 5 Qualitative estimation of microorganisms

3 5 1 Fungi

The young fungal colonies developed in dilution plates were transferred to potato dextrose agar medium (PDA) Pure cultures of fungi were obtained by hyphal tip isolation method and they were maintained in PDA

Morphological characters of the fungi in pure culture were studied by growing them in petri dishes slants and slide cultures On the basis of the morpho logical characters they were identified

3 5 2 Actinomycete

The single colonies of actinomycete developed in Kenknight s agar were transferred to test tube slants of the same medium and maintained in pure culture They were provisionally identified on the basis of their morphological characters

3 5 3 Bacteria

The bacterial colonies developed in the dilution plate method were streaked in nutrient agar (NA) and single colony isolation was made The pure cultures were maintained on NA slants Bacterial isolates were identified as gram positive and gram negative based on their gram reactions

The pure cultures of isolated fungi and actinomycete were sent to Indian type culture collection Indian Agricultural Research Institute New Delhi and the identification was got confirmed

3 6 Growth rate of antagonists and pathogen

3 6 1 Fungi

An aliquot of 15 ml of PDA was transferred into 90 mm petri dishes After solidification of the media 5 mm disc from actively growing zone of the young fungus culture on PDA was lifted by a sterile 5 mm cork borer and transferred to the centre of the media in petri dishes The plates were incubated at room temperatures ($28\pm2^{\circ}$C) and radial growth of the fungi was measured at intervals of 24 h upto 14 days to know their respective growth rates

3 6 2 Actinomycete

Actinomycete was grown in Kenknight s agar by adopting the method described in the case of fungi Observations on radial growth was taken at intervals of 24 h upto 18 days

3 6 3 Pathogen

For estimating the growth rate of *P aphanidermatum* the pathogen was grown in oat meal agar by employing the method as in fungi and measurements were taken for three days at intervals of 24 h

3 7 Screening the microorganisms for antagonistic properties against the pathogen

Qualitatively estimated microorganisms were subjected to antagonistic studies against the pathogen *P aphanidermatum* employing dual culture method (Johnson and Curl 1972)

3 7 1 Fungi

The organisms were inoculated as dual cultures after giving due considera tion for the growth rate of both the pathogen and the potential antagonists An aliquot of 15 ml of PDA was transferred into 90 mm petri dishes After solidifica tion of the media a 5 mm disc from an actively growing zone of the fungal isolate on PDA was removed by a sterile cork borer and transferred to near periphery of one half of petri dish A disc of 5 mm of the pathogen was similarly transferred from another plate and placed at the opposite half of petri dish towards the periphery The time of inoculation of the pathogen was decided after taking into account its growth rate with respect to antagonist

The growth measurements were taken at intervals of 24 h upto ten days The types of reactions exhibited were recorded Five replications were maintained for each antagonistic fungus The pathogen and the antagonist grown in monocul tures served as control The isolates possessing good antagonistic properties were identified

3 7 2 Actinomycete

In the case of antagonistic study of the actinomycete dual culture method was employed with the double agar technique (Johnson and Curl 1972) Nutrient glucose agar was used as basal medium and over that PDA was poured and the antagonist and pathogen were moculated as described in case of fungi The time of inoculation of the pathogen was delayed due to the slow growth of actinomycete The growth of antagonist and pathogen was recorded at 24 h intervals The type of

reaction exhibited was also recorded Replications and control were maintained as in the case of fungi

3 7 3 Bacteria

The antagonistic study with bacteria using the pathogen was done by the method as described in case of the actinomycete The bacterial antagonist was streaked horizontally against the pathogen towards the periphery of the dish in the double agar technique and observed for their antagonistic effects

3 8 Assay of culture filtrates of the promising antagonists selected from dual culture

The four isolates of fungi showing good antagonistic properties in dual cultures were grown in liquid cultures and were utilised for determining the production of secondary metabolites by the antagonists Fifty ml of the potato dextrose broth was taken in 250 ml conical flasks and sterilised at 15 lbs pressure for 20 min The broth in the flasks was then inoculated with 5 mm mycelial discs of each of the actively growing young cultures of promising antagonistic fungus grown on PDA They were then incubated at room temperature (28 ± 2 C) for 14 days in shake cultures

The cultures were filtered by coarse filtration using Buckner flasks This filtrate was again filtered through millipore filters (pore size 450 nm) and stored in vials for studying the production of secondary metabolites The culture filtrate containing secondary metabolites stored in vials were assayed for their antagonistic action against the pathogen by employing the poisoned food technique (Zentmayer 1955) A quantity of 0 3 ml of the culture filtrate and 20 ml of PDA were

poured into 90 mm sterile petri dishes The petri dishes were rotated well for mixing thoroughly After solidification a 5 mm disc of an actively growing culture of the pathogen was placed at the centre of the dish Growth measurements were recorded till the day when the test organism reached 90 mm in control Five replications were maintained in each case and control plates were also maintained by adding 0 3 ml of sterile distilled water

The inhibitory properties of culture filtrates of the antagonists were assayed against the pathogen and was expressed as per cent inhibition using the following formula suggested by Vincent (1927)

$$\frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100 = \text{Per cent inhibition}$$

3 9 *In vitro* effect of recommended fungicides on the antagonists and the pathogen

The following fungicides were used for the *in vitro* evaluation

Common name	Active ingredient	Concentration (per cent)
Bordeaux mixture	$CuSO_4 3Cu(OH)_2$	1 0
Fytolan	Copper oxychloride	0 2
Emisan 6	Methoxy ethyl mercuric chloride	0 1
Indofil M 45	Mancozeb	0 3

The effect of different fungicides on the growth of the fungi was tested by the "Poisoned food technique" (Zentmayer 1955) The required quantity of fungicides were added to the 100 ml sterile molten PDA so as to get the

required concentrations of the fungicides mixed well and poured into sterile petri dishes at the rate of 15 ml each Mycelial discs of 5 mm diameter were cut and placed in the centre of each petri dish containing the poisoned medium Control petri dishes of PDA medium alone inoculated with the fungus was also maintained Three replications were maintained for each of the chemicals The inoculated petri dishes were incubated at room temperature and the observations on the radial growth of fungus were taken when the control dishes completed full growth

3 10 Pot culture experiment for testing the efficacy of antagonists

Pot culture studies were carried out during the period from May to December 1995 to find out the efficacy of the four antagonistic microorganisms viz *Aspergillus niger Aspergillus fumigatus Aspergillus flavus* and *Trichoderma viride* selected from dual culture studies

Fifteen days old antagonists grown in milled rice were used for the studies Twenty five gram of milled rice was weighed and transferred to 250 ml conical flasks separately Sterilization of milled rice was done following the procedure given by Ahmed and Tribe (1977) Twenty five ml of water was added and autoclaved at 1 4 kg/cm^2 for 20 min Ten flasks were kept for each antagonist A 5 mm disc from an actively growing zone of the antagonistic fungi were removed separately by sterile cork borer and transferred to the growth media

The pot culture experiments were conducted without the sterilization of the potting mixture Eight kg of potting mixture containing sand dried and powdered cowdung and sick soil (soil from fields heavily infected with rhizome rot) in the ratio 1 1 1 was taken in earthern pots (30 cm) Ginger crop was raised by

planting one healthy sprouted rhizome (15 g) per pot The variety used was Rio-de Janeiro

The experimental design followed was CRD with 20 treatments and five replications The treatments were as follows

T_1 Healthy seed rhizomes pre inoculated with *A niger* by smearing the cultures shade dried and planting done in diseased soil

T_2 Do- using *A fumigatus*

T_3 Do- using *A flavus*

T_4 Do- using *T viride*

T_5 *A niger* grown in media was incorporated into the diseased soils at the rate of 60 g per pot in pits of 5 cm depth at the time of planting and then planting done

T_6 *A fumigatus* used as in T_5

T_7 *A flavus* used as in T_5

T_8 *T viride* used as in T_5

T_9 *A niger* incorporated into soil after first top dressing (60 days after plant ing)

T_{10} *A fumigatus* used as in T_9

T_{11} *A niger* used as in T_9

T_{12} *T viride* used as in T_9

T_{13} *A niger* incorporated into soil after first and second top dressings (60 and 120 DAP)

T_{14} *A fumigatus* used as in T_{13}

T_{15} *A flavus* used as in T_{13}

T_{16} *T viride* used as in T_{13}

T_{17} Treating the seed rhizomes as per Package of Practices recommendations (KAU 1993) and then planting

T_{18} Drenching the pots with copper oxychloride 0 3% when the disease incidence first noticed

T_{19} Drenching the pots with mancozeb 0 3% when the disease incidence first noticed

T_{20} Control (without antagonists/chemicals)

Cultural operations were carried out as per the Package of Practice Recommendations (KAU 1993) except fungicidal application

The following observations were made from the pot culture studies

(1) Number of infected tillers at monthly intervals from 120 days upto 180 days

(2) Yield

Statistical analysis of the data of the pot culture experiment was done with transformed values Treatment means were compared usmg Duncan s Multiple Range Test (DMRT)

Results and Discussion

RESULTS AND DISCUSSION

Rhizome rot a destructive disease of ginger assumes alarming propor tions during the monsoon periods resulting in yield loss as well as reduction in the quality and germinability of rhizomes In India eventhough a few workers have studied the control of rhizome rot of ginger with chemicals (Park 1935 Thomas 1940 Bhagwat 1960 Shahare and Asthana 1962 Kothari 1966 Sarma *et al* 1979 Sharma and Dohroo 1982) effective control has not been obtained so far In view of the serious nature of the disease the present study was undertaken to isolate the native antagonists and to screen them against the pathogen so as to select suitable biocontrol agents to be used for effective management of the disease

4 1 Isolation and Characterization of the pathogen

The rhizome rot pathogen was isolated from infected rhizomes and maintained as pure culture on potato dextrose agar and oat meal agar medium The pathogenecity of the isolate was also established

The morphological characters of the pathogen causing rhizome rot of ginger were studied and the pathogen was identified as *Pythium aphanidermatum* (Edson) Fitz The mycelium hyaline coenocytic and measured 3 6 μm hyphal thickness The growth of the fungus was rapid on oat meal agar medium with cottony white mycelium which covered 90 mm petri dish within 48 hours Sporangia sparse in solid media but abundant in the oat meal broth Large number of sporangia were observed within 24 hours when a culture disc from solid media was transferred to aerated water Sporangia filamentous in the initial stages later swollen

and produced lobulate inflated branches cut off by a cross wall from the vegetative hyphae lobes varied 1 9 (Plates 1 to 3) A long emission tube ending in a vesicle was formed from the sporangium prior to zoospore formation The oogonia terminal smooth spherical and measured 16 5 23 1 μm (mean 21 14 μm) in diameter (Plate 4) Antheridia mostly terminal rarely intercalary barrel shaped or broadly clavate measuring 12 5 23 1 μm x 3 3 8 7 μm (mean 16 2 x 6 4 μm) (Plate 4) Oospores spherical smooth and very loose in the oogonium The morphological characters agreed with the description of *P aphanidermatum* given by Fitzpatrick (1923) The identity of the fungus was further confirmed by the Head Division of Plant Pathology IARI New Delhi The ability of *P aphanider matum* to cause rhizome rot of ginger was reported by several workers (Butler 1907 Subramaniam 1919 Park 1937 Thomas 1938 Uppal 1940 Bertus 1942 and Indrasenan 1972) Apart from *P aphanidermatum* four other species of *Pythium viz P myriotylum* (Uppal 1940 Park 1941 and Bertus 1942) *P graminicolum* (Park 1935) *P deliense* (Haware and Joshi 1974) and *P vexans* (Ramakrishnan 1949) were also reported to cause rhizome rot of ginger However these species of *Pythium* were not found associated with the rhizome rot of ginger during the present investigation

4 2 Quantitative estimation of soil microorganisms

Soil microorganisms were isolated from the rhizosphere of healthy ginger plants in the rhizome rot affected fields for testing their efficacy in inhibiting *P aphanidermatum* the incitant of rhizome rot of ginger by employing standard methods as described in materials and methods

Plate 1 *Pythium aphanidermatum* showing single lobed sporangium (720x)

Plate 2 *P aphanidermatum* showing bi lobed sporangium (720x)

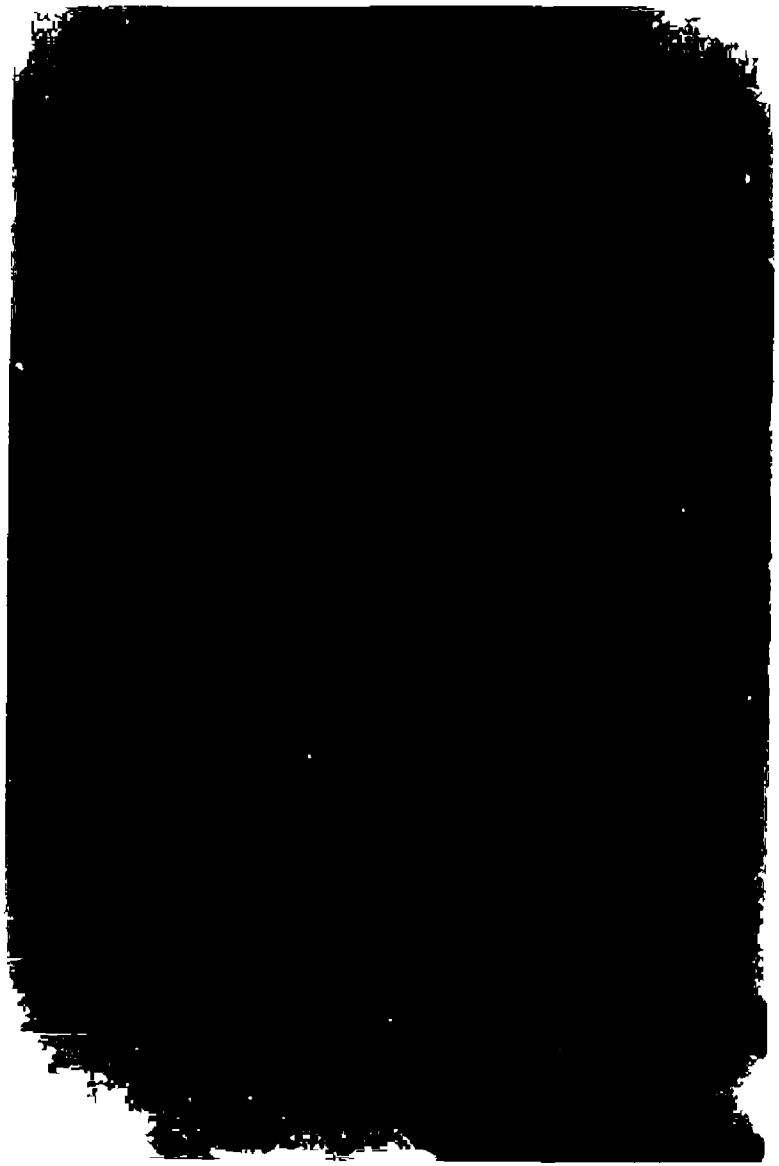


Plate 3 *P aphanidermatum* showing multilobed sporangia (720x)

Plate 4 *P aphan dermatum* sh vin_ ual reproductive structures (7 0x

The results of the quantitative estimation of soil microbes are presented in Table 1 The bacterial population was found to be maximum followed by actinomycetes and fungi The results agree with that of Skinner *et al* (1952) who reported several hundred thousands to hundred millions of bacteria per gram of dry soil Waksman (1952) also observed that the fungi were lesser in number than the actinomycetes and fungal population in soil ranged from a few to as many as ten lakhs per gram

4 3 Qualitative estimation of microflora

The results of the qualitative study of microflora revealed nine species of fungi belonging to four genera one actinomycete and four species of bacteria The fungi present were as follows

1 *Rhizopus* sp
2 *Aspergillus carneus*
3 *Aspergillus niger*
4 *Aspergillus fumigatus*
5 *Aspergillus flavus*
6 *Trichoderma viride*
7 *Aspergillus flavus* (sclerotial)
8 *Eupenicillium javanicum* ITCC No 4 505
9 *Eupenicillium javanicum* ITCC No 4 596

Only one genus of actinomycete was observed belonging to *Strep tomyces* and it comprised of flexuous sporophores The four bacterial spp obtained were distinguished from each other based on their colony characters shape and

Table 1 Quantitative estimation of rhizosphere microflora

Sl No	Name of the organism	Dilution	No of colonies/plate			Mean	Population/g of soil on dry weight basis
			R_1	R_2	R_3		
1	Bacteria	10^6	8 33	6 33	5 43	6 69	5 030 x 10^6
2	Actinomycetes	10^6	4 00	4 33	4 33	4 22	3 17 x 10^6
3	Fungi	10^4	15 33	9 66	16 33	13 77	10 35 x 10^4

gram reaction and were designated as B_1 B_2 B_3 and B_4 The colony characters observed were

B_1 rod shaped gram negative,yellowish on PDA

B_2 rod shaped gram negative slight brownish on PDA

B_3 rod shaped gram negative whitish on PDA

B_4 spherical gram negative slimy creamy white on PDA

4 4 Identification of soil microflora

The microorganisms isolated from the soil were brought into pure culture as described in materials and methods The slide culture of all the fungi and actinomycete were prepared and detailed morphological study was made and on that basis identification done The fungal and actinomycete cultures were sent to the Head Division of Plant Pathology IARI New Delhi and got the identification confirmed

4 5 Growth rate of antagonists and pathogen

A good understanding of the rate of growth of the antagonists and pathogen is highly essential for judging the time of inoculation of these organisms in dual cultures for studying the antagonistic properties The growth rate of the antagonists which comprised of nine fungi and one actinomycete and the pathogen were studied as described in materials and methods The results are presented in Table 2

4 5 1 Fungi

The growth rate of the fungi including nine antagonists and the pathogen

Table 2 Growth of antagonists and pathogen
(Mean radial growth in mm of the antagonists and pathogen of 3 replications)

Sl No	Name of organism	Days after inoculation																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Antagonistic fungi																		
1	*Rhizopus* sp	61	90	–	–		–	–	–			–		–	–			–	
2	*Aspergillus carneus*	9	14	20	25	29	45	53	61	68	76	81	87	90	–	–	–	–	–
3	*Aspergillus niger*	17	33	49	63	74	85	90	–	–	–	–	–	–	–	–	–		–
4	*Aspergillus fumigatus*	15	35	54	71	86	90	–	–			–	–		–	–	–	–	
5	*Aspergillus flavus*	17	31	38	46	54	64	73	82	90	–	–	–	–	–	–	–	–	–
6	*Trichoderma viride*	24	79	90	–	–	–	–	–	–	–	–		–	–	–	–	–	–
7	*Aspergillus flavus* (sclerotial)	17	31	48	60	70	83	90	–	–	–	–		–		–	–	–	–
8	*Eupenicillium javanicum* ITCC No 4 595	9	19	25	28	31	37	44	48	59	62	68	75	82	90	–	–	–	–
9	*Eupenicillium javanicum* ITCC No 4 596	8	11	24	32	41	53	61	71	80	90	–	–		–	–	–	–	–
	Antagonistic actinomycete																		
1	*Streptomyces* sp	2	5	8	11	15	18	20	23	25	26	28	29	31	33	35	37	37	38
	Pathogen																		
1	*P aphanidermatum*	58	82	90			–	–		–		–	–	–	–	–	–	–	–

varied widely The unidentified mucoraceous fungus *Rhizopus* sp was found to be fast growing covering the entire 90 mm petridish within two days However *Eupenicillium javanicum* (ITCC No 4 595) took 14 days for covering the 90 mm petri dish while *E javanicum* (ITCC No 4 596) covered it in 10 days (Table 2)

The growth rate of *Aspergillus* spp was found to be highly varying *A carneus* was found to be slow growing covering the 90 mm petridish in 13 days followed by *A flavus* in nine days *A flavus* (sclerotial) and *A niger* both in seven days and *A fumigatus* in six days (Table 2)

Trichoderma viride was found to be fast growing and covered the 90 mm petri dish in three days (Table 2)

4 5 2 Actinomycete

The growth rate of the actinomycete *Streptomyces* sp was found to be very slow and recorded only 38 mm within 14 days (Table 2)

4 5 3 Bacteria

From the results of a preliminary screening for the antagonistic property it was found that all the four isolates of bacteria did not possess antagonism against *P aphanidermatum* Hence growth rate of the isolated bacteria was not studied as it was not having relevance in the dual culture studies for determining antagonism

4 5 4 Pathogen

The growth rate of the pathogen *P aphanidermatum* was found to be very fast and covered the 90 mm petri dish in three days

4 6 Screening the microorganisms for antagonistic property against the pathogen

The microorganisms isolated from the rhizosphere of healthy ginger plant in the rhizome rot affected field were brought into axenic culture and tested for antagonistic properties against the pathogen *P aphanidermatum* employing the dual culture method described in materials and methods The growth rate of the antagonists as well as the pathogen was determined in monoculture (Table 2) and the antagonistic property was studied in dual culture by inoculating the pathogen in three different ways *viz* on the same day with the antagonist one day prior to the inocula tion of antagonist and after the inoculation of antagonist in case of slow growing antagonistic organisms The reactions of the organisms in dual culture were observed and recorded and results presented in Tables 3 to 12

Johnson and Curl (1972) has reported five types of reactions of mi croorganisms in dual plate technique *viz*

(1) Intermingling and over growth

(2) Inhibition at a distance and disintegration of pathogen

(3) Dieback and disintegration of pathogen

(4) Mutual inhibition on contact

(5) Mutual inhibition at a distance

The character of intermingling and overgrowth in dual culture cannot be considered a property of antagonism as these organisms have no adverse effect on the growth of the pathogen The antagonists showing similar type of reactions with the pathogen in dual culture are grouped together and discussed (Table 13)

The microorganisms which showed conspicuous antagonistic characters in the dual culture against the pathogen were then selected and their cell free culture filtrates were employed in poisoned food technique as described in materials and methods to determine the presence of inhibiting actions on pathogen The results are presented in Table 14

4 6 1 *Rhizopus* sp

The pathogen was inoculated with the antagonist in dual culture on the same day one day before and one day after the inoculation of the antagonist As the pathogen and antagonist were having almost same growth rate both the organisms grew over each other and covered the entire petri dish on the second and third days respectively (Table 3)

The reaction obtained in dual culture was only intermingling and over growth and no other antagonistic properties obtained

4 6 2 *Aspergillus carneus*

4 6 2 1 Inoculation on the same day

The pathogen and the antagonist were inoculated on the same day On the fourth day the pathogen came in contact with the antagonist and on fifth day overgrew the antagonist and on the eighth day it covered the petridish when the antagonist grew at a reduced rate being 52 mm Even after the pathogen completed its growth in the dish the antagonist grew further as in the monoculture and reached 63 mm on tenth day (Table 4)

Table 3 Growth of *P aphanidermatum* and *Rhizopus* sp in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation of pathogen	Days after inoculation																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	49	34	60	51	90	59	90	59	90	59	90	59																		
One day prior to antagonist	28	82	49	90	49	90	49	90	49	90	49	90																		
One day after antagonist	88	47	90	64	90	64	90	64	90	64	90	64																		

Table 4 Growth of *P aphanidermatum* and *A carneus* in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation of pathogen	Days after inoculation																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	6	19	9	28	14	42	19	67	24	74	31	79	39	87	52	90	59	90	63	90	63	90	63	90						
One day prior to antagonist	4	56	8	73	11	90	16	90	21	90	27	90	27	90	27	90	27	90	–											
One day after antagonist	13	21	19	35	24	49	28	62	37	78	44	82	55	90	55	90	55	90	55	90										

A Antagonist P Pathogen

4 6 2 2 Inoculation prior to the antagonist

On inoculation of the pathogen one day prior to the antagonist it over grew the antagonist on second day and covered the petridish on third day The antagonist ceased its growth in dual culture after sixth day (Table 4)

4 6 2 3 Inoculation after the antagonist

The pathogen was inoculated one day after the antagonist The antagonist initially grew at the same rate as in monoculture and ceased its growth after seventh day The pathogen overgrew the antagonist on fourth day and covered the petridish on seventh day (Table 4)

The observations revealed that in dual culture *A carneus* and *P aphanidermatum* can grow independently by intermingling without any interference showing no antagonistic property The results of the dual culture studies showing the reaction of the antagonists with the pathogen are presented in Table 13

Rhizopus sp and *A carneus* were also tested for their antibiotic properties against the pathogen by using cell free culture filtrates The results showed that the cell free culture filtrates have no effect on the pathogen *P aphanidermatum* This cell free culture filtrate technique further confirms the results of dual culture studies

4 6 3 *Aspergillus niger*

4 6 3 1 Inoculation on the same day

The pathogen and the antagonist were inoculated on the same day in dual culture Initially growth rate of both the organisms was slow when compared to

that in monoculture On the third day contact of the organisms was established (Plate 5) and thereafter the antagonist steadily grew reaching 66 mm on the ninth day while growth of the pathogen was reduced to 49 mm as against 70 mm on the fourth day showing disintegration from the growing tip (Plate 6) On subsequent days antagonist grew further causing disintegration of pathogen upto fifteenth day and diminishing its growth to 21 mm (Table 5)

The results revealed that *A niger* disintegrated the mycelial growth of *P aphanidermatum* on contact with each other finally bearing a clear zone in the cul ture dish on tenth day

4 6 3 2 Inoculation prior to the antagonist

The pathogen was inoculated one day prior to the antagonist On the fourth day of the inoculation of the antagonist the pathogen recorded a maximum growth of 89 mm while the antagonist recorded 15 mm growth The antagonist continued its growth upto seventh day of its inoculation attaining 30 mm and inhibit ing the pathogen at a distance The pathogen recorded 76 mm on the seventh day as against 89 mm on fourth day (Table 5)

4 6 3 3 Inoculation after the antagonist

The pathogen was inoculated one day after the antagonist On the second day both came in contact recording 59 mm and 35 mm growth respectively (Plate 7) On the fourth day antagonist grew at its normal rate whereas the pathogen showed reduction in its growth by recording 51 mm as against 59 mm on the second day (Plate 8) Antagonist still grew at the normal rate showing clear inhibition of the pathogen at a distance with a clear zone in culture dish by

Table 5 Growth of *P aphanidermatum* and *A niger* in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation of pathogen	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	12	41	25	59	31	67	37	70	44	65	52	62	58	58	62	55	66	49	71	42	74	39	77	34	80	29	82	24	82	21
One day prior to antagonist	3	58	8	69	11	81	15	89	21	84	27	78	30	76	30	76	30	76	30	76										
One day after antagonist	27	38	35	59	41	55	48	51	56	47	62	39	66	31	71	24	75	16	81	12	86	8	88	8	88	8	88	8	88	8

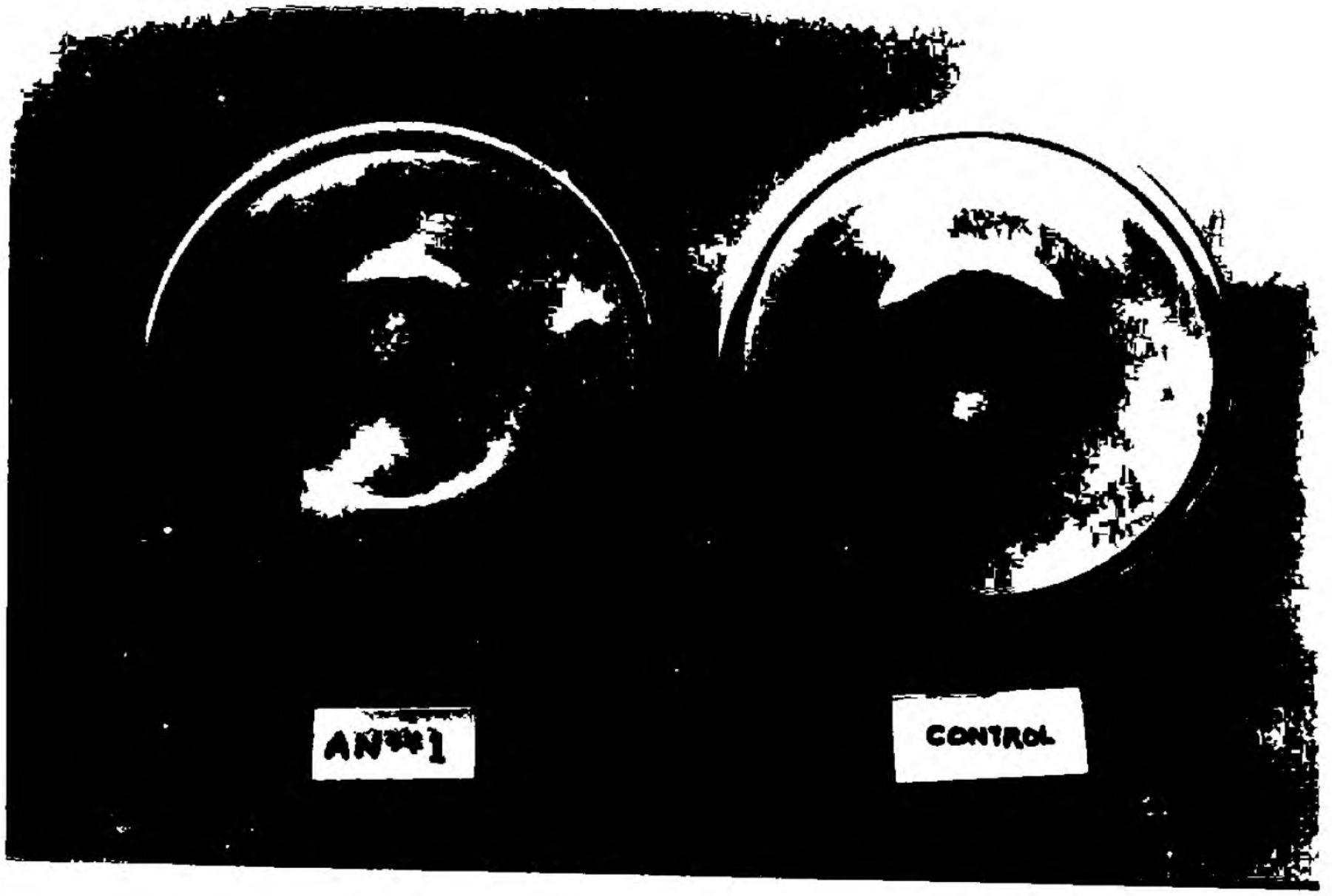
Table 6 Growth of *P aphanidermatum* and *A fumigatus* in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation of pathogen	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	2	49	9	58	13	70	17	79	21	85	26	78	31	72	34	68	37	65	40	62	44	58	46	51	48	49	48	49	48	49
One day prior to antagonist	2	73	4	88	6	90	6	90	6	90	6	90	6	90	6	90	6	90												
Three days after antagonist	20	38	24	69	28	82	30	71	34	62	37	53	40	47	45	40	48	36	51	33	55	30	61	28	61	28	61	28	–	

A Antagonist P Pathogen

Plate 5 *Aspergillus niger* x *P aphanidermatum* (inoculated on the same day) in dual culture on the third day

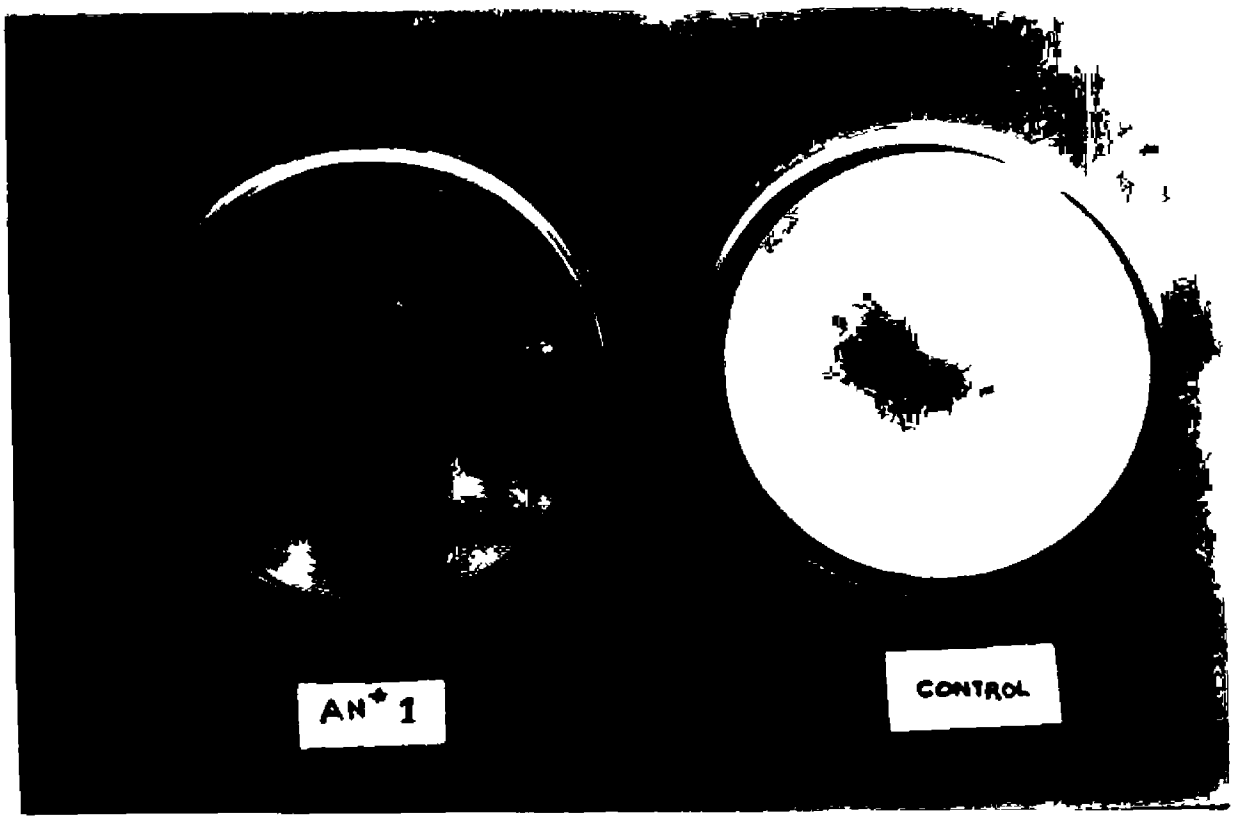
Plate 6 *A niger* x *P aphanidermatum* (inoculated on the same day) in dual culture on the ninth day


AN**1
CONTROL


AN**1

Plate 7 *A niger* x *P aphanidermatum* (inoculated one day after) in dual culture on the second day

Plate 8 *A niger* x *P aphanidermatum* (inoculated one day after) in dual culture on the fourth day


AN+ 1
CONTROL


AN+ 1

disintegrating the growth point of the pathogen The pathogen recorded only 8 mm growth on eleventh day (Table 5)

The results indicated definite antagonistic property of *A niger* against *P aphanidermatum* as it showed the dual culture reaction of inhibition at a distance and disintegration of pathogen (Table 13) The cell free culture filtrate studies using antagonist *A niger* also showed 100 per cent inhibition against the pathogen indicat mg the production of antibiotics by the antagonist which inhibited the pathogen completely (Table 14) This result of cell free culture filtrate study strongly supports the result of dual culture technique

The antagonist *A niger* have produced some diffusible metabolite into the medium which have strong antibiotic and some lytic activity against the pathogen *P aphanidermatum* The antagonist have produced the antibiotic which diffused into the substrate slowly and thus inhibiting only the growth of the pathogen nearer to the antagonist while growth of the pathogen in the periphery was not affected The disintegration of hyphae of the pathogen was also noticed indicating the lytic property of the metabolite The antibiotic producing ability of *A niger* is a well established fact and the properties of inhibition and lysis of the fungus was reported by many workers (Broadbent 1966 Bora 1977 Gokulapalan and Nair 1984 Wu *et al* 1986 Vinod 1988)

4 6 4 *Aspergillus fumigatus*

4 6 4 1 Inoculation on the same day

The pathogen was inoculated on the same day with the antagonist Initially while the pathogen showed same growth rate the antagonist recorded

reduced growth as against their growth in monoculture On the fifth day both came in contact and the antagonist and pathogen recorded growth of 21 mm and 85 mm respectively The antagonist further grew at a reduced rate over the pathogen causing its die back The growth of the pathogen was reduced considerably to 68 mm on eighth day 62 mm on tenth day and then further to 49 mm on 13th day onwards (Table 6)

4 6 4 2 Inoculation prior to the antagonist

Inoculation of the pathogen one day prior to antagonist showed complete coverage of petri dish on third day while the antagonist recorded 6 mm growth and thereafter remained constant (Table 6)

4 6 4 3 Inoculation after the antagonist

The pathogen was inoculated three days after the antagonist due to the slow growing nature of the latter In mono culture initial growth of both the organisms was almost same In dual culture they came in contact with each other on the third day and thereafter the antagonist grew at a reduced rate over the pathogen On sixth day the antagonist recorded 37 mm growth while that of pathogen decreased to 53 mm as against 82 mm on third day The antagonist grew over the latter by die back and disintegration The disintegration continued upto twelth day as the pathogen and antagonist recorded 28 mm and 61 mm respectively (Table 6)

The observations revealed that inoculation of the pathogen after the antagonist sufficiently inhibited the pathogen as the pathogen recorded 28 mm growth on 12th day as against 51 mm when the antagonist was inoculated on the same day with the pathogen

4 6 5 *Aspergillus flavus*

4 6 5 1 Inoculation on the same day

Inoculation of the pathogen on the same day with the antagonist initially showed reduced growth of the antagonist because of comparatively slow growth rate while the pathogen showed normal growth (Table 7) The pathogen after attaining a maximum growth of 87 mm on the third day (Plate 9) showed gradual reduction in its growth rate attaining a growth of 57 mm on 10th day as the antagonist grew over it (Table 7 Plate 10) Disintegration of the pathogen was noticed at the growing point @ 3 4 mm per day from fourth day onwards upto 10th day and after that it remained constant.

4 6 5 2 Inoculation prior to the antagonist

The pathogen was inoculated one day prior to the antagonist Because of the fast growing nature of the pathogen it covered the petri dish on the second day itself while the antagonist grew to a maximum of 13 mm on the third day and after that no further growth observed (Table 7)

4 6 5 3 Inoculation after the antagonist

The pathogen showed faster growth rate when compared to the antagonist and so it was inoculated three days after the antagonist The antagonist grew at a moderate rate while the pathogen showed slow growth rate and contact of the organisms were established on the second day (Plate 11) On the fourth day the antagonist overgrew the pathogen and the overgrown region of the pathogen was

Table 7 Growth of *P aphanidermatum* and *A flavus* in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	6	40	11	69	15	87	22	83	31	79	37	71	43	68	46	65	50	63	52	57	52	57	52	57	52	57				
One day prior to antagonist	6	69	9	90	13	90	13	90	13	90	13	90																		
Three days after antagonist	38	33	46	55	53	59	59	56	66	51	71	48	77	43	79	37	79	30	79	23	79	23	79	23						

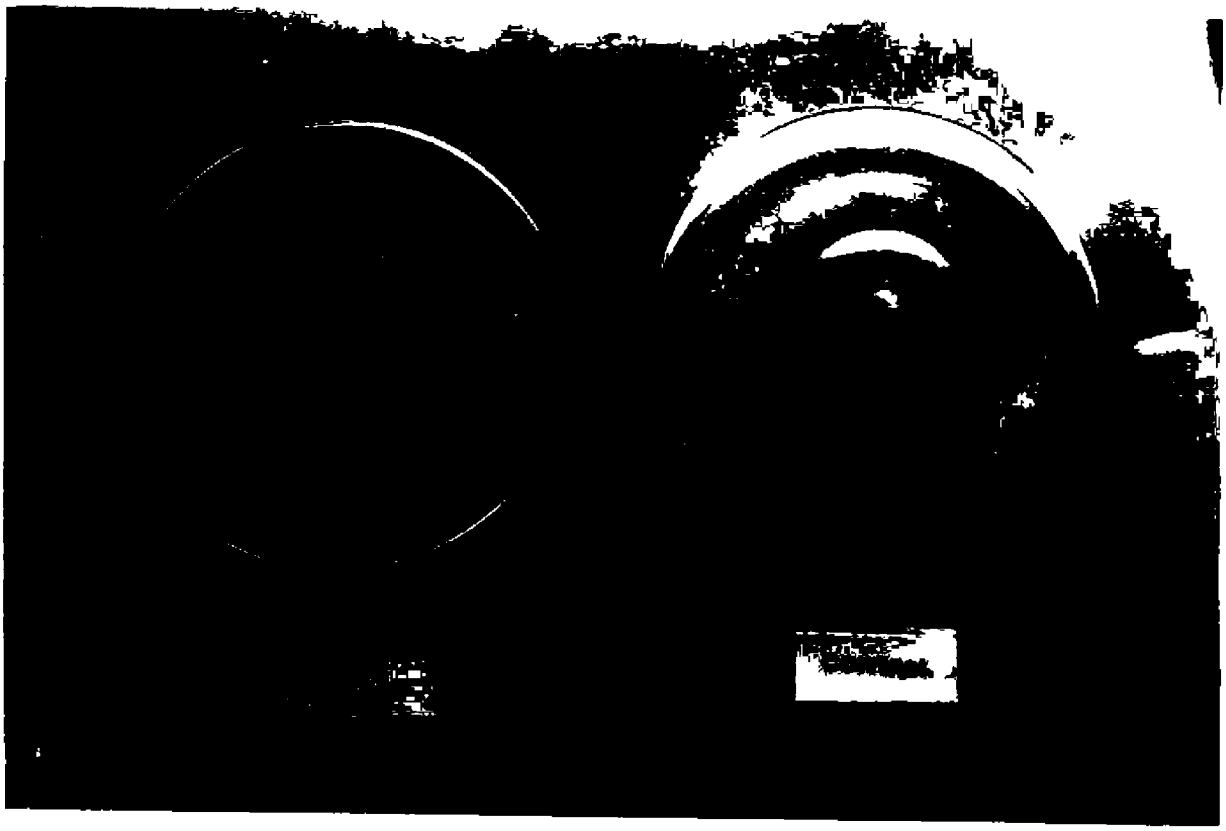
Table 8 Growth of *P aphanidermatum* and *T viride* in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation of pathogen	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	21	41	40	51	53	46	64	35	69	27	73	18	79	12	84	9	90	0	90	0										
One day prior to antagonist	14	67	23	82	31	68	48	49	69	26	85	14	90	0	90	0	90	0	90	0										
One day after antagonist	47	30	61	35	70	23	83	15	90	7	90	0	90	0	90	0	90	0	90	0										

A Antagonist P Pathogen

Plate 9 *A flavus* x *P aphanidermatum* (inoculated on the same day) in dual culture on the third day

Plate 10 *A flavus* x *P aphanidermatum* (inoculated on the same day) in dual culture on the tenth day



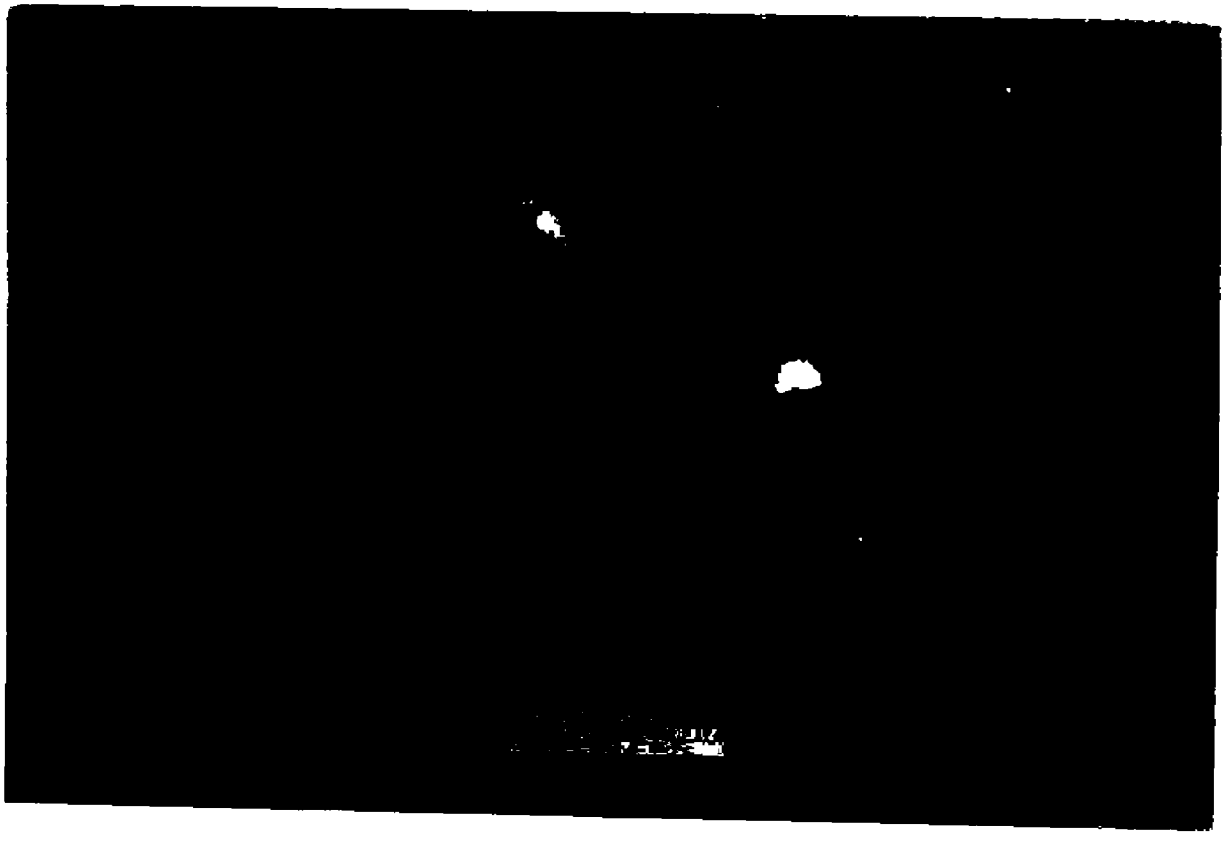
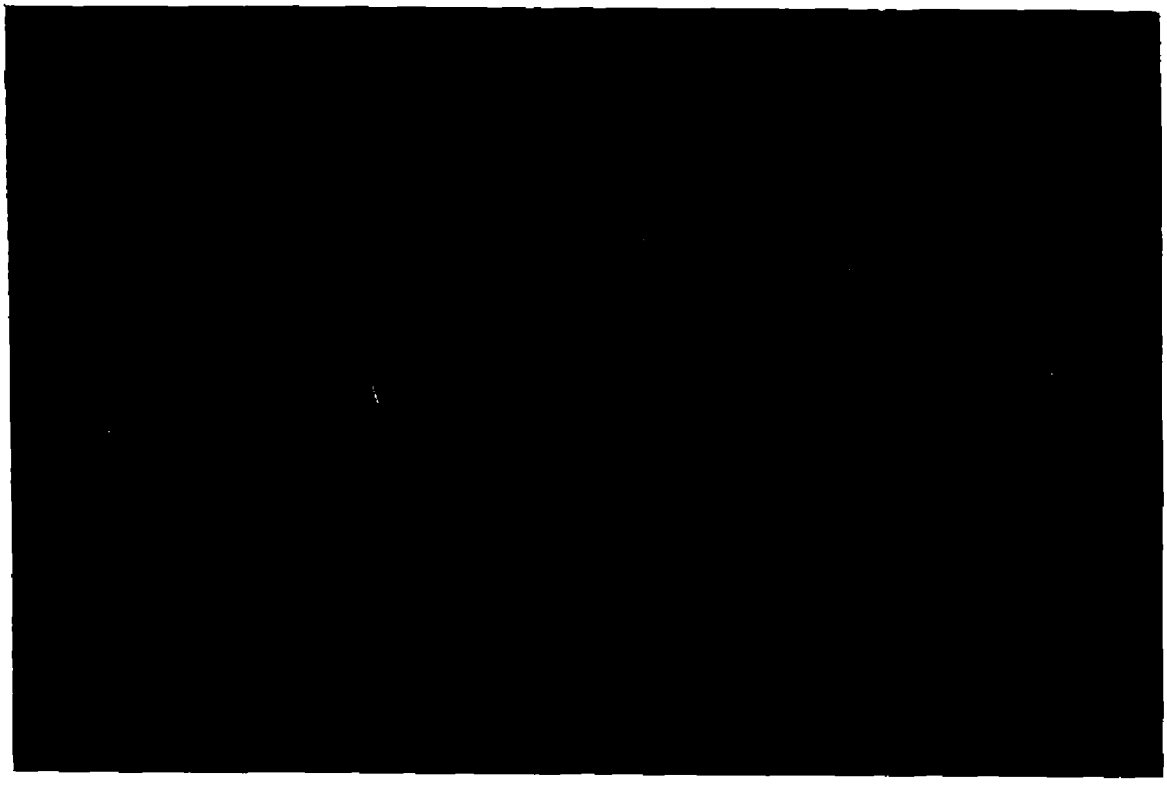


Plate 11 *A flavus* x *P aphanidermatum* (inoculated three days after) in dual culture on the second day

Plate 12 *A flavus* x *P aphanidermatum* (inoculated three days after) in dual culture on the tenth day



completely disintegrated The antagonist further grew over the pathogen diminishing the growth of the pathogen to 23 mm on 10th day (Table 7 Plate 12)

The results indicated that the inoculation of the antagonist prior to the pathogen was more effective in reducing the growth rate of the pathogen than when inoculated on the same day or one day prior to the antagonist Inoculation on the same day was also effective but there was no total destruction of the pathogen

4 6 6 *Trichoderma viride*

4 6 6 1 Inoculation on the same day

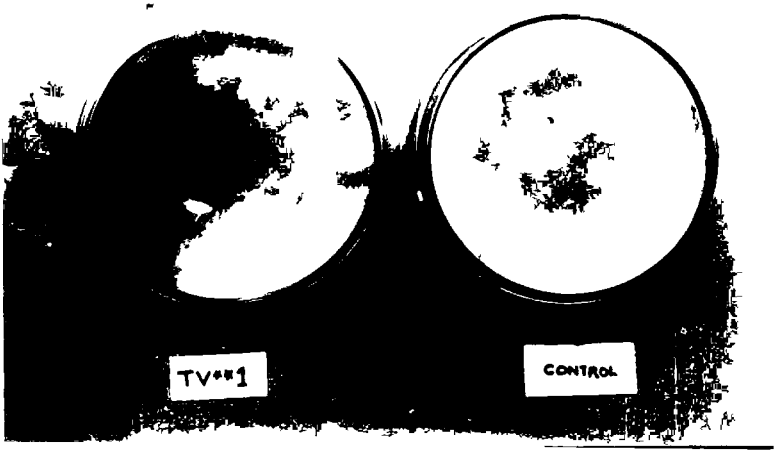
The pathogen and the antagonist were inoculated on the same day in dual culture Initially while the growth of the antagonist was same in mono and dual culture the pathogen showed reduced growth rate in dual culture On the second day though the antagonist and pathogen showed reduced growth rate than in mono culture the antagonist grew further at a reduced rate over the pathogen causing its die back (Plate 13) The growth of the pathogen was reduced considerably to 35 mm on fourth day 18 mm on sixth day and thereafter completely parasitised by the antagonist (Table 8 Plate 14)

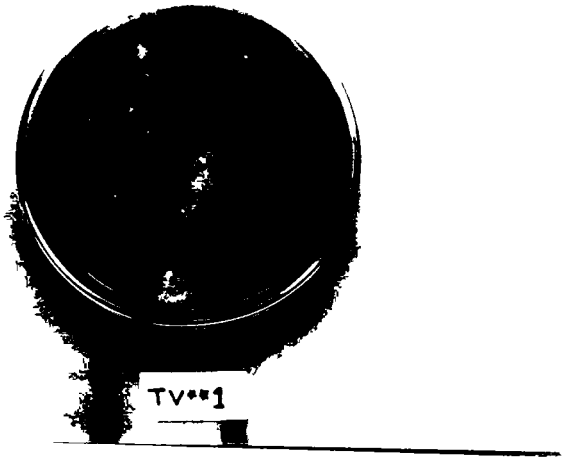
4 6 6 2 Inoculation prior to the antagonist

The pathogen was inoculated one day prior to the antagonist Initially the antagonist showed reduced growth rate when compared to mono culture Beyond it grew further over the pathogen and the growth of the pathogen was reduced to 65 mm on third day as against 8 mm on second day 14 mm on sixth day and thereafter completely parasitised by *T viride* (Table 8)

Plate 13 *Trichoderma viride* x *P aphanidermatum* (inoculated on the same day) in dual culture on the second day

Plate 14 *T viride* x *P aphanidermatum* (inoculated on the same day) in dual culture on the ninth day


TV**1
CONTROL


TV**1

4 6 6 3 Inoculation after the antagonist

The pathogen was inoculated one day after the antagonist The pathogen recorded a reduced growth rate of 35 mm on the second day (P a 5 and gradually completely parasitised by the antagonist on the sixth day (Tab e 8 Plate 16)

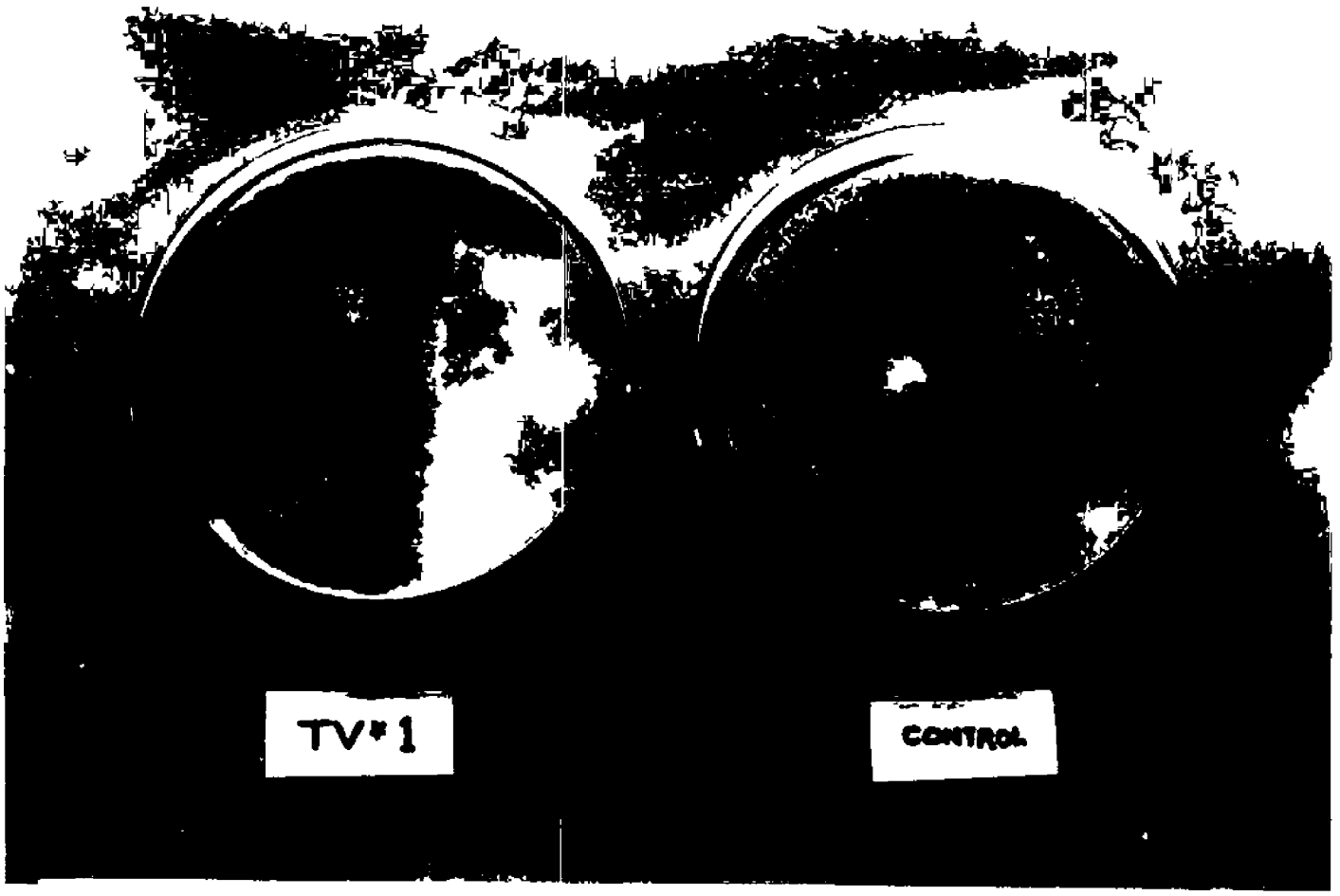
The results showed that the antagonist was effective when inocula ed on all the three ways

The dual culture reaction characterized by die back and disint ratu n of the fungal patho en after meeting the anta onist was shown by *A fun* 4 *flavus* and *I viride* against the pathogen *P aphanidermatum* (Table The antagonists when contacted the pathogen parasitised it and slowly cau d the d back and disintegration The biotic substances produced during the growth f the e antagonists inhibited the pathogen and also observed mycoparasitism re ulting in disintegration This is evident from the fact that the antogonist continued ts growth even after meetin the hyphae of the path gen causing the d back and d ntegra tion

A fumigatus caused the die back and disinte ration of the pa n *P aphanidermatum* by o er r wing and parasitisin it (Table 6) But th a n ons c rable nh bition of the patho en in the cell fre culture filtrate tud her it re orded 4 per cent nhibition of the patho en *P aphan n atum* Th pres nt n estigation indicates that the anta onistic property f 4 *n atu* agains t *P aphanidermatu n* is mainly by parasiti activity and t n h pr du e an stron nh b t ry sub tances aga n t the path gen Ther vere uc e

Plate 15 *I viride* x *P aphanidermatum* (inoculated one day after) in dual culture on the second day

Plate 16 *I viride* x *P aphanidermatum* (inoculated one day after) in dual culture on the sixth day


TV*1
CONTROL


TV*1

on the antagonistic property of *A fumigatus* on *Pythium* spp But Mukherjee and Sen (1992) observed *A fumigatus A terreus P citrinum* and *P simplicissimum* when tested for their antagonism towards *Macrophomina phaseolina* in *in vitro* studies only culture filtrate of *A fumigatus* inhibited fungal growth and sclerotial germination Mixed culture inoculation after different periods of incubation showed that competition for food was the primary mode of action and that antibiosis played a secondary role This is in conformity with the present study

A flavus has also shown the antagonistic property of die-back and disintegration against the pathogen *P aphanidermatum* (Table 7) This indicates that *A flavus* can parasitise *P aphanidermatum* causing disintegration and the biotic substances produced by the antagonist were found to be toxic to the pathogen The bioassay studies using cell free culture filtrates demonstrated 62 per cent inhibition (Table 14) The antagonistic property of *A flavus* against many pathogenic microorganisms has been reported by Masroor and Chandra (1987) and Deb (1990) though not specific against *Pythium* spp

The hyphal parasitism and production of inhibitory substances by different species of *Trichoderma* resulting in die back and disintegration of *Pythium* spp were reported by many workers (Wemdling and Emerson 1936 Brian and Mc Gowan 1946 Dennis and Webster 1971 Chet *et al* 1981 Papavizas 1984 Mukhopadhyay and Chandra 1986 Raju 1991 nomas *et al* 1991 Vinod *et al* 1991a) The present investigation also clearly demonstrated the ability of the *Tricho derma* sp to cause die back and disintegration of the pathogen

The cell free culture filtrate studies using *T viride* also showed evidence of production of biotic substances which inhibited the growth of the

pathogen to some extent (Table 14) The inhibition observed on the pathogen by *Trichoderma viride* was 20 per cent

The present investigation has proved that *Trichoderma viride* has the ability to show its antagonism against the pathogen and that it is mainly due to parasitism and to some extent due to the production of biotic substances which are toxic to the pathogen

4 6 7 *Aspergillus flavus* (sclerotial)

4 6 7 1 Inoculation on the same day

When the pathogen and antagonist were inoculated on the same day both came in contact on the third day On the fourth day the antagonist and the pathogen recorded 33 mm and 88 mm growth respectively On the fifth day the antagonist grew over the pathogen reducing its growth to 85 mm The pathogen and the antagonist recorded 40 mm and 82 mm growth respectively on the sixth day and showed no further growth thereafter (Table 9)

4 6 7 2 Inoculation prior to the antagonist

The pathogen was inoculated one day prior to the antagonist. Both the antagonist and the pathogen contacted each other on the fourth day and no further growth was observed (Table 9)

4 6 7 3 Inoculation after the antagonist

The pathogen was inoculated two days after the antagonist in dual culture to favour establishment of the latter Initially the growth rate of the antagonist was same as in mono culture and contacted pathogen on fifth day The antagonist grew

Table 9 Growth of *P aphanidermatum* and *A flavus* (sclerotial) in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	11	43	22	58	29	73	33	88	37	85	40	82	40	82	40	82	40	82	40	82										
One day prior to antagonist	5	77	10	90	12	90	14	90	14	90	14	90	14	90	14	90	14	90	14	90	–									
Two days after antagonist	29	32	41	49	47	62	51	73	57	88	59	86	62	86	65	84	67	82	67	82	67	82	67	82	67	82	67	82	67	82

Table 10 Growth of *P aphanidermatum* and *E javanicum* ITCC No 4 595 in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	7	12	18	29	26	46	30	64	32	69	34	71	34	71	34	71	34	61	34	71			–						–	
One day prior to antagonist	4	59	9	75	15	90	21	90	24	90	24	90	24	90	24	90	24	90			–				–					
One day after antagonist	15	23	19	38	27	52	39	63	39	69	39	69	39	69	39	69	39	69												

A Antagonist P Pathogen

further reducing the growth of the pathogen marginally On the ninth day the antagonist recorded 67 mm growth while the pathogen recorded 82 mm growth as against 88 mm on fifth day No further growth of either the antagonist or the pathogen was observed after ninth day (Table 9)

The results revealed that these two organisms were having mutual inhibition on contact and a demarcation can be seen at the point of contact (Table 13)

4 6 8 *Eupenicillium javanicum* ITCC No 4 595

4 6 8 1 Inoculation on the same day

Inoculation of the pathogen and the antagonist on the same day showed no growth after recording a maximum of 71 mm and 34 mm respectively on sixth day (Table 10)

4 6 8 2 Inoculation prior to the antagonist

The pathogen was inoculated one day prior to the antagonist On the third day the pathogen fully covered the petri dish while the antagonist recorded only 15 mm growth The antagonist grew upto 24 mm on fifth day and on succeeding days remained constant (Table 10)

From the data it reveals that there was no antagonism shown by *E javanicum* ITCC No 4 595 against *P aphanidermatum*

4 6 8 3 Inoculation after the antagonist

The pathogen was inoculated one day after the antagonist Initially the antagonist recorded equal growth rate in mono and dual culture On the fourth day

the antagonist contacted the pathogen and showed no further growth on succeeding days while the pathogen grew to 69 mm on fifth day and thereafter remained constant (Table 10)

The results indicated mutual inhibition on contact when the pathogen was inoculated on the same day with the antagonist and also one day latter (Table 13) Inoculation of the pathogen prior to the antagonist showed no mutual inhibition as it covered the petri dish on the third day before the antagonist could establish

4 6 9 *Eupenicillium javanicum* ITCC No 4 596

4 6 9 1 Inoculation on the same day

The pathogen was inoculated on the same day with the antagonist While the antagonist grew at normal rate in dual culture on the third day the pathogen grew only 62 mm as against 90 mm in the mono culture In dual culture the antagonist grew 28 mm and 36 mm on the fifth and sixth day respectively whereas the pathogen grew only 69 mm On subsequent days the growth of both the organisms remained constant in the dual culture (Table 11)

4 6 9 2 Inoculation prior to the antagonist

Inoculation of the pathogen prior to the antagonist by one day showed the pathogen completely covering the petridish on the third day while the antagonist showed 12 mm growth The antagonist grew further and reached 27 mm on the seventh day and thereafter remained constant (Table 11)

4 6 9 3 Inoculation after the antagonist

The pathogen was inoculated one day after the antagonist Both came in

Table 11 Growth of *P aphanidermatum* and *E javanicum* IICC No 4 506 in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	5	37	12	48	19	62	24	69	28	69	36	69	38	69	38	69	38	69	38	69										
One day prior to antagonist	4	69	8	81	12	90	17	90	20	90	23	90	27	90	27	90	27	90	27	90										
One day after antagonist	21	38	29	58	34	66	37	77	39	77	44	77	44	77	44	77	44	77	44	77										

Table 12 Growth of *P aphanidermatum* and *Streptomyces* sp in dual culture
(Mean radial growth in mm of 3 replications)

Time of inoculation	Days after inoculation																													
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P	A	P
Same day with antagonist	2	54	2	81	4	90	4	90	4	90	4	90	4	81																
One day prior to antagonist	2	79	2	90	2	90	2	90	2	90	2	90																		
Seven days after antagonist	18	37	20	54	23	62	23	69	23	74	23	74	23	74	23	74	23	74	23	74	23	74								

A Antagonist P Pathogen

contact on the third day The antagonist and the pathogen grew further to 37 mm and 77 mm respectively on fourth day While the pathogen showed no further growth the antagonist grew to 44 mm on sixth day and thereafter remained constant (Table 11)

The data clearly revealed that *E javanicum* ITCC No 4 596 and *P aphanidermatum* were having mutual inhibition on contact (Table 13)

4 6 10 *Streptomyces* sp

4 6 10 1 Inoculation on the same day

The pathogen and the antagonist were inoculated on the same day in dual culture Due to the slow growing nature of the latter it showed a maximum growth of 4 mm on third day when the pathogen completed its growth in petri dish and no further growth on succeeding days were observed (Table 12)

4 6 10 2 Inoculation prior to the antagonist

Inoculation of the pathogen one day prior to the antagonist favoured the growth of the pathogen while the antagonist grown to a maximum of 2 mm even after six days (Table 12)

4 6 10 3 Inoculation after the antagonist

The pathogen was inoculated seven days after the antagonist in dual culture The growth of both the organisms in dual culture upto two days was found to be normal On the fourth day both came in contact and recorded 23 mm and 69 mm growth respectively The antagonist ceased its growth on fourth day while the pathogen grew further to 74 mm and remained constant thereafter (Table 12)

Table 13 Reactions of the antagonists with the pathogen in dual culture

Sl No	Name of antagonist	Reaction with the pathogen
1	*Rhizopus* sp	Intermingling and overgrowth
2	*A carneus*	
3	*A niger*	Inhibition at a distance and disintegration of pathogen
4	*A fumigatus*	Dieback and disintegration of pathogen
5	*A flavus*	
6	*T viride*	
7	*A flavus* (sclerotial)	Mutual inhibition on contact
8	*E javanicum* (ITCC No 4 595)	
9	*E javanicum* (ITCC No 4 596)	
10	*Streptomyces* sp (flexuous sporophores)	

Table 14 Effect of cell free culture filtrates of antagonists on the growth of pathogen (Poisoned food technique)

Name of antagonist	*Pythium aphanidermatum*	
	Radial growth in mm measured two days after inoculation	Per cent inhibition
1 *A niger*	Nil	100
2 *A fumigatus*	52	42
3 *A flavus*	34	62
4 *T viride*	70	20
5 Control	90	–

Table 15 *In vitro* sensitivity of *P aphanidermatum* to selected fungicides

Sl No	Fungicide		*P aphanidermatum*	
	Name	Concentration (Per cent)	*Colony diameter (mm)	Inhibition over control
1	Indofil M 45	0 3	12	86 66
2	Emisan	0 1	12	86 66
3	Fytolan	0 2	0	100 00
4	Bordeaux mixture	1 0	0	100 00
5	Control		90	

* Mean of three replications

The growth character of these two organisms clearly showed that the *Streptomyces* sp and *P aphanidermatum* expressed the character of mutual inhibi tion on contact when the pathogen was inoculated after the antagonist (Table 13)

In the dual culture reaction of mutual inhibition on contact shown by three species of fungi *viz A flavus* (sclerotial) *E javanicum* ITCC No 4 595 and *E javanicum* ITCC No 4 596 and one species of actinomycete both the pathogen and the antagonists showed normal growth rate in dual culture but when they came in contact further growth of both was arrested The character of mutual inhibition on contact by the pathogen and the antagonist is of course an antagonistic property But it may not have much value in the biological control of the pathogen because till the contact of the antagonist and the pathogen they will have free growth and even on contact there was no disturbance of the pathogen except checking the further growth and that also at the expense of the antagonist being inhibited

The antagonistic effect of *Streptomyces* spp on *Pythium* was reported by several workers (Domsch *et al* 1980 Yehia *et al* 1981 Tahvonen 1982 1988 and Rath and Wolf 1992) Contradictory to the above reports in the present study the actinomycete did not show any antagonistic property towards *Pythium* There were no reports on the antagonistic properties of *Eupenicillium* spp

4 7 *In vitro* evaluation of fungicides against the pathogen and the antagonists

Plant disease control aims at prevention or reduction in the incidence or severity of the disease Among the various methods of plant disease control though use of chemicals offers comparatively more effectiveness and quick action in

prevention or reduction of disease encouragement of its use with antagonists favours reduced environmental hazards and cost effectiveness As rhizome rot of ginger is very serious during rainy seasons use of chemicals along with antagonists offer better control of the disease So in the present study *in vitro* effect of fungicides against *P aphanidermatum* and antagonists were evaluated with a view to assess the compatible nature of chemicals with biocontrol agents for the control of rhizome rot of ginger

Four fungicides viz Bordeaux mixture fytolan emisan and Indofil M-45 each at 1 0 0 2 0 1 and 0 3 per cent concentration respectively were screened for their inhibitory effect on the growth of the pathogen and the antagonists The bio assay studies carried out against the pathogen showed that Bordeaux mixture and fytolan completely inhibited the growth of the pathogen (Table 15) This finding on the effect of Bordeaux mixture against the pathogen is in full agreement with that of Singh (1985) who suggested soil application of Bordeaux mixture (5 5 50) at three weeks interval soon after germination against rhizome rot of ginger Chauhan *et al* (1994) found Bordeaux mixture at 3000 ppm most effective in reducing the per cent incidence of rhizome rot of ginger in pot under green house conditions even after 90 days of inoculation It was noted that Indofil M 45 and emisan exhibited comparatively low efficacy in inhibiting the fungus They showed only 86 66 per cent inhibition over control (Table 15) Out of the five fungicides tested *in vitro* against *P myriotylum* Sharma and Joshi (1979) got effective control of the fungus growth using Indofil M 45 bavistin ceresan and kitazin while benlate was not effective Sharma and Gupta (1988) reported complete inhibition of the growth of *P ultimum* causing collar rot of apple in Himachal Pradesh by emisan (methoxy ethyl mercuric chloride) and Indofil M 45 (mancozeb)

when tested *in vitro* In the present investigation also Indofil M-45 and emisan were found to be effective against *Pythium* though they were second to fytolan and Bor deaux mixture

Among the four fungicides tested against the antagonists for their sen sitivity Bordeaux mixture was found to be completely inhibiting the growth of all the antagonists while fytolan completely inhibited the growth of only *A flavus* and *A fumigatus* The per cent inhibition of *T viride* and *A niger* by fytolan was 56 66 and 1 11 respectively Though emisan completely inhibited the growth of *A fumigatus* it could inhibit only 85 55 per cent of *T viride* 23 33 per cent of *A flavus* and 14 44 per cent of *A niger* Considering the per cent inhibition Indofil M 45 was having comparatively low rate of inhibition of antagonist It recorded only 4 44 in the case of *T viride* 7 77 in *A niger* 36 66 for *A flavus* and 62 22 for *A fumigatus* (Table 16) The results showed that in general Indofil M 45 is compatible with *T viride A niger A flavus* and *A fumigatus* for chemical control of rhizome rot of ginger along with biocontrol agents

The effect of fungicides like fytolan emisan and Indofil M 45 on *A flavus* and *A niger* were studied both *in vitro* and *in vivo* and were reported by se eral workers (Prasad *et al* 1986 Zahar *et al* 1986 Bansal and Sobti 1988 Sundas and Raj 1989) Zahar *et al* (1986) reported less toxic effect of Cuprosan 311 SD (copper oxychloride + maneb + zineb) on *A niger* and *A flavus* However in the present study though fytolan recorded less efficacy in inhibiting the growth of *A niger* it completely inhibited the growth of *A flavus* Sundas and Raj (1989) noted that an isolate of the groundnut collar rot pathogen *A niger* tolerated blitox 50 (copper oxychloride) and Indofil M-45 (mancozeb) at 6000 ppm and

Table 16 *In vitro* sensitivity of antagonists to selected fungicides

Sl No	Fungicide		Antagonists							
	Name	Concentration (Per cent)	*A flavus*		*A fumigatus*		*T viride*		*A niger*	
			*Colony diameter (mm)	Per cent inhibition over control	*Colony diameter (mm)	Per cent inhibition over control	*Colony diameter (mm)	Per cent inhibition over control	*Colony diameter (mm)	Per cent inhibition over control
1	Bordeaux mixture	1 0	0	100 00	0	100 00	0	100 00	0	100 00
2	Fytolan	0 2	0	100 00	0	100 00	39	56 66	89	1 11
3	Emisan	0 1	69	23 33	0	100 00	13	85 55	77	14 44
4	Indofil M-45	0 3	57	36 66	34	62 22	86	4 44	83	7 77
5	Control		90		90		90		90	

* Mean of three replications

emisan 6 (2 methoxy ethyl mercury chloride) at 450 ppm when grown on PDA containing increasing concentration of fungicides Similar results were obtained in the present study also

4 8 Pot culture experiment for testing the efficacy of the antagonists

Pot culture studies were conducted to find out the efficacy of the antagonists selected from dual culture studies in reducing the rhizome rot incidence of ginger The details of the treatments are given in materials and methods Observations on the rhizome rot incidence were recorded from 120 days after planting and the results are presented in Tables 17a and 17b

The statistical analysis of the data on the rhizome rot incidence showed marked difference among the treatments at 120 150 and 180 days after planting

The rhizome rot incidence observed 120 DAP revealed significant difference among the treatments It was observed that the treatment T_1 was significantly superior to other treatments and was followed by T_3 T_{13} and T_{18} The treatment T_1 recorded the maximum efficiency over control in reducing the incidence Treatments T_4 T_5 and T_7 were on par with each other Treatments T_{10} T_{17} T_{11} T_{16} and T_{14} also showed no significant difference among them Treatment T_{20} recorded the maximum disease incidence which was closely followed by T_8 T_2 and T_6

Observations at 150 DAP showed T_{20} (control) recording maximum rhizome rot incidence and was on par with treatments T_8 T_2 T_6 T_{12} T_{15} and T_9 Treatments T_{11} and T_{14} did not vary significantly among themselves T_1 recorded minimum disease incidence followed by T_3 T_{13} and T_{18} as in previous

Table 17a Effect of antagonists and selected fungicides on the rhizome rot of ginger in pot culture experiment

Treatments	Percentage of rhizome rot incidence at monthly intervals from 120 DAP (Mean of 5 replications)		
	120 DAP	150 DAP	180 DAP
T_1	1 332 (1 354) f	3 15 (1 789) ı	11 658 (3 350) c
T_2	54 52 (7 447) ab	90 95 (9 569)a	100 00 (10 050) a
T_3	8 57 (2 470) ef	8 57 (2 470) hı	41 43 (5 363) bc
T_4	23 93 (4 108) bcdef	49 08 (6 473) bcdef	76 00 (8 030) ab
T_5	18 47 (4 395) bcdef	28 21 (5 383) efg	59 83 (7 739) ab
T_6	57 83 (7 644) ab	84 22 (9 227) a	100 00 (10 050) a
T_7	22 56 (4 486) bcdef	40 14 (6 150) cdefg	68 16 (8 195) ab
T_8	51 40 (7 214) ab	93 14 (9 692) a	100 00 (10 050) a
T_9	42 24 (6 495) abcd	79 88 (8 992) a	97 50 (9 921) a
T_{10}	34 86 (5 036) abcde	67 87 (8 013) abcd	95 29 (9 800) a
T_{11}	38 36 (5 235) abcde	77 00 (8 754) ab	96 92 (9 891) a
T_{12}	50 47 (7 162) abc	83 37 (9 162) a	100 00 (10 050) a
T_{13}	11 76 (3 543) def	16 16 (4 069) gh	47 50 (5 661) bc
T_{14}	38 15 (5 768) abcde	74 21 (8 672) ab	96 92 (9 890) a
T_{15}	47 45 (6 957) abcd	80 02 (8 996) a	100 00 (10 050) a
T_{16}	36 65 (5 671) abcde	69 43 (8 349) abc	96 36 (9 860) a
T_{17}	28 38 (5 216) abcde	56 76 (7 589) abcde	85 64 (9 276) a
T_{18}	12 43 (3 614) cdef	24 12 (4 848) fg	51 99 (7 045) ab
T_{19}	20 63 (4 626) abcdef	33 19 (5 827) defg	62 29 (7 941) ab
T_{20}	66 04 (8 178) a	93 71 (9 728) a	100 00 (10 050) a

DAP Days after planting
Figures given in parenthesis are transformed values ($\sqrt{x+1}$)
Treatment means followed by common letters do not significantly differ at 1% level

Table 17b Effect of antagonists and selected fungicides on the rhizome rot of ginger in pot culture experiment

Treatments	Per cent efficiency over control		
	120 DAP	150 DAP	180 DAP
T_1	97 98	96 63	88 34
T_2	17 44	2 94	0
T_3	87 01	90 85	58 57
T_4	63 76	47 62	24 00
T_5	72 03	69 89	40 00
T_6	12 43	10 12	0
T_7	65 83	57 16	31 84
T_8	22 16	0 60	0
T_9	36 03	14 75	2 50
T_{10}	47 21	27 57	4 71
T_{11}	41 91	17 83	3 08
T_{12}	23 57	11 03	0
T_{13}	82 19	82 75	52 50
T_{14}	42 23	20 80	3 08
T_{15}	28 14	14 60	0
T_{16}	44 50	25 90	3 64
T_{17}	57 02	39 43	14 36
T_{18}	81 17	74 26	48 01
T_{19}	68 76	64 58	37 71
T_{20}			

observation Treatments T_5 T_{19} T_7 and T_4 recorded less than 50 per cent disease incidence

The final observation at 180 DAP revealed that the treatment T_{20} (control) recorded 100 per cent disease incidence and was on par with the treatments T_2 T_6 T_8 T_{12} T_{15} T_9 T_{11} T_{14} T_{16} T_{10} and T_{17} Treatments T_1 T_3 and T_{13} showed less than 50 per cent disease incidence Among them T_1 recorded the least (11 65 per cent) and treatments T_3 and T_{13} were on par with each other Also treatments T_7 T_4 T_{19} T_5 and T_{18} showed no significant difference among them

From the data it is evident that among the twenty treatments T_1 (*A niger* seed inoculation) recorded the maximum efficiency over control in reducing the rhizome rot incidence and was followed by T_3 and T_{13} The minimum efficiency over control was recorded in treatments T_2 T_6 T_8 T_{12} and T_{15}

The results of pot culture study on the biocontrol of rhizome rot of ginger with antagonistic microorganisms indicated that in general the treatments T_1 (*A niger* seed inoculation) T_3 (*A flavus* seed inoculation) and T_{13} (*A niger* soil application after 60 and 120 DAP) had maximum efficiency in checking the incidence and severity of rhizome rot incidence than the other treatments Though T_5 (*A niger* soil application at planting) and T_{18} (copper oxychloride 0 3% soil drenching) initially recorded minimum disease incidence more than 50 per cent disease incidence was observed 180 DAP The rhizome rot incidence remained static throughout the study period in the treatments T_1 T_3 and T_{13} indicating the ability of the antagonists to prevent the further spread and development of the pathogen

The antagonistic properties of various species of *Aspergillus* were reported by several workers and were mainly attributed to the production of antimetabolites (Raistrick and Smith 1935 Marcus 1947 Zaehner *et al* 1963 Broadbent 1966) *In vitro* studies conducted by Vinod (1988) revealed that *A niger* showed the dual culture reaction of inhibition at a distance and disintegration of *P myriotylum* indicating the production of antibiotics by the antagonists which inhibited the pathogen completely Though there were no other reports of studies on the antagonistic property of *A niger* against *Pythium* sp its antagonistic property towards *Rhizoctonia solani* and *Phytophthora palmivora* was well established and was reported by Bora (1977) Gokulapalan and Nair (1984) and Vinod (1988)

A perusal of the literature revealed that there were no exhaustive study on the antagonistic properties of *A flavus* against *Pythium* spp However the antagonistic effect of *A flavus* against other pathogens was reported by Melgarejo *et al* 1986 Wokocha *et al* 1986 and Dwivedi *et al* 1993

4 8 1 Effect of treatments on yield in pot culture experiment

The maximum yield per plant (124 g) was recorded in the treatment T_{18} (copper oxychloride 0 3% soil drenching) and was closely followed by T_{19} (mancozeb 0 3% soil drenching) (112 g) T_1 (*A niger* seed inoculation) T_3 (*A flavus* seed inoculation) and T_{13} (*A niger* soil application after 60 and 120 DAP recorded 87 g 65 g and 55 g respectively Treatments T_5 (*A niger* soil application at planting and T_7 (*A flavus* soil application at planting) were inferior to T_1 T_3 and T_{13} and recorded 40 g and 24 g respectively Because of complete wilting yield could not be recorded in other treatments

Sarma *et al* (1979) obtained highest yield in ginger when blitane (Zinc + copper oxychloride) dithane Z 78 and difolatan were used as soil drench or drench plus seed treatment Sharma and Dohroo (1982) observed that seed dip in 0 2 per cent solution of either Indofil M-45 or dacomil was effective in controlling the rhizome rot as well as increasing yield in the field Doshi and Mathur (1987) noticed minimum incidence of pre and post drenching rotting and increased yield of rhizomes in treatments with aliette Bordeaux mixture mancozeb and captafol

The results of pot culture studies also revealed that the fungicidal treat ments T_{18} (copper oxychloride 0 3% soil drenching) and T_{19} (mancozeb 0 3% soil drenching) recorded highest yield than the other treatments

From the present study on the biocontrol of rhizome rot of ginger with antagonistic microorganisms the following facts emerged T_1 (*A niger* seed inoculation) T_3 (*A flavus* seed inoculation) and T_{13} (*A niger* soil application after 60 and 120 DAP) though performed well in reducing the disease incidence recorded comparatively lesser yield than T_{18} (copper oxychloride 0 3% soil drenching) and T_{19} (mancozeb 0 3% soil drenching) Among the treatments T_1 T_3 and T_{13} maximum efficiency over control (88 34%) was recorded in T_3 followed by T_1 (58 57%) and T_{13} (52 50%)

In vitro studies to find out the inhibition percentage of the antagonists *A niger* and *A flavus* by tytolan (copper oxychloride) showed 1 11 per cent inhibi tion of *A niger* and complete inhibition of *A flavus* Indofil M 45 (mancozeb) exhibited 7 77 and 36 66 per cent inhibition of *A niger* and *A flavus* respectively Hence seed inoculation of *A niger* in combination with either copper oxychloride

0 3% (or) mancozeb 0 3% was found to be most effective in checking the incidence and severity of rhizome rot of ginger and increasing the yield of rhizomes Seed inoculation of *A flavus* in combination with mancozeb 0 3% and soil application of *A niger* after 60 and 120 DAP with either copper oxychloride 0 3% or mancozeb 0 3% were also found to be promising treatments

Summary

1 Rhizome rot is one of the most destructive diseases of ginger inflicting heavy crop losses especially during rainy seasons Considering the seriousness of the disease the present study was undertaken to isolate the native antagonists and screen them against the pathogen so as to select suitable biocontrol agents to be used for effective management of the disease

2 The rhizome rot pathogen was isolated from infected rhizomes and its patho genecity established Cultural and morphological characters of the pathogen were studied Based on the cultural and morphological characters the causal organism of rhizome rot of ginger was identified as *Pythium aphanidermatum* (Edson) Fitz

3 Soil microorganisms were isolated from the rhizosphere of healthy ginger plants among the rhizome rot affected plants in the diseased field

4 Quantitative estimation of the rhizosphere soil of healthy ginger plants revealed maximum bacterial population (5 030 x 10^6) followed by actinomycetes (3 172 x 10^6) and fungi (10 35 x 10^4)

5 Qualitative study of microflora of rhizosphere soil of healthy ginger plants revealed nine species of fungi belonging to four genera one actinomycete and four species of bacteria The prominent genera of fungi were *Rhizopus Aspergillus Trichoderma* and *Eupenicillium* The actinomycete was observed belonging to *Streptomyces* sp and the four bacterial species obtained were

distinguished from each other based on their colony characters shape and gram reaction and were designated as B_1 B_2 B_3 and B_4

6 The antagonistic properties of all the isolates were studied against the pathogen *P aphanidermatum* by dual culture method

7 The reactions of the antagonists with the test pathogen studied in dual culture and observed as follows

(a) Intermingling and overgrowth
(b) Inhibition at a distance and disintegration of pathogen
(c) Die back and disintegration of pathogen
(d) Mutual inhibition on contact.

Of the above reactions intermingling and overgrowth did not show any antagonistic or antibiotic property and the reaction of mutual inhibition on contact showed only slight antagonistic property The dual culture reactions of inhibition at a distance and disintegration of pathogen and die back and disintegration of pathogen were considered to be strongly antagonistic

8 *Rhizopus* sp and *Aspergillus carneus* showed the dual culture reaction of inter mingling and overgrowth with the pathogen

9 *A niger* has shown the reaction of inhibition at a distance and disintegration of pathogen in dual culture

10 *A fumigatus A flavus* and *T viride* have shown the dual culture reaction of die back and disintegration of pathogen

11 *A flavus* (sclerotial) *Eupenicillium javanicum* ITCC No 4 595 *E javanicum* ITCC No 4 596 and *Streptomyces* sp have the reaction of mutual inhibition on contact with the pathogen in dual culture

12 The microorganisms which showed conspicuous antagonistic characters in dual culture against the pathogen were selected and further studied for production of antibiotic substances by means of assay of cell free culture filtrates by employ ing poisoned food technique

13 *A niger* produced very powerful toxic metabolite and inhibited 100 per cent growth of the pathogen *A fumigatus A flavus* and *T viride* inhibited the growth of *P aphanidermatum* by 42 20 and 62 per cent respectively

14 *In vitro* evaluation of the fungicides Indofil M-45, emisan, fytolan and Bordeaux mixture against the pathogen exhibited complete inhibition of the growth of the fungus by fytolan and Bordeaux mixture Indofil M-45 and emisan exhibited comparatively low efficacy in inhibiting the fungus

15 Among the four fungicides tested against the antagonists for their sensitivity, Bordeaux mixture completely inhibited the growth of all the antagonists while, Indofil M 45 exhibited less efficacy of inhibition against the antagonists

16 Pot culture studies were conducted to find out the efficacy of the antagonists selected from dual culture studies in reducing the rhizome rot incidence of ginger The results indicated that in general, treatments *A niger* seed inocula tion *A flavus* seed inoculation and *A niger* soil application after 60 and 120 DAP had minimum rhizome rot incidence than other treatments Among them *A niger* seed inoculation recorded the least

17 Studies on the effect of treatments on yield in pot culture experiment showed that the treatments copper oxychloride 0 3% soil drenching and mancozeb 0 3% soil drenching gave maximum yield per plant than other treatments

18 The present study revealed that seed inoculation of *A niger* in combination with either copper oxychloride 0 3% or mancozeb 0 3% was found to be most effective in checking the incidence and severity of rhizome rot of ginger and increasing the yield of rhizomes Seed inoculation of *A flavus* in combination with mancozeb 0 3% and soil application of *A niger* after 60 and 120 DAP with either copper oxychloride or mancozeb 0 3% were also found to be promising treatments

References

REFERENCES

Ahmed A H M and Tribe H T 1977 Biological control of white rot of onion (*Sclerotium cepivorum*) by *Coniothyrium minitans* *Pl Path* 26 75 78

Ainsworth G C 1977 *Ainsworth and Bisby s Dictionary of fungi* 6th (ed) Kew CMI pp 631

Akhtar C M 1977 Biological control of some plant diseases lacking genetic resist ance of the host crops in Pakistan *Ann Ny Acad Sci* 287 45 56

* Aristovskaya T V 1951 The quantitative relationship between aerobes and anaerobes in some soils *Pochvovedenier* 1 757 762

* Aristovskaya T V 1957 Some characteristics of the microflora of podzolic soils in the north west part of the U S S R *Sbcrn Rabbotsent Muz. Pochvoved Dokuchaeva* 2 228 249

Backman P A and Rodriguez Kabana R 1975 A system for growth and delivery of biological control agents to the soil *Phytopathology* 65 819 821

Baker K F and Cook R J 1974 *Biological Control of Plant Pathogens* Freeman W H and Company San Francisco pp 380

Balagopalan C Devi S B Indrasenan G and Wilson K I 1974 Varietal reac tions of ginger (*Zingiber officinale* L) towards soft rot caused by *Pythi um aphanidermatum* (Edson) Fitz *Agric Res J Kerala* 12 113 116

Bansal R K and Sobti A K 1988 Control of *Aspergillus flavus* associated with groundnut seeds *Indian Phytopath* 41 643 644

Bertus D D 1942 Report of the work of the Plant Pathology Division *Admn Rep Dis Agric* Ceylon 1941

Bhagwat V Y 1960 Control of soft rot of ginger *Poona Agric Coll Mag* 51 47 49

Bhardwaj S S and Gupta P K 1991 Biological approach to control rhizome rot of ginger with *Trichoderma* sp Abstr of papers presented in the National Seminar on Biological Control in Plantation Crops Rubber Res Inst. India Kottayam 27 28 June 1991, pp 24

Bhardwaj S S Gupta P K Dohroo N P and Shyam K R 1988 Biological control of rhizome rot of ginger in storage *Indian J Pl Path* 6 56-58

Blue W G Eno C F and Westigate P J 1955 Influence of soil profile characteristics and nutrient concentrations on fungi and bacteria in Leonfine sand *Soil Sci* 80 303 306

Bora T 1977 *In vitro* and *in vivo* investigations on the effect of some antagonistic fungi against the damping off disease of egg plant *J Turkish Phytopath* 6 17 23

Brian P W and McGowan J C 1946 Biological active metabolic products of the mould *Metarrhizium glutinosum Nature* 157 334

Broadbent D 1966 Antibiotics produced by Fungi *Bot Rev* 32 219 242

Broadbent P Baker K F and Waterworth Y 1971 Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils *Aust J Biol Sci* 24 925 944

Butler E J 1907 An account of genus *Pythium* and some Chytridiaceae *Mem Dep Agric India* (Bot Ser) 1 70

Chang Y C Chang Y C Baker R Kleifeld O and Chet I 1986 Increased growth of plants in presence of the biological control agent *Trichoderma harzianum Pl Dis* 70 145 148

Chauhan H L Patel M H and Patel D D 1994 Control of rhizome rot of ginger *Spice India* 7 10 17

Chet I Hadar Y Elad Y Katan J and Hems Y 1979 Biological control of soil borne plant pathogens by *Trichoderma harzianum Soil Borne Plant Pathogen* (Eds) B Schippers and W Gams Academic Press London p 585 591

Chet I Harman G E and Baker R 1981 *Trichoderma hamataum* its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp *Microbial Ecol* 7 28 38

Corke C T and Chase F E 1964 Comparative studies of actinomycete populations in acid podsolic and neutral mull forest soils *Soil Sci Soc Am Proc* 28 68 70

Deb P R 1990 *In vitro* inhibitory activity of some rhizosphere fungi of soybean against *Sclerotium rolfsii* Sacc growth *Acta Botanica Indica* 18 159 162

Dennis C and Webster J 1971 Antagonistic properties of species groups of *Trichoderma* 2 Production of volatile antibiotics *Trans Brit Mycol Soc* 57 41-48

Devaki N S Bhat S S Bhat S G and Manjunatha K R 1992 Antagonistic activities of *Trichoderma harzianum* against *Pythium aphanidermatum* and *Pythium myriotylum* on tobacco *J Phytopath* 136 82 87

Dhedhi B M Gupta O and Patel V A 1990 Antagonistic effect of microorganisms to *Fusarium oxysporum* f sp *ciceri Indian J Mycol Pl Path* 20 70-71

Dohroo N P and Sharma S L 1984 Biological control of rhizome rot of ginger in storage with *Trichoderma viride Indian J Pl Path* 2 185 186

*Domsch K H 1960 Das Pilzrpentrum emer bodenprobe 3 Nachtveis der unzei pilze *Arch Mikrobiol* 35 310-339

Domsch K H Gams W and Anderson T H 1980 *Compendium of Soil Fungi* Vol I Academic Press London pp 859

*Doshi A and Mathur S 1987 Symptamatology interaction and management of rhizome rot of ginger by xenobiotics *Korean J Pl Prot* 26 261 265

*Dumitras L and Fratilescusesan T 1979 Aspects of the antagonism of *Trichoderma viride* to *Pythium debaryanum* Hesse *Studii Si Cercetaride Biologie Biologie Vegetalia* 31 63 67

Dwivedi S K Ambasht R S and Dwivedi R S 1993 Toxicity of some antagonistic fungi on pathogenic fungi of two economic crops *Nat Acad Sci Lett* **16** 209 210

Elad Y Chet I and Henis Y 1982 Degradation of plant pathogenic fungi by *Trichoderma harzianum Can J Microbiol* **28** 719 725

Fajola A O and Alasoadura S O 1975 Antagonistic effect of *Trichoderma harzianum* on *Pythium aphanidermatum* causing the damping off disease of tobacco in Nigeria *Mycopathologia* **57** 47 52

Fitzpatrick H M 1923 Generic concepts in the Pythiaceae and Blastocladiaceae *Mycolgia* **15** 166

Garrett S D 1956 *Biology of Root Infecting Fungi* Cambridge University Press London pp 292

Gaur V K and Sharma L C 1991 Microorganisms antagonistic to *Fusarium udum* Butler Proceedings of the Indian Nat Sci Acad Part B *Biol Sci* **57** 85 88

Gokulapalan C and Nair M C 1984 Antagonism of a few fungi and bacteria against *Rhizoctonia solani Indian J Microbiol* **24** 57 58

Harman G E Chet I and Baker R 1980 *Trichoderma hamatum* effects on seed and seedling disease induced in radish and pea by *Pythium* spp or *Rhizoctonia solani Pytopathology* **70** 1167 1172

Haware M P and Joshi L K 1974 Studies on soft rot of ginger from Madhya Pradesh *Indian Phytopath* 27 158-61

Henis Y Elad Y Chet I and Hadar Y 1979 Control of soil borne plant pathogenic fungi in carnation strawberry and tomato by *Trichoderma harzianum Phytopathology* **69** 1031

Henis Y Ghaffar A and Baker R 1978 Integrated control of *Rhizoctonia solani* damping off of radish Effect of successive plantings PCNB and *Trichoderma harzianum* on pathogen and disease *Phytopathology* **68** 900 907

Henis Y and Inbar M 1968 Effect of *Bacillus subtilis* on growth and sclerotium formation by *Rhizoctonia solani* *Phytopathology* **58** 933 938

Hetherington A C and Raistrick H 1931 Studies in the biochemistry of microor ganisms *Philipp Trans R Soc London* Ser B **220** 1 367

Hoch H C and Abawi G S 1979 Biological control of *Pythium* root rot of table beet with *Corticium* sp *Phytopathology* **69** 417 419

Hoppe P E 1966 *Pythium* species still viable after twelve years in air dried muck soil *Phytopathology* **56** 1141

Indrasenan G 1972 *Studies on the soft rot of ginger (Zingiber officinale* Rosc) *caused by Pythium aphanidermatum* (Edson) Fitz *with special reference to the physiology of parasitism* M Sc (Ag) thesis Kerala University pp 118

Indrasenan G and Paily P V 1973 Studies on the soft rot of ginger (*Zingiber officinale* Rosc) caused by *Pythium aphanidermatum* (Edson) Fitz *Agric Res J Kerala* 2 53 56

* Jagnow G 1958 Investigations on microbial numbers and biological activity in meadow soils *Z Pflernahr Dung* **82** 50 67

* Jensen H L 1943 Observations on the vegetative growth of actinomycetes in soil *Proc Linn Soc N S W* **68** 67 71

Johnson L F and Curl E A 1972 *Methods for Research on the Ecology of Soil Borne Plant Pathogen* Burgess Publishing Company Minneapolis pp 247

Joseph Thomas Suseela Bhai R Vijayan A K and Naidu R 1991 Management of rot diseases of small cardamom through bio agents Abstr of papers presented in the National Seminar on Biological Control in Plantation Crops Rubber Res Inst India Kottayam 27 28 June 1991 pp 21

Kaiser W J and Hannan R M 1984 Biological control of seed rot and pre emer gence damping off of chickpea with *Penicillium oxalicum* *Pl Dis* **68** 806 811

Kannan K and Nair K P V 1965 *Zingiber officinale* (ginger) in Kerala *Madras Agric J* 52 168 76

Kerala Agricultural University 1994 *Package of Practices Recommendations Crops 93* Directorate of Extension Mannuthy Trichur p 126 128

Kommedahl T and Mew I C 1975 Biocontrol of corn root infection in the field of seed treatment with antagonists *Phytopathology* 65 296 300

Kommedahl T and Windels G E 1978 Evaluation of biological seed treatments for controlling root diseases of pea *Phytopathology* **68** 1087 1095

Kothari K L 1966 Effect of fungicides on the germination and growth of ginger plants *Sci Cult* 32 48-49

Krishnamoorthy A S and Bhaskaran R 1991 Screening of fungal antagonists against *Pythium indicum* causing damping off of tomato *Madras Agric J* 78 127 128

* Laudelout H D Hoore J L and Fripiat J J 1949 Influence of microorganisms on certain physiochemical properties of Yangambi soils *Bull Agric Congo Nelgie* **40** 339 354

Lewis J A and Papavizas G C 1984 A new approach to stimulate population proliferation of *Trichoderma* spp and other potential biocontrol fungi introduced into natural soils *Phytopathology* 74 1240 1244

Lumsder R D Locke J C Adkins S T Walter J F and Ridout C J 1992 Isolation and localization of the antibiotic gliotoxin produced by *Gliocladium virens* from alginate prill in soil and soil less media *Phytopathology* 82 230 235

Mahadevamurthy S Prakash H S and Shetty H S 1988 Effect of some antagonists on the sclerotial germination of *Claviceps fusiformis J Biol Cont* 2 120 122

Maiti D and Sen C 1985 Integrated biocontrol of *Sclerotium rolfsii* with nitrogenous fertilizers and *Trichoderma harzianum Indian J agric Sci* 55 464 468

Mangenot F and Diem H G 1979 Fundamentals of biological control *Ecology of Root Pathogens* (Eds) S V Krupa and Y R Dommergues Elsevier Scientific Amsterdam p 207 265

Marcus S 1947 Antibacterial activities of Geodin and Erdin *Biochem J* 41 462 463

Martin S B Abawi G S and Hoch H C 1984 Influence of the antagonist *Laeti saria arvalis* on infection of table beets by *Phoma betae Phytopathology* 74 1092 1096

Masroor M K and Chandra S 1987 Antagonism between *Aspergillus* spp and *Xanthomonas campestris* pv *citri* (Hasse) Dye incitant of citrus canker *Nat Acad Sci Lett* 10 233 236

Masroor M K and Chandra S 1989 A modified medium for antibiotic production by *Aspergillus* spp antagonistic to citrus canker pathogen *Nat Acad. Sci Lett* 12 103 106

McRae W 1911 Soft rot of ginger in the Rangpur district, East Bengal *Agric J India* 6 139-46

Melgarejo P Carrillo R and Sugasta E M 1986 Potential for biological control of *Monilinia laxa* in peach twigs *Crop Prot* 5 422-426

Merriman P R Price R D and Baker K F 1974 The effect of inoculation of seed with antagonists of *Rhizoctonia solani* on the growth of wheat *Aust J Agric Res* 25 213 218

* Milosevic R 1958 Microbiological analysis of the sandy soils of Deliblato *Xemlj Bilj* 7 259 265

Mitchell R and Hurwitz E 1965 Suppression of *Pythium debaryanum* by lytic rhizosphere bacteria *Phytopathology* 55 156 158

Mitra M and Subramaniam L S 1928 Fruit rot disease of cultivated Cucurbitaceae caused by *Pythium aphanidermatum* (Eds) Fitz *Mem Dep Agric India* (Bot Ser) 15 79 84

Moody A R and Gindrat D 1977 Biological control of cucumber black root rot by *Gliocladium roseum* *Phytopathology* **67** 1159 1162

Mukharjee B Khatua D C and Sen C 1987 Potential antagonists of *Mac rophomina phaseolina* and biocontrol of seedling blight of jute Abstr of papers presented in Workshop on Biological Control of Plant Diseases (Eds) R Jayarajan R Narayanasamy and K Sivaprakasam Department of Plant Pathology Centre for Plant Protection Studies T N A U Coimbatore 10 12 March 1987 pp 21

Mukherjee B and Sen C 1992 *Aspergillus* and *Penicillium* species Potential agents for biocontrol of *Macrophomina phaseolina* *Indian Phytopath* 45 39 43

Mukhopadhyay A N 1991 Biological control of soil borne fungal plant pathogens Abstr of papers presented in the National Seminar on Biological Control in Plantation Crops Rubber Res Inst India Kottayam 27 28 June 1991 pp 21

Mukhopadhyay A N Brahmbhatt A and Patel G J 1986 *Trichoderma harzia num* A potential biocontrol agent for tobacco damping-off *Tob Res* 12 26 35

Mukhopadhyay A N and Chandra I 1986 Biocontrol of sugarbeet and tobacco damping off by *Trichoderma harzianum* Abstr of papers presented in Seminar on Management of Soil Borne Diseases of Crop Plants (Eds) R Jayarajan R Bhaskaran and N Shanmugam Department of Plant Pathology Centre for Plant Protection Studies T N A U Coimbatore pp 34

Nagarajan K and Reddy T S N 1986 Role of *T viride* and *T harzianum* in the biological control of *P aphanidermatum* Abstr of papers presented in Seminar on Management of Soil Borne Diseases of Crop Plants (Eds) R Jayarajan R Bhaskaran and N Shanmugam Department of Plant Pathology Centre for Plant Protection Studies T N A U Coimbatore pp 33

Nybe E V and Nair P C S 1979 Field tolerance of ginger types to important pests and diseases *Indian Arecanut Spices and Cocoa J* 2 109 111

* Ordentlich A and Chet I 1989 Biological control of soil borne plant pathogenic fungi by antagonistic *Trichoderma Israel Agro Res* 3 137 152

Padmanaban P and Alexander K C 1984 Biological control of cane seedling root rot *Sugarcane* (1) 11

Padmanaban P and Alexander K C 1986 Biological control of *Pythium graminicolum* incitant of root rot of sugarcane seedlings Abstr of papers presented in Seminar on Management of Soil Borne Diseases of Crop Plants (Eds) R Jayarajan R Bhaskaran and N Shanmugam Department of Plant Pathology Centre for Plant Protection Studies T N A U Coimbatore pp 35

Padmanaban P and Alexander K C 1987 Control of root rot of sugarcane seedling by antagonistic organism *Trichoderma viride* sub Abstr of papers presented in Workshop on Biological Control of Plant Diseases (Eds) R Jayarajan R Narayanasamy and K Sivaprakasam Department of Plant Pathology Centre for Plant Protection Studies T N A U Coimbatore 10 12 March 1987 pp 10

Papavizas G C 1984 Soil borne plant pathogen new opportunities for biological control *British Crop Protection Conference Pests and Diseases 1* British Crop Protection Council p 371 378

Parashar R D Sindhan G S and Hooda I 1992 Biological control of bacterial blight (*Xanthomonas campestris* pv *cyamopsidis*) of cluster bean by epiphytes present on leaf surface *Crop Res* 5 551 558

Park M 1935 Report on the work of the Mycological Division *Admn Rep Div Agric* Ceylon 1934 p 124 131

Park M 1937 The seed treatment of ginger *Trop Agric Ceylon* 39 3 7

Park M 1941 Report of the Division of Plant Pathology *Admn Rep Div Agric* Ceylon 1939 p 20 22

Park D 1961 Morphogenesis fungistasis and cultural stating in *Fusarium oxysporum* Snyder & Harsen *Trans Brit Mycol Soc* 44 377 390

Popova T E 1963 Distribution of microorganisms in the profile of irrigated typical serozems *Mikrobiologia* 32 94 98

Potter M C 1908 On a method of checking parasitic disease in plants *J Agric Sci* 3 102 107

Prasad B K Sinha T S P Shanker U and Kumar S 1986 Decay of garlic bulb in the field a new disease report *Indian Phytopath* 39 622 624

Purseglove J W 1972 Tropical crops Monocotyledons Longman Group Ltd London pp 607

Raistrick H and Smith G 1935 Studies in the biochemistry of microorganisms 62 The metabolic products of *Aspergillus terreus* Thom A new mould metabolic product Terrein *Biochem J* 29 606-611

Raju C A 1991 Survey of antagonism in *Trichoderma* species against *Pythium aphanidermatum* Abstr of papers presented in the National Seminar on Biological Control in Plantation Crops Rubber Res Inst India Kottayam 27 28 June 1991, pp 25

Ramakrishnan T S 1949 The occurrence of *Pythium vexans* de Bary in South India *Indian Phytopath* 2 27 30

Rath M and Wolf G 1992 Biological control of seed rot and damping off of sugarbeet with microbial antagonists *Bull OILB/SROP* 15 113 114

Riker A J and Riker R S 1936 *Introduction to research on plant diseases* John Swift Co St Louis Chicago pp 117

Robinson P M and Park D 1966 Citrinin A fungistatic antibiotic and narrowing factor *Nature* 211 883 884

Rose R E 1954 Contributions to the biology of pumice soils (a summary) *Proc 1st Conf NZ Soc Soil Sci* pp 23

Sahare K C and Asthana R P 1962 Rhizome rot of ginger and its control *Indian Phytopath* 15 77 78

Sanford G R 1926 Some factors affecting the pathogenicity of *Actinomyces scabies* *Phytopathology* **16** 525 547

Sarma Y R Nambiar K K N and Brahma R N 1979 Studies on rhizome rot of ginger and its control *Proc Placrosym* II 386 97

Sawant I S and Mukhopadhyay A N 1991 Integeration of metalaxyl with *T harzianum* for the control of *Pythium* damping off in sugarbeet *Indian Phytopath* 43 535 541

Sharif F M Al Fadhal F A H Okash A N and Jamil W N 1988a Biological control of *Pythium aphanidermatum* (Edson) Fitz on cucumber in the plastic green house *J Agric Water Resource Res Pl Prod* 7 99 109

*Sharif F M Okasha A M and Kazem K T 1988b *Penicillium stipitatum* and *Trichoderma harzianum* in the biological control of cucumber damping off disease caused by *Pythium aphanidermatum* *J Univ Kuwait Sci* 15 107 113

Sharma S L and Dohroo N P 1982 Efficacy of chemicals in controlling rhizome rot of ginger (*Zingiber officinale* Rosc) *In Proceedings of National Seminar on Ginger and Turmeric* Kasaragod 1980

Sharma I M and Gupta V K 1988 Effect of fungicides and fertilizers on the control of *Pythium ultimum* Trow causing collar rot of apple in HImachal Pradesh a new pathogen *Indian J Pl Path* 6 110 114

Sharma N D and Joshi L K 1979 *In vitro* evaluation of certain fungicides against eleven seed borne fungi and control of rhizome rot of ginger during storage *Pesticides* 13 37 39

Shekunova E G and Volkova K A 1977 Accumulation of antagonistic microflora in field soil under crop rotation *Byulleten Vsesoyuznogo Nauchno Issledovatelskoga Instituta Zashchity Rastenii* 41 56 59

Sindhan G S Hooda I and Parashar R D 1991 Effect of antagonist spray on the incidence of bacterial blight and biochemical constituents of guar plants *Pl Dis Res* **6** 28 31

Singh R S 1985 *Diseases of Vegetable Crops* Oxford and IBH Publishing Co New Delhi p 319 321

Sivan A Elad Y and Chet I 1984 Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum* *Phytopathology* **74** 498 501

Skinner F A Jones P C T and Mollison J E 1952 A comparison of a direct and a plate counting technique for the quantitative estimation of soil microorganisms *J Gen Microbiol* **6** 261 271

Smith K P Hawey M J and Handelsman J 1993 Suppression of cotton leak of cucumber with *Bacillus cereus* Strain UW 85 *Pl Dis* **77** 139 142

Starc A 1942 Microbiological investigations in some podzots in Croatia *Arch Microbiol* **12** 329 352

Subramaniam L S 1919 A Pythium disease of ginger tobacco and papaya *Mem Dep Agric India* (Bot Ser) **10** 181 194

Sundas D K and Raj S K 1989 Growth morphological and cultural characteris tics of fungicide adapted isolate of *A niger* Van Tieghem *Indian J Mycol Res* **27** 173 180

*Suslow T V Kloepper J W Schroth N N and Burr T J 1979 Beneficial bacteria enhance plant growth *Calif Agric* November/December p 15 17

*Tahvonen R 1982 Preliminary experiments into the use of *Streptomyces* spp isolated from peat in the biological control of soil and seed borne disease in peat culture *J Scint Agric Soc Finland* **54** 357 359

Tahvonen R T 1988 Microbial control of plant disease with *Strepto myces* spp *Bull OEPP* **18** 55 59

Tedla T and Stanghellini M E 1992 Bacterial population dynamics and interac tions with *Pythium aphanidermatum* in intact rhizosphere soil *Phyto-pathology* **82** 652 656

Thomas K M 1938 *Adm Rep Govt Mycologist* Madras 1937 38 pp 21

Thomas K M 1940 *Detailed Adm Rep Govt Mycologist* Madras 1939-40 p 18

Thomas J Suseela Bhai R Vijayan A K and Naidu R 1991 Management of rot diseases of small cardomom through bioagents Abstr of papers presented in the National Seminar on biological control in plantation crops Rubber Res Inst Indian Kottayam 27 28 June 1991 pp 21

Trevinoo G F and Espinosa R G 1981 Effectiveness of some microorganisms antagonistic to *Phytophthora palmivora* in controlling black rot of cocoa pods *Revista Mexicana de Fitopatologica* 1 16 20

Truong H X Salinas M D Obien A S and Carasi R C 1988 Biological control of *Sclerotium rolfsii* and *Pythium aphanidermatum* damping off on Tobacco with *Trichoderma harzianum* culture In Brighton Crop Prot Conf Pests and Dis p 1189 1194

Tsao C B Khoa V S and Yu C F 1959 The microbiological characteristics of rice soils I Study of the number and activity of microorganisms in the fundamental types of rice soils at varying levels of fertility in Eastern and Central China *Acta Pedol Sin* 7 218 226

Uppal B N 1940 Appendix K Summary of the work done under the Plant Pathol ogist to the Government of Bombay Poona for the year 1938 39 *Rep Dep Agric* Bombay 1938 39

Venkatesan R 1964 The influence of soil depth on the microbial population of paddy soil *Annamalai Univ Agric Mag* 4 53 54

Vincent J M 1927 Distribution of fungal hyphae in the presence of certain inhibi tors *Nature* 159 8.0

Vinod P B 1988 Antibiotic producing and antagonistic microorganisms in the forest soils of Kerala M Sc (Ag) thesis Kerala Agricultural University Vellanikkara, Thrissur

Vinod P B Sukumara Varma A and Abi Cheeran 1991a Antagonistic and antibiotic properties of three species of *Trichoderma* isolated from the forest soils of Kerala Abstr of papers presented in the National Seminar on Biological Control of Plantation Crops Rubber Res Inst India Kottayam 27 28 June 1991 pp 24

Vinod P B Sukumara Varma A and Abi Cheeran 1991b Antagonistic and antibiotic properties of *Bacillus subtilis* isolated from the forest soils of Kerala Abstr of papers presented in the National Seminar on Biological Control in Plantation Crops Rubber Res Inst India Kottayam 27 28 June 1991 pp 26

Waksman S A 1952 *Soil Microbiology* John Wiley and Sons Inc New York

Waksman S A and Curtis R E 1916 The actinomycetes of the soil *Soil Sci* 1 99 134

Waksman S A and Curtis R E 1918 The occurrence of actinomycetes in the soil *Soil Sci* 6 309 319

Weindling R 1932 *Trichoderma lignorum* as a parasite of other soil fungi *Phytopathology* 22 837 845

Weindling R 1934 Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi *Phytopathology* 24 1153 79

Weindling R 1937 Isolation of toxic substances from the culture filtrates of *Trichoderma* and *Gliocladium Phytopathology* 27 1175 7

Weindling R and Emerson O H 1936 The isolation of a toxic substance from the culture filtrate of *Trichoderma Phytopathology* 26 1068 70

Wokocha R C Ebenebe A C and Erinle I D 1986 Biological control of the basal stem rot disease of tomato caused by *Corticium rolfsii* (Sacc) Curzi in Northern Nigeria *Trop Pest Mgmt* 32 35 39

Wu W S Liu S D Chang Y C and Tschen J 1986 Hyperparasitic relationships between antagonists and *Rhizoctonia solani* *Pl Prot Bull* Taiwan 28 91 100

*Xu T Zhong J P and Li D B 1993 Antagonism of *Trichoderma harzianum* T82 and *Trichoderma* sp NF 9 against soil borne fungus pathogens *Acta Phytopathologica Sinica* 23 63 67

*Yehia A El Hassan S A and Ismail F K 1981 Studies on damping off disease of tomato seedlings and its biological control *Mesopotamia J Agric* 16 115 124

*Zachner H Keller Schierlein W Huetter R Hess Leisinger K and Deer A 1963 Stoffwechselprodukte von Mikroorganismen 40 Sideramine Aus Aspergillaceen *Arch Microbiol* 45 119 135

* Zahar E Barakat F M Osman A R and El Khaleely M I 1986 Effect of fungicides on *Ulocladium botrytis* and epiphytic microflora of tomato *Egyp J Phytopath* 18 1 10

* Zaspel I and Suss R 1992 Biological control of damping off in *Pinus sylvestris* with bacterial antagonists *Bull OILB/SROP* 15 118 120

Zentmayer G A 1955 A laboratory method for testing some fungicides with *Phytophthora cinnamomi* as test organism *Phytopathology* 45 398

* Zhukova R A 1956 Microbiological investigations of virgin soils of the Kolapeninsula *Mikrobiologia* 25 569 576

*Originals not seen

Appendices

APPENDIX I
Potato dextrose agar medium

Peeled and sliced potato	200 g
Dextrose	20 g
Agar agar	20 g
Distilled water	1000 ml

APPENDIX II
Oat meal agar medium

Rolled oats	100 g
Agar agar	15 g
Distilled water	1000 ml
pH	6 0 to 6 5

APPENDIX III
Nutrient agar medium

Beef extract	1 gm
Yeast extract	2 gm
Peptone	5 gm
NaCl	5 gm
Agar agar	15 gm
Distilled water	1000 ml

APPENDIX IV
Nutrient glucose medium

Beef extract	3 0 g
Peptone	5 0 g
Glucose	5 0 g
NaCl	5 0 g
Agar agar	15 0 g
Tap water	1000 ml
pH	6 8 to 7 2

APPENDIX V
Martin s Rosebengal streptomycin agar medium

(Martin 1950)

Peptone	5 0 g
Dextorse	10 0 g
KH_2PO_4	1 0 g
$MgSO_4\ 7H_2O$	0 5 g
Agar agar	15 0 g
Rose bengal	1 part in 30 000 parts of the medium
Distilled water	1000 ml
Streptomycin	30 0 mg

APPENDIX VI
Kenight s agar medium

Glucose	1 0 g
KH_2PO_4	0 1 g
$NaNO_3$	0 1 g
KCl	0 1 g
$MgSO_4$ $7H_2O$	0 1 g
Agar agar	20 g
Distilled water	1000 ml

APPENDIX VII
Analysis of variance table Effect of antagonists and selected fungicides on the rhizome rot of ginger in pot culture experiment

Source	df	Mean square		
		120 DAP	150 DAP	180 DAP
Treatment	19	16 573**	29 770**	18 930**
Error	80	3 300	1 683	3 271
Total	99			

** Significant at 1 per cent level
DAP Days after planting

BIOCONTROL OF RHIZOME ROT OF GINGER (*Zingiber officinale*) BY ANTAGONISTIC MICROORGANISMS

BY

SHANMUGHAM V.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF HORTICULTURE
Vellanikkara Thrissur 680 654
Kerala India

1996

ABSTRACT

Rhizome rot is one of the most destructive diseases of ginger in Kerala The pathogen was isolated from infected rhizomes and its pathogenecity established The pathogen was characterized and identified as *Pythium aphanider matum* (Edson) Fitz based on its cultural and morphological characters

Soil microorganisms were isolated from the rhizosphere of healthy ginger plants among the rhizome rot affected plants in the diseased field and were quantitatively and qualitatively estimated The population of bacteria was maximum followed by actinomycetes and fungi A diversified group of soil microflora consist ing of nine species of fungi belonging to four genera viz *Rhizopus Aspergillus Trichoderma* and *Eupenicillium* one actinomycete of *Streptomyces* sp and four species of bacteria which were distinguished from each other based on their colony characters as B_1 B_2 B_3 and B_4

Antagonistic properties of the microorganisms isolated were studied against the pathogen *P aphanidermatum* in dual culture

Rhizopus sp and *Aspergillus carneus* showed the dual cu ture reaction of intermingling and overgrowth with the pathogen which is not considered as an antagonistic reaction *A niger* exhibited the reaction of inhibition at a distance and disintegration of pathogen while die back and disintegration of the pathogen was shown by *A fumigatus A flavus* and *Trichoderma viride* Both these characters are considered strong antagonism *A flavus* (sclerotial) *Eupenicillium javanicum* ITCC No 4 595 and *E javanicum* ITCC No 4 596 have the reaction of mutual inhibition

on contact with the pathogen in dual culture which is not considered as promis ing antagonistic property

Inhibitory properties of antagonists usmg cell free culture filtrates were estimated and found that *A niger* inhibited 100 per cent growth of the pathogen While *A fumigatus T viride* and *A flavus* inhibited the growth of *P aphanider matum* by 42 20 and 60 per cent respectively

Among the different fungicides screened in *in vitro* fytolan and Bordeaux mixture completely inhibited the growth of the pathogen Indofil M-45 and emisan exhibited comparatively less inhibitory effect While Bordeaux mixture showed complete inhibition of the growth of all the antagonists Indofil M-45 exhibited less efficacy of inhibition against the antagonists

Result of the pot culture studies indicated that treatments *A niger* seed inoculation *A flavus* seed inoculation and *A niger* soil application after 60 and 120 DAP had minimum rhizome rot incidence The studies on the effect of treatments on yield in pot culture experiment showed that copper oxychloride 0 3% as soil drenching and mancozeb 0 3% as soil drenching recorded maximum yield per plant

Thus the present study revealed that seed inoculation of *A niger* in combination with either copper oxychloride 0 3% or mancozeb 0 3% was found to be most effective in checking the incidence and severity of rhizome rot of ginger and increasing the yield of rhizomes Seed inoculation of *A flavus* in combination with mancozeb 0 3% and soil application of *A niger* after 60 and 120 DAP with either copper oxychloride 0 3% or mancozeb 0 3% were also found to be promising treat ments