BIOCONTROL OF RHIZOME ROT OF GINGER (*Zingiber officinale* Rosc.) USING SELECTED ANTAGONISTS

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled '**Biocontrol of rhizome rot of ginger (*Zingiber officinale* Rosc.) using selected antagonists**' 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.

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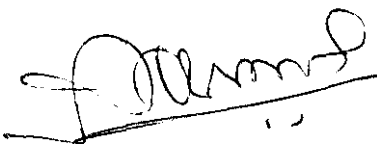
Certified that the thesis entitled **'Biocontrol of rhizome rot of ginger (*Zingiber officinale* Rosc.) using selected antagonists'** is a record of research work done independently by **Ms. Julie George K.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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We, the undersigned members of the Advisory Committee of **Ms. Julie George K.,** a candidate for the degree of **Master of Science in Agriculture,** with major in **Plant Pathology,** agree that the thesis entitled **'Biocontrol of rhizome rot of ginger (*Zingiber officinale* Rosc.) using selected antagonists'** may be submitted by Ms. Julie George K., in partial fulfilment of the requirement for the degree.

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JULIE GEORGE K.

Dedicated to my parents

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Introduction

INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) belonging to the family Zingiberaceae is an important crop known from ancient times for its use both as spice and medicine. In India, it is cultivated in an area of 62,090 ha with an-annual production of 1.86 lakh tonnes. Kerala alone contributes about 40 per cent of India's production.

One of the major constraints in the cultivation of ginger in Kerala is the rhizome rot caused by *Pythium aphanidermatum* (Edson). Fitzpatrick. Apart from the crop damage in the field, the stored rhizomes also are affected by this pathogen. Because of the multiplicity of factors involved in the disease, satisfactory control is rarely achieved by a single measure. Although the fungicide application are often thought to provide single-shot control, they usually do not. In India, even though a few researchers have attempted the control of rhizome rot of ginger with chemicals, (Park, 1935; Thomas, 1940; Bhagwat, 1960; Sahare and Asthana, 1962; Kothari, 1966; Sharma and Dohroo, 1982; Abraham *et al.*, 1988a; Abraham *et al.*, 1988b; Das *et al.*, 1990; Anandam *et al.*, 1996.) the absolute control has not been obtained so far. The application of chemicals also leads to environmental pollution. In this context the importance of biological control in the management of the soil borne diseases becomes significant.

The concept of biological control embodies the introduction of antagonists into cropping systems and also manipulation of the environment to favour resident beneficial micro-organisms by crop rotation, residue management and a wide range of other cultural practices. (Nigam and Mukherjee, 1988.). Biological control of plant diseases is a promising alternative to chemical control and in recent years, considerable success have been achieved by various workers in checking the population of pathogenic, *Pythium* spp. in the soil by native antagonists. (Kuruchev, 1980; Yehia *et al.*, 1981; Sesan, 1986; Nair, 1990; Bhardwaj and Gupta, 1991; Abada, 1994; Vilasini, 1996; Jose, 1997). Shanmugham (1996) reported that three antagonistic fungi, *Trichoderma viride*, *Aspergillus niger* and *Aspergillus flavus* were effectively reduced the incidence of rhizome rot of ginger in pot culture experiments. Compared to chemical control, this method is usually long lasting and economical. Moreover, a living multiplying biocontrol agent potentially provides a continuous, non-chemical control of the pathogen.

The present study was undertaken to evaluate the efficacy of *T. viride*, *A. niger* and *A. flavus* in the management of rhizome rot of ginger in field conditions. The programme of research envisaged the following:

1. Standardisation of mass multiplication of the selected antagonists using cheaper and commonly available materials like rice hull, rice bran, sawdust and soil + cowdung for field application.
2. Quantitative estimation of soil microflora in the experimental field before planting and after treatments at bimonthly intervals.
3. Field experiment for evaluation of the biocontrol agents in sick plot.

Review of literature

REVIEW OF LITERATURE

The most serious disease of ginger is the rhizome rot caused by *Pythium* species. Butler (1907) first reported the prevalence of this disease from Surat (Gujarat) in India caused by a species of *Pythium* which was identified as *P. gracile* Sehrenk. Subramanian (1919) studied the disease from Pusa and he was in full agreement with Butler's description about the pathogen and renamed it as *P. butleri* Subram. Later Mitra and Subramanian (1928) from their cross inoculation studies came to the conclusion that *P. butleri* is a strain of *P. aphanidernatum* (Edson) Fitzpatrick. Sen (1930) reported this pathogen from Assam causing rhizome rot ginger.

Ramakrishnan (1949) first reported *P. aphanidermatum* as the major pathogen causing rhizome rot of ginger in Kerala. Many attempts were made by several workers to control rhizome rot of ginger by various means. The relevant literature available in India and abroad on various aspects related to the present study is reviewed in this chapter.

2.1 Effect of food bases on the multiplication of selected antagonists

Formulation and application methods of antagonists are often of paramount importance in biological control. The effect of various substrates on the multiplication of antagonists before, was studied by many workers.

Backman and Kabna (1975) found that a diatomacious earth granule impregnated with a 10 per cent molasses solution was suitable for the growth and delivery of *Trichoderma harzianum* to groundnut fields. They also found that, the damage caused by *Sclerotium rolfsii* was controllable with *Trichoderma* granules at 140 kg ha^{-1} which was found equivalent to that with 10 per cent PCNB granules at the rate of 112 kg ha^{-1}. The yield was also high in plots treated with the antagonist.

Sundheim (1977) reported the application of bark pellets containing *Trichoderma* spp. to glass house soil infested with ***Phomopsis sclerotoides*** reduced bark root rot of cucumber for two months. The wheat bran cultures of *T. harzianum* protected radish seedlings from damping - off induced by *R. solani* and also increased germination when applied to soil (Henis *et al.*, 1978)

From various experiments conducted in carnation, strawberry and tomato to control *R. solani* with the antagonist *T. harzianum*, the results showed that the antagonist preparation with wheat bran as a carrier was a long term effective biocontrol agent in artificially and naturally infested soil (Henis *et al.*, 1979).

Hardar *et al.* (1979) reported that in glasshouse, the application of *T. harzianum* wheat bran culture to soil infested with *R. solani* effectively controlled damping-off of bean, tomato and plant seedling. Elad *et al.* (1980) reported that the wheat bran preparation of *T. harzianum* increased the growth of bean plants and yield in a non-infested soil. It controlled *Sclerotium rolfsii* and *Rhizoctonia solani* more effectively than a conidial suspension of antagonist.

The control of *Sclerotium cepivorum* causing white rot disease of onion was maximum, when the antagonist *T. harzianum* was grown on barley grain, was added at the time of planting (Abd-El-Moity and Shatla, 1981). A wheat bran based culture of *T. harzianum* was tested against *R. solani* in carnation field by Elad *et al.*, (1981). Incidence was reduced by 70 per cent when preparation was applied at 150g dry wt/m^2. The antagonist *T. harzianum* isolated from soil when applied in the form of wheat bran culture to soil infested with *S. rolfsii* or *R. solani* in the glass house resulted the control of damping off of bean, groundnut and egg plant (Chet and Elad, 1982)

In a preliminary pot tests on the control of *S. rolfsii* on sunflower, the incorporation of finger millet seeds colonized by *T. harzianum* into soil containing straws precolonised with *S. rolfsii* or of straw pieces colonized by *T. harzianum* into soil containing sclerotia reduced survival of the pathgen. (Anilkumar and Gowda, 1983). Jones *et al.* (1984) tested a lignite - stillage carrier system for applying *T. harzianum* and *Gliocladium virens*. Root rot ratings and root and shoot dry weights revealed positive effects of the biocontrol agent and carrier.

Lewis and Papavizas (1984) observed that the population densities of isolates of *T. viride* and *T. harzianum* increased about 10^4 and 10^3 fold respectively in natural soil during the first three week of incubation when the two antagonists were added as a mycelial preparation in sterile bran-sand-water at the ratio of 1:1:2 w/w/v and the population did not increase when the conidia were added without bran. Sivan *et al.* (1984) reported that a wheat bran + peat mixture(1:1 v/v) was the most efficient of the

raw plant material substrate found suitable for growing a new isolate of *T. harzianum*. Fravel *et al.* (1985) developed a method to encapsulate microrganisms such as *Talaromyces flavus*, *Gliocladium virens*, *Penicillium oxalicum* and *T. viride* with aqueous solutions containing one per cent sodium alginate and 10 per cent pyrax to control plant diseases.

Sivan and Chet (1986) reported that one isolate of *T. harzianum* in wheat bran-peat preparation, when applied to artificially infested soil under glass house condition, reduced the Furarium wilt in cotton, wheat and muskmelon. The application of *T. harzianum* isolate as a wheat bran-peat preparation, or as a seed coating decreased the diseases incidence caused by *F. oxysporum* f.sp. *radicis lycopersici* in tomato and *F. oxysporum* f.sp. *niveum* in water melon under field conditions.

Upadhyay and Mukhopadhyay (1986) reported that the application of *T. harzianum* as infested sorghum grains to *S. rolfsii* infested soil, of green house, gave upto 76 and 88 per cent disease control in first and second cycles of sugarbeet seedlings respectively. Peat bran preparation of *T. harzianum* reduced the disease incidence caused by *M. phaseolina* in green house planted beans by 37-74 per cent (Elad *et al.*, 1986).

Seed pelleting with different antagonists such as *T. viride* and *T. harzianum* could increase the germination rate and reduce the post emergence mortality of LRA 5166 cotton caused by *R. solani* when compared to control significantly. (Alagarswamy *et al.*, 1987). Lewis and Papavizas (1987) prepared alginate pellets from a wet fermentor biomass of 11 isolates of *Trichoderma* spp. and *G. virens* with wheat bran as a food base carrier. All the isolates significantly reduced growth of the pathogen *R. solani* from infested beet seed into natural soil.

Gangadharan and Jeyarajan (1990) reviewed the various growth media to identify suitable substrates among the agricultural bye-products and waste for mass multiplication of *T. viride* and *T. harzianum*. Both the antagonists produced maximum number of colony forming units on tapioca rind and was on par with tapioca refuse and with decomposed farm yard manure. Krishnamoorthy and Bhaskaran (1990) mulitplied the culture of *T. viride* and *T. harzianum* in wheat bran - peat soil medium for soil as well as seed treatment against *Pythium indicum* causing damping off in tomato.

Nair (1990) reported that the isolates of *T. longibrachiatum* was found to grow well in rice bran, whereas for *T. harzianum* and *Aspergillus terreus*, milled rice was found to be the best substrate. Taylor (1991) reported that the liquid coating formulation of *T. harzianum* enhanced its efficacy, when the treated cucumber seeds were sown in a *Pythium* infested soil and that the coating provided a physical barrier that delayed the pathogen attack and resulted in a conducive environment for *T. harzianum* growth. Panicker and Jeyarajan (1993) observed that of the five substrates tested, farmyard manure was the best for multiplication of *T. viride* and *T. harzianum* followed by wheat and rice bran.

Ramakrishnan *et al.* (1994) reported that the application of talc based formulation of *Trichoderma* significantly reduced root rot of urd bean, which was caused by *M. phaseolina*. The antagonists, *T. harzianum T. viride* and *G. virens* prepared in wheat bran-saw dust-tap water mixture was most effective in reducing seedling mortality induced by *Sclerotinia sclerotiorum* in chick pea (Sharma, 1994). Singh (1994) mass cultured these isolates of *T. harazianum* on sterilized wheat bran-saw dust-water mixture in the ratio 3:1:3.5 w/w/v to treat the setts of sugarcane to inhibit pathogen *Colletotrichum falcatum*.

Agarwal *et al.*, (1995) multiplied the species of *Trichoderma* and *Gliocladium* on pre-soaked and sterilised wheat seeds and applied in soil infected by *Neovossia indica*. The pre-emergent damping off in tomato caused by *P. aphanidermatum* was inhibited by the soil application of *T. viride* and *T. harzianum* which were multiplied in wheat bran peat mixture (Karpagavally and Ramabadran, 1995).

Jeyarajan and Ramakrishnan (1995) developed a talc based formulation of *T. viride* for dry seed treatment of oil seed and pulses against *M. phaseolina*. Desai (1995) pelletized the fermented biomass of *T. harzianum* in different substances alginate, alginate + clay and alginate + clay + wheat bran. Alginate + clay + bran was found to be suitable for pelletization. The seed treatment with talc based formulation of *T. harzianum* and *T. viride* significantly reduced the root rot incidence of sesamum (Sankar and Jeyarajan, 1996).

Lewis *et al.* (1996) formulated alginate prills with a biomass of isolates of *G. virens* and *Trichoderma* spp. in various food bases viz., wheat bran, maize cobs,

ground nut hulls, soyafibre, castor pomace, cocoa hulls and chitin. Prills with all food bases except cocoa hulls significantly reduced damping off of zinnia caused by *R. solani* and *Pythium ultimum* in a soil less mixture.

2.2 Use of biocontrol agents in the management of soil borne diseases

2.2.1 *Trichoderma* as biocontrol agent

Trichoderma spp. are well documented as effective biological agent of plant diseases caused by soil borne fungi especially *Pythium* spp. They are reported as the most promising biocontrol agents and the biocontrol potential has been studied extensively.

Docea *et al.* (1974) obtained complete inhibition of *Pythium debaryanum* by *Trichoderma lignorum* and *T. viride*. An active substance trichodermicin was obtained from *T. viride* both in *in vitro* and in studies with tomato, cucumber and begonia plants in green house. The addition of *T. viride* and *Streptomyces* spp. to two commercial soil less mixtures infested with *Pythium splendens* delayed by 22-28 days the expression of severe disease symptoms in rooted geranium cuttings (Bolton, 1978).

Dumitras and Sesan (1979) reported that of the 31 isolates of *T. viride* investigated against *Pythium debaryanum*, 25 were strongly antagonistic to the pathogen *P. debaryanum* infected in sugarbeet, pea and cotton seedlings. In *in vitro* tests, *T. viride* protected beet and cotton seedlings against the pathogen as effectively as fungicidal products. Bolton (1980) achieved a good protection of Poinsettia from sudden wilting caused by *Pythium aphanidermatum* by incorporation of spores and mycelium of *T. viride*. The degree of protection was directly proportional to the amount of antagonists added. Harman *et al.* (1980) reported that the treatment of radish seeds with conidia of *T. hamatum* protected seeds and seedlings from the infection of *Pythium* spp. nearly as effectively as fungicide.

Yehia *et al.* (1981) also reported a good control of *Pythium* infection by seed treatment with the antagonists. In trials with tomato wet seeds coated with *T. viride* significantly reduced both pre and post emergence occurrence of damping off disease and increased seedlings survival.

Tang and Uung (1983) reported that damping off of *Pinus marsonia* was reduced by 3 of 5 strains of *Trichoderma* tested. Incorporation of the antagonists before sowing into soil infested with *P. spinosum* increased germination. After emergence, the disease was reduced by sprinkling the seed bed with a spore suspension. Ruppel *et al.* (1983) observed that, a pre-planting row application of *T. harzianum* in a wheat bran carrier slightly but significantly reduced the severity of *Rhizoctonia* root rot in sugarbeet as compared with the non-treated control.

The application of *T. harzianum* in the wheat bran + peat mixture preparation, efficiently controlled damping off induced by *P. aphanidermatum* in peas, cucumber, tomatoes, pepper and gypsophila. *T. harzianum* broadcasting as a seed coating mixture was also effective in sandy soil (Sivan *et al.*, 1984). Dohroo and Sharma (1984) reported that the treatment of rhizomes with *T. viride* resulted in more than 80 per cent control of rhizome rot in ginger caused by *Pythium pleuroticum* (wet rot) and *Fusarian equisetti* (dryrot) in storage. Hardar *et al.* (1984) observed that the 2 isolation of *Trichoderma* viz, *T. koningii* and *T. harzianum* protected the seeds of against seed rots naturally infested with *Pythium* spp. when applied either as a seed coating in various adhesive or in gels used for fluid seed drilling.

Padmanabhan and Alexandar (1984) reported that when *T. viride* was incorporated in soil the seedlings root rot of sugar cane caused by *Pythium graminicolum* was completely inhibited compared to 8.5 to 21.5 per cent disease incidence in untreated control. Teyes and Dirks (1985) observed that the isolates of *Gliocladium catenulatum, G. virens* and *T. hamatum* significantly suppressed root rot of peas caused by *P. ultinum* in both steamed and unsteamed soil. The results revealed that the tested antagonist may be considered as potential biological control agent for *Pythium* root rot in the field.

Sesan (1986) obtained good results by the application of biopreparation of *T. viride* in soil as well as seed treatment against *P. debaryanum, R. solani, Furarium* spp. and *S. sclerotiorum* in sunflower and leguminous plants and also against *Botrytis cinerea* in grapevine. Application of conidia of isolates of *T. harzianum* or *T. koningii* to pea seed reduced the incidence of pre-emergence damping off induced by *Pythium* spp. (Lifshitz *et al.*, 1986). The fungal antagonist *T. harzianum* controlled *S. rolfsii* and *P. aphanidermatum* causing damping off in toacco when applied in a mixture of

rice bran : rice hull : water at the rate of 3:1:1.2 v/v/v (Truong *et al.*, 1988). Jacob *et al.* (1988) tested eight fungal antagonists *in vitro* against *P. aphanidermatum* causing pre-emergence damping off of brinjal and promising treatment *viz.* with *T. harzianum* was used for seed treatment @ 5 x 10 conidia / ml.

Bhardwaj *et al.* (1988) obtained good control of rhizome rot of ginger caused by *P. aphanidermatum* and *Fusarium equiseti* during storage by steeping inoculated rhizomes in spore suspension of *T. viride* or smearing with *T. harzianum*. Sharif *et al.* (1988) reported that the efficacy of biological control with *Penicillium septatum* and *T. harzianum* was equivalent to that obtained with ridomil against *P. aphanidermatum* causing cucumber damping off. Ahmad and Baker (1988) reported that the seed treatment with conidia of rhizosphere-competent mutants of *T. harzianum* reduced the incidence of pre-emergence damping off of barley, cucumber, pea, radish and tomato induced by *Pythium ultimum*

Sivan and Chet (1989) observed that, the seed treatment with preparations from *T. viride* prevented white rot due to *S. sclerotiorum* on sunflower and reduced infection by *P. debaryanum* in cotton in glass house and field. In green house tests it was seen that isolates of *T. harzianum* obtained from field soils were effective for control of diseases of various crops caused by *Pythium* spp, *R. solani*, *S. rolfsii* etc., when grown in a semisolid fermentation medium in wheat bran by different application techniques. (Ordentilich and Chet, 1989).

Viswakumar (1989) isolated few species of *Trichoderma* from rhizosphere and phylloplane of rice plants to test against *R. solani* causing sheath blight of rice. Of these *T.viride* and *T. harzianum* were found antagonistic in *in vitro* and *in vivo*. Soil inoculation with *T. viride*, *T. harzianum* and *Laetisaria arvalis* gave good control of *Pythium indicum* and in treated pots gave 78.2, 90.9 per cent and 72.2 per cent seed germination in tomato respectively. Seed treatment with each of biological control agents was equally effective (Krishnamoorthy and Bhaskaran, 1990). Padmanabhan and Alexander (1990) reported that the root rot incidence of sugarcane during different seasons, was drastically reduced following the application of *T. viride* to soil. Zacharia (1990) isolated 13 species of fungal antagonists to *R. solani* from rice soils of Thiruvananthapuram district of Kerala. Among them, *T. harzianum* was found as the best followed by *T. koningii*.

Bhardwaj and Gupta (1991) reported that three species of *Trichoderma viz.*, *T. viride*, *T. harzianum* and *T. hamatum* suppressed the infection by *Pythium aphanidermatum* on ginger during storage. The seed rhizomes were treated by smearing with the spore suspension of antagonists or steeping inoculated rhizomes in spore suspension. Rathore *et al.* (1992) observed that *T. viride* produced non-volatile substance which inhibited the growth of the ginger rhizome rot pathogen, *Pythium myriotylum* and *Fusarium solani* by 70 and 10 percentage respectively, when the organisms were grown on media plates previously used for *T. viride*.

Devaki *et al.* (1992) reported significant decrease in disease incidence in tobacco caused by *Pythium* spp. by the incorporation of *T. harzianum* into sterile soil before infection with *Pythium* spp. Xu *et al.* (1993) reported that soil treatment with 0.6 per cent (w/w) *T. harzianum* culture (107c.f.u g^{-1}) reduced the incidence of disease caused by *C. rolfsii*, *R. solani* and *P. aphanidermatum* by 46.5 per cent, 28.4 per cent and 81.2 per cent respectively in cucumber, in green house experiments 20 days after inoculation with the pathogen. The efficiency of *Trichoderma* as a biocontrol agent against fungi causing damping off and root rot was investigated in pot and field experiments by Abada (1994) *T. harziamum* caused a great reduction in the infection level of damping-off and root rot diesease.

Inbar *et al.* (1994) applied *T. harzianum* to cucumber and pepper seedlings as a peat-bran preparation incorporated into the propagation mixture in a commercial production nursery. They found that the *Trichoderma* tested plants were more resistant to damping-off disease caused by *Pythium* spp. and *R. solani*. Wu (1995) reported that seed treatment with *T. harzianum* in controlling *P. aphanidermatum*, *Pythium splendens* and *R. solani* the causal agents of seedling blight of cucumber and poinsettia was more effective than soil treatment. Xue *et al.* (1995) reported that the isolates TR-5 of *Trichoderma* sp. isolated from soil controlled damping off of cucumber caused by *P. aphanidermatum* by 88.2 per cent. Zacharia (1995) studied the effectiveness of utilising *Trichoderma* as biological antagonist against storage pathogens in tomato, brinjal and chilli viz., *F. solani*, *R. solani* and *Colletotrichum capsici* respectively. The study revealed that *T. viride* was effective in reducing fruit rot of brinjal and tomato by 52 and 50 per cent respectively upto 12 days in storage and in chilli 43 per cent. Ebenezer *et al.* (1996) obtained the reduction of damping off diseases of mustard caused

by *P. aphanidermatum* upto 63.7 per cent by the seed treatment with *T. viride* and upto 71.3 and 64.4 per cent by the soil application of *T. viride* and *T. harzianum* respectively.

2. 2 .2 *Aspergillus* spp. as biocontrol agent

The studies on the application of *Aspergillus* spp. as biocontrol agent in the management of *Pythium* spp. is limited. However, the antagonistic property of various species of *Aspergillus* against the pathogens other than *Pythium* were reported by several workers. The antimetabolic production by various species of *Aspergillus* was reported by Zachner *et al.* (1963) and Knaphur, (1964). In laboratary experiments conducted by Truszkowska *et al.* (1969) it was revealed that *A. fumigatus* was the most important antagonist to *Verticillium albo-atrum*, pathogenic fungi of tomato. Raicu and stain (1975) reported the antagonism of *A. flavus* and *A. niger* against *Phytophthora nicotianae* var. *parasitica* and *V. dahliae* in tomato.

Bora (1975) reported that the development of microsclerotia of *V. dahliae* was inhibited by root extracts of lucerne *A. niger* a strong antagonist was isolated from lucerne root cuttings. Abdalla and El Tayeb (1976) observed that the metabolic products of *A. flavus* had a toxigenic effect upon *Sclerotium bataticola* decreasing its linear growth and dry weight. Wu (1977) reported that the growth of *P. graminicolum* was inhibited by *A. niger* in dual culture method in *in vitro* studies. The fourteen isolates of *A. flavus* were antagonistic to *Fusarium oxysporum* f.sp. *albedinie*. The intensity of the action being related to the colour of the colony and its diffusable pigments in the culture medium (Sabaou, 1977).

Bora (1977) observed that *A. niger* has shown greatest antagonism against *R. solani* from egg plant, when its antagonistic property was estimated among other soil fungi. Bastor (1978) reported the antagonistic effect of *A. terreus* against *Crinipellis perniciosa* the causal agent of witches-broom of cocoa. Yadav and Dayal (1978) found that culture filtrates of *A. flavus* contained volatile metabolite which delayed and inhibited germination of macroconidia of *F. solani* f.sp. var. *coeruleum*. In a study conducted by Bailey *et al.*, (1978) it was found that of the 15 antagonists tested *A. terreus*, *A. flavipes* and *A. flavus oryzae* inhibited sporulation of *P. palmivora* from cocoa pods. *A. flavus oryzae* also prevented healthy pods from being infected by mycelium potential of the pathogen.

Tashleeva (1979) developed a method of preparing a spore suspension of *Thielaviiopsis basicola* the cotton pathogen, and while developing this method, it was found that the fungi, *Aspergillus* spp. especially *A. terreus*, str. 803, *A. flavus* etc. were most antagonistic. Khan *et al.* (1979) reported that *A. niger*, *A. candidus*, *A. flavus* and *A. fumigatus* were active against the plant pathogenic bacteria tested in culture. It has been reported that in the culture filtrates of *A. niger*, *A. flavus*, and *A. candidus* the growth of *R. solani* isolates from forest soil was suppressed (Shukla and Dwivedi, 1979)

Atac (1979) observed that the inhibition of the pathogen *Phytophthora capsici* by *A. flavus* was upto 50 per cent and in pot experiments in the glass house, the inhibition of *Phytophthora capsici* infection by the antagonists was complete. *Sclerotium rolfsii* the pathogen of root disease of lentil overgreen was inhibited by all the rhizosphere isolates except *T. harzianum* and *A. niger* (Arora and Dwivedi, 1979).

Zakhi (1980) reported that the antagonistic activity of *Aspergillus*, *Penicillium* and *Trichoderma* against pathogen, *F. solani*, *F. moniliformae*, *F. oxysporum* which caused the disease in tomato. Of the 114 fungal isolates investigated against *Corynespora cassicola* (soybean root rot causing fungi). *Aspergillus japonica* was found to be the most antagonistic to the pathogen (Zhukovskaya, 1989)

Trevinov and Epinosa (1981) applied conidial suspension of different species of *Aspergillus* with potato saccharose agar to cocoa against *Phytophythora palmivora.* Only *Aspergillus terreus* was found to retard the start of disease by 30 days. Infestation of soil with pathogens like *F. solani*, *R. solani* and *S. rolfsii* caused more serious disease in bean. Of 110 fungal isolates investigated thirteen were antagonistic to *R. solani* and to nine to *F. solani*. The most effective antagonists against all the three pathogens were three *Trichoderma* spp. and two *Aspergillus* spp. (Newelgy *et al.*, 1982). Babushkina (1982) reported the antagonism of *A. flavus*, *A. fumigatus*, *A. lutescens*, *A. nidulans* and *A. terreus* against *V. dahliae* and *V. tricorpus* in cotton.

Fedoseeva *et al.* (1983) reported that two isolates of *Trichoderma lignorum*, two of *Aspergillus niger* and one of *Trichothecium roseum* had lytic activity or inhibited *Ustilago maydis*, pathogen of maize smut. It has been reported that *Aspergillus terreus* isolated from soil inhibited the growth of *R. solani* f.sp. *sasakii* (*Corticium sasakii*) the

rice sheath blight fungus in *in vitro*. When one month old seedlings of the cultivar Pusa 2.21 were grown in pots of soil infested with *C. sasaki*, sheath blight incidence was 4.4 per cent compared with 0.3 per cent when *A. terreus* was also present (Roy, 1984) The inhibition of growth of *R. solani* by parasiting the hyphae by *A. niger* was reported by Gokulapalan and Nair, 1984.

The inhibition of germination of sclerotia of *S. rolfsii* by *A. flavus* was reported by Khetmalas *et al.*, 1984, during screening with soil fungi. The suppression of sporangia in *Phytophthora parasitica* var. *piperina* in *Piper betle* by culture filterates of *A. niger* and *A. flavus* was reported by Vyas *et al.*, 1981.

Pande (1985) noticed that the culture filtrate of three species of *Aspergillus* and *T. viride* retarded the growth of *Alternaria alternata, Dreschlera* sp., *Fusarium oxysporum, R. bataticola* and *S. rolfsii.* Gupta *et al.* (1985) reported that soil isolates of *A. fumigatus, A. luchnensis* and *A. niger* were antagonistic in *in vitro* to the rice sheath blight pathogen *S. oryzeae.* The culture filtrate also inhibited the pathogen growth. The antagonistic effect *A. flavus* in peach twigs was assayed by Melgarijo *et al.* (1986) and found that *A. flavus* caused a transient delay in symptom development by multiplying profusely. The *in vitro* studies by Razik *et al.* (1985) revealed that, *Aspergillus candidus* was the most inhibiting antagonist of *S. cepivorum.* Application of *A. candidus* to infested soil @ 2 $x10^5$ propagules per 1 ml decreased the percentage of white rot significantly.

Manandhas *et al.* (1987) studied the interaction between the pathogenic and saprophytic fungi isolated from soybean roots and seeds and observed the antagonism of *A. terreus* against many soybean pathogens *viz., Cercospora sojina, Macophomina phaseolina, Phomopsis sojae* and *Septoria glycinea.* In field studies, the seeds coated with the conidial suspension of *A. terreus* produced a better stand than the control.

Masroor and Chandra (1987) reported that of 27 fungal isolates tested, against *Xanthomonas campestris* pv. *citri*, the incitant of citrus canker, the highest activity was shown by *A. flavus, A. clavatus* and *A. niger.* Mahadevamoorthy *et al.* (1988) noticed that germination of sclerotia of *Claviceps fusiformis* causing ergot of *Pennisetum americanum* was inhibited in soil amended with *A. niger, T. harazianum, T. viride* and *B. subtilis.* The sclerotia colonized by the antagonists disintegrated and when fragments

were incubated, colonies of the antagonists were obtained. The germination of the host seed was not affected.

Palakshappa (1989) observed that *T. viride*, *Penicillium* sp., *A. flavus*, *A niger* *Bacillus subtilis* and *Streptomyces* sp. isolated from amended soil were antagonistic to *Corticium rolfsii*. Viswakumar (1989) reported that in *in vitro* *A. niger* caused granulation, vacuolation and disintegration of *R. solani*, the sheath blight pathogen of rice. Dhedhi *et al.* (1990) observed *A. fumigatus* as an effective antagonist to *F.oxysporum* f.sp. *ciceris* causing vascular wilt of *Cicer arietinum* in *in vitro* studies. The suppression of the growth of *S. rolfsii* a pathogen of soyabean, by *A. flavus* by overgrowing it, was reported by Deb and Dutta, (1991).

Bopalah *et al.* (1991) investigated the effect of *A. niger*, *A. fumigatus* and *Penicillium islandicum* isolated from the phyllosphere of sorghum on the growth of *Helminthosporium oryzae*, *Phytophthora arecae* and *Pyricularia oryzae* and found the inhibition by *A. fumigatus* and *A. niger* . The antagonists property of white sterile fungus, *A. niger*, *Penicillium citrinum* and *B. subtilis* against *X. campestris* pv. *cyamopsidis* causing bacterial blight of *Cyamopsis tetragonoloba* was reported by Sindhan *et al.* (1991).

Mukherjee and Sen (1992) isolated *A. fumigatus*, *A. terreus*, *Penicillium citrinum*, *P. simplicissimum* from soil and investigated for their antagonism against *M. phaseolina* in *in vitro*. Only culture filterate of *A. fumigatus* inhibited fungal growth and scleorotial germination. When applied to soil as a food base, all of the test antagonists reduced the intensity of seedling blight of jute. Devenhage and Kotze (1993) observed that *A. candidus* reduced root rot and root colonization by *Phytophthora cinnamomi* when used with the pathogen to inoculate the nursery planting medium.

The non-volatile activity of *A flavus* inhibited *F. oxysporum* f.sp. *lini* from tomato by 28.2 and 26 per cent respectively (Dwivedi *et al.* 1993). Zacharia (1995) found that *A. niger* and *A. flavus* were suitable antagonists against *F. solani* causing fruit rot of tomato in storage. Supression of *P. aphanidermatum* causing rhizome rot of ginger by the fungi *A. niger*, *A. terreus*, *Penicillium* spp. and *Absidia cylindrospora* was reported by Balakrishnan *et al.* (1997). The sorghum grain preparation of respective antagonist was applied in *Pythium* infested soil and results indicated that the antagonists can be effectively exploited for the control of pathogen.

2.3 Use of fungicides in the management of rhizome rot of ginger

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 oxychloridie), Dithane Z-78 and Difolatan when used as soil drench or drench plus seed treatments (Sarma and Joshi, 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.

In a study conducted by Doshi and Mathur (1987) reported that the pre and post emergence rotting of ginger was minimum with the drenching of fungicides Aliette, Bordeaux mixutre, Dithane M-45 and Difolatan. Rathaiah (1987) tested the fungicide Ridomil (metalaxyl) against rhizome rot (*P. myriotylum*) of ginger, alone and in combination with captafol and mancozeb. Dipping or wetting of seed rhizomes one day before planting and soil drenching with a mixture of Ridomil + captafol three months after planting controlled the disease and significantly increased the yield of ginger. Abraham *et al*, (1988a) carried out a field experiment to manage the soft rot of ginger. The results revealed that the maximum germination percentage was observed in plots receiving Captaf 5G treatment. The minimum post-emergence rot, less partially infected

rhizome and maximum yield of ginger were obtained from plots treated with Dithane M-45. Abraham *et al,* (1988b) reported that among the various chemical tested Captaf 0.2%, Captafol 0.2 %, Dithane M-45 0.3%) to study the effect of seed treatment for the control of the rhizome rot of ginger. The maximum percentage of germination was obtained with Captafol treatment. The disease incidence in the field one month after germination was also very less in the above treatment compared to others.

Ramachandran *et al.,* (1989) evaluated four fungicides viz., Fosetyl–Al, metalaxyl, oxadixyl, propamocarb and ethazole against rhizome rot (*P. aphanidermatum*) of ginger. The fungicides were tested as soil and seed treatments by raising plants in fungus infested soil. The result indicated that, the metalaxyl formulations (Ridomil 5 G and Apron 35 W.S) gave best control of disease. In a study, conducted by Das (1990), to control the rhizome rot of ginger caused by *Pythium* spp., the lowest incidence of the disease and highest percentage of germination was obtained by the seed treatment with captan 0.2 % ai for 30 minutes. Anandam (1996) reported that, of the seven fungicides tested against the rhizome rot of turmeric (*P. aphanidermatum*) soil application of Ridomil, effectively controlled the disease followed by mancozeb and alliete.

2.4 Compatibility of selected antagonists with fungicides

There are reports where the compatible nature of chemicals were evaluated with biocontrol agents both *in vitro* and *in vivo*. Papavizas *et al.* (1982) evaluated new biotypes of *T. harzianum* for tolerance to benomyl. Out of 36 colonies of *T. harazianum* that survived the 3 irradiations, 19 colonies tolerated high concentrations of benomyl (100-500 mg l^{-1}) as indicated by growth germination tests on benomyl - amended agar. Kraft and Papavizas (1983) studied the effect of *T. harazianum* with checking the root rot and damping off of peas caused by *P. ultimum* and *F. solani* f.sp. *pisi* and increasing the seed yield. The result showed that, the highest seed yield with dark skin perfection were obtained with the seed treatment combining metalaxyl and *T. harzianum* spores. In a study, conducted by Sesan *et al.* (1986), the cotton and sunflower seeds were treated with the preparation of *T. viride* and the phytotron to prevent the white rot due to *S. sclerotiorum* on sunflower and infection by *P. debaryanum* on cotton. The result indicated that, the seed treatment with specific fungicides combined with preparation from *T. viride* at low doses effectively prevented the pathogens on both crops. Zahar

et al. (1986) reported the less toxic effect of cuprosan 311 SD (Copper oxychloride + maneb + zineb) on *A. niger* and *A. flavus*. In a study conducted by Viswakumar (1989) to control the sheath blight of rice in Kerala the combination of *T. viride* and Edefenphos was found to be very effective. Sundas and Raj (1989) noted that the groundnut collar rot pathogen *A. niger* tolerated blitox 50 (copper oxychloride) and Indofil M-45 (mancozeb) at 6000 ppm and Emisan-6 (2-methoxy ethyl mercury chloride) at 450 ppm when grown on PDA containing increasing concentration of fungicides.

Sawant and Mukhopadhyay (1991) obtained 100 per cent control of damping of caused by *Pythium* spp. in sugar beet when *T. harzianum* and metalaxyl were used in combination. Pavashar *et al.* (1992) reported that, the fungal antagonist *A. niger* was sensitive to the fungicides, Blitox, Dithane Z-78 or Dithane M-45. Shanmugam (1996) evaluated four fungicides, Bordeaux mixture, Fytolan, Emisan and Indofil M-45 against the antagonists, *A.flavus* and *A. niger* both in *in vitro* and *in vivo*. He found that Indofil M-45 exhibited less efficacy in inhibiting the growth of *A. flavus* in *in vitro*. However, it completely inhibited the growth of *A. niger*. In field experiment, seed inoculation of *A. niger* in combination with either copper oxychloride 0.3 per cent or mancozeb 0.3 per cent was found to be most effective in checking the incidence and severity of rhizome rot of ginger.

Materials and methods

MATERIALS AND METHODS

The present study on biocontrol of rhizome rot of ginger using selected antagonist was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period from April 1997 to February 1998. The details of materials used and methodology followed for the investigation are described in this session

3.1 Isolation and purification of pathogen

The pathogen causing rhizome rot of ginger was isolated from naturally infected ginger rhizomes by applying standard isolation methods (Ricker and Ricker, 1936). The pathogen was identified as *Pythium aphanidermatum* (Edson) Fitzpatrick by comparing the characters of the isolate with the type culture available in the Department of Plant Pathology, College of Horticulture, Vellanikkara. The pure culture of fungus was maintained on potato dextrose agar slants by periodic subculturing. Koch's postulates were confirmed on ginger variety, Rio-de-Janeiro.

3.2 Standardisation of the mass multiplication of the antagonist

The antagonists *Trichoderma viride* Pers. exFr., *Aspergillus flavus* Link and *Aspergillus niger* Van Tiegh which were found effective against the ginger rhizome rot pathogen *P. aphanidermatum* in a previous study (Shanmugham, 1996) were selected for the present investigation. The growth of these antagonists were compared in the following four food bases which were cheaper and commonly available.

1. Rice bran
2. Rice hull
3. Saw dust
4. Soil + cowdung (1:1)

3.2.1 Preparation of growth media

Twenty five grams each of the various food bases viz., rice bran, rice hull and sawdust were weighed and transferred to 250 ml conical flasks separately. A quantity of 50 ml of distilled water was added to each conical flask and shaken well to wet the media. In the case of soil and dried cowdung, 12.50 g of each of them was taken in a conical flask,

and 50 ml of water was added. The prepared growth media were autoclaved at 1.04 $kgcm^{-2}$ pressure for 1 h for two successive days. Six replications were kept for each treatment.

3.2.2 Inoculation of food bases with antagonists

A 5 mm disc of 15 days old culture of each antagonist grown on PDA was transferred to the flask containing food bases under aseptic condition, which was sterilized earlier. The antagonists were incubated for 15 days to estimate the population.

3.2.3 Estimation of antagonist population in food bases

The population of the antagonists in various food bases was estimated by serial dilution plate technique (Johnson and Curl, 1972). The serial dilutions of the antagonists were prepared upto one in 10^8. One ml. of the dilution was pipetted to sterile petriplates and molten but cooled Martin's rose bengal streptomycin agar media was added. The colonies of *T. viride* were counted on the second day of plating and that of *A. niger* and *A. flavus* on the third day. The population of the antagonists in different food bases was expressed as number of colony forming units (c.f.u.) per gram substrate.

3.3 Field experiment

A field experiment was conducted to test the efficacy of the antagonists *T. viride*, *A. flavus* and *A. niger* in controlling the rhizome rot of ginger. The area of field trial has a typical warm humid tropical climate and located at an altitude of 22.5 m above MSL and at 10^0 32^1 N latitude and 76^o 16^1 E longitude. The soil of the experimental field is of loamy laterite type of moderate fertility with a pH of 5.3.

The details of the experiment were as follows :-

Crop	:	Ginger
Variety	:	Rio-de-Janeiero
Design	:	R.B.D
Spacing	:	25 x 25 cm
No of plants per plot	:	32
Replication	:	3
Plot size	:	2 x 1 m
No. of treatments	:	28

Treatments

1. Treating seed rhizomes with *T. viride* and planting
2. Treating seed rhizomes with *A. niger* and planting.
3. Treating seed rhizomes with *A. flavus* and planting
4. Treating seed rhizomes with mancozeb 0.3% and planting
5. Treating seed rhizome with copper oxychloride 0.3% and planting
6. Application of *T.viride* in pits at the time of planting
7. Application of *A. niger* in pits at the time of planting
8. Application of *A. flavus* in pits at the time of planting
9. Application of *T. viride* + *A. niger* in pits at the time of planting
10. Application of *T. viride* + *A. flavus* in pits at the time of planting
11. Application of *A. niger* + *A. flavus* in pits at the time of planting
12. Application of *T. viride* + A. niger + A. flavus in pits at the time of planting
13. Application of *T. viride* into soil at 60 and 120 days after planting (DAP)
14. Application of *A. niger* into soil at 60 and 120 DAP
15. Application of *A. flavus* into soil at 60 and 120 DAP
16. Application of *T.viride* + *A. niger* into soil at 60 and 120 DAP
17. Application of *T.viride* + *A.flavus* into soil at 60 and 120 DAP
18. Application of *A. niger* + *A. flavus* into soil at 60 and 120 DAP
19. Application of *T.viride* + *A.niger* + *A. flavus* into soil at 60 and 120 DAP
20. Application of mancozeb 0.3% at 60 and 120 DAP
21. Application of copper oxychloride 0.3% at 60 and 120 DAP
22. Application of mancozeb 0.3% at 60 DAP followed by incorporation of *T. viride* at 90 DAP
23. Application of mancozeb 0.3% at 60 DAP followed by incorporation of *A. niger* at 90 DAP
24. Application of copper oxychloride 0.3% at 60 DAP followed by incorporation of *A. flavus* at 90 DAP
25. Application of copper oxychloride 0.3% at 60 DAP followed by incorporation of *T. viride* at 90 DAP
26. Application of copper oxychloride 0.3% at 60 DAP followed by

incorporation of *A. niger* at 90 DAP

27. Application of copper oxychloride 0.3% at 60 DAP followed by incorporation of *A. flavus* at 90 DAP
28. Control

3.3.1 Mass multiplication of antagonists for field application

Based on the results of the experiment on standardisation of the mass multiplication of antagonists, *T. viride* and *A. flavus* were mass multiplied in rice hull where as *A. niger* in rice bran. The sterilized food bases were inoculated with actively growing cultures of antagonists and incubated for two weeks before application.

3.3.2 Application of antagonists and fungicides

The time and type of application of antagonists and fungicides varied according to the treatments. The seed rhizomes were treated with the antagonists @ 5 g kg^{-1} seed for 30 minutes. The fungicides, mancozeb and copper oxychloride were used for seed rhizome treatment @ 3 g l^{-1} for 30 minutes. The plots were drenched with 0.3 per cent mancozeb and 0.3 per cent copper oxychloride @ 6 l m^{-2}. The antagonists were incorporated into soil @ 250 g m^{-2}. The incorporation of fungicides / antagonists were done after collecting soil samples for estimation.

3.3.3 Planting

The ginger, var. Rio-de-Janiero, obtained from Regional Agricultural Research Station, Ambalavayal was used for the study. The seed rhizomes were treated with fungicides as per Package of Practices Recommendations for Crops (KAU, 1993) and stored on sawdust till sowing. Seed rhizomes (15-20 g) were planted in raised beds of size 2 x 1 m at a spacing of 25 x 25 cm. All cultural practices except fungicidal application were carried out as per Package of Practices Recommendations for Crops.

3.4 Germination

The number of rhizomes germinated in each plot were counted upto 45 days from planting, and the germination percentage was worked out.

3.5 Pre-emergence rotting

The non-germinated rhizomes were taken out after 45 days of planting and the isolations were carried out, to know the association of rhizome rot pathogen and the percentage of pre-emergence rotting was worked out.

3.6 Post-emergence rotting (rhizome rot)

The number of plants showing typical symptoms of rhizome rot was recorded at fortnightly intervals till harvest. The percentage incidence was worked out at each fortnight.

3.7 Quantitative estimation of mycoflora

The quantitative estimation of total mycoflora and *P. aphanidermatum* was done at the time of planting and at bimonthly intervals upto harvest. Similarly, the population of antagonists *T. viride*, *A. flavus* and *A. niger* was also found out.

The soil samples were collected from different parts of each plot from the various treatments. The samples from each plot were mixed thoroughly and shade dried. The samples were then used for estimation of total mycoflora, the pathogen and the antagonists.

3.7.1 Estimation of the population of *Pythium aphanidermatum*

Fifty mg of soil was weighed from each sample separately. This was sprinkled uniformly in a sterile petriplate and 20 ml of cooled selective medium (Peethambaran and Singh, 1977) was poured over it. The plates were swirled before solidification of the medium in order to get a uniform distribution of soil particles. The plates were incubated at room temperature and colonies were counted after 48h. Three replications were kept for each treatment.

3.7.2 Estimation of total fungal population

The quantitative estimation of fungal population was carried out by serial dilution plate technique (Johnson and Curl, 1972) using Martins rose bengal agar. The fungal colonies developed after 48 h of inoculation were counted and expressed as c.f.u. per g dry weight of soil. Three plates were kept for each treatment.

3.7.3 Estimation of antagonist population

The petriplates from which the total fungal population were taken were kept undisturbed. The number of colonies formed by the antagonist *T. viride*, *A. flavus* and *A. niger* were recorded. The identity of above antagonists were established by its colour, growth and morphological characters. The population count was expressed as c.f.u. per g dry weight of soil.

3.8 Statistical analysis of data

The results were statistically analysed by ANOVA/ANOCOVA as the case may be. The treatment means were compared using Duncan's Multiple Range Test (DMRT).

Results

RESULTS

The results on present investigation "biocontrol of rhizome rot of ginger using selected antagonists" are presented in this chapter.

4.1 Isolation and purification of pathogen

The pathogen causing soft rot of ginger was isolated from naturally infected ginger rhizomes. The isolate was purified and maintained on potato dextrose agar slants by periodic subculturing. Koch's postulates were confirmed on ginger variety Rio-de-Janeiro. Based on the characters, the fungus causing rhizome rot of ginger was identified as *Pythium aphanidermatum* (Edson) Fitzpatrick.

4.2 Standardisation of mass multiplication of antagonists

Three antagonistic fungi, viz., *T. viride*, *A. flavus* and *A. niger* were grown in four different food bases. The results on population of antagonists, are given in Table 1, 2 and 3. The data pertains to the number of colony forming units (c.f.u.) of the antagonists per gram weight of food bases, 15 days after inoculation, is presented.

4.2.1 *Trichoderma viride*

After 15 days of inoculation, the highest population count of *T. viride* recorded in the food base, rice hull (5.36×10^8, c.f.u. g^{-1}). All the treatments were significantly different from each other (Table 1). The food base, sawdust recorded the least count (0.86×10^8, c.f.u. g^{-1}) (Plate 1).

4.2.2 *Aspergillus flavus*

Among the food bases tried, rice hull was found to be the best medium for the growth of *A. flavus* (12.86×10^8 c.f.u. g^{-1}) followed by rice bran (8.86×10^8 c.f.u. g^{-1}). Soil + Cowdung was ranked third in promoting the growth of *A. flavus* (5.76×10^8 c.f.u. g^{-1}). The minimum population of *A. flavus* was recorded in saw dust (3.98×10^8 c.f.u. g^{-1}) (Plate 3).

Table 1 Population of *T. viride* in different food bases

Food bases	No. of c.f.u x 10^8
Rice bran	*3.66 [b]
Rice hull	5.36 [a]
Soil + cowdung (1 : 1)	2.56 [c]
Saw dust	0.86 [d]

* Treatment mean followed by same letter do not differ significantly at 5 % level

Table 2. Population of *A. flavus* in different food bases

Food bases	No. of c.f.u x 10^8
Rice bran	*8.86 [b]
Rice hull	12.86 [a]
Soil + cowdung (1 : 1)	5.76 [c]
Saw dust	3.98 [d]

* Treatment mean followed by same letter do not differ significantly at 5 % level

Table 3 Population of *A. niger* in different food bases

Food bases	No. of c.f.u x 10^8
Rice bran	*13.60 [a]
Rice hull	9.43 [b]
Soil + cowdung (1 : 1)	9.81 [b]
Saw dust	4.43 [c]

* Treatment mean followed by same letter do not differ significantly at 5 % level

Plate 1. Growth of *Trichoderma viride* in different food bases 15 days after inoculation

Plate 2. Growth of *Aspergillus niger* in different food bases 15 days after inoculation


RICE HULL
RICE BRAN
SAWDUST
SOIL + COWDUNG

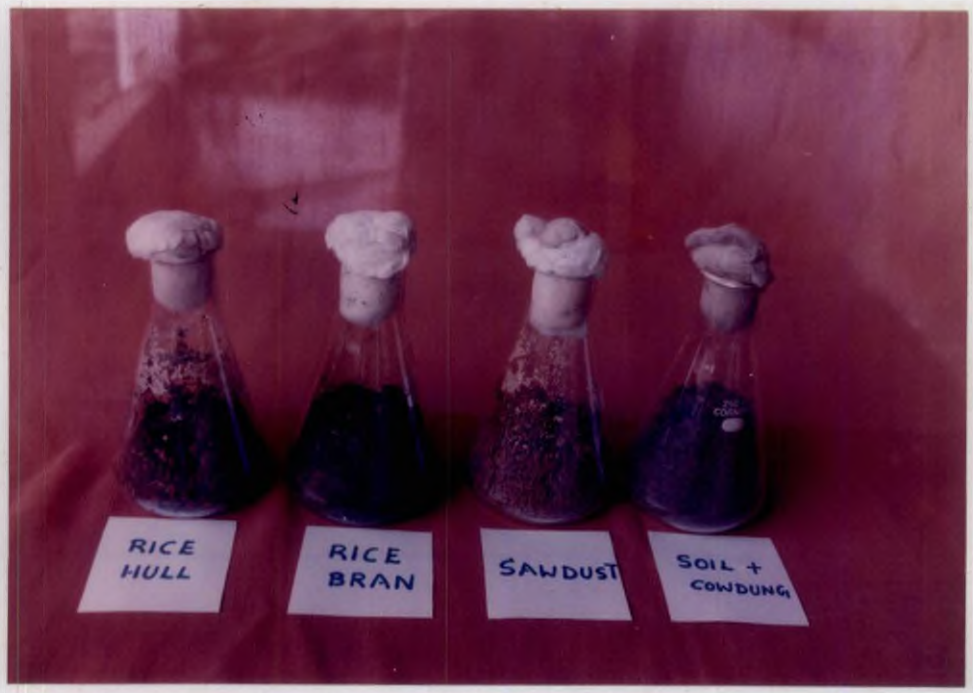
RICE HULL
RICE BRAN
SAWDUST
SOIL + COWDUNG

Plate 3. Growth of *Aspergillus flavus* in different food bases 15 days after inoculation


RICE HULL
RICE BRAN
SAWDUST
SOIL + COWDUNG

4.2.3 *Aspergillus niger*

Rice bran was found to be superior to all other food bases in promoting the growth of *A. niger* (13.60 x10^8c.f.u. g^{-1}). The difference was not significant for the growth of *A. niger* in food bases rice hull and soil + cowdung (Table 3). As in the case of other antagonists, sawdust was the poorest substrate for the growth of *A. niger* (4.43 x 10^8 c.f.u. g^{-1}) (Plate 2).

4.3 Germination

The result on the germination revealed that in all the plots, the germination was completed within 45 DAP. There was no significant difference between the treatments (Table 4). Among the treatments where seed rhizomes were treated with antagonists T_1 (seed treatment with *T. viride*), T_2 (seed treatment with *A. niger*), T_7 (application of *A. niger* at time of planting), T_{10} (application of *T. viride* and *A. flavus* at the time of planting), T_{13} (application of *T. viride* at 60 and 120 DAP) and fungicides, T_4 (Seed treatment with mancozeb 0.3 %) recorded 100 per cent germination. The least percentage of germination recorded was 83.3 by T_{20} (application of mancozeb 0.3 % at 60 and 120 DAP.)

4.4 Pre-emergence rotting

There was considerable variation in pre-emergence rotting among the treatments (Table 5). The maximum pre-emergence rotting was observed in T_{20} (application of mancozeb 0.3 % at 60 and 120 DAP) (8.3 %) followed by T_{25} (application of copperoxychloride 0.3 % at 60 DAP followed by incorporation of *T. viride* at 90 DAP), T_{22} (application of mancozeb 0.3 % at 60 DAP, followed by incorporation of *T. viride* at 90 DAP) (6.3 %). In most of the plots the pre-emergence rotting was nil.

4.5 Post-emergence rotting (rhizome rot)

The observations on rhizome rot were recorded at fortnightly intervals, to find out the efficacy of selected antagonists and fungicides in reducing the incidence of the disease. The results are presented in Tables 6 and 7.

Table 4. Germination of ginger in different treatments 45 DAP

Treatments	Germination (%)	Treatments	Germination (%)
T_1	100[a]	T_{15}	88.5[a]
T_2	100[a]	T_{16}	95.8[a]
T_3	97.9[a]	T_{17}	97.9[a]
T_4	100[a]	T_{18}	91.7[a]
T_5	99[a]	T_{19}	97.9[a]
T_6	85.4[a]	T_{20}	83.3[a]
T_7	100[a]	T_{21}	96.9[a]
T_8	97.9[a]	T_{22}	92.7[a]
T_9	95.8[a]	T_{23}	95.8[a]
T_{10}	100[a]	T_{24}	95.8[a]
T_{11}	91.7[a]	T_{25}	91.7[a]
T_{12}	95.8[a]	T_{26}	93.8[a]
T_{13}	100[a]	T_{27}	96.9[a]
T_{14}	94.8[a]	T_{28}	92.7[a]

The treatments folowed by same letter are not significantly different at 5 % level

Table 5. Percentage pre-emergence rot of ginger in different treatments

Treatments	Pre-emergence rot (%)	Treatments	Pre-emergence rot (%)
T_1	0	T_{15}	1.0
T_2	0	T_{16}	0
T_3	0	T_{17}	1.0
T_4	0	T_{18}	3.1
T_5	0	T_{19}	0
T_6	4.2	T_{20}	8.3
T_7	0	T_{21}	0
T_8	1.0	T_{22}	6.3
T_9	3.1	T_{23}	0
T_{10}	0	T_{24}	1.0
T_{11}	0	T_{25}	6.3
T_{12}	0	T_{26}	0
T_{13}	0	T_{27}	0
T_{14}	3.1	T_{28}	4.2

The disease incidence was first noticed during the fourth fortnight after planting and continued upto tenth fortnight. During the fourth fortnight the maximum disease incidence was in control (8.6 %) followed by T_2 (Seed treatment with *A. niger*) (8.30 %), In some plots, the disease was not observed (Table 6).

During the fifth fortnight, the disease incidence was observed in all the treatments. The minimum disease incidence of 1.04 per cent was observed in T_{10} (application of *T. viride* and *A. flavus* at the time of planting) which was significantly superior to all treatments. The control (T_{28}) recorded the maximum percentage of disease incidence. There was a gradual increase in the disease during sixth fortnight. The treatment T_{19} (application of *T. viride, A. niger* and *A. flavus* 60 and 120 DAP) was found to be significantly superior to other treatments in reducing the disease incidence followed by T_{16} (application of *T. viride* and *A. niger* at 60 and 120 DAP), T_9 (application of *T. viride* and *A. niger* at the time of planting), T_{24} (application of mancozeb 0.3 % at 60 DAP followed by *A. flavus*) and T_{23} (application of mancozeb 0.3 % at 60 DAP followed by *A. niger* at 90 DAP) which were on par with each other (Table 6). The maximum percentage of disease incidence was in control (55.17 %).

During the seventh fortnight, the minimum disease incidence was recorded in T_{19} (6.51 %) and the maximum in control (67.25 %). The treatments T_{21} (soil application of copper oxychloride 0.3 % at 60 and 120 DAP) (8.86 %), T_{16} (10.02 %), T_{23} (10.01 %) and T_9 (10.07 %) were also good in reducing the incidence of the disease.

The observation on eighth fortnight showed that the percentage of disease incidence ranged from 7.65 to 72.79. Here also the treatment T_{19} recorded the minimum disease incidence. (7.65 %) and was significantly superior to all other treatments. This was followed by T_{16} (10.01 %). The highest disease incidence was in control (72.79 %).

During ninth fortnight, there was an increase in the disease incidence. The treatment T_{19} recorded the least disease incidence (7.65 %). The treatments T_{16}, T_{24}, T_{25} (application of copper oxychloride 0.3 % at 60 DAP followed by *T. viride* at 90 DAP), and T_{17} (application of *T. viride and A. flavus* at 60 and 120 DAP) recorded a disease incidence of 10.02, 12.40, 13.99 and 14.29 per cent respectively. These treatments were significantly different from each other. The maximum disease occurred in control plot (77.1 %).

Table 6. Effect of the selected antagonists and fungicides on the rhizome rot of ginger

Treatments	Rhizome rot incidence (%)						
	Fortnight 4	Fortnight 5	Fortnight 6	Fortnight 7	Fortnight 8	Fortnight 9	Fortnight 10
T_1	5.20 (0.227) ^{abc}	10.69 (0.327) ^{bcdefghi}	23.73 (0.501) ^{bcdefg}	35.15 (0.625) ^{c}	41.9 (0.700) ^{bcd}	49.00 (0.778) ^{g}	52.9 (0.822) ^{d}
T_2	8.30 (0.291) ^{a}	18.67 (0.438) ^{abcde}	28.81 (0.558) ^{bcd}	28.64 (0.559) ^{f}	31.1 (0.589) ^{bcdefg}	35.87 (0.639) ^{j}	37.33 (0.656) ^{g}
T_3	6.30 (0.249) ^{ab}	23.28 (0.501) ^{ab}	27.51 (0.547) ^{bcde}	30.5 (0.581) ^{d}	39.4 (0.675) ^{bcde}	42.56 (0.707) ^{o}	42.56 (0.707) ^{e}
T_4	5.20 (0.217) ^{abcd}	22.03 (0.468) ^{abcd}	40.35 (0.681) ^{ab}	45.81 (0.739) ^{b}	48.7 (0.771) ^{b}	56.80 (0.855) ^{z}	56.8 (0.855) ^{b}
T_5	2.10 (0.143) ^{bcde}	12.70 (0.359) ^{bcdefgh}	19.8 (0.460) ^{cdefghi}	19.93 (0.462) ^{k}	24.0 (0.510) ^{defgh}	26.83 (0.544) ^{w}	26.83 (0.544) ^{h}
T_6	3.10 (0.172) ^{abcde}	8.94 (0.287) ^{bcdefghij}	16.5 (0.414) ^{defghij}	17.66 (0.431) ^{n}	21.5 (0.475) ^{efghi}	25.22 (0.519) ^{q}	25.22 (0.519) ^{l}
T_7	0.00 (0.884) ^{e}	3.13 (0.146) ^{hijk}	15.09 (0.385) ^{defghij}	18.68 (0.440) ^{l}	19.8 (0.457) ^{efghi}	22.25 (0.489)	25.12 (0.522) ^{l}
T_8	5.40 (0.221) ^{abcd}	8.73 (0.294) ^{bcdefghij}	14.28 (0.387) ^{defghij}	17.9 (0.434) ^{m}	23.2 (0.493) ^{defghi}	24.63 (0.516) ^{c}	24.63 (0.516) ^{m}
T_9	0.00 (0.090) ^{e}	4.31 (0.169) ^{ghijk}	7.82 (0.275) ^{hij}	10.07 (0.310) ^{t}	13.8 (0.361) ^{hi}	17.43 (0.425) ^{s}	18.73 (0.441) ^{s}
T_{10}	1.00 (0.118) ^{cde}	1.04 (0.065) ^{k}	11.55 (0.341) ^{fghij}	13.43 (0.375) ^{p}	14.6 (0.319) ^{fghi}	18.40 (0.442) ^{f}	21.29 (0.474) ^{p}
T_{11}	0.00 (0.098) ^{de}	8.33 (0.283) ^{cdefghij}	14.81 (0.389) ^{defghij}	22.35 (0.488) ^{l}	23.5 (0.504) ^{defghi}	23.54 (0.505) ^{v}	23.54 (0.505) ^{n}
T_{12}	5.40 (0.226) ^{abc}	7.65 (0.261) ^{defghijk}	13.69 (0.367) ^{defghij}	21.19 (0.476) ^{j}	21.2 (0.476) ^{efghi}	22.58 (0.491) ^{y}	22.58 (0.491) ^{o}
T_{13}	5.20 (0.217) ^{abcd}	14.92 (0.396) ^{bcdef}	28.29 (0.560) ^{bcd}	24.62 (0.512) ^{g}	33.1 (0.612) ^{bcdef}	36.10 (0.644) ^{x}	36.1 (0.644) ^{h}
T_{14}	0.00 (0.090) ^{e}	9.86 (0.316) ^{bcdefghi}	20.72 (0.472) ^{cdefgh}	22.4 (0.493) ^{l}	22.4 (0.492) ^{defghi}	27.80 (0.549) ^{l}	30.61 (0.583) ^{j}
T_{15}	1.00 (0.124) ^{cde}	8.37 (0.292) ^{bcdefghij}	17.49 (0.425) ^{cdefghij}	17.83 (0.429) ^{mn}	22.5 (0.490) ^{defghi}	22.48 (0.490) ^{r}	22.51 (0.49) ^{o}
T_{16}	0.00 (0.090) ^{e}	2.38 (0.095) ^{jk}	7.81 (0.271) ^{ij}	10.02 (0.319) ^{t}	10.01 (0.318) ^{hi}	10.02 (0.390) ^{a}	10.02 (0.319) ^{w}
T_{17}	1.10 (0.120) ^{cde}	8.61 (0.296) ^{bcdefghij}	9.76 (0.314) ^{ghij}	13.1 (0.369) ^{q}	13.1 (0.368) ^{jhi}	14.29 (0.386) ^{d}	14.29 (0.386) ^{u}
T_{18}	0.00 (0.090) ^{e}	9.32 (0.299) ^{bcdefghij}	18.22 (0.434) ^{cdefghij}	24.13 (0.506) ^{h}	24.1 (0.506) ^{defghi}	21.31 (0.455) ^{h}	25.32 (0.523) ^{l}
T_{19}	0.00 (0.090) ^{e}	4.23 (0.204) ^{fghijk}	6.51 (0.252) ^{j}	6.51 (0.252) ^{v}	7.7 (0.279) ^{l}	7.65 (0.279) ^{e}	7.65 (0.279) ^{x}
T_{20}	5.20 (0.229) ^{abc}	22.85 (0.491) ^{abc}	33.6 (0.613) ^{bc}	45.93 (0.742) ^{b}	50.7 (0.791) ^{b}	51.95 (0.805) ^{b}	55.21 (0.568) ^{l}
T_{21}	0.00 (0.089) ^{e}	3.22 (0.111) ^{ijk}	10.2 (0.318) ^{ghij}	8.86 (0.287) ^{u}	13.7 (0.370) ^{ghi}	19.95 (0.446) ^{k}	19.95 (0.446) ^{r}
T_{22}	1.10 (0.121) ^{cde}	14.46 (0.381) ^{bcdefg}	26.31 (0.530) ^{bcdef}	29.16 (0.566) ^{e}	46.0 (0.742) ^{bc}	36.90 (0.650) ^{f}	40.07 (0.684) ^{f}
T_{23}	0.00 (0.090) ^{e}	5.49 (0.233) ^{efghijk}	8.87 (0.300) ^{hij}	10.01 (0.322) ^{t}	15.1 (0.395) ^{fghi}	15.12 (0.395) ^{p}	15.12 (0.395) ^{t}
T_{24}	0.00 (0.090) ^{e}	7.63 (0.278) ^{cdefghijk}	8.78 (0.290) ^{hij}	12.4 (0.354) ^{r}	12.4 (0.353) ^{hi}	12.40 (0.354) ^{m}	15.17 (0.39) ^{t}
T_{25}	0.00 (0.090) ^{de}	7.29 (0.263) ^{defghijk}	12.13 (0.352) ^{efghij}	12.14 (0.353) ^{s}	13.99 (0.375) ^{ghi}	13.99 (0.376) ^{u}	13.95 (0.375) ^{v}
T_{26}	5.90 (0.229) ^{abc}	18.27 (0.432) ^{abcde}	23.28 (0.499) ^{bcdefg}	24.58 (0.513) ^{g}	27.3 (0.546) ^{cdefgh}	28.61 (0.563) ^{t}	30.79 (0.587) ^{l}
T_{27}	2.10 (0.144) ^{bcde}	4.42 (0.209) ^{fghijk}	12.96 (0.364) ^{defghij}	14.11 (0.382) ^{o}	20.4 (0.463) ^{efghi}	20.40 (0.464) ^{h}	20.4 (0.464) ^{q}
T_{28}	8.60 (0.294) ^{a}	34.57 (0.626) ^{a}	55.17 (0.837) ^{a}	67.25 (0.969) ^{a}	72.79 (0.032) ^{a}	77.10 (1.099) ^{o}	77.13 (0.099) ^{a}

Figures given in paranthesis are in the values in the original scale Treatment means followed by common letters do not differ significantly at 5 % level

Table 7. Effect of selected antagonists and fungicides on the rhizome rot of ginger

Treatments	Percentage efficiency of the treatments over control						
	Fortnight 4	Fortnight 5	Fortnight 6	Fortnight 7	Fortnight 8	Fortnight 9	Fortnight 10
T_1	39.53	69.07	56.98	51.64	42.47	36.47	36.47
T_2	3.48	45.99	47.79	57.41	57.27	53.49	51.60
T_3	26.74	32.65	49.65	54.64	45.91	44.82	54.82
T_4	39.53	36.27	26.86	31.88	33.09	26.35	26.32
T_5	75.58	63.06	64.11	70.36	67.05	65.21	65.21
T_6	63.95	74.13	70.09	77.73	70.53	67.30	67.30
T_7	100.00	90.94	72.64	72.22	72.77	68.06	67.43
T_8	37.20	74.74	74.11	73.88	67.65	77.40	77.40
T_9	100.00	87.53	85.82	85.02	80.98	76.14	75.71
T_{10}	88.37	96.99	79.06	80.02	79.98	76.14	72.39
T_{11}	100.00	75.90	73.15	66.76	67.67	69.48	69.48
T_{12}	37.20	77.87	75.18	68.49	70.88	70.72	70.72
T_{13}	79.95	56.84	48.72	63.39	54.56	53.19	53.19
T_{14}	100.00	33.97	62.44	66.69	69.24	63.95	60.31
T_{15}	88.37	75.78	68.20	73.48	69.11	70.85	70.81
T_{16}	100.00	93.11	85.84	85.10	86.24	87.00	87.00
T_{17}	87.20	75.09	82.30	80.52	82.00	81.47	81.49
T_{18}	100.00	73.04	66.97	64.11	66.86	72.37	67.17
T_{19}	100.00	87.43	88.20	90.34	89.49	90.09	90.09
T_{20}	79.95	33.90	39.09	31.70	30.40	32.64	56.25
T_{21}	100.00	90.68	81.51	86.82	81.17	74.13	74.13
T_{22}	88.37	58.17	52.31	56.63	36.80	52.15	48.04
T_{23}	100.00	84.13	83.92	85.10	79.24	80.39	80.39
T_{24}	100.00	77.92	84.08	81.56	82.97	83.92	80.33
T_{25}	100.00	78.91	77.94	81.94	80.79	81.86	81.86
T_{26}	31.39	47.15	57.80	63.44	62.50	62.80	60.10
T_{27}	75.58	87.21	76.50	79.01	72.00	73.55	73.55

Table 8a. Effect of selected antagonists and fungicides on the rhizome rot of ginger

Treatments	Percentage of rhizome rot incidence at monthly intervals from 120 DAP		
	120 DAP	150 DAP	180 DAP
T_1	35.15 (0.625) ^{c}	49.00 (0.778) d	52.90 (0.822) c
T_2	28.64 (0.559) ^{f}	35.87 (0.639) h	37.33 (0.656) f
T_3	30.50 (0.581) ^{d}	42.56 (0.707) e	42.56 (0.707) d
T_4	45.81 (0.739) ^{b}	56.80 (0.855) b	56.80 (0.855) b
T_5	19.93 (0.462) ^{k}	26.83 (0.544) k	26.83 (0.544) j
T_6	17.66 (0.431) ^{n}	25.22 (0.519) l	25.22 (0.519) k
T_7	18.68 (0.440) ^{i}	22.25 (0.489) p	25.12 (0.522) k
T_8	17.90 (0.434) ^{m}	24.63 (0.516) m	24.63 (0.516) l
T_9	10.07 (0.310) ^{t}	17.43 (0.425) u	18.73 (0.441) r
T_{10}	13.43 (0.375) ^{p}	18.40 (0.442) t	21.29 (0.474) o
T_{11}	22.35 (0.488) ^{j}	23.54 (0.505) h	23.54 (0.505) m
T_{12}	21.19 (0.476) ^{l}	22.58 (0.491) o	22.58 (0.491) n
T_{13}	24.62 (0.512) ^{g}	36.10 (0.644) g	36.10 (0.644) g
T_{14}	22.40 (0.493) ^{j}	27.80 (0.549) j	30.61 (0.583) i
T_{15}	17.83 (0.429) ^{mn}	22.48 (0.490) o	22.51 (0.490) n
T_{16}	10.02 (0.319) ^{t}	10.02 (0.319) z	10.02 (0.319) v
T_{17}	13.10 (0.369) ^{q}	14.29 (0.386) w	14.29 (0.386) t
T_{18}	24.13 (0.506) ^{h}	21.31 (0.455) q	25.32 (0.523) k
T_{19}	6.49 (0.252) ^{v}	7.64 (0.279)	7.64 (0.279) w
T_{20}	45.93 (0.742) ^{b}	51.95 (0.805) c	33.74 (0.568) h
T_{21}	8.86 (0.287) ^{u}	19.95 (0.446) s	19.94 (0.446) q
T_{22}	29.16 (0.566) ^{e}	36.90 (0.650) f	40.07 (0.684) e
T_{23}	10.03 (0.322) ^{t}	15.12 (0.395) v	15.12 (0.395) s
T_{24}	12.40 (0.354) ^{r}	12.40 (0.354) y	15.17 (0.390) s
T_{25}	12.14 (0.353) ^{s}	13.99 (0.376) x	13.95 (0.375) u
T_{26}	24.58 (0.513) ^{g}	28.69 (0.563) l	30.77 (0.587) i
T_{27}	14.11 (0.382) ^{o}	20.40 (0.464) r	20.40 (0.464) p
T_{28}	67.25 (0.969) ^{a}	77.13 (1.099) a	77.13 (0.099) a

Treatment means followed by common letters do not significantly differ at 5% level
The figures given in parenthesis are transformed values (logarithmic transformation)
DAP - Days after planting

Table 8b. Effect of antagonist population on the incidence of rhizome rot of ginger

	Correlation coefficients		
	T. viride	*A. flavus*	*A. niger*
120 DAP	-0.119	-0.212*	-0.199
180 DAP	-0.156	-0.249*	-0.248*

* correlation coefficient significant at 5 % level

After ninth fortnight, there was not much increase in the disease in most of the treatments. The final observation at tenth fortnight revealed that the disease incidence ranged from 7.65 per cent in T_{19} to 77.13 per cent in control. Other treatments which recorded less disease incidence were T_{16} (10.02 %) T_{25} (13.95 %) and T_{17} (14.29%) and the difference was significant.

From the Table 7, it is evident that among the twenty eight treatments T_{19} recorded the maximum efficiency over control in reducing per cent disease incidence from sixth to tenth fortnight. At fifth fortnight the maximum efficiency over control was in T_{10} (96.99 %).

4.5.1 Effect of antagonist population on the incidence of rhizome rot

The influence of the antagonist population on the incidence of rhizome rot was determined and found that a negative correlation exists between them (Table 8a and 8b). The result revealed that the correlation between the population of *T. viride* and per cent incidence of disease was not significant both at 120 DAP ($r = -0.119$) and 180 DAP ($r = -0.156$). Similar correlation studies made between the percentage of disease incidence and the population of *A. flavus*. The correlation was significant both at 120 DAP ($r = -0.212$) and 180 DAP ($r = -0.249$). The population of *A. niger* and per cent disease incidence showed a non-significant relationship at 120 DAP. ($r = -0.199$). However, at 180 DAP the correlation was significant ($r = -0.268$) (Table 8b).

4.6 Population status of *P. aphanidermatum* in the experimental field

The population of *P. aphanidermatum* in experimental plots was 173.92 c.f.u. g^{-1} soil at the time of planting of ginger. After 60 days of planting the soil population of *P. aphanidermatum* showed an increase and the various treatments showed significant difference in the popoulation (Table 9). Then the least population of the pathogen recorded was 148.7 c.f.u. g^{-1} in T_{24}, the plot where mancozeb 0.3 % was applied at 60 DAP followed by incorporation of *A. flavus* at 120 DAP. The treatment T_2 (seed treatment with *A. niger*) recorded the maximum number of propagules of pathogen (415.5 c.f.u. g^{-1} soil).

After 120 days of planting, the population of *P. aphanidermatum* varied differently in the different treatments. A minimum count of 114.0 c.f.u. g^{-1} soil was observed in T_{13} (application of *T. viride* and *A. flavus* at 60 and 120 DAP) and was on par with T_{12} and

Table 9 Population of *Pythium aphanidermatum* in the experimental field at different intervals

Treatments	No. of colony forming units of (c.f.u) *P aphanidermatum* of soil				
	60 DAP	120 DAP	180 DAP	At the time of harvest	Mean value
T_1	343.70 abc	384.50 a	232.70 abc	153.30 bcd	278.55
T_2	415.50 a	244.50 bcdefg	286.40 ab	50.00 ef	249.10
T_3	300.30 abcd	282.20 abcdef	184.20 bc	86.67 def	213.34
T_4	353.30 abc	320.00 abcd	208.70 abc	146.70 bcd	257.17
T_5	268.90 cd	304.50 abcdef	208.90 abc	189.80 b	243.02
T_6	268.40 cd	278.90 abcdef	263.90 abc	149.50 bcd	240.17
T_7	264.30 cd	196.70 cdefgh	216.20 abc	110.00 bcdef	196.80
T_8	286.70 bcd	297.80 abcdef	192.90 bc	134.50 bcd	227.97
T_9	286.60 bcd	258.70 abcdefg	226.20 abc	90.00 def	215.37
T_{10}	257.50 cde	303.30 abcdef	244.00 abc	187.80 bc	248.15
T_{11}	281.90 bcd	256.00 abcdefg	266.00 abc	115.30 bcdef	229.80
T_{12}	287.70 bcd	130.00 gh	183.90 bc	118.70 bcdef	180.07
T_{13}	324.00 abcd	174.50 efgh	161.30 c	86.67 def	186.62
T_{14}	310.70 abcd	312.30 abcde	191.10 bc	76.67 def	222.61
T_{15}	286.40 bcd	237.80 bcdefg	279.30 abc	115.30 bcdef	229.70
T_{16}	301.70 abcd	212.20 cdfgh	166.60 bc	38.67 f	179.79
T_{17}	239.50 cde	114.00 h	248.80 abc	144.00 bcd	186.57
T_{18}	250.90 cde	135.50 gh	277.70 abc	143.30 bcd	201.85
T_{19}	281.80 bcd	166.70 fgh	243.70 abc	141.30 bcd	208.38
T_{20}	392.20 ab	224.00 cdefg	204.40 abc	116.70 bcdef	234.32
T_{21}	306.00 abcd	292.70 abcdef	223.10 abc	131.10 bcde	238.22
T_{22}	275.50 bcd	230.00 cdefg	182.00 bc	85.33 def	193.20
T_{23}	243.90 cde	253.30 acdefg	217.30 abc	76.67 def	197.79
T_{24}	148.70 e	191.10 cdefgh	208.90 abc	100.00 def	162.17
T_{25}	244.40 cde	224.50 cdefg	203.30 bc	140.00 bcd	203.05
T_{26}	333.10 abcd	326.70 abc	222.10 abc	135.50 bcd	254.35
T_{27}	221.30 de	179.80 defgh	239.50 abc	106.70 cdef	186.82
T_{28}	394.70 ab	376.50 ab	324.70 a	280.00 a	343.97

Treatment means followed by common letters do not significantly differ at 5% level

T_{18} and other four treatments. The maximum count of the pathogen was in T_1 (seed treatment with *T. viride*) i.e., 384.5. c.f.u. g^{-1} soil followed by 376.5 c.f.u. g^{-1} soil in control and was not significantly different.

The population of *P. aphanidermatum* after 180 DAP, ranged from 161.3 to 324.7 c.f.u. g^{-1} soil. The minimum count of pathogen was in T_{13} (application of *T. viride* at 60 and 120 DAP) and the maximum in control.

At the time of harvest a general reduction in the population of *P. aphanidermatum* was observed in all the treatments. The population varied among the treatments ranging from 38.67 (T_{16}) to 280.0 (control) c.f.u. g^{-1}soil (Table 9).

4.7 Population status of selected antagonists in the treatment plots at different intervals

The population of the antagonists, *T. viride*, *A. flavus* and *A niger* was determined by serial dilution plate method at the time of planting at bimonthly intervals up to harvest. The mean population of antagonists was expressed as c.f.u. g^{-1} dry weight of soil, the data are presented in Tables 10, 12 and 14. The difference in the population of the antagonists at various intervals from that of the initial observation was taken for each treatment. Analysis of covariance done using transformed data (logarithmic transformation) taking the initial population count as the covariate (Tables 11, 13 and 15) The treatment means were compared using DMRT.

4.7.1 *Trichoderma viride*

At the time of planting the population of *T. viride* under different treatments ranged from 0 to 2.21 x 10^3 c.f.u. g^{-1}). At 60 DAP, T_9 (application of *T. viride* and *A. niger* at the time of planting) recorded the maximum count (8.26 x 10^3 c.f.u.) which was on par with T_6 (application of *T. viride* at the time of planting) and T_{12} (application of *T. viride*, *A. niger* and *A. flavus* at the time of planting). The maximum increase in the population from the initial count was also observed in T_9 (7.71 x 10^3 c.f.u., Table 11).

The result on the population of *T. viride* at 120 DAP, showed that, the maximum count of 22.42 x 10^3 c.f.u. was recorded by T_{22} (application of mancozeb 0.3 % at 60 DAP followed by incorporation of *T. viride* at 90 DAP). The result of analysis of covariance

Table 10. Population of *T.viride* under different treatment at different intervals

Treatments	No. of colony forming units (c.f.u) of *T. viride* in soil at 10^{-3} dilution				
	At the time of planting	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	0.65 ab	4.30 bcd	10.37 bcd	7.77 def	3.12 efg
T_2	0.00 b	0.00 f	0.23 f	3.01 gh	0.00 g
T_3	1.36 ab	0.32 ef	2.77 f	0.00 h	0.00 g
T_4	0.23 ab	0.00 f	0.91 f	0.85 h	0.00 g
T_5	0.83 ab	0.43 ef	1.94 f	0.00 h	0.76 fg
T_6	0.50 ab	8.02 a	9.91 bcde	3.64 fgh	4.22 def
T_7	0.00 b	0.32 ef	1.13 f	0.00 h	0.00 g
T_8	0.00 b	1.08 ef	4.71 def	0.00 h	0.00 g
T_9	0.55 ab	8.26 a	11.93 b	5.91 efg	6.65 bcd
T_{10}	0.32 ab	5.02 bc	11.23 b	8.86 cde	10.22 ab
T_{11}	0.73 ab	0.61 ef	2.01 f	0.00 h	1.86 fg
T_{12}	0.00 b	6.89 ab	9.21 bcde	11.88 bcd	7.01 bcd
T_{13}	0.00 b	1.93 def	14.24 b	12.70 bc	11.97 a
T_{14}	0.73 ab	0.00 f	0.15 f	0.00	0.00 g
T_{15}	0.00 b	0.00 f	0.78 f	0.00 h	0.00 g
T_{16}	0.33 ab	0.10 f	5.15 cdef	10.82 bcd	7.03 bcd
T_{17}	0.00 b	0.00 f	12.16 b	9.24 cde	8.07 bc
T_{18}	1.81 ab	0.00 f	0.00 f	0.00 h	0.00 g
T_{19}	0.00 b	0.00 f	10.17 bcde	20.45 a	6.81 bcd
T_{20}	1.89 ab	2.61 cdef	4.49 ef	2.41 gh	0.00 g
T_{21}	0.55 ab	0.30	2.09 f	0.00 h	0.68 fg
T_{22}	1.50 ab	4.61 ef	22.42 a	14.14 b	11.70 a
T_{23}	0.90 ab	3.29 bcd	2.06 f	3.28 gh	0.28 g
T_{24}	2.21 a	2.72 cde	0.28 f	1.40 gh	0.00 g
T_{25}	0.00 b	0.00 cdef	10.88 bc	10.20 bcde	5.82 cde
T_{26}	0.79 ab	0.00 f	0.00 f	0.55 h	0.35 g
T_{27}	0.57 ab	0.00 f	0.00 f	0.00 h	0.37 g
T_{28}	0.44 ab	0.74 f	1.55 f	0.00 h	0.12 fg

Treatment means followed by common letters do not significantly differ at 5% level

Table 11. Increase in population of *T.viride* from initial count in terms of c.f.u. at different intervals

Treatments	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	1.373 (3.65) bcd	1.436 (7.34) ab	1.433 (7.12) de	1.352 (2.47) efg
T_2	1.286 (0.00) g	1.334 (3.60) b	1.345 (3.01) fgh	1.288 (0.00) h
T_3	1.296 (-1.04) efg	1.339 (-0.01) b	1.289 (-1.36) h	1.286 (-1.36) h
T_4	1.287 (-0.23) g	1.324 (2.09) b	1.304 (0.62) gh	1.288 (-0.23) h
T_5	1.297 (-0.40) efg	1.330 (1.11) b	1.288 (-0.83) h	1.304 (-0.07) gh
T_6	1.435 (7.52) a	1.409 (6.80) ab	1.358 (3.15) fgh	1.369 (3.73) def
T_7	1.293 (0.32) fg	1.341 (3.74) b	1.285 (0.00) h	1.288 (0.00) h
T_8	1.307 (1.08) efg	1.357 (4.71) ab	1.285 (0.00) h	1.288 (0.00) h
T_9	1.439 (7.71) a	1.431 (8.17) ab	1.398 (5.36) ef	1.413 (6.10) bcd
T_{10}	1.384 (4.71) abc	1.454 (9.42) ab	1.446 (8.54) cde	1.468 (9.90) ab
T_{11}	1.301 (-0.13) efg	1.373 (4.11) ab	1.288 (-0.73) h	1.323 (1.11) fgh
T_{12}	1.413 (6.89) ab	1.424 (8.79) ab	1.485 (11.88) bcd	1.415 (7.01) bcd
T_{13}	1.325 (1.93) defg	1.458 (11.04) ab	1.499 (12.70) bcd	1.491 (11.98) a
T_{14}	1.287 (-0.73) fg	1.379 (4.74) ab	1.288 (-0.73) h	1.287 (-0.73) h
T_{15}	1.286 (0.00) g	1.282 (0.58) b	1.285 (0.00) h	1.288 (0.00) h
T_{16}	1.289 (-0.23) fg	1.352 (3.49) ab	1.476 (10.50) cd	1.420 (6.70) bcd
T_{17}	1.286 (0.00) g	1.402 (7.39) ab	1.448 (9.24) cde	1.431 (8.09) bc
T_{18}	1.290 (-1.81) fg	1.424 (4.50) ab	1.291 (-1.81) h	1.286 (-1.81) h
T_{19}	1.286 (0.00) g	1.375 (6.12) ab	1.589 (20.45) a	1.412 (6.81) bcd
T_{20}	1.343 (0.72) cdefg	1.428 (3.59) ab	1.341 (0.52) fgh	1.286 (-1.89) h
T_{21}	1.294 (-0.25) fg	1.383 (4.60) ab	1.287 (-0.55) h	1.303 (0.18) gh
T_{22}	1.385 (3.11) abc	1.534 (13.08) a	1.534 (12.64) b	1.500 (10.20) a
T_{23}	1.355 (2.38) cde	1.451 (8.99) ab	1.356 (2.39) fg	1.293 (-0.63) gh
T_{24}	1.349 (0.51) cdef	1.337 (-1.93) b	1.322 (-0.81) gh	1.280 (-2.21) h
T_{25}	1.286 (0.00) g	1.393 (7.47) ab	1.462 (10.20) cd	1.399 (5.82) cde
T_{26}	1.288 (-0.79) fg	1.293 (-0.79) b	1.300 (-0.24) gh	1.295 (-0.44) gh
T_{27}	1.287 (-0.57) fg	1.286 (-0.57) b	1.287 (-0.57) h	1.295 (-0.19) gh
T_{28}	1.302 (0.30) efg	1.313 (1.08) b	1.287 (-0.44) h	1.310 (0.68) gh

Treatment means followed by common letters do not significantly differ at 5% level

The data are transformed values (logarithmic transformation) and values in paranthesis are in original scale

showed that there was an increase of 13.08 x 10^3 c.f.u. from the initial count in T_{22} which was significantly superior to all other treatments.

The population of *T. viride* recorded at 180 DAP ranged from 0 to 20.45 x 10^3 c.f.u. the maximum being in T_{19}, followed by T_{22} (14.14 x 10^3 c.f.u.) (Table10). On analysis with tranformed data it was found that, the increase was also maximum in T_{19} (20.45 x 10^3 c.f.u.) from the initial population to that at 180 DAP, followed by T_{22}(12.64 x 10^3 c.f.u.)

The population of *T. viride* was maximum in T_{13} (11.97 x 10^3 c.f.u.) at the time of harvest, which was on par with T_{22} (11.70 x 10^3 c.f.u.). Analysis of covariance of tranformed data, also revealed the same result. The maximum increase over initial count at the time of harvest was in T_{13} (11.98 x 10^3 c.f.u.) followed by T_{22} (10.20 x 10^3 c.f.u.) and which was significantly different from T_{13}.

4.7.2 *Aspergillus flavus*

The initial count of *A. flavus* in plots ranged from 0 to 10.36 x 10^3 c.f.u. the highest value being recorded by T_8 (application of *A. flavus* at the time of planting). The count obtained at 60 DAP revealed that the population in T_{12} (application of *T. viride*, *A. niger* and *A. flavus* at the time of planting) was 7.06 x 10^3 c.f.u. being significantly superior to the rest followed by T_8 (5.32 x 10^3 c.f.u.) which was on par with T_3, T_{10} and T_{11}. The highest increase at 60 DAP from initial count was in T_{12} (6.88 x 10^3 c.f.u.) followed by T_8 (5.04 x 10^3 c.f.u.) which were significantly different from each other (Table 13).

During 120 DAP the maximum population count of *A. flavus* (37.40 x 10^3 c.f.u.) was recorded in T_{24} (application of mancozeb 0.3 % at 60 DAP followed by incorporation of *A. flavus* at 90 DAP) which was significantly superior to the rest (Table 12). This was followed by T_{27} (application of copper oxychloride 0.3 % at 60 DAP, followed by incorporation of *A. flavus* at 90 DAP) and T_{18} (application of *A. niger* and *A. flavus* at 60 and 120 DAP) which recorded 28.25 x 10^3 c.f.u. and 13.82 x 10^3 c.f.u. respectively. The significant increase in the population at 120 DAP compared to initial one was in T_{24} (37.40 x 10^3 c.f.u.) which was on par with T_{27} (28.25 x 10^3 c.f.u.) (Table 13).

The population of *A. flavus* in different treatments at 180 DAP varied widely from 0 to 56.30 x 10^3 c.f.u., the maximum being in T_{12} (application of *T. viride*, *A. niger* and *A. flavus* at the time of planting), followed by T_{15} (application of *A. flavus* at 60 and

Table 12. Population of *A. flavus under* different treatments at different intervals

Treatments	No. of colony forming units of *A.flavus* (c.f.u) in soil at 10^{-3} dilution				
	At the time of planting	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	0.06 ^{b}	0.09 ^{c}	0.00 ^{d}	0.77 ^{f}	0.71 ^{b}
T_2	1.23 ^{b}	0.23 ^{c}	1.94 ^{d}	0.16 ^{f}	0.54 ^{b}
T_3	0.54 ^{b}	3.79 ^{b}	5.21 ^{d}	16.36 ^{bcdef}	0.09 ^{b}
T_4	0.00 ^{b}	0.38 ^{c}	0.78 ^{d}	0.58 ^{f}	0.55 ^{b}
T_5	0.00 ^{b}	0.00 ^{c}	0.09 ^{d}	0.92 ^{f}	0.40 ^{b}
T_6	0.40 ^{b}	0.00 ^{c}	0.00 ^{d}	0.00 ^{f}	0.09 ^{b}
T_7	0.43 ^{b}	0.00 ^{c}	0.00 ^{d}	0.00 ^{f}	0.44 ^{b}
T_8	10.36 ^{a}	5.32 ^{b}	0.00 ^{d}	2.37 ^{def}	2.67 ^{b}
T_9	0.42 ^{b}	0.00 ^{c}	0.56 ^{d}	0.00 ^{f}	0.83 ^{b}
T_{10}	0.17 ^{b}	4.48 ^{b}	0.37 ^{d}	1.20 ^{f}	1.95 ^{b}
T_{11}	0.00 ^{b}	5.21 ^{b}	3.44 ^{d}	0.00 ^{f}	1.76 ^{b}
T_{12}	0.18 ^{b}	7.06 ^{a}	6.03 ^{d}	56.30 ^{a}	10.27 ^{b}
T_{13}	0.00 ^{b}	0.00 ^{c}	0.17 ^{d}	1.47 ^{ef}	0.53 ^{b}
T_{14}	0.00 ^{b}	0.00 ^{c}	0.36 ^{d}	0.00 ^{f}	0.44 ^{b}
T_{15}	0.00 ^{b}	0.16 ^{c}	2.86 ^{d}	33.55 ^{b}	29.80 ^{a}
T_{16}	0.18 ^{b}	0.39 ^{c}	0.00 ^{d}	2.09 ^{cdef}	0.00 ^{b}
T_{17}	0.00 ^{b}	1.41 ^{c}	0.30 ^{d}	10.89 ^{cdef}	16.18 ^{ab}
T_{18}	0.07 ^{b}	0.36 ^{c}	13.82 ^{d}	20.84 ^{bcd}	30.47 ^{a}
T_{19}	1.63 ^{b}	0.41 ^{c}	8.55 ^{cd}	21.42 ^{bc}	4.20 ^{b}
T_{20}	0.20 ^{b}	0.00 ^{c}	0.00 ^{d}	2.22 ^{cdef}	0.07 ^{b}
T_{21}	1.33 ^{b}	0.56 ^{c}	0.55 ^{d}	0.46 ^{f}	1.58 ^{b}
T_{22}	0.00 ^{b}	0.00 ^{c}	0.00 ^{d}	5.29 ^{cdef}	2.45 ^{b}
T_{23}	0.00 ^{b}	0.15 ^{c}	0.00 ^{d}	0.72 ^{f}	0.44 ^{b}
T_{24}	0.00 ^{b}	1.12 ^{c}	37.40 ^{a}	20.64 ^{bcdef}	3.99 ^{b}
T_{25}	0.53 ^{b}	1.14 ^{c}	0.00 ^{d}	0.52 ^{f}	0.74 ^{b}
T_{26}	0.14 ^{b}	0.16 ^{c}	0.40 ^{d}	0.00 ^{f}	0.43 ^{b}
T_{27}	0.00 ^{b}	0.19 ^{c}	28.25 ^{b}	6.52 ^{cdef}	5.94 ^{b}
T_{28}	0.00 ^{b}	0.57 ^{c}	0.00 ^{d}	0.54 ^{f}	0.40 ^{b}

Treatment means followed by common letters do not significantly differ at 5% level

Table 13 Increase in population of *A. flavus* from initial count in terms of c.f.u. at different intervals

Treatments	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	1.285 (0.02) ^{jk}	1.278 (-0.06) ^{d}	1.297 (0.71) ^{e}	1.300 (0.64) ^{c}
T_2	1.294 (-1.00) ^{h}	1.339 (0.71) ^{cd}	1.295 (-1.07) ^{e}	1.298 (-0.68) ^{c}
T_3	1.362 (3.24) ^{e}	1.409 (5.99) ^{bc}	1.482 (15.81) ^{bcde}	1.288 (-0.46) ^{c}
T_4	1.291 (0.38) ^{hij}	1.293 (0.78) ^{d}	1.292 (0.58) ^{e}	1.297 (0.55) ^{c}
T_5	1.283 (0.00) ^{k}	1.278 (0.09) ^{d}	1.299 (0.92) ^{e}	1.294 (0.40) ^{c}
T_6	1.285 (-0.40) ^{jk}	1.283 (-0.40) ^{d}	1.284 (-0.40) ^{e}	1.288 (-0.31) ^{c}
T_7	1.285 (-0.43) ^{jk}	1.283 (-0.43) ^{d}	1.284 (-0.43) ^{e}	1.296 (0.01) ^{c}
T_8	1.404 (5.04) ^{b}	1.249 (-10.36) ^{d}	1.323 (-8.00) ^{e}	1.298 (-7.70) ^{c}
T_9	1.285 (-0.42) ^{jk}	1.295 (0.14) ^{d}	1.284 (-0.42) ^{e}	1.288 (-0.34) ^{c}
T_{10}	1.371 (4.31) ^{d}	1.287 (0.20) ^{d}	1.306 (1.03) ^{e}	1.324 (1.78) ^{c}
T_{11}	1.383 (5.21) ^{c}	1.341 (3.44) ^{cd}	1.280 (0.00) ^{e}	1.319 (1.76) ^{c}
T_{12}	1.415 (6.88) ^{a}	1.279 (-0.18) ^{d}	1.857 (56.12) ^{a}	1.286 (-0.18) ^{c}
T_{13}	1.283 (0.00) ^{k}	1.280 (0.17) ^{d}	1.309 (1.47) ^{e}	1.296 (0.53) ^{c}
T_{14}	1.283 (0.00) ^{k}	1.284 (0.36) ^{d}	1.280 (0.00) ^{e}	1.295 (0.44) ^{c}
T_{15}	1.286 (0.16) ^{jk}	1.332 (2.86) ^{cd}	1.644 (33.56) ^{b}	1.590 (29.59) ^{a}
T_{16}	1.292 (0.21) ^{hi}	1.279 (-0.18) ^{d}	1.321 (1.92) ^{e}	1.286 (-0.18) ^{c}
T_{17}	1.312 (1.41) ^{f}	1.283 (0.30) ^{d}	1.466 (10.89) ^{bcde}	1.539 (16.18) ^{ab}
T_{18}	1.291 (0.29) ^{hij}	1.494 (13.75) ^{b}	1.587 (20.77) ^{bc}	1.644 (33.56) ^{a}
T_{19}	1.301 (-1.22) ^{g}	1.463 (6.92) ^{b}	1.587 (19.79) ^{bc}	1.362 (2.57) ^{c}
T_{20}	1.284 (-0.20) ^{k}	1.280 (-0.20) ^{d}	1.324 (2.03) ^{e}	1.287 (-0.13) ^{c}
T_{21}	1.302 (0.77) ^{g}	1.308 (-0.77) ^{d}	1.304 (-0.87) ^{e}	1.317 (0.25) ^{c}
T_{22}	1.283 (0.00) ^{k}	1.276 (0.00) ^{d}	1.365 (5.29) ^{de}	1.334 (2.45) ^{c}
T_{23}	1.286 (0.17) ^{jk}	1.276 (0.00) ^{d}	1.295 (0.72) ^{e}	1.295 (0.44) ^{c}
T_{24}	1.306 (1.12) ^{g}	1.720 (37.40) ^{a}	1.562 (20.64) ^{bcd}	1.364 (3.99) ^{c}
T_{25}	1.289 (-0.39) ^{hijk}	1.285 (-0.53) ^{d}	1.296 (-0.01) ^{e}	1.302 (0.21) ^{c}
T_{26}	1.287 (0.02) ^{ijk}	1.285 (0.26) ^{d}	1.281 (-0.14) ^{e}	1.295 (0.29) ^{c}
T_{27}	1.287 (0.19) ^{ijk}	1.654 (28.25) ^{a}	1.390 (6.52) ^{cde}	1.398 (5.94) ^{bc}
T_{28}	1.295 (0.56) ^{h}	1.276 (0.00) ^{d}	1.291 (0.54) ^{e}	1.294 (0.40) ^{c}

Treatment means followed by common letters do not significantly differ at 5% level

The data are transformed values (logarithmic transformation) and values in paranthesis are in original scale

120 DAP) 33.55 x 10^3 c.f.u. The difference was significant in T_{12} from other treatments. The increase of population from initial count in T_{12} was 56.12 x 10^3 c.f.u. which was the maximum increase among the treatments followed by T_{15} (33.56 x 10^3 c.f.u.).

At the time of harvest, the population of *A. flavus* was decreased in almost all the treatments. The highest population was recorded by T_{18} which was on par with T_{15} and T_{17} (application of *T. viride* and *A. flavus* at 60 and 120 DAP) being 30.47 x 10^3, 29.80 x 10^3 and 16.18 x 10^3 c.f.u. respectively. Result on the increase of population compared to initial count, at the time of harvest showed that it was maximum in T_{18} (33.56 x 10^3 c.f.u.) followed by T_{15} (29.59 x 10^3 c.f.u.). These two treatments were not differed significantly between them (Table 13).

4.7.3. *Aspergillus niger*

The population of *A. niger* in various plots, at the time of planting, ranged from 0 to 4.03 x 10^3 c.f.u. At 60 DAP, the maximum population of 20.43 x 10^3 c.f.u. was recorded in T_9 (application of *T. viride* and *A. niger* at the time of planting) followed by 6.64 x 10^3 c.f.u. in T_{18} (application of *A. niger* and *A. flavus* at 60 and 120 DAP). The treatments T_7 (*A. niger* applied at the time of planting), T_{14} (*A. niger* applied at 60 and 120 DAP) and T_{12} (application of *T. viride*, *A. niger* and *A. flavus* at the time of planting) were on par with T_{18}. The maximum increase in population over initial count was in T_9 (17.58 x 10^3 c.f.u.) (Table 15).

The maximum population count of 65.49 x 10^3 c.f.u. was recorded in T_{14} followed by 45.48 x 10^3 c.f.u. in T_{16} (application of *T. viride* and *A. niger* at 60 and 120 DAP) at 120 DAP which was on par with T_{26} (37.94 x 10^3 c.f.u.). The maximum increase of population from the initial count at 120 DAP was recorded in T_{14} (65.03 x 10^3 c.f.u.) followed by T_{16} (45.48 x 10^3 c.f.u.) which was on par with T_{26} (37.87 x 10^3 c.f.u.).

During 180 DAP, the highest population of *A. niger* was in T_{14} (61.89 x 10^3 c.f.u.) which was on par with T_{16} (55.72 x 10^3 c.f.u.). There was a significant increase in the population in T_{14} (61.33 x 10^3 c.f.u.) which was on par with T_{16} (55.72 x 10^3 c.f.u.).

At the time of harvest, the maximum population was recorded by T_{19} (45.59 x 10^3 c.f.u.) followed by T_{16} (35.36 x 10^3 c.f.u.) and there was no significant difference between

Table 14. Population of *A. niger* under different treatments at different intervals

Treatments	No. of colony forming units of *A.niger* (c.f.u) in soil at 10^{-3} dilution				
	At the time of planting	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	0.73 [b]	0.52 [d]	0.78 [ef]	9.69 [cd]	4.16 [d]
T_2	0.00 [b]	1.52 [d]	11.17 [de]	11.33 [cd]	2.58 [de]
T_3	0.31 [b]	1.63 [d]	1.17 [f]	2.00 [d]	0.09 [e]
T_4	0.00 [b]	0.00 [d]	0.39 [f]	1.44 [d]	1.11 [e]
T_5	0.00 [b]	0.53 [d]	0.96 [f]	4.36 [d]	0.18 [e]
T_6	0.00 [b]	0.55 [d]	0.56 [f]	0.83 [d]	0.00 [e]
T_7	0.10 [b]	6.27 [bc]	1.40 [ef]	0.00 [d]	0.59 [e]
T_8	0.00 [b]	0.00 [d]	0.69 [f]	6.76 [cd]	0.66 [e]
T_9	2.85 [ab]	20.43 [a]	5.30 [ef]	7.57 [cd]	0.28 [e]
T_{10}	0.00 [b]	0.83 [d]	0.29 [f]	0.46 [d]	3.90 [de]
T_{11}	0.00 [b]	2.15 [bcd]	5.11 [ef]	0.18 [d]	3.46 [de]
T_{12}	0.00 [b]	3.83 [bcd]	5.54 [ef]	17.91 [c]	0.00 [e]
T_{13}	0.00 [b]	0.27 [d]	0.16 [f]	1.33 [d]	1.64 [de]
T_{14}	0.46 [b]	4.89 [bcd]	65.49 [a]	61.89 [a]	14.33 [cd]
T_{15}	0.00 [b]	0.00 [d]	0.92 [f]	0.43 [d]	0.00 [e]
T_{16}	0.00 [b]	1.46 [d]	45.48 [b]	55.72 [a]	35.36 [ab]
T_{17}	0.85 [b]	1.05 [d]	0.81 [f]	0.70 [d]	1.28 [e]
T_{18}	0.00 [b]	6.64 [b]	17.63 [d]	39.96 [b]	24.45 [bc]
T_{19}	0.00 [b]	0.60 [b]	17.02 [d]	30.75 [b]	45.59 [a]
T_{20}	4.03 [b]	0.00 [d]	0.33 [f]	0.56 [d]	2.65 [de]
T_{21}	0.00 [a]	0.00 [d]	0.67 [f]	4.32 [d]	1.29 [e]
T_{22}	0.00 [b]	2.15 [cd]	0.30 [f]	0.63 [d]	2.19 [de]
T_{23}	0.45 [b]	0.00 [d]	29.15 [e]	10.36 [cd]	0.61 [e]
T_{24}	0.00 [b]	0.00 [d]	0.85 [f]	1.12 [d]	4.17 [de]
T_{25}	0.08 [b]	0.68 [d]	2.47 [ef]	2.91 [d]	0.65 [e]
T_{26}	0.00 [b]	1.07 [d]	37.94 [b]	36.70 [b]	13.03 [b]
T_{27}	0.00 [b]	1.77 [cd]	0.29 [f]	0.96 [d]	2.83 [de]
T_{28}	0.000	0.00 [d]	0.44 [f]	0.90 [d]	2.10 [de]

Treatment means followed by common letters do not significantly differ at 5% level

Table 15. Increase in population of *A. niger* from initial count in terms of c.f.u. at different intervals

Treatments	60 DAP	120 DAP	180 DAP	At the time of harvest
T_1	1.309 (-0.21) ^{cd}	1.311 (0.06) ^{fgh}	1.425 (8.97) ^{fgh}	1.374 (3.43) ^{cd}
T_2	1.321 (1.52) ^{cd}	1.476 (11.17) ^{de}	1.494 (11.36) ^{ef}	1.335 (2.58) ^{cd}
T_3	1.327 (1.32) ^{cd}	1.317 (0.87) ^{fgh}	1.336 (1.70) ^{gh}	1.294 (-0.22) ^{d}
T_4	1.290 (0.00) ^{d}	1.300 (0.39) ^{gh}	1.330 (1.44) ^{h}	1.310 (1.11) ^{cd}
T_5	1.301 (0.53) ^{cd}	1.311 (0.96) ^{fgh}	1.378 (4.36) ^{fgh}	1.292 (0.18) ^{d}
T_6	1.301 (0.55) ^{cd}	1.304 (0.57) ^{fgh}	1.302 (0.08) ^{h}	1.288 (0.00) ^{d}
T_7	1.403 (6.17) ^{b}	1.320 (1.30) ^{fgh}	1.299 (-0.10) ^{h}	1.302 (0.49) ^{d}
T_8	1.290 (0.00) ^{d}	1.306 (0.69) ^{fgh}	1.403 (6.76) ^{fgh}	1.302 (0.66) ^{d}
T_9	1.635 (17.58) ^{a}	1.411 (2.22) ^{ef}	1.394 (4.69) ^{fgh}	1.323 (-2.57) ^{cd}
T_{10}	1.307 (0.82) ^{cd}	1.298 (0.29) ^{gh}	1.310 (0.46) ^{h}	1.362 (3.90) ^{cd}
T_{11}	1.333 (2.14) ^{cd}	1.384 (5.11) ^{efgh}	1.305 (0.18) ^{h}	1.349 (3.46) ^{cd}
T_{12}	1.362 (3.82) ^{bc}	1.397 (5.54) ^{efg}	1.571 (17.91) ^{de}	1.288 (0.00) ^{d}
T_{13}	1.295 (0.27) ^{d}	1.295 (0.16) ^{gh}	1.328 (1.33) ^{h}	1.322 (1.64) ^{cd}
T_{14}	1.391 (4.47) ^{b}	1.931 (65.03) ^{a}	1.905 (61.33) ^{a}	1.461 (13.88) ^{c}
T_{15}	1.290 (0.00) ^{d}	1.311 (0.92) ^{fgh}	1.310 (0.43) ^{h}	1.288 (0.00) ^{d}
T_{16}	1.318 (1.46) ^{cd}	1.802 (45.48) ^{b}	1.877 (55.72) ^{ab}	1.723 (35.36) ^{ab}
T_{17}	1.321 (0.20) ^{cd}	1.312 (-0.04) ^{fgh}	1.296 (-0.15) ^{h}	1.325 (0.43) ^{cd}
T_{18}	1.412 (6.64) ^{b}	1.562 (17.63) ^{d}	1.772 (39.96) ^{bc}	1.629 (24.45) ^{b}
T_{19}	1.302 (0.60) ^{cd}	1.557 (17.02) ^{d}	1.680 (30.75) ^{cd}	1.803 (45.59) ^{a}
T_{20}	1.290 (0.00) ^{d}	1.298 (0.32) ^{gh}	1.312 (0.56) ^{h}	1.340 (2.65) ^{cd}
T_{21}	1.286 (-4.03) ^{d}	1.287 (-3.36) ^{h}	1.309 (0.29) ^{h}	1.329 (-2.74) ^{cd}
T_{22}	1.334 (2.15) ^{cd}	1.298 (0.30) ^{gh}	1.314 (0.63) ^{h}	1.329 (2.19) ^{cd}
T_{23}	1.290 (0.00) ^{d}	1.662 (29.15) ^{c}	1.467 (10.36) ^{efg}	1.301 (0.61) ^{d}
T_{24}	1.294 (-0.45) ^{d}	1.311 (0.40) ^{fgh}	1.313 (0.67) ^{h}	1.373 (3.72) ^{cd}
T_{25}	1.304 (0.68) ^{cd}	1.340 (2.47) ^{fgh}	1.353 (2.91) ^{gh}	1.301 (0.65) ^{d}
T_{26}	1.312 (0.99) ^{cd}	1.754 (37.87) ^{b}	1.752 (36.62) ^{c}	1.689 (30.96) ^{ab}
T_{27}	1.326 (1.78) ^{cd}	1.298 (0.29) ^{gh}	1.320 (0.96) ^{h}	1.339 (2.83) ^{cd}
T_{28}	1.298 (0.39) ^{d}	1.301 (0.44) ^{gh}	1.319 (0.90) ^{h}	1.328 (2.10) ^{cd}

Treatment means followed by common letters do not significantly differ at 5% level

The data are transformed values (logarithmic transformation) and values in paranthesis are in original scale

them. The maximum increase in population over initial count was recorded by T_{19} (45.59 x 10^3 c.f.u.) followed by T_{16} (35.36 x 10^3 c.f.u.).

4.8 Yield data

The plots treated with *A. niger* and *A. flavus* at 60 and 120 DAP (T_{16}) recorded the maximum yield (6250 g per plot). When *T. viride*, *A. niger* and *A. flavus* were applied together at 60 and 120 DAP (T_{19}) the yield recorded was 5237 g per plot. The treatment were 0.3 per cent mancozeb was applied at 60 DAP, followed by incorporation of *A. flavus* at 90 DAP (T_{24}) recorded an yield of 5017 g per plot. However, in respect of yield T_{19} and T_{24} were on par with T_{16}. The minimum yield recorded was 451.7 g per plot, by the control plot

Table 16. Effect of the antagonists and fungicides on yield of ginger

Treatments	Yield (g / plot)
T_1	1607.0 ^{hijk}
T_2	1967.0 ^{fghijk}
T_3	1800.0 ^{ghijk}
T_4	936.0 ^{jk}
T_5	2543.0 ^{defghij}
T_6	3137.0 ^{cdefghi}
T_7	3307.0 ^{bcdefghi}
T_8	3000.0 ^{cdefghi}
T_9	3600.0 ^{bcdefgh}
T_{10}	3833.0 ^{bcdefg}
T_{11}	3983.0 ^{bcdef}
T_{12}	3483.0 ^{bcdefgh}
T_{13}	1803.0 ^{ghijk}
T_{14}	2217.0 ^{efghijk}
T_{15}	3100.0 ^{cdefghi}
T_{16}	6250.0 ^{a}
T_{17}	4183.0 ^{bcde}
T_{18}	3083.0 ^{cdefghi}
T_{19}	5327.0 ^{ab}
T_{20}	1317.0 ^{ijk}
T_{21}	4090.0 ^{bcde}
T_{22}	1783.0 ^{ghijk}
T_{23}	3700.0 ^{bcdefgh}
T_{24}	5017.0 ^{abc}
T_{25}	4367.0 ^{bcd}
T_{26}	2617.0 ^{defghij}
T_{27}	3787.0 ^{bcdefg}
T_{28}	451.7 ^{k}

Treatment means followed by common letters do not significantly differ at 5% level

Discussion

DISCUSSION

One of the important threats to ginger cultivation in Kerala is the rhizome rot caused by *Pythium spp.* Butler (1907) first recorded this disease of ginger in Gujarat caused by a species of *Pythium* which was later identified as *Pythium gracile* Sehrenk. Ramakrishnan (1949) first reported *Pythium aphanidermatum* as the major pathogen causing rhizome rot of ginger in Kerala. Attempts to control rhizome rot of ginger using chemicals were made by several workers (Park, 1935; Thomas, 1940; Bhagwat, 1960; Kothari, 1966; Sharma and Dohroo, 1982., Abraham *et al.* 1988a., Abraham *et al,* 1988b., Das *et al,* 1990. Anandam *et al*, 1996.). But the chemical control methods of this disease are known to leave harmful effects in soil and environment. More over it is very difficult to eliminate this soil borne pathogen, which is known to survive in soil for periods of 2 -12 years (Hoppe, 1966). Now a days it is widely recognised that ecofriendly methods to control plant pathogens is a distinct possibility for the future and can be successfully exploited especially within the frame work of Integrated Disease Management System (Muthamilan and Jeyarajan, 1996). Manipulation of the existing soil microflora to the disadvantage of the pathogen or by addition of non-resident antagonist offers an alternative method to combat the ravages of soil borne diseases (Cook, 1982).

The potential use of different species of *Trichoderma* and *Aspergillus* as bio-control agents against the pathogen *Pythium* spp. is well established (Padmanabhan and Alexander, 1984; Krishnamoorthy and Bhaskaran, 1990; Mukherjee and Sen, 1992; Devenhage and Kotze, 1993; Ebenezer *et al.*, 1996; Balakrishnan *et al.*, 1997). Shanmugham (1996) reported the biocontrol efficiency of *T. viride*, *A. flavus* and *A. niger* against *P. aphanidermatum* causing rhizome rot of ginger. So in the present study attempts were made to evaluate the efficacy of these biocontrol agents along with their combinations in checking the rhizome rot of ginger under field condition.

One of the critical obstacles in biological control of plant pathogens is the paucity of methods for mass culturing and it is application in the soil. A variety of substrates such as cereal grains, agricultural bye-products, peat, farm yard manure and soil have been put to evaluation in *in vitro* for multiplication of antagonists by several workers (Backman and Kabna, 1975; Sundheim, 1977; Henis *et al.*, 1978; Elad *et al.*, 1986). In

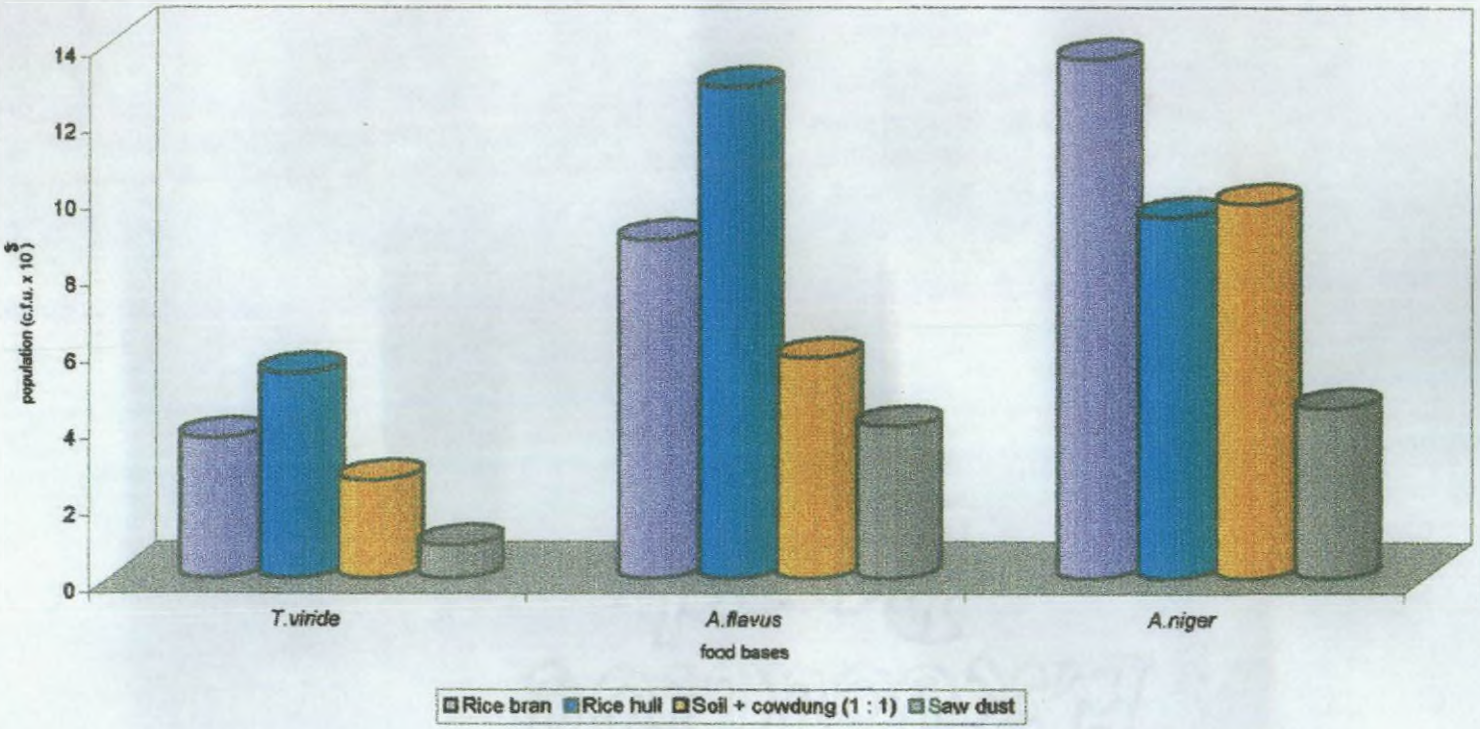

Fig.1 Population of selected antagonists in different food bases

the present study various food bases were evaluated for the mass multiplication of the selected antagonists.

Among the food bases tested, rice hull was found to be significantly superior to all others to obtain maximum growth for *T. viride* and *A. flavus* (Table 1, 2), (Fig. 1). Rice hull was reported to be a good substrate for the multiplication of the antagonists. Gangadharan and Jeyarajan (1990) observed that, paddy chaff supported good growth and sporulation of *T. viride* and *T. harzianum*. The result also revealed that the growth of the antagonists in rice bran was good. For *A. niger*, rice bran was the best medium for maximum growth (Table 1) and for *T. viride* and *A. flavus* it ranked second (Table 2, 3), (Fig. 1). The use of rice bran as a food base for the multiplication of antagonists was reported by Henis *et al.* (1979). Nair (1990) observed that the isolates of *T. longibracheatum* grew well in rice bran. There are reports regarding the use of farm yard manure or dried cowdung as good substrates for the multiplication of antagonists. Panicker and Jeyarajan (1993) observed that from among five substrates they tested, farm yard manure was the best for multiplication of the antagonists. K.A.U. (1996) also recommends dried neem cake and cowdung for the mass multiplication of *Trichoderma* spp. However during the present study, soil + dried cowdung was not found to be as good as rice hull and rice bran for supporting the multiplication of antagonists. But in the case of *A. niger*, soil + cowdung was as good as rice hull.

In the present study, sawdust was found to be the poorest substrate for all the three antagonists. All antagonists recorded the least population in sawdust (Table 1,2 and 3), (Fig. 1). This is in conformity with findings of Gangadharan and Jeyarajan (1988) who observed poor growth of *T. harzianum* and *T. viride* on sawdust.

The effect of various treatments on germination percentage was studied and the data revealed no significant difference among the treatments. Among the various treatments, hundred per cent germination was recorded in T_1 (seed treatment with *T. viride*), T_2 (seed treatment with *A. niger*), T_4 (seed treatment with mancozeb 0.3%), T_7 (application of *A. niger* at the time of planting), T_{10} (application of *T. viride* and *A. flavus* at the time of planting) and T_{13} (application of *T. viride* at 60 and 120 DAP). The minimum germination of 83.3 per cent was observed in T_{20} (application of mancozeb 0.3% at 60 and 120 DAP). This may be because the treatment T_{20} received the fungicide (mancozeb) only at later stages ie at 60 and 120 DAP. According to Tang and Uung

(1983), the seed treatment as well as soil incorporation of the antagonist *Trichoderma* sp. before sowing into soil infected with *Pythium spinosum* increased the germination of pinus seedlings. Ebenezer *et al.* (1996) reported an increased percentage of germination by seed treatment with *T. viride* and soil application of *T. viride* and *T. harzianum*. The effect of various treatments on germination percentage of ginger was studied and the data revealed that there was no significant difference among the treatments.

The occurrence of pre-emergence rotting varied considerably between treatments (Table 5). The data reveals that, in plots where the treatments with antagonists (*T. viride*, *A. flavus* or *A. niger*) or fungicides (mancozeb 0.3% or copper oxychloride 0.3 %) were applied either as seed treatment or as soil incorporation at the time of planting, the pre-emergence rotting was not observed. The effectiveness of *Trichoderma* spp. as seed treatment for the control of pre-emergence damping off caused by *P. aphanidermatum* was reported by several workers in various crops (Jacob *et al.*, 1988; Troung *et al.*, 1988; Xue *et al.*, 1995; Ebenezer *et al.*, 1996). Sharma and Dohroo (1982) observed that seed dip in 0.2 per cent solution of Dithane M-45 was effective in controlling the pathogen *P. aphanidermatum* in ginger. The present study also confirms these findings. The maximum pre-emergence rotting was observed in T_{20} (application of mancozeb 0.3 % at 60 and 120 DAP) where the fungicide was not applied at the time of planting.

The efficacy of the antagonists and common fungicides in reducing the incidence of rhizome rot of ginger was studied. Data on the incidence of disease recorded at fortnightly intervals revealed significant difference among the treatments.

Even though, the disease was first noticed during the fourth fortnight its occurrence in all the treatments was observed during fifth fortnight. In spite of the seed treatment with *A. niger* at the time of planting, the maximum percentage of disease incidence was observed in T_2 (8.3) during fourth fortnight. Though *A. niger* is an efficient antagonist against *P. aphanidermatum* the multiplication and establishment of it was not sufficient to suppress the pathogen as evidenced from the data (Table 14). The antagonist applied as seed treatment probably could not multiply and establish in sufficient quantity at 60 DAP, when the pathogen population was maximum (Table 9) and hence the maximum disease incidence.

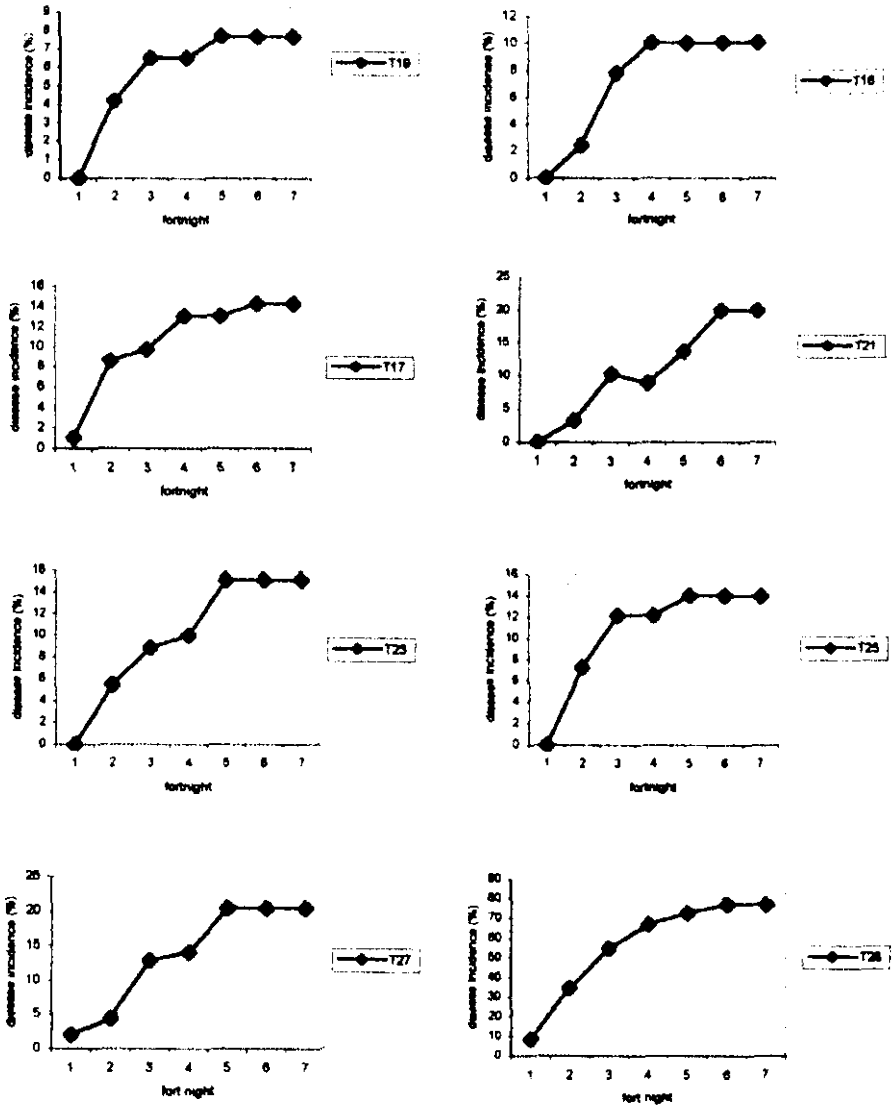

Fig. 2 Rhizome rot incidence (%) in different treatments at fortnightly intervals (4th fortnight onwards)

From fifth fortnight onwards, an increasing trend in percentage of disease incidence was noticed in all the plots; which continued up to ninth fortnight (Fig. 2). Initially, the disease incidence ranged from 0 to 8.6 per cent and at ninth and tenth fortnight it ranged from 7.64 to 77.13 per cent. The plot in which the minimum percentage of disease incidence recorded was T_{19} (Soil incorporation of *T. viride*, *A. niger* and *A. flavus* at the time of planting). Even though T_{10} (soil incorporation of *T. viride* and *A. flavus* at the time of planting) recorded minimum disease incidence initially the disease increased gradually (Table 6). In general, the result revealed that, the treatment T_{19} was found to be significantly superior to all treatments, in controlling the disease (Table 6),(Fig. 2).

Treatment T_{16} was also good in checking the disease and it ranked second to T_{19}. The effectiveness of soil application of *Trichoderma* spp. in checking the soil born diseases caused by *Pythium* spp. has been reported by several workers (Sivan *et al.*, 1984; Krishnamoorthy and Bhaskaran, 1990; Whipps and Lumsden, 1991; Xu *et al.*, 1993). The use of *A. niger* as an antagonist against *P. aphanidermatum*, the causal agent of rhizome rot of ginger was reported by Shanmugham (1996). The suppression of *P. aphanidermatum* causing rhizome rot of ginger by *A. niger* and *A. terreus* was observed by Balakrishnan *et al.* (1997). The antagonists multiplied in food base sorgum grains when applied in *Pythium* infested soil, obtained good control. However, the information on *A. flavus* as an antagonist against *P. aphanidermatum* is limited. But the antagonistic properties of *A. niger* and *A. flavus* against other pathogens were reported. (Gokulapalan and Nair, 1984; Wokocha *et al.*, 1986; Vinod, 1988; Dwivedi *et al.*, 1993; Bora, 1997).

Joshi and Keshwal (1969) reported that the soil application of the culture filtrates of *Aspergellus niveus* and *A. tamarii* considerably reduced pre and post emergence death of tomato seedlings caused by *P. debaryanum*.

In the present study, it has been found that, incorporation of a combination of the antagonist, *T. viride*, *A. niger* and *A. flavus* at 60 and 120 DAP (T_{19}) is more effective in checking the disease than applying them alone, indicating the compatibility of the antagonists which in turn helped in reducing the incidence of the disease (Fig. 2). Bisiach *et al.* (1985) reported the advantages of using mixture of isolates of *Trichoderma* spp. against *P. ultimum*. The compatibility of bio-control agents *Gliocladium virens* and

Enterobacter cloacae in controlling the lettuce damping off caused by *P. ultimum* was reported by Lynch *et al.* (1991).

Bolten (1980) observed that, the degree of protection by *T. viride* against *P. aphanidermatum* will be directly proportional to the amount of antagonist added. The present study also revealed that the plots in which the antagonists were applied twice ie at 60 and 120 DAP, (T_{19} and T_{16}) the disease incidence was low compared to the plots with only one time application of antagonist (T_6, T_7, T_8, T_9, T_{10}, T_{11} and T_{12}).

The maximum efficiency over control was also recorded by the treatment T_{19} from sixth to tenth fortnight (Table 7). At fifth fortnight, the maximum efficiency over control was in T_{10}

In order to find out the efficacy of various treatments including antagonist, on the population of *P. aphanidermatum*, the quantitative estimation of the pathogen was done at the time of planting and also at bimonthly intervals up to harvest. The average native population of *Pythium aphanidermatum* in the soil was 173.92 c.f.u. g^{-1} soil at the time of planting. The data on pathogen population at 60 DAP revealed a general increase compared to the initial one (Table 9). Among the treatments, the minimum population was observed in T_{24} (148.7 c.f.u. g^{-1} soil) and maximum in T_2 (415.5 c.f.u. g^{-1} soil). A general increase in the population of *P. aphanidermatum* at 60 DAP in treated as well as untreated plots may be due to the favourable environmental conditions prevailing at that period for the growth and multiplication of pathogen coinciding with the peak south west monsoon.

After 120 DAP, there was either increase or decrease on the population of the pathogen compared to 60 DAP. The maximum and minimum population of pathogen was observed in treatments T_1 (384.50 c.f.u. g^{-1} soil) and T_{17} (114.0 c.f.u. g^{-1} soil) respectively. It is also noticed that the treatment T_{17} exerted a maximum percentage reduction in the population of *P. aphanidermatum* compared to other treatments during this period (66.59 %). The treatments T_{12} and T_{18} also showed 54.81 and 45.99 per cent reduction in the population of *P. aphanidermatum* respectively. The treatment T_{12}, T_{17} and T_{18} were the plots in which the antagonists were incorporated into soil in combinations.

The result on the population of *P. aphanidermatum* at 120 DAP revealed that the soil application of the combination of the antagonist either at the time of planting (T_{12}) or at 60 and 120 DAP (T_{17}, T_{18}) has an effect on checking the population of *P. aphanidermatum*. The combined effect of the antagonists *T. viride*, *A. flavus* and *A. niger* may be resulted in the reduction in the pathogen population in these treatments compared to the other ones.

At 180 DAP, a decrease in the population of the pathogen, was noticed in 18 treatments. At this stage, population ranged from a minimum of 161.3 c.f.u. g^{-1} soil in treatments T_{13} to a maximum of 324.7 c.f.u. g^{-1} soil in control. However, the treatments which recorded comparatively low population of the pathogen at 120 DAP had higher pathogen population compared to earlier stages.

The population count taken at the time of harvest indicated a drastic reduction compared to the other previous three stages. The minimum count of 38.67 c.f.u. g^{-1} soil in T_{16} and maximum in control (Table 9). As the crop was harvested during February, the adverse conditions to the pathogen during this period might have contributed the reduction in the population.

The minimum population of the pathogen at different periods, varied among the treatments. However, on an average of four observations (60, 120, 180 DAP and at the time of harvest), the treatment T_{24} (soil application of *A. flavus* with mancozeb) recorded the minimum population of 162.17 c.f.u. g^{-1} soil which is followed by T_{16} (179.79 c.f.u. g^{-1} soil) and T_{12}(180.07 c.f.u. g^{-1} soil). The compatibility of *A. flavus* with mancozeb in *in vitro* was reported by Shanmugham (1996). In the present study, the maximum inhibition of *P. aphanidermatum* was obtained in plot where *A. flavus* was applied in combination with mancozeb, which supports result of the previous study. The soil application of the different species of *Trichoderma* against *P. aphanidermatum* has been reported in various crops. (Bolten, 1980; Sivan *et al.* 1984; Sharif *et al.*, 1988; Xu *et al.*, 1993; Ebenezer *et al.*, 1996). The antagonistic property of *A. flavus* and *A. niger* against *P. aphanidermatum* in *in vitro* was reported by Shanmugham (1996). The suppression of *P. aphanidermatum* causing rhizome rot of ginger by *A. niger* was observed by Balakrishnan *et al.* (1997). In the present study T_{16} (Soil application of *A. flavus* and *A. niger* at 60 and 120 DAP) and T_{12} (application of *T. viride*, *A. flavus* and *A. niger* at the time of planting) were also found as promising treatments. It is also noted that, the

T_{16} is one of the treatments which has been found to be recording the minimum disease incidence in the experiment. The results of the present study revealed that in general the soil application of the selected antagonists as combination either at the time of planting (T_{12}) or at 60 and 120 DAP. (T_{16} and T_{24}) has the advantage in decreasing the population of the pathogan in soil.

The native population of *T. viride* in the experimental plots was considerably low at the time of planting ranging from zero to 2.21 x 10^3 c.f.u.g^{-1} dry weight of soil. In general the result on the population, indicated that, in plots when *T. viride* was applied either alone or in combination with other antagonists or fungicides resulted in higher population than in plots where *T. viride* was not applied indicating its multiplication in soil (table 10). Similar results obtained by Nair (1990), when *T. harzianum* multiplied in wheat bran and applied to potted plants yielded increased number of propagules in rhizosphere of ginger, pepper and cowpea.

At 60 DAP, the population of *T. viride* in applied plots increased drastically. In plots when *T. viride* was applied in soil at the time of planting either alone (T_6) or with other antagonists (T_9, T_{12}), there observed a considerable multiplication of the antagonists (Table 10). The data on the population of *T. viride* at 120 DAP, showed that even though, the treatments T_{16}, T_{17} and T_{19} received the application of *T. viride* twice, ie at 60 and 120 DAP, the treatment T_{22} (application of *T. viride* with mancozeb) recorded the maximum increase in population over initial count and was significantly superior to all other treatments (Table 11). The population of *T. viride* was also high in T_{22} at 180 DAP and at the time of harvest. At 180 DAP, the treatment T_{22} was second to T_{19} and at the time of harvest, T_{22} was on par with the treatment T_{13} which recorded the maximum population of *T. viride*. The increase in propagules of *T. viride* in treatment where *T. viride* and mancozeb were applied in combination revealed that the fungicide mancozeb is not adversely affecting the growth and multiplication of *T. viride*. The compatibility of *T. viride* and mancozeb has already been established in *in vitro* by Shanmugham (1996).

The application of *T. viride* alone (T_6 and T_{13}) resulted in the increase in the populaiton of *T. viride*. However, the application of *T. viride* twice in the plot (T_{13}) showed better results than applying it only once (Table 10), (Fig. 3) indicating that the repeated incorporation of *T. viride* will help to increase the number of propagules. From

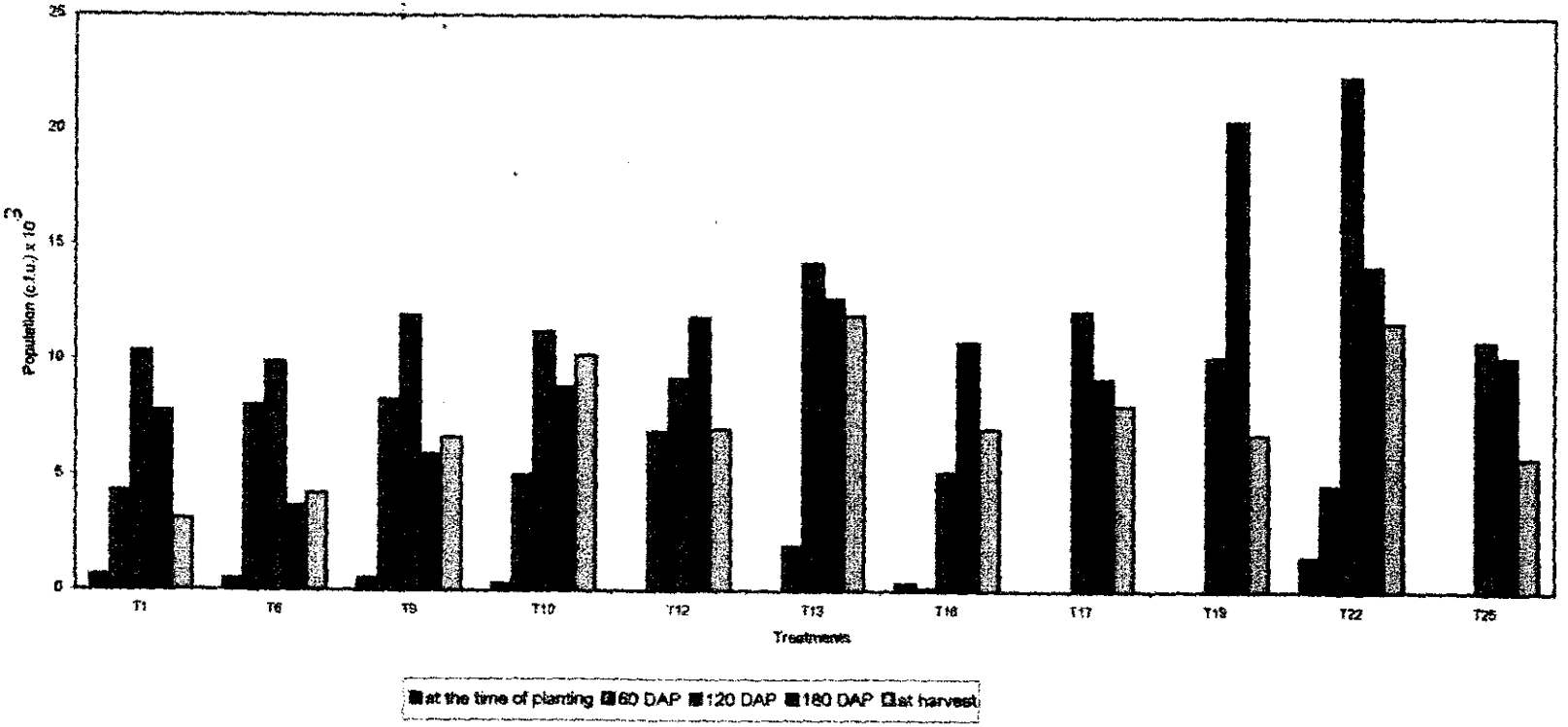

Fig. 3 Population of *T.viride* under different treatments at different intervals

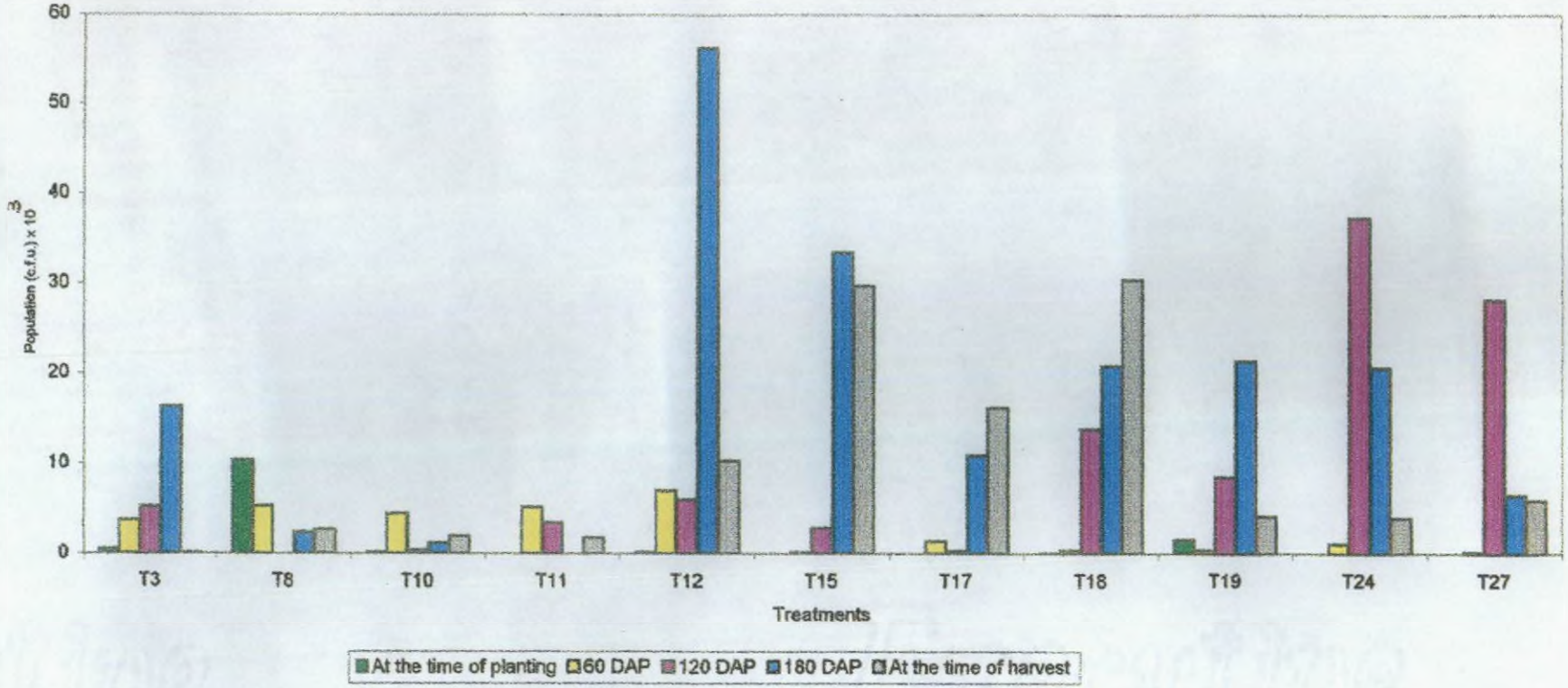

Fig. 4 Population of *A.flavus* under different treatments at different intervals

the result on 60 as well as 180 DAP it is evident that, the application of antagonist, *A. niger* and *A. flavus* will not affect the multiplication of *T. viride* either applied at the time of planting (T_9 and T_{12}) or at 60 and 120 DAP (T_{19}) (Fig. 3).

The population of *A. flavus* and *A. niger* was estimated and the influence of different treatments in their multiplication in soil was determined. The maximum population of *A. flavus* at the time of planting, 10.36 x 10^3 c.f.u.g^{-1} dry weight of soil was in T_8. The other treatments differed significantly. The native population of *A. niger* in the experimental plots before the application of antagonist was very low ranging from zero to 4.03 x 10^3 c.f.u.g^{-1} dry weight of soil. The maximum population was recorded in T_{20}

The plots where the seed rhizomes were treated with *A. flavus* (T_3) recorded a population of 3.78 x 10^3 c.f.u. at 60 DAP, which increased in next bimonth and attained maximum at 180 DAP (Table 12). However the antagonist failed to multiply after 180 DAP as revealed by the lesser count of propagules (Fig. 4). The lesser count of the antagonist recorded in the study at the time of harvest might be due to the effect of adverse soil and climatic conditions prevailing during the period.

The antagonistic effect of *A. flavus* against the pathogen, *Monilinia laxa* on peach twigs was assayed by Melgarijo (1986) and found that *A. flavus* caused a transient delay in symptom development by multiplying profusely. The data on the population of *A. flavus* revealed that the soil application of *A. flavus* at the time of planting (T_8) could not increase the population at 60 DAP. However the application of *A. flavus* with other antagonist *T. viride* and *A. niger* (T_{12}) was found to be helping in multiplication of *A. flavus*. This was probably due to the synergistic effect on the growth and multiplication of one fungus by the combining effect of other fungi The homogenized cultures of *Trichoderma* and *Gliocladium* sp. reduced the karnalbunt of wheat by multiplying profusely (Sharma, *et al.*, 1996).. There was significant increase in the population in T_{12} at 60 and 180 DAP, compared to the initial count (Table 13). Among the treatments when *A. flavus* was applied twice, (T_{15}, T_{17}, T_{18} and T_{19}), T_{18} was found to be superior in recording the population of the antagonist (Fig. 4). Compared to initial count, the increase of population was maximum in T_{18} at the time of harvest (Table 13).

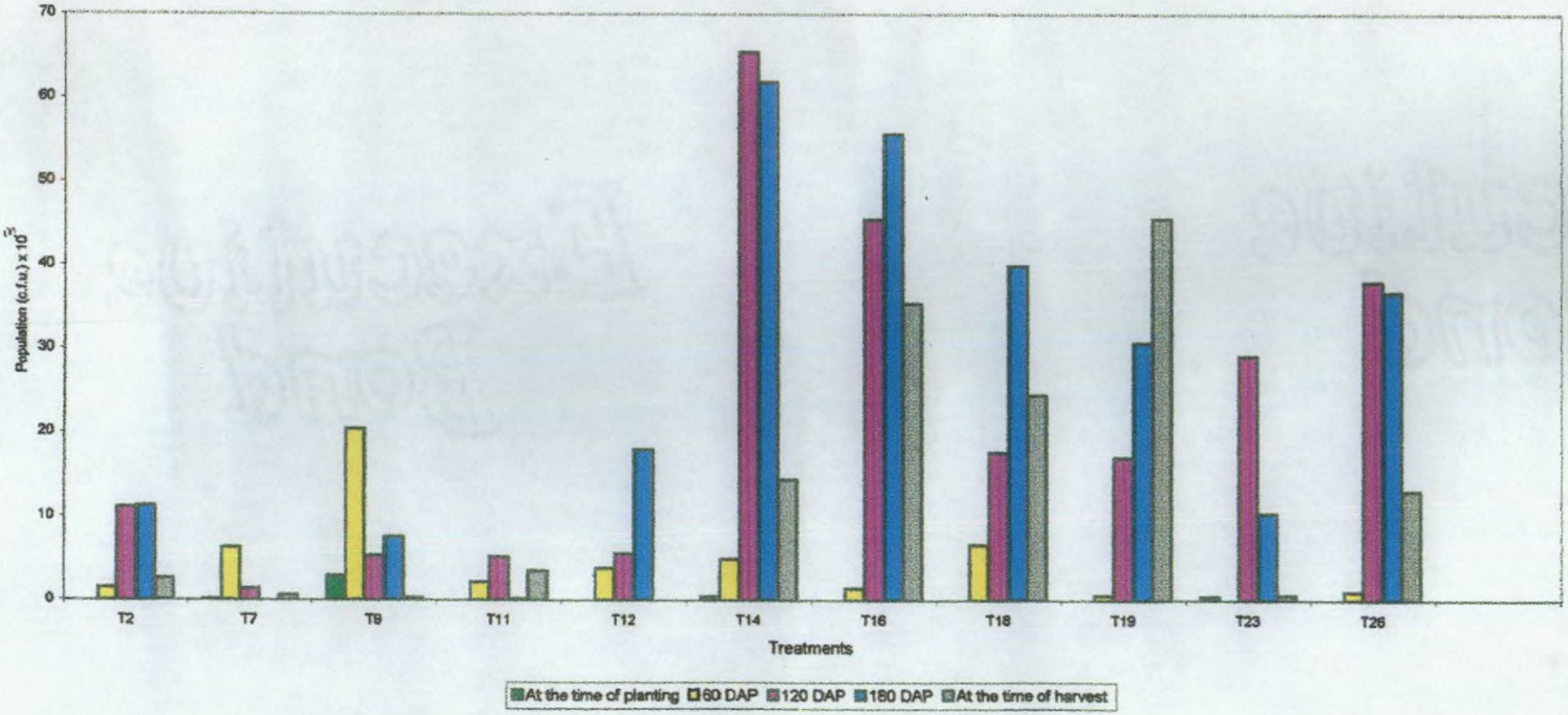

Fig. 5 Population of *A.niger* under different treatments at different intervals

The multiplication of *A. niger* was very less in plots where it was applied either alone (T_7) or with other antagonists (T_9, T_{11} and T_{12}) at the time of planting. Even though, the increase in the population from the initial value was maximum in T_9, at 60 DAP, it decreased in the following periods. In the case of antagonist *A. niger*, the incorporation of the antagonist to the plots twice was found to be superior than applying it only at the time of planting. The treatment T_{14} (application of *A. niger* at 60 and 120 DAP) was found to be recording the maximum population of 65.49 x 10^3 c.f.u.at 120 DAP (Table 14). During 180 DAP also T_{14} recorded the maximum population of *A. niger* (61.89 x 10^3 c.f.u.) and a significant increase in population from initial count (61.5 x 10^3 c.f.u.).

Among the treatments where *A. niger* was applied with other antagonists in T_{16} (application of *T. viride* and *A. niger* at 60 and 120 DAP) was found to be good in recording considerable higher population at 120 and 180 DAP and at the time of harvest (Fig. 5). The treatment T_{16} was next to T_{14} both at 120 and 180 DAP in recording the maximum increase in population of *A. niger* compared to initial count (Table 15). The treatment T_{19} (application of *T. viride*, *A. niger* and *A. flavus* at 60 and 120 DAP) recorded the maximum population of *A. niger* (45.59 x 10^3 c.f.u.) at the time of harvest followed by T_{16} (35.36 x 10^3 c.f.u.), (Fig. 5).

The compatibility of *Aspergillus* spp. with fungicides has been studied by several workers. Zahar *et al.* (1986) reported less toxic of cuprosan 311 SD (copper oxychloride + manaeb + zineb) on *A. niger* and *A. flavus*. In the present study, there showed an increased number of colony forming units of *A. flavus* in both plots (T_{24} and T_{27}), (Fig.4) in combination with fungicide (Table 12). The data showed that, the maximum population of *A. flavus* was in T_{24} at 120 DAP followed by T_{27}. The result on the population of *A. niger* revealed that it was higher in plots where *A. niger* was applied in combination with the fungicides (T_{23} and T_{26}), (Fig. 5). This result is in conformity with the reports of Shanmugham (1996). In his study with *A. flavus* and *A. niger* he found that these antagonists were quite compatible with mancozeb under *in vitro* conditions. He also reported that, though fytolan(copper oxychloride) recorded less efficacy, in inhibiting the growth of *A. niger*, it completely inhibited the growth of *A. flavus*.

The treatments T_{16} (soil incorporation of *T. viride* and *A. niger* at 60 and 120 DAP) and T_{19}(soil incorporation of *T. viride*, *A. niger* and *A. flavus* at 60 and 120 DAP) which were found to be superior in minimising the rhizome rot also recorded the

maximum yield of ginger (Table 16). There are reports in getting higher yield by the application of antagonist (Backman and Kabna, 1975; Elad *et al.*, 1980). The present study indicated that, this environmentally desirable management strategy is also economically viable in recording the maximum yield of ginger. The integration of the chemical and antagonist application (T_{24}) was also found to be good in producing higher yield of ginger in the present experiment.

Summary

SUMMARY

1. Rhizome rot of ginger caused by *Pythium aphanidermatum* (Edson) Fitzpatrik is one of the most destructive diseases of ginger. Considering the seriousness of the disease, the present study was undertaken to test the efficacy of fungal biocontrol agents *T. viride, A. flavus* and *A. niger* to manage the disease in field conditions so that a suitable management practice with biocontrol agent may be evolved.

2. The rhizome rot pathogen was isolated from naturally infected ginger rhizomes and its pathogenicity established by Koch's postulates in ginger variety Rio-de-Janeiro. Based on the characters, the fungus causing rhizome rot of ginger was identified as *Pythium aphanidermatum* (Edson) Fitzpatrick.

3. The various food bases were evaluated for the mass multiplication of the selected antagonists. Among the food bases tested, rice hull was found to be significantly superior to all others to obtain maximum growth for *T. viride* and *A. flavus*. For *A. niger* rice bran was found to be good in obtaining maximum growth

4. In general, treating seed rhizome with antagonists *T. viride* and *A. niger* or with fungicides (mancozeb) will be good for better germination.

5. The occurrence of pre-emergence rotting varied considerably between treatments. In plots where the treatments with antagonists (*T. viride, A. flavus* or *A. niger*) or fungicides (mancozeb or copper oxychloride) were applied either as seed treatment or as soil incorporation at the time of planting, the pre-emergence rotting was not observed.

6. The incorporation of the combination of the antagonists, *T. viride A. niger* and *A. flavus* 60 and 120 DAP (T_{19}) has a significant effect in checking the rhizome rot incidence when compared to other treatments.

7. The present study also revealed that the plots in which the antagonists were applied twice i.e., at 60 and 120 DAP, (the treatments T_{19} and T_{16}) the rhizome rot incidence was minimum compared to the plots with only one time application of antagonists.

8. The minimum population of the pathogen *P. aphanidermatum* recorded at different periods varied among the treatments. On an average of four observations, (60, 120, 180 DAP and at the time of harvest), the maximum inhibition of *P. aphanidermatum* was observed in plot where *A. flavus* was applied in combination with mancozeb.

9. The treatments T_{16} (soil application of the antagonists *A. flavus* and *A. niger* at 60 and 120 DAP) and T_{12} (soil application of *T. viride*, *A. flavus* and *A. niger* at the time of planting) were also found promising in checking the pathogen *P. aphanidermatum.*

10. The multiplication of the selected antagonists, *T. viride*, *A. flavus* and *A. niger* in soil was found out by estimating their population at different stages. The result on the population of the antagonists indicated that, in plots where *T. viride* was applied either alone or in combination with other antagonists or fungicides resulted in higher population than in plots where the antagonists were not applied indicating their multiplication in soil.

11. The observations on the population of *T. viride* at different stages indicated that, the significant increase from the initial count was maximum in plots where *T. viride* was applied in combination with mancozeb (T_{22}) in most of the period.

12. The increase in the propagules of *T. viride* in treatment where *T. viride* and mancozeb were applied in combination established that the fungicide mancozeb is not affecting the growth and multiplication of *T. viride.*

13. The study revealed that application of the antagonists in combination will not affect the multiplication of any one of them.

14. The application of *T. viride* with other antagonists, *A. niger* and *A. flavus* did not affect its multiplication in soil.

15. The data on the population of the antagonist *A. flavus* revealed that, application of this antagonist with other antagonists *T. viride* and *A. niger* was found to be helping in the mulitplication of *A. flavus* than applying it alone.

16. In plots where the seeds treatment was done with the antagonists *T. viride*, *A. flavus* and *A. niger*, the multiplication was very less compared to plots where the antagonists were incorporated into soil.

17. The multiplication of the antagonists *A. flavus* and *A. niger* was very less in plots, where, the application was done at the time of planting.

18. The incorporation of the antagonists, *A. flavus* or *A. niger* to the plots, twice was found to be superior than applying them only once, for getting maximum multiplication of propagules in soil.

19. It was found that both the tested fungicides, mancozeb and copper oxychloride were compatible with *A. flavus* and *A. niger* in field condition.

20. The treatments T_{16} (Soil incorporation of *T. viride* and *A. niger* at 60 and 120 DAP) and T_{19} (Soil incorporation of *T. viride*, *A. niger* and *A. flavus* at 60 and 120 DAP) which were found to be superior in minimising the incidence of rhizome rot also recorded the maximum yield of ginger.

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BIOCONTROL OF RHIZOME ROT OF GINGER (*Zingiber officinale* Rosc.) USING SELECTED ANTAGONISTS

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ABSTRACT OF A THESIS

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ABSTRACT

Rhizome rot of ginger casused by *Pythium aphanidermatum* (Edson) Fitzpatrick is one of the most destructive diseases of ginger in Kerala. The pathogen was isolated and its pathogenecity was established by Koch's postulates in ginger variety Rio-de-Jenerio. Among the various food bases evaluated, for the mass multiplication of selected antagonists, rice hull was found to be significantly superior to all others to obtain maximum growth for *Trichoderma viride* and *Aspergillus flavus*. For *Aspergillus niger*, rice bran was found to be significantly superior.

The results on the effect of various treatments on germination showed that there was no significant difference among the treatments. The effect of antagonists on the pre-emergence rotting and post-emergence rotting (rhizome rot) were studied. In plots where antagonists (*T. viride*, *A.flavus* or *A. niger*) or fungicides (mancozeb or copper oxychloride) were applied either as seed treatment or as soil incorporation at the time of planting the pre-emergence rotting was not observed. The plot in which the antagonists *T. viride*, *A. niger* and *A. flavus* were applied in combination at 60 and 120 DAP, recorded the minimum rhizome rot incidence compared to other treatments. The plots in which the antagonists were applied twice, i.e., 60 and 120 DAP, the rhizome rot incidence was minimum compared to the plots with only one time application of antagonists. The maximum population of the pathogen *P. aphanidermatum*, was observed in plot where *A. flavus* was applied in combination with mancozeb.

The multiplication of selected antagonists, *T. viride*, *A. flavus* and *A. niger* in soil was found out by estimating their population at different stages. The population of *T. viride* was maximum in plots where it was applied in combination with mancozeb in most of the period. The soil application of the combination of antagonists *T. viride*, *A. flavus* and *A. niger* did not affect the multiplication of any one of them in soil. In general, soil incorporation of antagonists was found to helping in their mulitplication profusely compared to the seed treatment. The incorporation of the antagonists *A. flavus* and *A. niger* to the plots, twice was found to be superior than applying them only once, for getting maximum multiplication of propagules in soil. The result on the compatibility of antagonist with fungicide in field condition revealed that the antagonist *T. viride* was quite compatible with mancozeb whereas *A. flavus* and *A. niger* were compatible with

both fungicides tested (mancozeb and copper oxychloride). The treatments which was found to be superior in minimising the incidence of rhizome rot of ginger viz., T_{16} (soil incorporation of *T. viride*, *A. niger* at 60 and 120 DAP) and T_{19} (soil incorporation of *T. viride*, *A. niger* and *A. flavus*) also recorded the maximum yield of ginger.