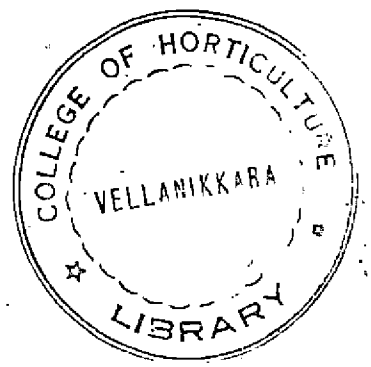
STANDARDISATION OF FOOD BASES FOR SELECTED ANTAGONISTIC MICROFLORA AGAINST SOIL-BORNE PATHOGENS



By

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THESIS

Submitted in partial fulfilment of
the requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF HORTICULTURE
Vellanikkara, Trichur

1990

DECLARATION

I hereby declare that this thesis entitled "Standerdisation of food bases for selected antagonistic microflora against soil-borne pathogens" 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, associate-ship, fellowship or other similar title, of any other University or Society.

Vellanikkara,
25-5-1990.

MINI.S. NAIR

CERTIFICATE

Certified that this thesis entitled "Standerdisation of food bases for selected antegonistic microflora against soil-borne pathogens" is a record of research work done independently by Kumari Mini.S.Nair under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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ACKNOWLEDGEMENTS

I express my deep sense of gratitude and indebtedness to Dr. P.V.Nair, Professor, Department of Plant Pathology and Chairman of my Advisory Committee for his expert guidance, immense help and constant encouragement throughout the course of this investigation and preparation of the thesis.

My profound gratitude is due to Dr.Abi Cheeran, Associate Director of Research, Kerala Agricultural University and member of the Advisory Committee for the keen interest, constant encouragement and critical suggestions received from him during the course of this study.

I express my heartfelt thanks to Dr. A.I.Jose, Professor and Head, Department of Soil Science and Agricultural Chemistry and member of the Advisory Committee for the valuable suggestions rendered during the preparation of the thesis.

My sincere thanks are due to Dr. Sally K. Mathew, Associate Professor, Department of Plant Pathology and member of the Advisory Committee for her help and valuable suggestions throughout the course of this investigation.

With all regards, I acknowledge the co-operation and help rendered by all the staff members of the Department

of Plant Pathology during the period of the study.

I am thankful to my friends particularly Sherina George, Ambika, S. and Madhu, S. for the help and co-operation rendered in the successful completion of thesis.

Thanks are also due to Sri.V.P.Asokan for the neat typing of the thesis. The award of Junior Research Fellowship for my post graduate programme by the I.C.A.R. is also gratefully acknowledged.

Vellanikkara,
25.5.'90

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Introduction

INTRODUCTION

Biological control is regarded as one of the basic components of the integrated disease management system in modern agriculture. Intensive search on basic aspects of the interaction of the biocontrol agents with other soil microflora has gained momentum the world over. However, because of the extremely complex nature of the soil microbiota little of this work has led to practical application. In order to make biological control adoptable under field conditions research on certain basic aspects of the pathogen as well as the antagonists are needed.

Promoting growth of antagonistic organisms in non-sterile soil is basic to practical biological disease control which can be accomplished through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists. Addition of antagonists to non-treated soil has the best chance of initiating successful biocontrol when applied in such quantity as to swamp the resident microbiota or when the population of resident microorganism has been severely diminished by treatment.

The efficacy of biocontrol is dependent upon the adaptability of the introduced antagonist under the prevailing environment as well as its ability to persist in the soil for longer periods. To establish the antagonist at population level high enough to produce the desired effect by direct massive soil augmentation, the organism being added must have additional selective nutrients to overcome the fungistatic effects of the native microflora. Presence of appropriate food bases in soil will thus result in the increased growth and colonization of the antagonist and in turn will lead to antibiosis at the desired level.

Once the effectiveness of any antagonist is established the most critical obstacle to be circumvented would be to develop method for mass culturing and delivery to field. The antagonistic preparation formulated should be in a form which can be handled conveniently by the farmers and at the same time it should be effective and economic. Thus the significance of food bases in formulating antagonistic preparation is two fold. Firstly the food bases provide additional nutrients for the establishment of introduced organism in soil. Secondly the currier based preparation is convenient for transport and use in the field.

In recent years several soil-borne diseases have become serious causing heavy damage to many important crops in the state. Management of these diseases by chemical methods is not often successful. Recent studies conducted at the College of Horticulture, Vellanikkara proved the strong antagonism of five fungi and one bacterium isolated from the forest soils of Kerala against the soil-borne pathogens viz., Rhizoctonia solani Kuhn, Pythium myriotylum Drechsler and Phytophthora palmivora (Butler) Butler. Having demonstrated the ability of these isoletes to control these pathogens it was considered worthwhile to standardise a technique suitable for the preparation of antagonist inocula which could be successfully used in the biocontrol of some of the important soil-borne disease prevalent in the state. Therefore, the present investigation was undertaken with the objective of standardising a technique for mass multiplication and production of antagonistic microflora recently isolated from the forest soils of Kerala in the biocontrol of soil-borne pathogens viz., Rhizoctonia, Pythium and Phytophthora.

Review of Literature

REVIEW OF LITERATURE

The history of biological control dates back to 1908 when Potter showed that plant pathogens could be inhibited by their own metabolic products. Garrett (1956) defined biological control of plant diseases as "any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of the disease caused by the pathogen". This involves the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists (Baker and Cook, 1974).

Biological control of plant pathogens accomplished through host plant resistance and cultural practices continues to be a predominant disease control strategy. In contrast, biological control accomplished through introduction or encouragement of microorganisms antagonistic to plant pathogens has been slow to develop even though a few

successful attempts to control disease in naturally infested soil have been reported. Biological control by introduction of antagonist into nontreated soil is difficult to achieve because it attempts to establish an alien antagonist in a biologically buffered community (Baker and Cook, 1974). Although this is difficult it can be done when the right organisms are obtained and properly used. In attempting to introduce antagonists into nontreated soil, microorganisms isolated from soil have to be screened for their antagonistic properties, and selected individuals have to be grown in mass culture.

1. Importance of food bases for multiplication and production of antagonistic microflora

Direct introduction of antagonistic micro-organisms into the soil were effective against certain soil-borne plant pathogens in previously sterilized soils (Garrett, 1956). But in natural soils, the selected micro-organisms, antagonistically active against pathogens in pure culture were not able to exert any biological control on pathogens when mixed in soil. The success in sterile soils may be due to their abundant nutrients and to freedom from competition by other micro-organisms.

The major problem of applying antagonists to soil is their inability to become established in the ecosystem and to overcome the resistance of soil microflora to the introduction of new micro-organisms (Alexander, 1971 and Boosalis and Mankau, 1970). Lockwood (1977) reported that when naked spores of most fungi are added to natural soils, fungistasis prevents their germination and proliferation. Biocontrol agents introduced into the soil in the absence of easily utilizable organic matter may lyse or revert to a resting stage. This temporary inactivation could depress the impact of massive introduction of antagonists that are expected to overcome soil-borne pathogens while the antagonists are still viable in soil. Addition of the proper food base to soil with the antagonist might overcome fungistasis and thus enhence the chances of the antagonist to grow and colonize the food base in soil.

Growth and colonization by antagonists would be essential if biological control depended on production of toxic substances, including antibiotics. Appropriate food bases in soil are essential for production of antibiotics (Wright, 1955 and 1956). For antibiotic production by introduced organisms to be of significance in the control of pathogens, the antagonist must be established, the antibiotic

must be produced, the toxin must accumulate to levels that are inhibitory, and it must then persist for periods sufficiently long for effectiveness to be assured (Alexander, 1971).

Direct soil augmentation with biological control agents for an impact on soil-borne plant pathogens has a greater chance of success when the agent is introduced with rather than without, the proper food base (Akhtar, 1977; Mangenot and Diem, 1979; Wells et al., 1972). Wells et al. (1972) controlled Sclerotium rolfsii on tomatoes by temporarily over whelming the infection court with Trichoderma harzianum and a fresh food base and they stressed the importance of food bases for successful biocontrol. Hadar et al. (1979) found that bran was the best food base, not only for growth and sporulation of the antagonist, but also for suppression of damping-off caused by Rhizoctonia solani on beans, tomatoes and egg plants.

Lab grown conidia of Trichoderma spp. and Gliocladium virens were sensitive to soil fungistasis (Beagle-Ristaino and Papavizas, 1985). They observed that chlamydospores from a liquid fermentation system or from potato dextrose broth germinated readily in soil. They also observed that the number of propagules increased hundred fold when either of the

two genera was added in fermentor biomass containing traces of a food base and consisting mostly of chlamydospores. Mukharjee et al. (1987) obtained more promising results when the antagonists were applied to the soil on a food base.

2. System of growth and delivery of antagonists into soil

One of the most critical obstacles to biological control by direct massive soil augmentation has been the lack or scarcity of methods for mass culturing and delivering antagonists to soil. Despite the limited progress, scientists are attempting to develop effective experimental systems of growth and delivery of antagonists into soil. For bacterial antagonists, nutrient broth has been extensively used (Mitchell and Hurwitz, 1965; Merriman et al., 1974; Mangenot and Diem, 1979). Broadbent et al. (1971) grew potential bacterial antagonists in shake cultures of yeast mannitol broth or nutrient broth. Bacillus subtilis was cultured on potato dextrose broth for applying to kernels of corn (Kommedahl and Mew, 1975). Sun and Huang (1978) experimentally controlled watermelon wilt (caused by Fusarium oxysporum f. sp. niveum). They grew an Arthrobacter sp. on a mixture of sugarcane bagasse and urea and added the cultures to the soil before planting the susceptible host.

Formulation of biocontrol agents as powders or granules is frequently possible. Iswaren et al. (1969) and Rao (1977) reported that peat-like material available in India is a good carrier for Rhizobium, a nitrogen fixing bacterium. Lignite is another carrier which is widely used for preparation of Rhizobium inoculant (Kandasamy and Prasad, 1971; Rao, 1977). Peatmoss, milled to a very fine powder, formulated with buffers and adjuvants and adjusted to 30 to 50 per cent moisture is the classical formulation for Rhizobium (Roughley, 1976). Of all the carriers tested, powdered and sterilized farm yard manure (FYM) + soil, FYM alone or FYM + charcoal supported the survival of Azospirillum upto 31 weeks (Lakshmi et al., 1977). Tilak and Rao (1978) observed that among the different carriers compared combinations of Indian peat soil, farm yard manure, compost or prasemud with charcoal (1:1) gave higher rhizobial count than individual carrier. Paczkowski and Berryhill (1979) demonstrated that coal-based carrier containing Rhizobium are acceptable inoculants. Suslow et al. (1979) suggested that formulation of Pseudomonas may be prepared by coating the cells with gums and polysaccharides that stabilize them so they can be formulated as a dry powder.

Development of growth media for large-scale production of antagonists and of commercially acceptable carriers for

their production into soils is brighter for fungi than for bacteria. Wells et al. (1972) were the first to report use of Trichoderma harzianum preparations for field control of Sclerotium rolfsii. They grew Trichoderma harzianum on a rye-grass soil medium. A diatomaceous earth granule impregnated with a molasses solution was found suitable for growth and delivery of T. harzianum (Backman and Rodriguez-Kabana, 1975).

Verticillium dahliae on cotton was best controlled by introducing oats infected by Trichoderma (Marupov, 1976). He also reported that the mustard plant litter ploughed into the soil acted as a good substrate for development of Trichoderma. Akhter (1977) used wheat straw as the growth medium for Trichoderma viride.

Many workers reported the use of wheat bran as a growth medium for T. harzianum (Henis et al., 1978; 1979; Chet et al., 1979; Maiti and Sen, 1985; Mukherjee et al., 1987). Henis et al. (1978) observed that after 5-7 days of incubation wheat bran preparation contained 4.1×10^9 conidia/g of the substrate. They applied this preparation to soil for controlling damping-off of radish seedlings caused by Rhizoctonia solani. Hadar et al. (1979) reported that wheat bran was the best medium for the growth and sporulation of

T. harzianum. Fungal preparations contained 2.9 x 10^9 spores/g dry wt after 8 days of incubation of the medium inoculated with T. harzianum. Henis et al. (1979) concluded that T. harzianum preparation with wheat bran as a carrier is a long-term effective biocontrol agent in artificially and naturally infested soil and can be used to protect fumigated soils from reinfection with the pathogen. Maiti and Sen (1985) reported that when wheat bran formulation of T. harzianum was added to infested soil, it reduced the viability of sclerotia of S. rolfsii in the soil. Adding a very high dose of T. harzianum (hyperparasite) in soil on a food base (wheat bran) reduced seedling blight of jute (Mukharjee et al., 1987).

Elad et al. (1980) grew T. harzianum on a wheat bran: saw dust: tap water mixture (3:1:4 v/v). Abl-el-moity and Shatla (1981) reported that control of white rot of onion (Sclerotium cepivorum) was best when T. harzianum grown on barley grain was added to soil at the time of planting. Anilkumar and Gowda (1983) obtained control of S. rolfsii on sunflower by the incorporation of finger millet seeds colonized by T. harzianum into soil containing straws pre-colonized with S. rolfsii. They also reported that incorporation of straw pieces colonized by T. harzianum into

soil containing sclerotia reduced survival of the pathogen.

A lignite-stillage carrier system was tested by Jones et al. (1984) for applying biocontrol agents to the soil. Gliocladium virens and T. harzianum were used as test organisms. After storage of granules for four months at 20°C fungal viability remained > 90 per cent as determined by plating of granules. When colonized lignite-stillage carrier granules were applied to soil in growth boxes artificially infested with R. solani root rot ratings and root and shoot dry weights revealed positive effects of biocontrol agents and carrier.

Lewis and Papavizas (1984) used a mixture of wheat bran, sand and water to grow the antagonists viz., T. viride, T. harzianum, Trichoderma hamatum, Gliocladium roseum, G. virens, G. catenulatum, Talaromyces flavus and Aspergillus ochraceous. Sivan et al. (1984) studied the growth potential of T. harzianum on organic food bases including several agricultural wastes like wheat bran, wheat straw compost, ground wheat straw, ground cotton straw, peat and a wheat bran/peat mixture (1:1 v/v). They found that wheat bran/peat was the best medium for the growth and survival of T. harzianum. Wheat bran/peat mixture was utilized for the preparation of T. harzianum inoculum

(Elad et al., 1986; Sivan and Chet, 1986a; 1986b; Sivan et al., 1987).

Fermentor biomass containing traces of a food base was used for the growth of Trichoderma spp. and G. virens (Beagle-Ristanio and Papavizas, 1985). They also reported that populations of both the antagonists increased in soil planted with cotton to which fermentor biomass was added. Pelletized formulations of wheat bran or kaolin clay in an alginate gel containing conidia, chlamydospores or fermentor biomass (FB) of several isolates of the biocontrol fungi Trichoderma spp. and G. virens were prepared by Lewis and Papavizas (1985a and 1987). Higher population densities were obtained when alginate pellets added to soil contained chlamydospores rather than conidia and bran rather than kaolin as the bulking agent.

A medium consisting of equal volumes of wheat bran, peatmoss and water was used for culturing T. harzianum (Chang et al., 1986). Mukhopadyay et al. (1986) prepared T. harzianum in a wheat bran: saw dust: tap water mixture. Biological control of sugarbeet and tobacco damping-off was achieved in the glass house by the application of wheat bran sawdust preparation of T. harzianum to Pythium infested soils

(Mukhopadyay and Chandra, 1986). Mukhopadyay (1987) also used wheat bran saw dust preparation of T. harzianum and T. koningii for the control of damping-off in tomato and brinjal and wilt and root rots in lentil and chickpea.

Mass multiplication of T. viride in sand sorghum medium (sand 100 g and sorghum 20 g) was reported by Padmanaban and Alexander (1986). T. viride was fully grown after 10 days of incubation. Parakhia and Vaishnav (1986) prepared T. harzianum inoculum in wheat husk bran and incorporated into soil for controlling Rhizoctonia bataticola on Cicer arietinum. Conidia of Trichoderma were produced on autocleved barley and sprayed on flowering plants (Tronsmo, 1986).

Trichoderma harzianum was multiplied on sorghum grain and applied to soil in field at 30 g/m row before sowing for controlling S. rolfsii causing root rot in sugar beets (Upadhyay and Mukhopadyay, 1986). Of eighteen agricultural wastes and bye-products tested as substrates for T. harzianum and T. viride, tapioca rind, tapioca thippi, well decomposed farm yard manure, gobar gas slurry, mushroom spent bed, paddy chaff and wheat bran were found to be suitable for mass multiplication (Gangadharan and Jeyarajan, 1988).

Potato dextrose agar was used for culturing Chaetomium globosum (Kommedahl and Mew, 1975) and Penicillium oxalicum (Kommedahl and Windels, 1978). Turner and Tribe (1975) and Ahmed and Tribe (1977) prepared the inoculum of biocontrol agent (Coniothyrium minitans) of white rot of onion in milled rice. Huang (1976) used barley, rye and sunflower seed in the ratio 1:1:1 for the production of C. minitans inoculum.

G. roseum was prepared in a mixture of peat, soil and nutrients (Moody and Gindrat, 1977). The antagonist Corticium sp. first grown on corn leaf meal (CLM) and incorporated into Pythium infested field gave reduction in the incidence of damping-off of table beet (Hoch and Abawi, 1979).

Laetisaria arvalis was grown on wheat bran and used for the control of damping-off of tomato by Pythium sp. and root rot of black gram caused by R. betaticola (Martin et al., 1984). Venkatasubbaiah and Safeeulla (1984) grew Aspergillus niger on rawa meal sand medium with 2 per cent sucrose solution to facilitate profuse growth. This was mixed at 5 per cent (w/w) level with soil artificially infested with R. solani.

Cardoso and Chandi (1985) grow avirulent, Rhizoctonia-like binucleated fungi (BN) on sterilized oat kernels and mixed with soil at the rate of 3 g of kernels per litre of steamed soil. Ramert (1985) discussed the importance of providing supplementary nutrients for the antagonists.

3. Control of soil-borne plant pathogens through introduced antagonists

i) Antagonistic fungi

After the well known experiments of Weindling (1932), many studies were made to explore the possibilities of using microbial antagonists to control plant diseases.

Weindling and Fawcett (1936) showed that Trichoderma lignorum introduced into acidified soil as spores suppressed damping-off of citrus seedlings caused by R. solani. Volovik et al. (1974) reported that treatment of seed potatoes with a spore suspension of T. lignorum (T.viride), one per cent polyoxin and one per cent trichothecin reduced infection by Rhizoctonia. A sterile basidiomycete was used as an antagonist against the charcoal root rot (Macrophomina phaseolina) disease of slash pine seedlings (De La Cruz and Hubbell, 1975).

T. viride reduced Rhizoctonia infection of Phaseolus lunatus and peas (Mall, 1975), controlled bleck rot of lettuce caused by R. solani (Bedlan, 1985 and 1988), and reduced seedling disease of cotton incited by R.solani (Alagarsamy et al., 1987).

Integrated control of R. solani damping-off of redish by PCNB and T. harzianum was reported by Henis et al. (1978). Kommedahl and Windels (1978) and Windels and Kommedahl (1978) found that seed treatment with spore suspension of Penicillium oxalicum was as effective as captan in controlling root diseases of pea caused by Fusarium solani and R. solani in green house experiments.

T. harzianum reduced seedling disease of bean, tomato and peanut caused by R. solani (Hadar et al., 1979). Henis et al. (1979) reported that wheat bran preparation of T. harzianum added to methyl bromide fumigated soils protected carnations and straw berry plants from R. solani. They also reported that Trichoderma preparation added to soil protected tomato seedlings from S. rolfsii. Wheat bran + saw dust preparation of T. harzianum introduced into the soil delayed the progress and incidence of damping-off of beans caused by S. rolfsii and R. solani in the field for

nine weeks, decreased the severity of disease and increased yield by twenty per cent (Elad et al., 1980a). Elad et al. (1980b) reported that incidence of R. solani in a strawberry nursery was reduced by 20 to 46 per cent by applying T. harzianum after methyl bromide treatment.

In lab studies, treatment of radish and pea seeds with conidia of T. hamatum in a Methocel slurry protected seeds and seedlings from Pythium spp. or R. solani nearly as effectively as fungicide seed treatments (Harman et al., 1980). Lewis and Papavizas (1980) obtained control of cucumber fruit rot caused by R. solani by augmenting soil with T. harzianum. Someng (1980) observed that T. viride, T. aureoviride and Penicillium funiculosum reduced the count of gram wilt fungus (Rhizoctonia bataticola) in the rhizosphere of gram.

Tests in the field to suppress root rot and blight of beans caused by R. solani and Pythium spp., respectively with T. harzianum in combination with chemical seed treatment was reported by Papavizas and Lewis (1981). Tu and Vaartaja (1981) showed that the presence of G. virens in soil artificially infested with R. solani reduced at planting the severity of Rhizoctonia root rot in Phaseolus vulgaris. They found

that root rot severity decreased with increasing concentrations of G. virens.

Marshall (1982) opined that biocontrol of the bean (Phaseolus vulgaris) disease depends on soil reaction and inoculum concentration of the pathogen. Reduction in disease incidence was observed when seeds coated with conidia of T. harzianum was planted in acidified R. solani infested soils. Neweigy et al. (1982) obtained control of damping-off in broad bean incited by Fusarium solani, R. solani and S. rolfsii by seed treatment with Bacillus sp., Streptomyces sp. and Trichoderma spp. Jager and Velvis (1984) reported biological control of R. solani on potatoes by antagonist Verticillium biguttatum.

Mew and Rosales (1984) reported that when T.harzianum was introduced into rice field soil under rainfed conditions, it decomposed rice straw and by deplenishing the substrates reduced the survival of R. solani, causal agent of sheath blight of rice. Venkatasubbaiah and Safeeulla (1984) reported that incorporation of Aspergillus niger inoculum to the soil infested with R. solani reduced the incidence of collar rot under glasshouse and field conditions. Soil incorporation of T. harzianum inoculum significantly reduced

collar rot of coffee seedlings incited by R. solani (Venkatesubbaiah et al., 1984).

The bean plants were protected from root rot caused by R. solani when avirulent, Rhizoctonia-like binucleated fungi grown on sterilized oat kernels were incorporated in the soil (Cardoso and Chendi, 1985). Ichielevich-Auster et al. (1985) reported that a nonpathogenic isolate of R. solani (AG-4) suppressed damping-off of cotton, radish and wheat seedlings caused by virulent isolates of R. solani and R. zeae.

Mycelial preparations of most isolates of Trichoderma spp. and G. virens prevented damping-off of cotton, sugarbeet and radish seedlings caused by R. solani (Lewis and Papavizas, 1985b). Lifshitz et al. (1985) reported that seed treatment with conidial suspensions of T. harzianum was effective in reducing incidence of Rhizoctonia damping-off of radish. Strashnow et al. (1985a) obtained complete control of R. solani in bean seedlings with T. harzianum + a reduced dose of methyl bromide. Application of T.harzianum to soil or coating tomato fruits reduced R. solani fruit rot by up to 43 per cent and 85 per cent respectively under laboratory conditions (Strashnov et al., 1985b).

Bhaskaran and Seetharaman (1986) reported that seed treatment with T. harzianum reduced the disease incidence of black gram. Addition of wheat bran preparation of T. harzianum in green house planted beans (Phaseolus) and coating melon seeds with T. harzianum conidia reduced disease incidence caused by M. phaseolina by 37 to 74 per cent and 37.5 to 46.3 per cent respectively (Elad et al., 1986). Sekhar and Anahosur (1986) reported that safflower cake + T. viride significantly suppressed the saprophytic survival of M. phaseolina causal agent of charcoal rot of sorghum in soil. Under green house conditions Vyas and Khare (1986) completely controlled dry root rot (R. bataticola) of soyabean seedlings with T. harzianum and a reduced dose of carbendazim.

In laboratory, green house and field experiments, T. viride performed well in reducing the growth of M.phaseolina and root rot disease in green gram (Arjunan et al., 1987). Amendment of soil with Trichoderma aureoviride controlled rice sheath blight disease (Manian and Paulsamy, 1987). Mukhopadhyay (1987) reported control of damping-off in tomato and brinjal and wilt and root rots in lentil and chickpea under glass house and/or field conditions using Trichoderma spp.

Mukherjee et al. (1987) tested the potentiality of certain antagonists viz., Aspergillus fumigatus, A. terreus,

Penicillium citrinum, P. simplicissimum, T. harzianum, Streptoverticillium sp. and Bacillus subtilis against M. phaseolina. Most effective among the fungal antagonists was P. citrinum. Cole and Zvenyika (1988) achieved biological control of R. solani and F. solani infections in tobacco transplants by adding T. harzianum to methyl bromide fumigated seed beds before sowing of seeds. Bran preparations of Laetisaria arvalis, C. minitans, Dendrostilbella sp. and Cladorrhinum sp. prevented damping-off of cotton caused by R. solani (Lewis and Papavizas, 1988).

T. harzianum has been used by many scientists against S. rolfsii. Wells et al. (1972) obtained control of S. rolfsii diseases of lupines, tomatoes and peanuts by use of T. harzianum in green house tests and on tomatoes in the field. The inoculum of T. harzianum applied in the form of diatomaceous earth granules to peanut fields 70 to 100 days after planting reduced southern blight (S. rolfsii) of peanuts by 42 per cent and increased crop yields (Beckman and Rodriguez-Kabana, 1975). In pot experiments Agrawal et al. (1977) obtained control of collar rot of lentil caused by S. rolfsii by seed treatment with spore suspension of T. harzianum. Wheat bran preparation of T. harzianum applied to the soil reduced peanut disease caused by S. rolfsii (Chet et al., 1979; Grinstein et al., 1979).

Anilkumar and Gowda (1983) observed reduction in the survival of S. rolfsii when T. harzianum was added to soil. Addition of T. harzianum to soil reduced the viability of sclerotia of S. rolfsii in the soil (Maiti and Sen, 1985). Upadhyay and Mukhopadhyay (1986) reported that application of T. harzianum as infested sorghum grains to S. rolfsii infested soil gave upto 76 and 88 per cent disease control in the first and second cycles of sugarbeet seedlings respectively. They found that degree of control increased with increasing amount of Trichoderma inoculum.

Lozano and Pineda (1977) reported control of S. rolfsii on tomato seedlings by inoculating seedlings with Penicillium sp. and then with S. rolfsii. Pineda and Polanco (1981) obtained reduction in the incidence of S. rolfsii on bean by the addition of Penicillium notatum grown on dry milled Dicanthium aristatum seeds to the soil. Maiti and Sen (1987) tested the ability of an isolate of Gliocladium virens to reduce stem-rot of groundnut caused by S. rolfsii both in green house and field and it was found to be potent enough to reduce the disease.

Huang (1976) reported that C. minitans caused a 97 per cent reduction in the survival of sclerotia of Sclerotinia sclerotiorum in soil 100 days after the

mycoparasite was added to soil artificially infested with the pathogen. Lee and Wu (1984) suggested that Trichoderma spp. and G. virens may have potential as agents for biological control of S. sclerotiorum by reducing survival of sclerotia in soil. Soil and seed treatments with T. viride reduced infection of sunflowers by S. sclerotiorum and Botrytis cinerea in the glass house and prevented infection in the field (Sesan et al., 1984).

Application of mycelium and spores of T.harzianum decreased Sclerotium cepivorum infection of onion in pots, glasshouse plots and in the field (Abd-el-moity and Shatla, 1981). El-Razik et al. (1985) reported that application of suspensions of Penicillium godlewskii and Aspergillus candidus (2×10^5 and 4×10^5 propagales/ml) to soil infested with S. cepivorum 15 d before transplanting onions decreased the percentage of white rot in glasshouse tests.

Wright (1956) obtained control of Pythium infection of white mustard by seed inoculation with T.viride and Liu and Vaughan (1965) controlled damping-off of beet caused by Pythium ultimum by seed treatment with T. viride. Reduction of tobacco damping-off (Pythium aphenidermatum) by T. harzianum was reported by Fajola and Alasoadura (1975).

Kommedahl and Mew (1975) suggested biocontrol of corn root infection in the field by seed treatment with Chaetomium globosum.

An isolate of Corticium sp. was effective in controlling pre-and post-emergence damping-off of table beets caused by Pythium ultimum (Hoch and Abawi, 1979). Yehia et al. (1981) reported that T. viride, Streptomyces griseus and Bacillus subtilis reduced damping-off of tomato caused by Pythium deberyanum, Phytophthora (nicotianae) var. parasitica and Fusarium oxysporum f. sp. lycopersici in the glasshouse. Seed treatment with conidia of P. oxalicum reduced seed rot and damping-off of chickpea caused by P. ultimum in naturally infested soils (Kaiser and Hannen, 1984).

Seedling root rot caused by Pythium graminicola in canes did not occurred when T. viride was incorporated in the soil (Padmanaban and Alexander, 1984; 1986; 1987). Sivan et al. (1984) obtained efficient control of damping-off induced by P. aphanidermatum in peas, cucumbers, tomatoes and peppers by application of wheat bran/peat preparation of T. harzianum to soil. Teyes and Dirks (1985) reported that isolates of Gliocladium catenulatum, G. virens, Myrothecium verrucaria and T. hamatum suppressed root rot caused by P. ultimum in both steamed and unsteamed soil, but they were

not effective in reducing root rot caused by F. solani f.sp. pisi in steamed soil.

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 sp. (Lifshitz et al., 1986b).

Martin et al. (1986) observed that decrease in disease incidence in beet seedlings and final P. ultimum inoculum densities were linearly related to increasing population density of the antagonist Leptosphaeria arvalis in raw field soils and infested steamed soils. Mukhopadhyay and Chandra (1986) achieved control of damping-off of sugarbeet and tobacco incited by P. aphanidermatum by the application of wheat bran saw dust preparation of T. harzianum at different layers to soil. Incorporation of the antagonists (T. harzianum and T. viride) to the soil 9 days before sowing protected tobacco seedlings from damping-off up to 25 days (Nagarajan and Reddy, 1986).

Seed treatment with conidia of rhizosphere competent mutants of T. harzianum reduced the incidence of disease in barley, cucumber, pea, radish and tomato induced by P.ultimum (Ahmad and Baker, 1988). Out of 17 Trichoderma strs tested,

three were effective against damping-off of sugarbeat caused by Pythium and Aphanomyces spp. (Camporota et al., 1988).

Khare (1968) observed that A. fumigatus, A. orchreceous, Chaetomium globosum, Gliocladium deliquescens and Penicillium sp. were antagonistic to Phytophthora fragariae both in vitro and in vivo. Better control of foot rot (Phytophthora parasitica var. piperina) of Piper betle was obtained when an antagonistic strain of T. viride was inoculated with the pathogen after fumigation of the soil with carbon disulphide (Tiwari and Mehrotra, 1973). Organic amendments of cotton seed meal and groundnut cake completely suppressed Phytophthora palmivora causing black pepper wilt. Among the antagonists isolated from treated soil, the most effective were Talaromyces wortmanii and Penicillium variable (Dutta and Hegde, 1987). Smith et al. (1988) reported that Phytophthora root rot and crown rot of apple seedlings were controlled in green house trials by addition of selected isolates of Trichoderma to soil.

ii) Antagonistic bacteria

Cordon and Haenseler (1939) were among the first to use an antagonistic strain of Bacillus simplex to inhibit R. solani. Addition of a bacterial suspension to green house soil gave control of seed decay and damping-off in cucumber

and pea seedlings. Working with lettuce seedlings Wood (1951) achieved a significant control of damping-off of lettuce caused by R. solani by addition of cultures of Streptomyces and Bacillus sp. Naim (1966) reported control of damping-off of cotton seedlings caused by R. solani with B. subtilis str.II. Direct inoculation of B. subtilis isolate to pre-steamed soil depressed damping-off of radish caused by R. solani (Olsen and Baker, 1968).

Soaking wheat grains in B. subtilis suspension and then planting in pasteurized green house soil or in field soil protected plants from infection by R. solani (Merriman et al., 1974). In field tests, treatment of seed pieces and whole tubers with B. subtilis reduced the frequency of charcoal rot (M. phaseolina) and Botryodiplodia solani tuberosi at harvest (Thirumalachar and O'Brien, 1977). They suggested that biological control with a bacterial antagonist may supplement the cultural practices used in control of the disease. Vargas and Ramirez (1983) reported reduction in the seedling damage from 69 to 49 per cent in soil inoculated with R. solani by treatment of cotton seeds with Bacillus megaterium.

Tschen and Kuo (1985) obtained control of damping-off of mung bean (Vigna radiata) caused by R. solani by

adding B. subtilis to the soil. Treatment of coffee seeds with B. subtilis increased percentage seed germination and reduced disease incidence in field and green house tests with soils naturally and artificially infested with R.solani (Venkatasubbaiah, 1985). Mew and Rosales (1986) noticed that the fluorescent and nonfluorescent bacteria isolated from the rhizosphere of rice plants when used for seed bacterization suppressed the disease and protected the rice plant from infection by R. solani (sheath blight of rice).

Eight spp. of bacterial antagonists (Bacillus cereus, Enterobacter cloacae, Flavobacterium balustinum, Janthinobacterium lividum, Pseudomonas fluorescens biovar III, P. putida, P. stutzeri and Xanthomanas maltophila induced suppression of Rhizoctonia damping-off in container media amended with composted hardwood tree bark (Kwok et al., 1987).

In in vivo tests there was a considerable improvement in wheat germination and stand in soil infested with S. rolfsii after seed treatment or soil drenches with B. subtilis (Hegde et al., 1980). Ordentlich et al. (1987) found that Serratia marcesans was the best control agent of S. rolfsii under green house conditions (up to 75 per cent disease reduction). They also reported that S. marcesans significantly reduced damping-off incidence of bean caused by R. solani by 50 per cent.

Seed treatment with B. subtilis gave protection of onion against Sclerotium cepivorum (white rot of onion) under field conditions (Utkhede and Rahe, 1980; 1983). Zazzerni and Tosi (1985) reported that treatment of sunflower seed with a str. of B. subtilis and an unidentified bacterium reduced infection by Sclerotinia sclerotiorum (Lib.) de Bary in glasshouse trials in which soil was inoculated with the pathogen.

Podile and Dube (1983) suggested that amendment of wilt sick soils with an isolate of B. subtilis might provide biological control of fungal wilt disease.

In the greenhouse, damping-off of tomato caused by P. deberyanum was controlled by soaking seeds in cell suspension of Arthrobacter sp. (Mitchell and Hurwitz, 1965). Howell and Stipanovic (1980) reported that treatment of cotton seed with pyoluteorin (from cultures of str. Pseudomonas fluorescens - 5) or P. fluorescens at planting in P. ultimum infested soil increased seedling survival from 33 to 65 per cent and from 28 to 71 per cent respectively.

Out of 21 B. subtilis isolates from local and exotic sclerotia of S. cepivorum, six isolates provided significant reductions of infection by Phytophthora cactorum

(crown rot of apple) on McIntosh apple seedlings in greenhouse (Utkhede, 1984). Lifshitz et al. (1986a) reported that strains of Pseudomonas putida or P. fluorescens reduced root rot of soybean when the seedlings were inoculated with 10^8 zoospores of Phytophthora megasperma f. sp. glycinea.

The foregoing amount of literature indicates that there is great potential to bring about reduction of the soil-borne plant pathogens (Rhizoctonia, Pythium and Phytophthora) by selecting correct antagonists and applying it to the field at the correct time. Therefore it seems that search for a suitable technique of mass multiplication and delivery of antagonists to the field is relevant.

Materials and Methods

MATERIALS AND METHODS

1. Antagonistic microorganisms

The antagonists viz., Trichoderma harzianum Rifai, T. longibrachiatum Rifai, Aspergillus terreus Thom, Penicillium citrinum Thom, P. simplicissimum (Oudem.) Thom and a bacterial isolate Bacillus subtilis Cohn, emend available at the division of Plant Pathology, College of Horticulture, Vellanikkara were used for the study. These organisms were isolated from the forest soils of Idukki and Wynad districts of Kerala. The fungal isolates were maintained on potato dextrose agar (20 g potato, 20 g dextrose, 15 g agar and volume made to 1000 ml with distilled water) and the bacterial isolate on nutrient agar (5 g peptone, 3 g beef extract, 15 g agar and volume made to 1000 ml with distilled water).

1.1. Growth of antagonist in various food bases

The following seven food bases were selected for the study.

1. Rice
2. Wheat bran
3. Paddy straw
4. Rice bran
5. Cowpea
6. Forest soil
7. Soil + dried cowdung (1:1)

1.1.1. Preparation of growth media

Twenty five g each of various food bases were weighed and transferred to 250 ml conical flasks seperately. Wheat bran, rice bran and ground paddy straw were sterilized following the method adapted by Menis et al. (1979) with slight modifications. Fifty ml of water was added to each flask and autoclaved at 1.04 kg/cm^2 pressure for 1 h on two successive days.

For sterilization of cowpea and milled rice, the procedure given by Ahmed and Tribe (1977) was followed. Twenty five ml of water was added and autoclaved at 1.4 kg/cm^2 for 20 min. Soil + dried cowdung (1:1) and forest soil were sterilized by autoclaving at 1.04 kg/cm^2 pressure for 4 h (Rao, 1977).

1.1.2. Inoculation of food bases with antagonists

Spore suspensions of T. harzianum, T. longibrechiatum, A. terreus and P. citrinum were prepared from 12 day old colonies growing on potato dextrose agar. Since the growth of P. simplicissimum was slow three week old colonies on potato dextrose agar were used for preparing spore suspension. The suspension contained approximately 7 x 10^4 conidia per ml. The bacterial isolate was grown in nutrient broth (5 g peptone, 3 g beef extract and 1000 ml distilled water) for 72 h.

Each food base was inoculated with one ml suspension of antagonist and incubated for two weeks at room temperature till maximum growth was noticed. Six flasks were kept for each treatment.

1.1.2.1. Population dynamics of the antagonist in food bases

The survival of antagonist in various food bases was estimated by serial dilution and plate count technique (Stanier et al., 1977) at 15 days, 45 days, 75 days, 105 days, 135 days and 165 days after inoculation.

Serial dilutions of the antogonistic cultures were prepared up to one in 10^8 depending on the growth of the antagonist in various food bases. One ml of the appropriate dilution was pipetted to sterile petriplates and molten but cooled medium was added. The plates were then incubated at room temperature. Martin's rose bengal streptomycin agar (10 g dextrose, 5 g peptone, 1 g potassium dihydrogen phosphate, 0.5 g mangnesium sulphate, rose bengal (1 part in 30,000 parts of the medium), 30 mg streptomycin, 20 g agar and 1000 ml water) (Martin, 1950) for fungal antagonists and nutrient agar for bacterial isolate were used. Colonies of T. harzianum and T. longibrachiatum were counted on the third day of plating and colonies of A. terreus, P. citrinum and P. simplicissimum on the fourth day. Bacterial colonies

were counted after 48 h of incubation. The population of antagonists in different food bases were expressed as number of colony forming units (cfu) per g substrate on oven dry basis. The data were analysed statistically.

1.2. Selection of promising food bases

The most promising food bases for each antagonist were selected based on the growth and sporulation of antagonists for two weeks in various food bases.

Antagonists	Food bases
Trichoderma harzianum	Rice, wheat bran, paddy straw
Trichoderma longibrachiatum	Rice, rice bran, cowpea
Aspergillus terreus	Wheat bran, cowpea, rice
Penicillium citrinum	Wheat bran, cowpea, rice
Penicillium simplicissimum	Rice, wheat bran, cowpea
Bacillus subtilis	Rice, wheat bran, rice bran

2. Effect of carrier based antagonists in controlling soft rot of ginger (*Zingiber officinale* Rosc.) caused by *Pythium myriotylum*, collar rot and web blight of cowpea (*Vigna unguiculata* (L) Walp) caused by *Rhizoctonia solani* and quick wilt of black pepper (*Piper nigrum* L.) caused by *Phytophthora palmivora*.

2.1. Experimental details

A pot culture experiment was laid out during the period from March to September, 1989 at the College of Horticulture, Vellanikkara.

For each crop four antagonistic cultures grown in three different food bases were used. An untreated control was also kept. Thus a total of 13 treatments were there for each crop (Table 1).

The experimental design followed was CRD with five replications.

The exppriment was conducted under non-sterile conditions. About 8 kg of potting mixture containing sand, dried and powdered cowdung and top soil in the ratio 1:1:1 was taken in earthern pots (30 cm).

Cowpea seeds of the variety 'Kanakamony' were sown at the rate of 10 seeds per pot. Ginger crop was raised by planting three rhizome bits with sprouted buds per pot. The variety 'Maran' was used. One year old Panniyur-1 variety of pepper was used for planting.

Cultural operations were carried out as per the Package of Practices recommendations (Anon, 1986).

2.2. Application of antagonists to soil

The antagonists were grown in selected food bases for 15 days. This carrier based antagonists were applied at the rate of 50 g per pot after 110 days of planting of pepper and ginger and after 30 days of sowing of cowpea.

Table 1. Antagonists and food bases selected for biocontrol of *Rhizoctonia* in cowpea, *Pythium* in ginger and *Phytophthora* in black pepper

Crop	Pathogen	Antagonists	Food bases
Cowpea	*Rhizoctonia*	*Trichoderma harzianum*	Rice, wheat bran, paddy straw
		T. longibrachiatum	Rice, rice bran, cowpea
		Aspergillus terreus	Wheat bran, cowpea, rice
		Bacillus subtilis	Rice, wheat bran, rice bran
Ginger	*Pythium*	*T. harzianum*	Rice, wheat bran, paddy straw
		T. longibrachiatum	Rice, rice bran, cowpea
		Penicillium simplicissimum	Rice, wheat bran, cowpea
		B. subtilis	Rice, wheat bran, rice bran
Black pepper	*Phytophthora*	*T. harzianum*	Rice, wheat bran, paddy straw.
		T. longibrachiatum	Rice, rice bran, cowpea
		Penicillium citrinum	Wheat bran, cowpea, rice
		B. subtilis	Rice, wheat bran, rice bran

2.3. Inoculation with the pathogen

Inoculation with pure cultures of the respective pathogens were done one week after the introduction of antegonist. Pythium was grown on oat meal agar in petriplates for 7 days and mycelial mat was mixed with the soil. Rhizoctonia was grown in chopped paddy straw for 15 days and this preparation containing sclerotia were used for artificial inoculation. Zoospore suspension of Phytophthora was used for artificial inoculation. This was prepared by placing one week old culture in sterile water for 3 days.

2.4. Observations

2.4.1. Assay for colony forming units of antegonists introduced into the soil

The population count of the antagonistic microflora introduced into the soil was assessed at intervals following serial dilution and plate count technique. Composite rhizosphere soil samples were obtained by pooling samples from five replications under each treatment at 7, 30 and 60 days after the incorporation of antagonists into soil for the estimation. Populations were expressed as colony forming units per g dry weight of soil.

2.4.2. The intensity of disease was record as percentage of infected plants.

Results

RESULTS

Six antagonistic microflora viz., *Trichoderma harzianum*, *T. longibrachiatum*, *Aspergillus terreus*, *Penicillium citrinum*, *P. simplicissimum* and *Bacillus subtilis* were grown in seven different food bases as described in Materials and Methods.

1. Population dynamics of antegonists in food bases

1.1. *Trichoderma harzianum*

Results on the population count of *T. harzianum* in different growth media are summarised in Table 2 and Fig.1. The data contain the number of colony forming units (cfu) per g dry weight of food base at 15, 45, 75, 105, 135 and 165 days after inoculation.

After 15 days of growth, the highest population count was recorded by rice (4827.58×10^6 cfu per g of substrate) followed by wheat bran (833.33×10^6 cfu per g of substrate) but soil + cowdung recorded the least count (70.55×10^6 cfu per g of substrate). The growth of the antagonist showed different trends at 45 days of inoculation. In rice the count was increased from 4827.58×10^6 to

Table 2. Growth and survival of *Trichoderma harzianum* in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time of					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	4827.58 (8.482)	7966.10 (8.983)	3855.41 (8.257)	16.69[a] (2.814)	3.00 (1.098)	0.40 (-0.916)
Wheat bran	833.33 (6.725)	1016.39 (6.924)	0.02 (-3.912)	0.019 (-3.963)	0.005 (-5.298)	0.003 (-5.809)
Paddy straw	473.43 (6.160)	471.54 (6.156)	348.65[a] (5.854)	313.33 (5.747)	293.47 (5.681)	251.70 (5.528)
Rice bran	248.72 (5.516)	573.33 (6.351)	271.19[a] (5.602)	199.22 (5.294)	148.14 (4.998)	108.70 (4.688)
Cowpea	279.07 (5.631)	52.47 (3.960)	0.0008 (-7.130)	0.00	0.00	0.00
Forest soil	239.74 (5.479)	39.15 (3.667)	18.00[b] (2.890)	10.66[a] (2.366)	10.33[a] (2.335)	1.66 (0.506)
Soil + dried cowdung (1:1)	70.55 (4.256)	32.64 (3.485)	17.77[b] (2.877)	16.00[a] (2.772)	9.67[a] (2.269)	10.00 (2.302)

Values of each column followed by the same letter do not differ significantly (P= 0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

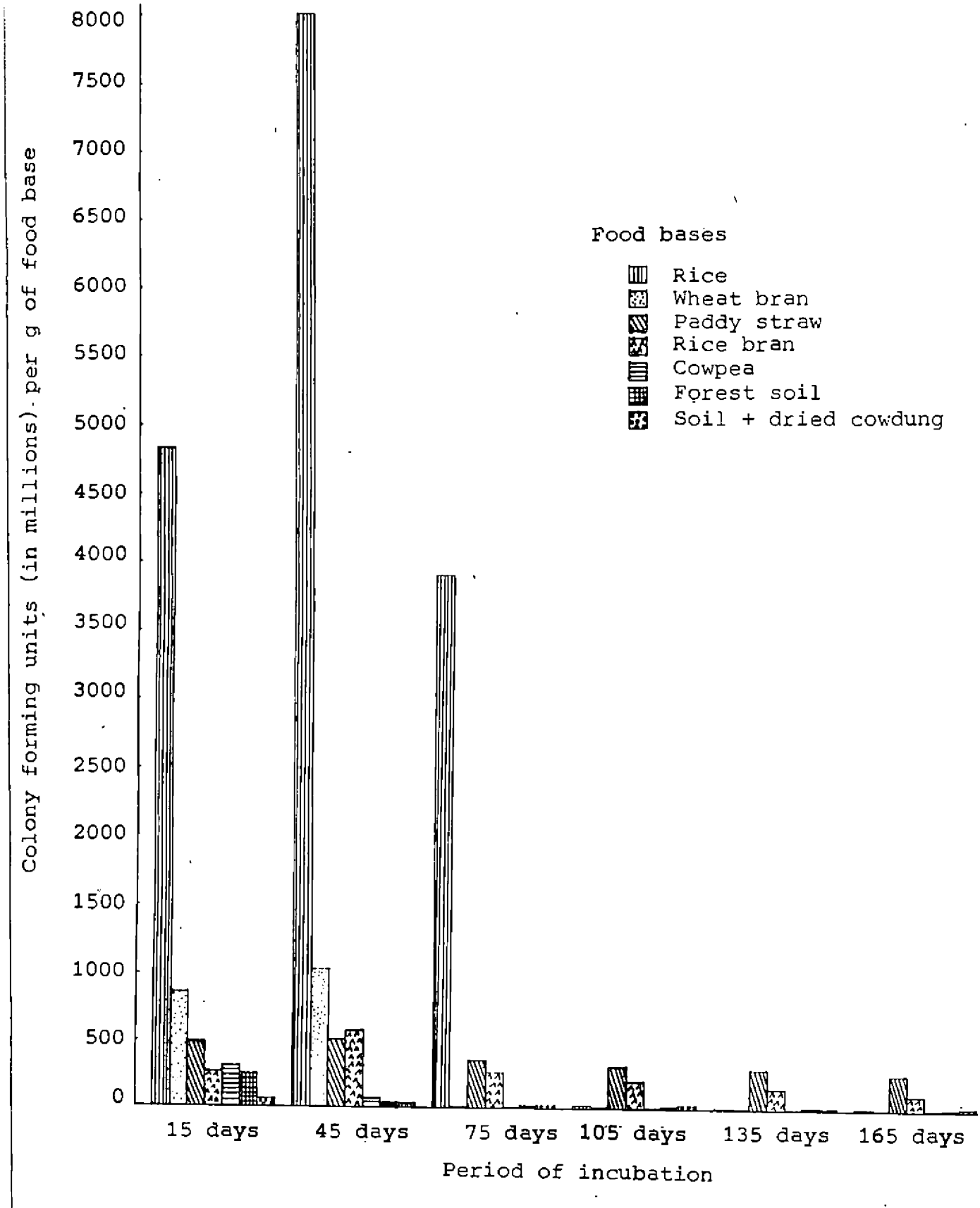


Fig.1. Population of Trichoderma harzianum at different intervals of incubation in various food bases.

7966.10 x 10^6 cfu per g of substrate. The same trend was observed in rice bran and wheat bran but in all other food bases the population count decreased. The reduction was very less in paddy straw (473.43 x 10^6 to 471.54 x 10^6 cfu per g of substrate) while it was very much pronounced in cowpea (279.07 x 10^6 to 52.47 x 10^6 cfu per g of substrate) and forest soil (239.74 x 10^6 to 39.15 x 10^6 cfu per g of substrate). In the case of soil + cowdung, more than 50 per cent of reduction was observed.

In all the food bases, the viable count considerably reduced at 75 days and it was much pronounced in cowpea (52.47 x 10^6 to 0.0008 x 10^6 cfu per g of substrate) and wheat bran (1016.39 x 10^6 to 0.02 x 10^6 cfu per g of substrate). Least reduction in the vieble count was observed in paddy straw (26.07 per cent) followed by soil + cowdung (45.56 per cent). In rice, rice bran and forest soil, the reduction in viable count were found to be more than 50 per cent.

The vieble count was very much reduced in all the food bases except paddy straw and rice bran at 105 days of inoculation. No vieble count was observed in cowpea but it was very negligible in wheat bran (0.019 x 10^6 cfu per g of substrate). In rice, forest soil and soil + cowdung, the viable count recorded was between 10 x 10^6 and 16 x 10^6 cfu

per g of substrate. The same trend was observed at 135 and 165 days of incubation.

Among the substrates tested, rice was found to be superior to all other food bases tried up to 75 days of incubation but maximum growth was obtained at 45 days. Next to rice, wheat bran was found superior to other food bases at 15 and 45 days of incubation. Later it was found to be very poor in maintaining the viable count. Even though the initial count was not high, paddy straw was found to be a good food base for the survival of the antagonist for a long period of incubation. The viable count of 473.43×10^6 at 15 days of incubation showed slow reduction to 251.70×10^6 cfu per g of substrate at 165 days. Almost the same trend was noticed in rice bran. The maximum colony count was recorded at 45 days of incubation (573.33×10^6 cfu per g of substrate) and it gradually declined to 108.70×10^6 cfu per g of substrate at 165 days of incubation.

This study revealed that rice was the best food base for obtaining maximum number of propagules at 45 days. Paddy straw was found to be superior to all others for the survival of antagonist even at 165 days of incubation.

1.2. Trichoderma longibrachiatum

Data on the number of cfu in different growth media at different intervals are presented in Table 3 and

Table 3. Growth and survival of *Trichoderma longibrachiatum* in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time of					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	277.78[a] (5.626)	20.00[c] (2.995)	5.00 (1.609)	1.67 (0.512)	0.97 (-0.030)	0.08 (-2.525)
Wheat bran	4.00 (1.386)	83.33 (4.422)	16.32[a] (2.792)	5.00[a] (1.609)	0.90 (-0.105)	0.05 (-2.995)
Paddy straw	21.66 (3.075)	25.33[ab] (3.231)	26.26 (3.268)	15.60 (2.747)	8.33[a] (2.119)	5.56[a] (1.715)
Rice bran	294.11[a] (5.683)	234.04[c] (5.455)	50.00 (3.912)	31.34 (3.444)	12.73 (2.543)	5.61[a] (1.724)
Cowpea	125.00 (4.828)	20.00[c] (2.995)	0.00	0.00	0.00	0.00
Forest soil	12.20 (2.501)	23.16[bc] (3.142)	14.66[a] (2.685)	3.66[a] (1.297)	3.66 (1.297)	3.66[a] (1.297)
Soil + dried cowdung (1:1)	28.64 (3.354)	30.22[a] (3.408)	14.51[a] (2.674)	10.20 (2.322)	10.00[a] (2.302)	5.66[a] (1.733)

Values of each column followed by the same letter do not differ significantly (P= 0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

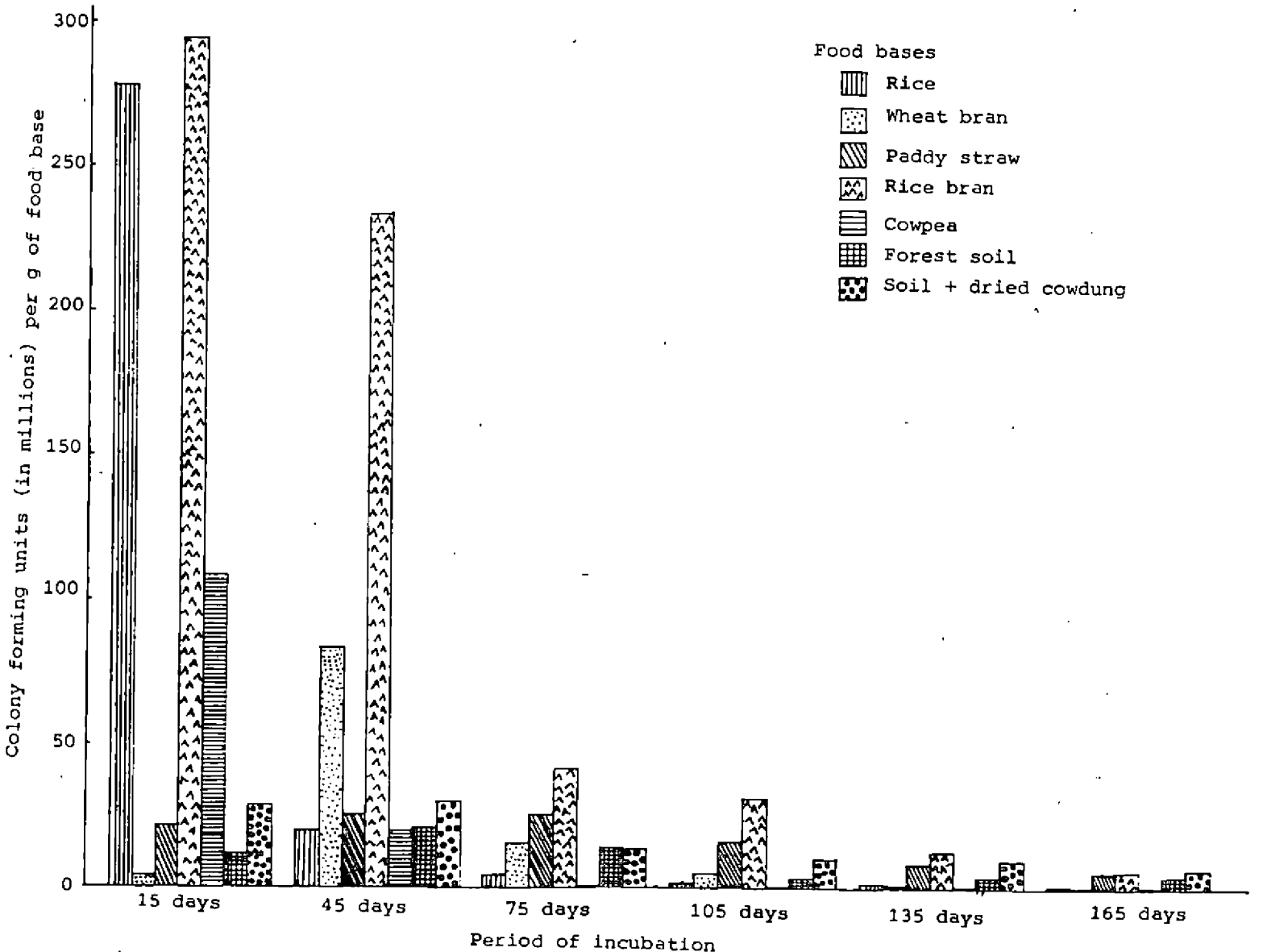


Fig.2. Population of Trichoderma longibrachiatum at different intervals of incubation in various food bases.

Fig.2. Rice bran and rice were found equally good and superior to all other food bases tried at 15 days of incubation. The growth of T. longibrachiatum in other food bases differed significantly. The population count in rice bran and rice were 294.11 x 10^6 and 277.78 x 10^6 cfu per g of substrate respectively. In the case of rice, there was sudden decline in the number of viable propagules after 15 days of inoculation. Though a reduction in population count was observed in rice bran also, rate of decline was very less at 45 days. Among all the food bases, this was found to harbour maximum number of propagules at 45 days of incubation.

The rice bran and rice were followed by cowpea (125.0 x 10^6 cfu per g of substrate) at 15 days of incubation but the population count in this medium declined to great extent (20.0 x 10^6 cfu per g of substrate) at 45 days and no viable count was recorded after 75 days of incubation.

The growth of the antagonist in soil + cowdung, paddy straw, forest soil and wheat bran was poor at 15 days and then increased up to 45 days of incubation. The increase was much pronounced with wheat bran where the population count increased from 4.0 x 10^6 to 83.33 x 10^6 cfu per g of substrate at 45 days. In the case of paddy straw, a slight

increase in the count was observed up to 75 days of incubation and thereafter the viable count declined. At 105th and 135th days of incubation, rice bran was found superior to all other food bases but at 165 days it was on par with paddy straw and soil + cowdung.

Among the food bases tried, rice bran was found to be superior throughout the period of observation.

1.3 Aspergillus terreus

Among the food bases tried, wheat bran was found to be the best medium even though no statistical difference could be observed among wheat bran, cowpea and rice (Table 4 and Fig.3) after two weeks of incubation. Population count of 2469.13 x 10^6 cfu per g of substrate was recorded by wheat bran followed by cowpea and rice with 2058.82 x 10^6 and 2000.0 x 10^6 cfu per g of substrate respectively. The least population count was observed in soil + cowdung (32.81 x 10^6 cfu per g of substrate) followed by forest soil (40.00 x 10^6 cfu per g of substrate) and rice bran (462.96 x 10^6 cfu per g of substrate at 15 days of incubation.

In the case of rice bran, paddy straw and soil + cowdung, growth of the antagonist increased after 15 days but the latter showed the highest per cent of increase in

Table 4. Growth and survival of Aspergillus terreus in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time of					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	2000.00[a] (7.690)	793.65[a] (6.676)	650.41 (6.477)	299.62 (5.702)	186.67[a] (5.229)	136.66 (4.917)
Wheat bran	2469.13[a] (7.811)	882.35[a] (6.782)	499.23 (6.213)	30.77 (3.426)	11.52 (2.444)	0.98 (-0.020)
Paddy straw	1250.00 (7.130)	2088.35 (7.644)	1941.74 (7.571)	966.18 (6.873)	416.66 (6.032)	210.52[a] (5.349)
Rice bran	462.96 (6.137)	697.67[a] (6.547)	830.41 (6.721)	670.88 (6.508)	203.51[a] (5.315)	176.66[a] (5.174)
Cowpea	2058.82[a] (7.629)	0.00	0.00	0.00	0.00	0.00
Forest soil	40.00[b] (3.688)	26.33 (3.270)	26.00 (3.258)	21.66 (3.075)	18.66 (2.926)	13.33 (2.590)
Soil + dried cowdung (1:1)	32.81[b] (3.490)	337.71 (5.822)	248.96 (5.517)	223.33 (5.408)	160.00[a] (5.075)	160.00[a] (5.075)

Values of each column followed by the same letter do not differ significantly (P = 0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

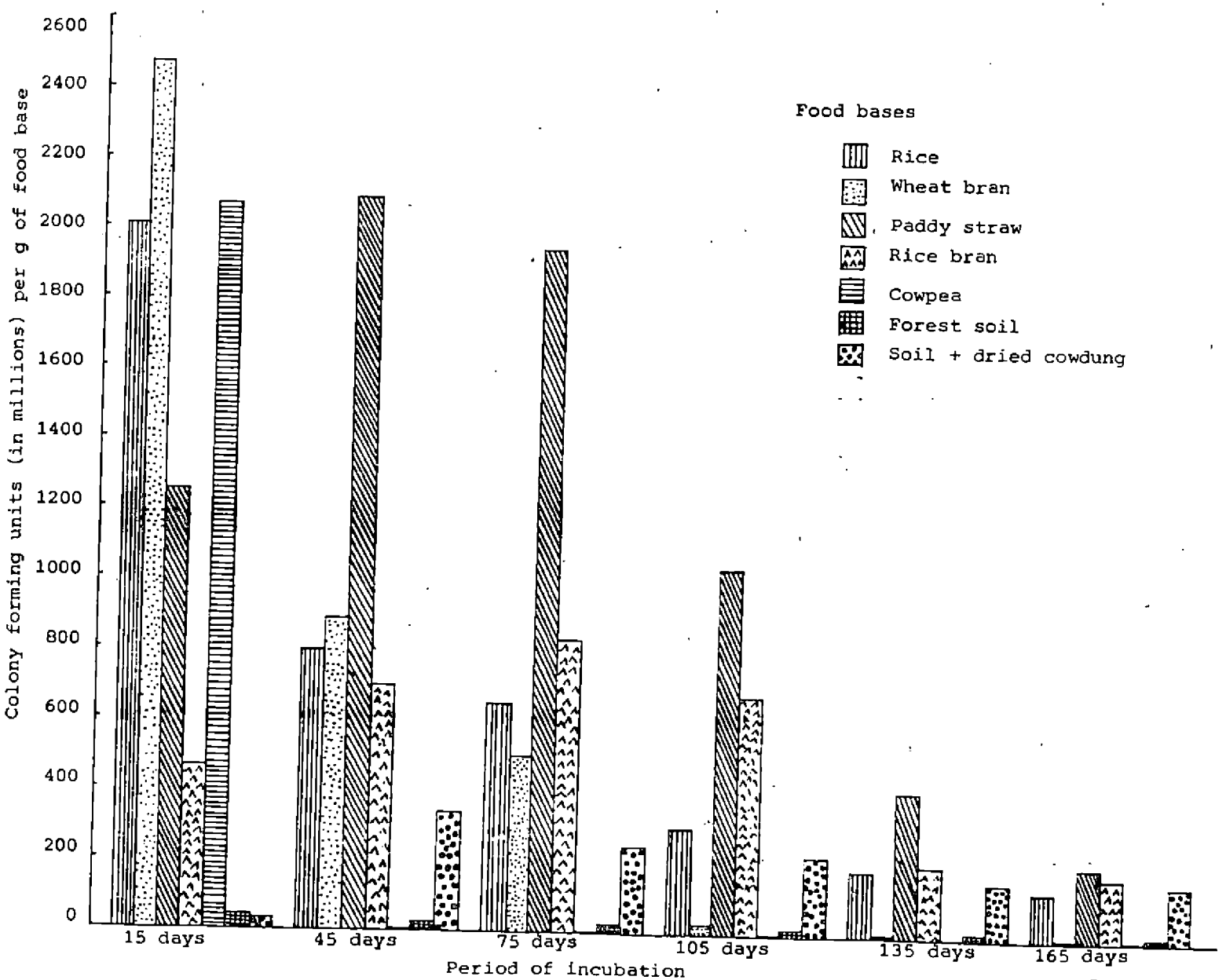


Fig.3. Population of Aspertillus terreus at different intervals of incubation in various food bases.

population count. In all other cases, reduction in viable count was noticed but in cowpea no viable colony was obtained after 15 days of incubation. The rate of decline was slow and steady in paddy straw. At 165 days, the viable count in paddy straw was 210.52 x 10^6 cfu per g of substrate followed by rice bran (176.66 x 10^6 cfu per g of substrate) and was on par with soil + cowdung.

A perusal of the data revealed that the maximum cfu per g of substrate was obtained in wheat bran at 15 days of incubation but it was found to be on par with cowpea and rice. Even though growth of the antagonist in paddy straw (1250.0 x 10^6 cfu per g of substrate) was not as much in cowpea and wheat bran, population count in the former increased up to 2088.35 x 10^6 cfu per g of substrate at 45 days and maintained its superiority during the remaining period of incubation.

1.4. Penicillium citrinum

Results of the population estimation of P.citrinum in different food bases at different intervals are presented in Table 5 and Fig.4. Wheat bran recorded the maximum population of 5833.33 x 10^6 cfu per g of substrate after two weeks of incubation. This was followed by cowpea

Table 5. Growth and survival of *Penicillium citrinum* in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	2038.09 (7.619)	863.94 (6.761)	197.65 (5.286)	126.61 (4.841)	19.92[b] (2.991)	5.61 (1.724)
Wheat bran	5833.33 (8.671)	1989.66 (7.595)	1285.91 (7.159)	956.52 (6.863)	420.76[a] (6.042)	224.47 (5.413)
Paddy straw	166.66 (5.115)	666.66 (6.502)	759.33 (6.632)	702.29 (6.554)	578.54 (6.360)	503.54 (6.221)
Rice bran	527.77[a] (6.268)	598.66 (6.394)	627.70 (6.442)	590.27 (6.380)	406.66[a] (6.007)	280.00 (5.634)
Cowpea	2571.42 (7.852)	323.10 (5.777)	0.00	0.00	0.00	0.00
Forest soil	54.23 (3.993)	29.33 (3.378)	28.33 (3.343)	25.66 (3.244)	25.00[b] (3.218)	17.33 (2.852)
Soil + dried cowdung (1:1)	582.01[a] (6.366)	91.67 (4.518)	40.66 (3.705)	29.33 (3.378)	18.66[b] (2.926)	7.66 (2.036)

Values of each column followed by the same letter do not differ significantly (P=0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

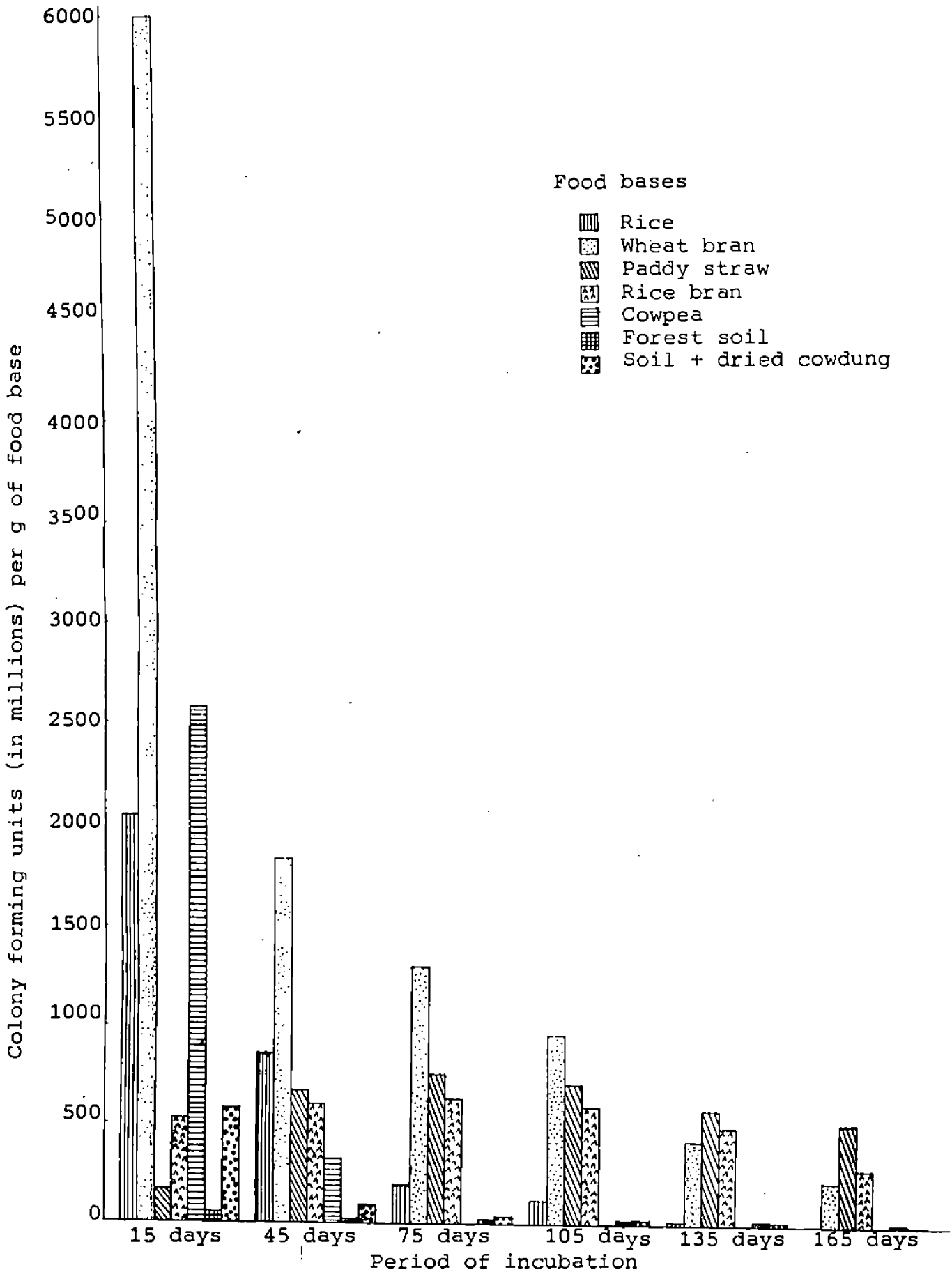


Fig.4. Population of Penicillium citrinum at different intervals of incubation in various food bases.

(2571.42 x 10^6 cfu per g of substrate) and rice (2038.09 x 10^6 cfu per g of substrate). Statistical analysis revealed that wheat bran was superior to all other food bases at 15 days of incubation. The least population count was observed in forest soil (54.23 x 10^6 cfu per g of substrates) followed by paddy straw (166.66 x 10^6 cfu per g of substrate), rice bran (527.77 x 10^6 cfu per g of substrate) and soil + cowdung (582.01 x 10^6 cfu per g of substrate). The fungal population in wheat bren medium was found to decline to one third at 45 days and the reduction was gradual in subsequent observations. A sudden decline in population was noticed with cowpea after two weeks of incubation (2571.42 x 10^6 to 323.10 x 10^6 cfu per g of substrate at 45 days) and no colony count was obtained in subsequent observations. A similar reduction was found in rice, soil + cowdung and forest soil after two weeks of incubation. The growth rate of the antagonist in paddy straw showed an increasing trend up to 75 days of incubation and thereafter a gradual decline was noticed. The same trend was noticed in rice bran also.

The maximum colony count per g of substrate was recorded by wheat bran at 15 days of incubation. This food base was found to be superior to all other food bases up to 105 days. Even though paddy straw showed a slow initial

growth, it was found superior to all other substrates at 135 and 165 days of incubation.

1.5. Penicillium simplicissimum

The observations on the population count are embodied in Table 6 and Fig.5. Out of seven substrates tried, rice was found to be the best with a population count of 5137.84 x 10^6 cfu per g of substrate followed by wheat bran (3956.56 x 10^6 cfu per g of substrate) and then cowpea (385 x 10^6 cfu per g of substrate) at 15 days of incubation. The growth was very poor in rice bran, forest soil and paddy straw. There was no significant difference among these food bases. The soil + dried cowdung recorded the minimum population count (4.1 x 10^6 cfu per g of substrate) after two weeks of incubation.

In all the growth media tried, the viable count of the antagonist was found to decrease after two weeks of incubation except in cowpea and rice bran. In cowpea and rice bran, population counts increased up to 45 days and thereafter a declining trend was noticed. In soil + cowdung, no count was recorded in last three estimations (105, 135 and 165 days of incubation).

The data revealed that rice was the best medium for yielding maximum cfu per g of substrate and survival

Table 6. Growth and survival of *Penicillium simplicissimum* in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time of					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	5137.84 (8.544)	1552.20[a] (7.347)	859.16[a] (6.755)	593.35[a] (6.385)	428.57[a] (6.060)	249.12[a] (5.517)
Wheat bran	3956.56 (8.283)	1597.82[a] (7.376)	862.43[a] (6.759)	586.66[a] (6.374)	362.33[a] (5.892)	195.65[a] (5.276)
Paddy straw	35.08[a] (3.557)	19.20 (2.954)	10.15 (2.317)	5.63[b] (1.728)	3.87[b] (1.353)	1.39[b] (0.329)
Rice bran	41.66[a] (3.729)	90.37 (4.503)	34.70 (3.546)	18.35 (2.909)	14.54 (2.676)	4.33 (1.465)
Cowpea	385.33 (5.954)	435.18 (6.075)	361.58 (5.890)	225.35 (5.417)	71.16 (4.264)	37.41 (3.621)
Forest soil	37.64[a] (3.628)	35.39 (3.566)	13.66 (2.614)	4.66[b] (1.539)	4.33[b] (1.465)	2.00[b] (0.693)
Soil + dried cowdung (1:1)	4.10 (1.410)	1.18 (0.165)	0.24 (-1.427)	0.00	0.00	0.00

Values of each column followed by the same letter do not differ significantly (P= 0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

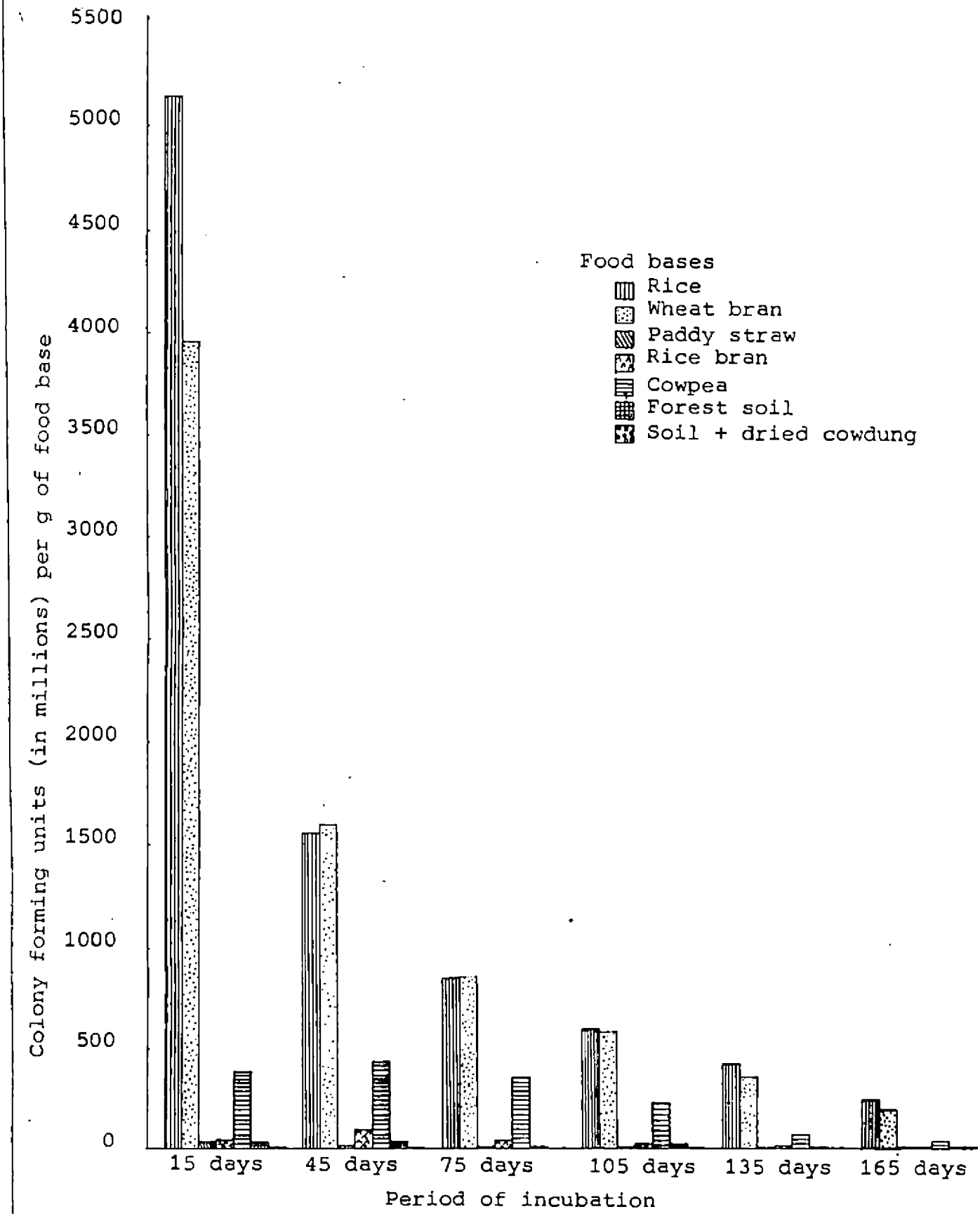


Fig.5. Population of Penicillium simplicissimum at different intervals of incubation in various food bases

of the antagonist for prolonged period of incubation (165 days). Even though wheat bran was found to be a best food base only next to rice at 15 days of incubation, in later observations it was found to be on par with rice.

1.6. Bacillus subtilis

The data on the growth and survival of B.subtilis in different food bases are presented in Table 7 and Fig.6. Observation at 15 days of incubation indicated that rice was the most suitable medium for the growth of antagonistic bacterium followed by wheat bran. Rice bran was ranked as third with regard to its efficacy as a growth medium followed by cowpea. No significant difference between soil + cowdung and paddy straw could be observed at 15 days of incubation. Forest soil was found to be the least effective as a food base.

Maximum growth of bacterium was observed in rice (3137.25×10^6 colonies per g of substrate) followed by wheat bran (1249.34×10^6 colonies per g of substrate) and rice bran (729.97×10^6 colonies per g of substrate). Forest soil recorded the least bacterial count of 5.84×10^6 colonies per g of substrate at 15 days of incubation. The bacterial count declined after 15 days in all the food bases except paddy straw and forest soil. In the case of paddy straw, maximum count was noticed at 45 days of incubation

Table 7. Growth and survival of *Bacillus subtilis* in different food bases

Food base	Colony forming units (in millions) per g dry weight of food base after incubation time of					
	15 days	45 days	75 days	105 days	135 days	165 days
Rice	3137.25 (8.051)	2913.90 (7.977)	884.95 (6.785)	630.18 (6.446)	347.43[a] (5.850)	270.00[a] (5.598)
Wheat bran	1249.34 (7.130)	649.35 (6.475)	429.40[a] (6.062)	228.24 (5.430)	156.94 (5.055)	87.62[b] (4.473)
Paddy straw	180.26[a] (5.194)	499.00 (6.212)	471.09[a] (6.155)	386.20 (5.956)	342.46[a] (5.836)	319.14[a] (5.765)
Rice bran	727.97 (6.590)	367.34 (5.906)	228.20 (5.430)	157.93 (5.062)	112.22[b] (4.720)	62.43 (4.134)
Cowpea	308.24 (5.730)	290.52 (5.671)	134.83 (4.904)	123.33 (4.814)	113.33[b] (4.730)	93.33[b] (4.536)
Forest soil	5.84 (1.764)	6.00 (1.791)	6.33 (1.845)	13.66 (2.614)	16.66 (2.813)	13.33[c] (2.590)
Soil + dried cowdung (1:1)	208.69[a] (5.340)	185.59 (5.223)	100.00 (4.605)	50.00 (3.912)	43.33 (3.768)	16.33[c] (2.793)

Values of each column followed by the same letter do not differ significantly (P=0.05) using Duncan's multiple range test.

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

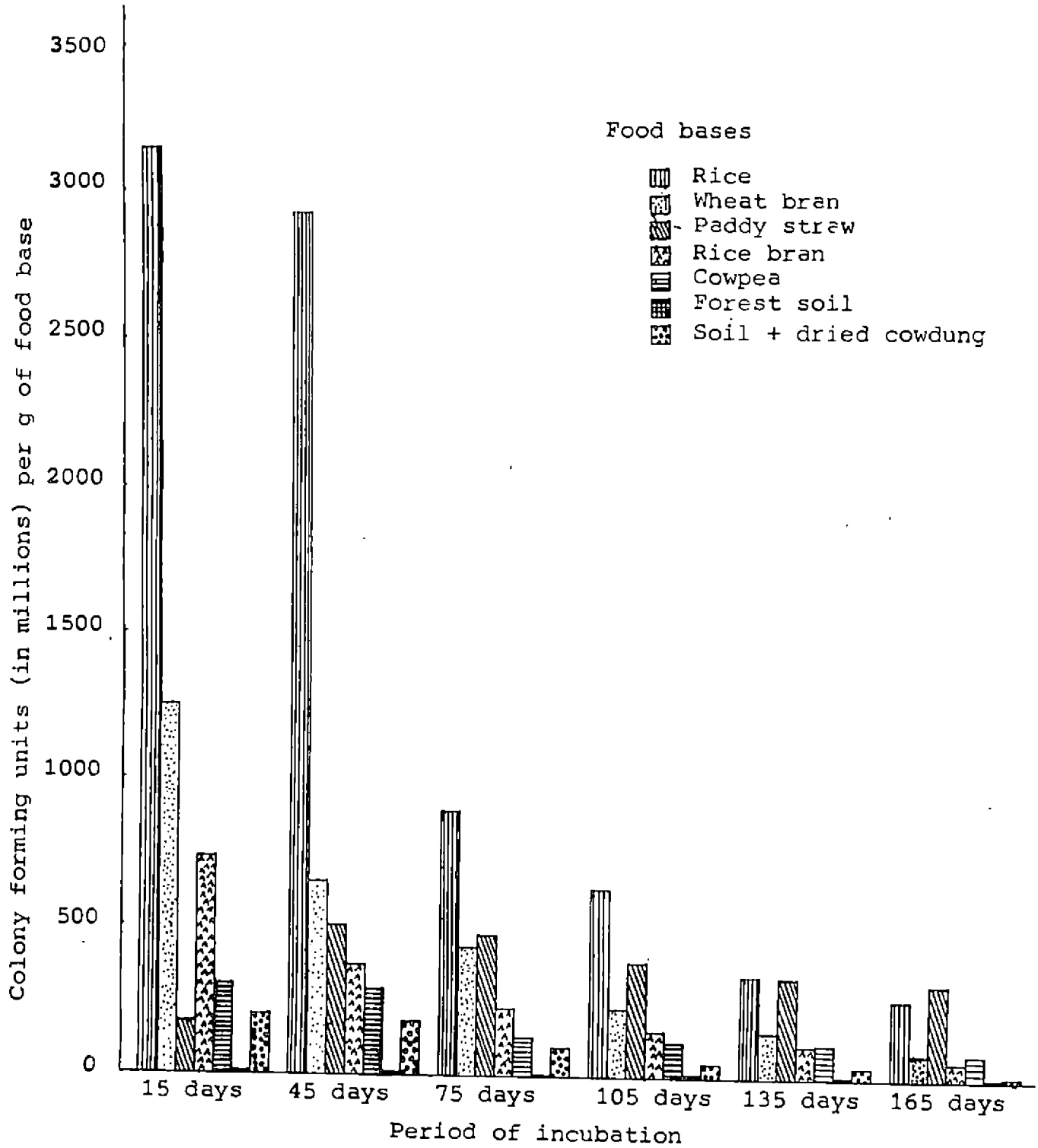


Fig.6. Population of Bacillus subtilis at different intervals of incubation in various food bases

and in forest soil at 135 days of incubation. Though an increase in population was noticed after three months of incubation, forest soil recorded the minimum number of viable propagules throughout the period of observation.

Among the food bases tested, rice was found to be significantly superior to all other food bases during the period of incubation followed by wheat bran.

2. Population dynamics of antagonists in the rhizosphere of cowpea, ginger and black pepper as affected by different carrier based inoculants

Based on the growth and multiplication of the antagonists as observed in in vitro studies, three food bases were selected for each antagonist for mass cultivation. The carrier based antagonists were tested in pot culture trials to study the population dynamics of the antagonist in the rhizosphere of cowpea, ginger and black pepper.

A comparison of the quantitative estimates of the antagonist in the treated and untreated rhizosphere samples were made and the results are presented in Tables 8, 9 and 10. In all the cases, the rhizosphere amended with food based antagonist yielded the colonies of the respective antagonist. But the number of colony forming units varied depending on the food base, crop and period of incubation. The control,

where the rhizosphere was not amended with antagonist did not yield any of the antagonist tried in this study. This clearly indicated that the rhizosphere of test plants were almost free from any of the antagonist used in this study.

2.1. Enumeration of antagonistic microflora in cowpea rhizosphere

Results on the estimation of introduced antagonist associated with the rhizosphere of cowpea are summarised in Table 8. The data embodies the number of cfu per g of soil at one week, one month and two months of inoculation in the rhizosphere amended with antagonist grown in different food bases.

2.1.1. Trichoderma harzianum

Among the three food bases tried, the rhizosphere amended with wheat bran-T. harzianum preparation yielded the maximum number of cfu after one week of application, i.e., 528.0 x 10^4 in the serial dilution and plate count method (Table 8 and Fig.7). An estimated number of 78.43 x 10^4 cfu per g of soil was recorded with rice-T. harzianum preparation while paddy straw gave the minimum count of 16.88 x 10^4 cfu per g of soil. The treatments were found

Table 8. Colony forming units of *Trichoderma harzianum*, *T. longibrachiatum*, *Aspergillus terreus* and *Bacillus subtilis* in the rhizosphere of cowpea amended with food based antagonist.

Antagonists	Food bases	Colony forming units (in 10,000s) per g of soil		
		7 days after application	30 days after application	60 days after application
Trichoderma harzianum	Paddy straw	16.88 (2.826)	11.26 (2.421)	6.79 (1.915)
" "	Wheat bran	528.00 (6.269)	77.30 (4.347)	9.67 (2.269)
	Rice	78.43 (4.362)	76.30 (4.334)	34.90 (3.552)
CD (0.05)		0.057	0.069	0.075
Trichoderma longibrachiatum	Rice bran	58.00 (4.060)	12.63 (2.536)	2.08 (0.732)
" "	Rice	28.90 (3.360)	8.33 (2.119)	3.10 (1.131)
	Cowpea	0.97 (-0.030)	0.82 (-0.198)	0.75 (-0.287)
CD (0.05)		0.097	0.057	0.083
Aspergillus terreus	Cowpea	2615.00 (7.869)	1057.00 (6.963)	52.17 (3.954)
" "	Wheat bran	8409.00 (9.037)	1222.49 (7.108)	115.03 (4.745)
	Rice	897.43 (6.799)	879.17 (6.778)	109.16 (4.692)
CD (0.05)		0.039	0.039	0.086
Bacillus subtilis	Rice bran	377.59 (5.933)	200.66 (5.301)	73.26 (4.294)
" "	Rice	721.15 (6.580)	294.12 (5.683)	23.28 (3.147)
	Wheat bran	651.42 (6.479)	140.15 (4.942)	42.09 (3.739)
CD (0.05)		0.039	0.021	0.112
Untreated control		0.00	0.00	0.00

Logarithmic transformation was used for analysis. Transformed values are given in parentheses

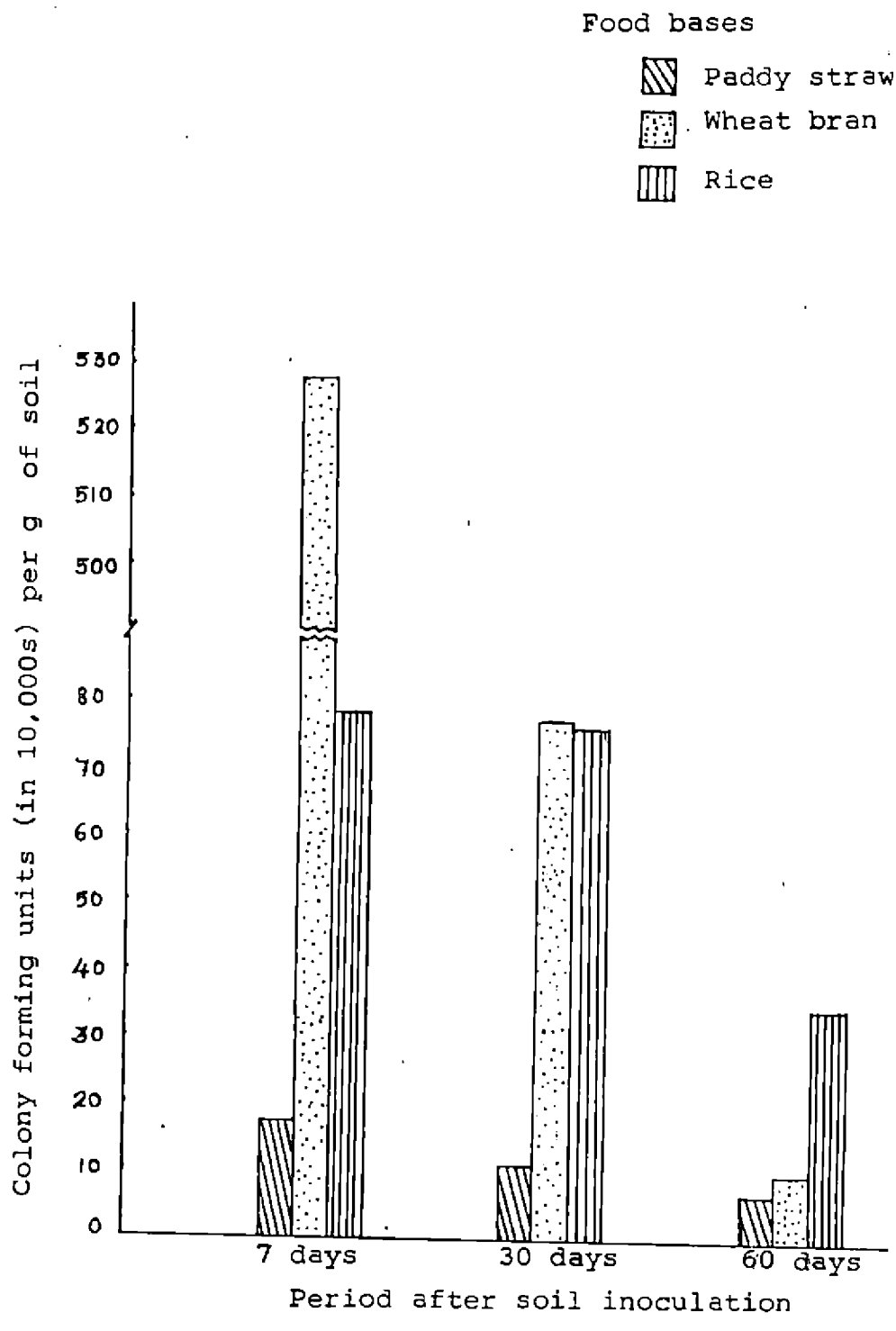


Fig.7. Population of T. harzianum at different intervals in cowpea rhizosphere amended with food based antagonist

to be statistically significant. The same trend was observed at 30 days of introduction. But the estimated number of T. harzianum propagules in the rhizosphere at 60 days of introduction indicated the superiority of rice as a food base over wheat bran and paddy straw.

A comparison of the population at different intervals revealed that the rate of survival differed considerably among the three food bases. The rate of decline of the antagonist in the rhizosphere amended with rice T. harzianum preparation was low when compared to paddy straw and wheat bran during the period of observation. After 30 days a faster rate of decline in the population count was noticed in the rhizosphere amended with wheat bran-antagonist preparation but it was less with paddy straw and negligible with rice.

2.1.2. Trichoderma longibrachiatum

The estimation of rhizosphere soil amended with rice bran-antagonist preparation gave a count of 58.0×10^4 cfu per g of soil at 7 days (Table 8 and Fig.8). This was followed by rice yielding a count of 28.90×10^4 cfu per g of soil. Cowpea antagonist preparation gave the lowest count (0.97×10^4 cfu per g of soil).

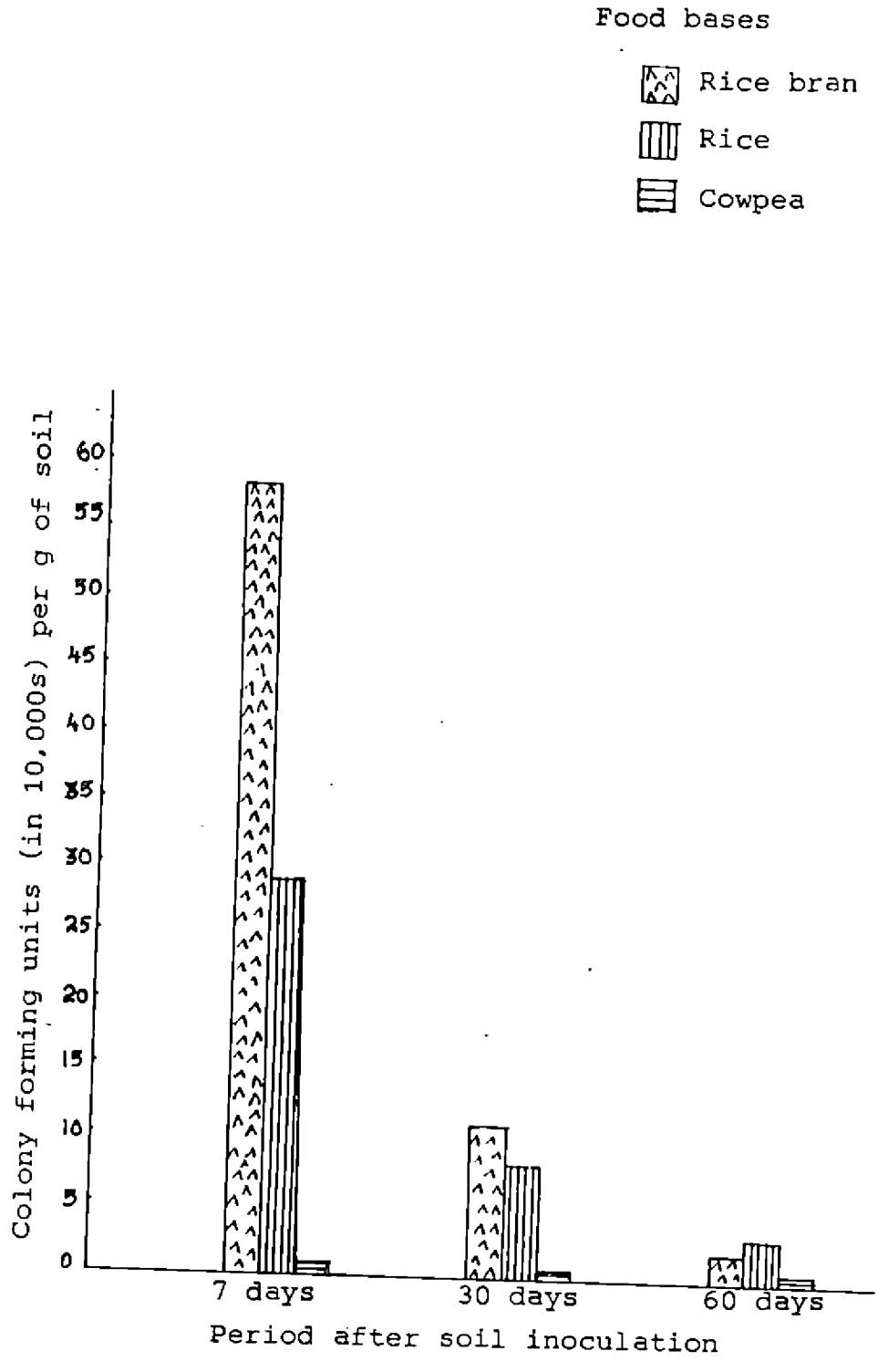


Fig.8. Population of T. longibrachiatum at different intervals in cowpea rhizosphere amended with food based antagonist.

Statistical analysis revealed that there was significant difference among the treatments. T. longibrachiatum grown in rice bran was found superior to rice and cowpea at one week and one month of introduction to soil. In all the treated pots, the antagonist population was found to decline after one week of application but it was more pronounced in the case of rice bran T. longibrachiatum preparation. On 60th day of application, rice antagonist preparation yielded maximum count followed by rice bran and cowpea.

2.1.3. Aspergillus terreus

At 7 days of inoculation, the rhizosphere soil amended with the antagonist grown in wheat bran gave the maximum population count (8409.0 x 10^4 cfu per g of soil) followed by cowpea (2615.0 x 10^4 cfu per g of soil) and rice (897.43 x 10^4 cfu per g of soil). Thereafter a sudden decline in population count was observed in rhizosphere soil amended with wheat bran (1222.49 x 10^4 cfu per g of soil) and and cowpea (1057.0 x 10^4 cfu per g of soil) antagonist preparations. But the rhizosphere soil treated with rice antagonist mixture showed a slight reduction of 18.26 x 10^4 cfu per g of soil in population count at 30 days of inoculation (Table 8 and Fig.9). On the sixtieth day after introduction of food based antagonist preparation, further reduction was observed but

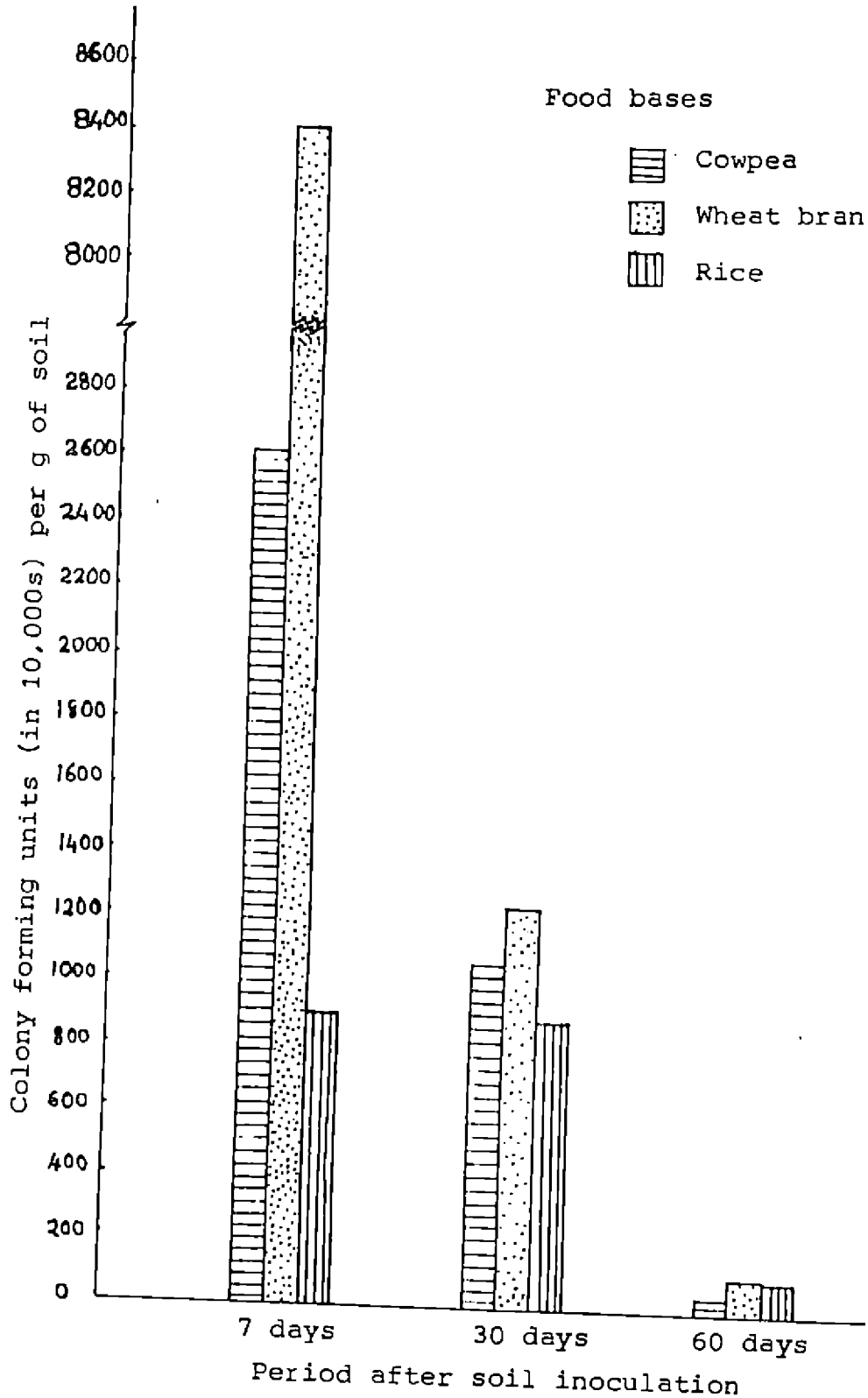


Fig.9. Population of A. terreus at different intervals in cowpea rhizosphere amended with food based antagonist.

maximum count was obtained with wheat bran (115.03 x 10^4 cfu per g of soil) followed by rice (109.16 x 10^4 cfu per g of soil) and cowpea (52.17 x 10^4 cfu per g of soil).

The difference among the treatments were significant with regard to the population count estimated at 7 days and one month of introduction of food based antagonist preperation to soil. At two months of introduction of carrier based antagonist wheat bran yielded the maximum count but it was on par with rice.

2.1.4. Bacillus subtilis

Among the three food bases tried, the antagonistic bacterium grown in rice survived better in the rhizosphere soil of cowpea than in wheat bran or rice bran (Table 8 and Fig.10). The population count was 721.15 x 10^4 colonies per g of soil for rice antagonist preperation followed by wheat bran (651.42 x 10^4 colonies per g of soil) and rice bran (377.59 x 10^4 colonies per g of soil). In all the cases population count declined after 7 days of inoculation. The decline was much pronounced with rice and wheat bran based B. subtilis where the count reduced to 294.12 x 10^4 and 140.15 x 10^4 colonies per g of soil respectively at 30 days of inoculation. But the reduction in colony count was very less with rice bran antagonist preparation. At

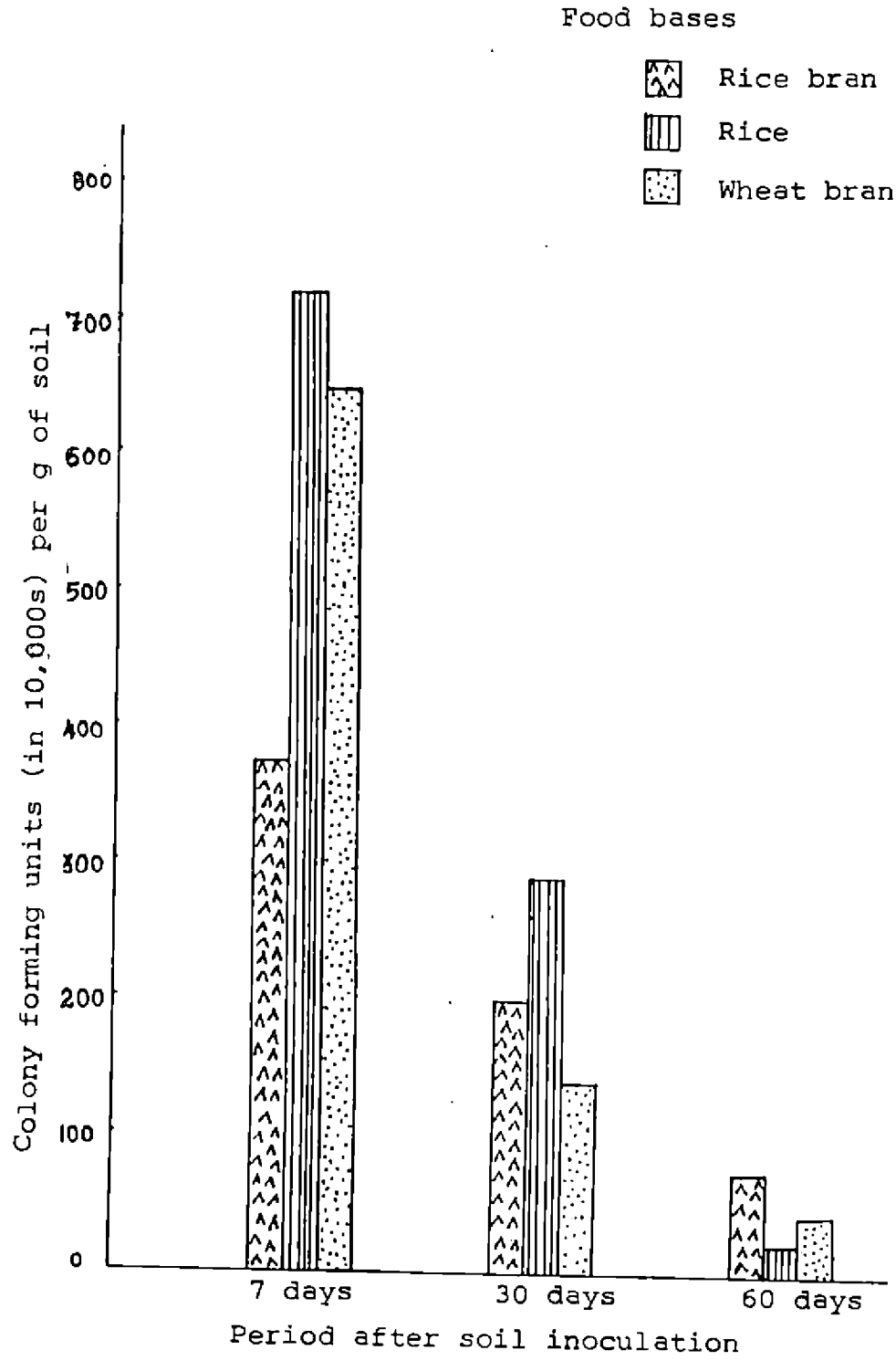


Fig.10. Population of B. subtilis at different intervals in cowpea rhizosphere amended with food based antagonist.

60 days after inoculation, the maximum count was obtained with rice bran antagonist preparation (73.26 x 10^4) followed by wheat bran (42.09 x 10^4 colonies per g of soil). The antagonist preparation which yielded the maximum count in the early periods recorded the least count (23.28 x 10^4 colonies per g of soil) at 60 days.

The treatments were significant throughout the period of observation. Rice was superior to wheat bran and rice bran based antagonistic preparation in the first two observations. But at 60 days rice bran based preparation was better than wheat bran and rice.

2.2. Enumeration of antagonistic microflora in ginger rhizosphere

Data on the estimation of population of the antagonist in the rhizosphere of ginger are presented in Table 9, Fig.11, 12, 13 and 14.

2.2.1. Trichoderma harzianum

Among the three food bases used, wheat bran T. harzianum preparation yielded the maximum count in the rhizosphere of ginger (4952.56 x 10^4 cfu per g of soil) at 7 days of introduction of soil. The paddy straw and rice based cultures gave much lesser count than wheat bran.

Table 9. Colony forming units of *Trichoderma harzianum*, *T. longibrachiatum*, *Penicillium simplicissimum* and *Bacillus subtilis* in the rhizosphere of ginger amended with food based antagonist

Antagonists	Food bases	Colony forming units (in 10,000s) per g of soil		
		7 days after application	30 days after application	60 days after application
Trichoderma harzianum	Paddy straw	292.03 (5.676)	15.95 (2.769)	8.24 (2.109)
" "	Wheat bran	4952.56 (8.507)	225.99 (5.420)	171.05 (1.41)
" "	Rice	143.88 (4.968)	74.91 (4.316)	53.96 (3.988)
CD (0.05)		0.081	0.105	0.055
Trichoderma longibrachiatum	Rice bran	118.10 (4.771)	12.18 (2.499)	15.39 (2.733)
" "	Rice	3.05 (1.115)	48.72 (3.886)	10.15 (2.317)
" "	Cowpea	4.46 (1.495)	11.17 (2.413)	10.18 (2.320)
CD (0.05)		0.033	0.056	0.767
Penicillium simplicissimum	Cowpea	2.84 (1.043)	0.85 (-0.162)	0.009 (-4.710)
" "	Wheat bran	633.20 (6.450)	19.10 (2.949)	0.72 (-0.328)
" "	Rice	142.86 (4.961)	21.37 (3.061)	2.46 (0.900)
CD (0.05)		0.104	0.304	0.146
Bacillus subtilis	Rice bran	134.40 (4.900)	44.12 (3.786)	12.05 (2.489)
" "	Rice	421.94 (6.044)	240.00 (5.480)	15.64 (2.749)
" "	Wheat bran	727.13 (6.589)	327.64 (5.791)	15.00 (2.708)
CD (0.05)		0.056	0.194	0.082
Untreated control		0.00	0.00	0.00

Logarithmic transformation was used for analysis. Transformed values are given in parentheses

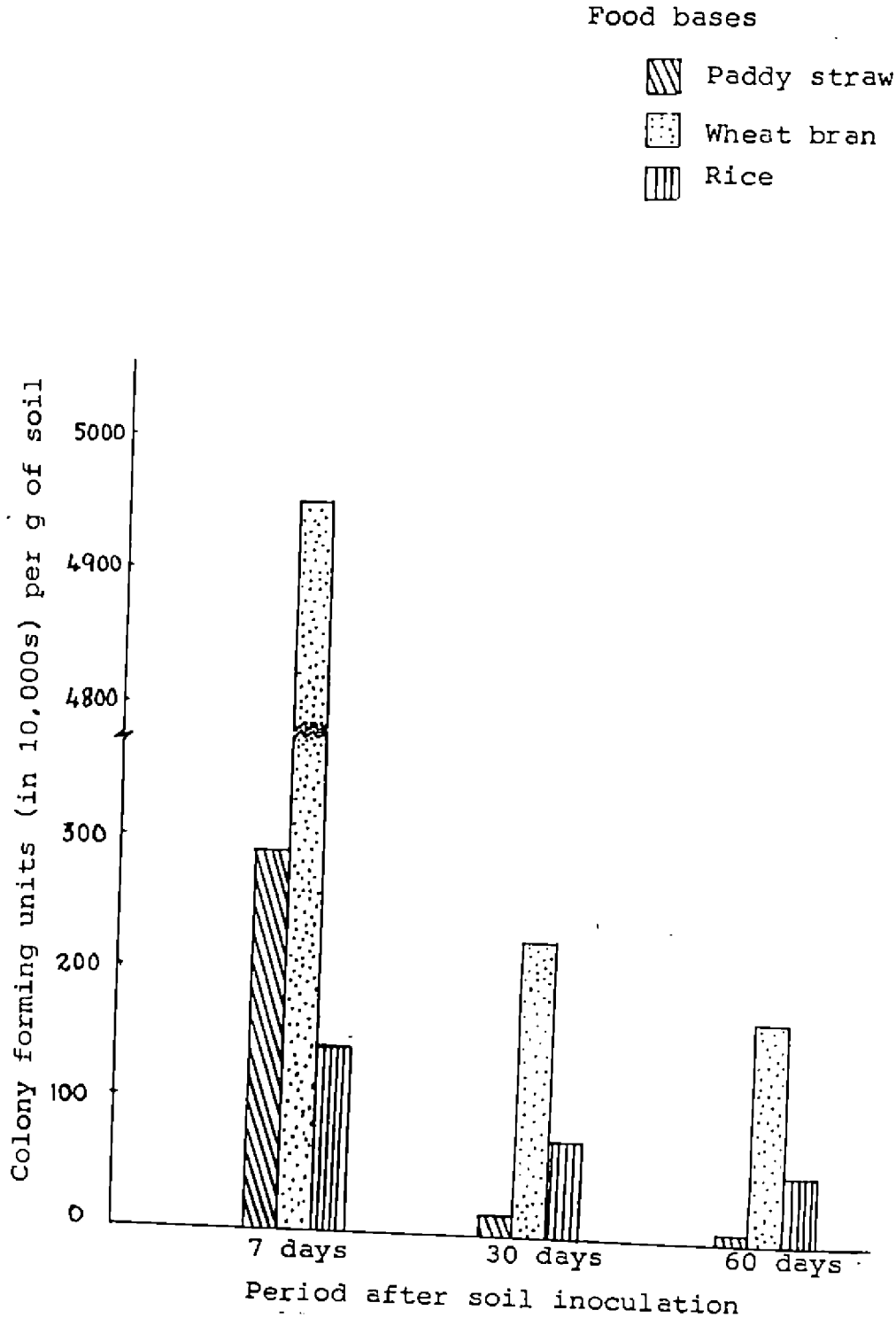


Fig.11. Population of T. harzianum at different intervals in ginger rhizosphere amended with food based antagonist.

The counts were 292.03 x 10^4 and 143.88 x 10^4 cfu per g of soil respectively. A substantial reduction in population count was noticed in the case of wheat bran and paddy straw antagonist preparations while the reduction in rice-antagonist preparation was very less. The former preparation recorded five per cent of the initial count as against 52 per cent by the latter. At 60 days of incculation, the maximum count was observed with wheat bran (171.05 x 10^4 cfu per g of soil) but per cent of reduction in count was only about 25 when compared to the count at 30 days. This was followed by rice where the count was 53.96 x 10^4 cfu per g of soil. The maximum reduction in population count at 60 days was observed in paddy straw based antagonist (8.24 x 10^4).

Statistical analysis revealed that wheat bran was superior to rice and paddy straw based antagonists throughout the period of observation.

2.2.2. Trichoderma longibrachiatum

The rhizosphere of ginger treated with rice bran T. longibrachiatum preparation yielded the maximum population count (118.10 x 10^4 cfu per g of soil) at 7 days of inoculation (Table 9 and Fig.12). The population counts in the rhizosphere amended with rice-and cowpea-antagonist mixtures

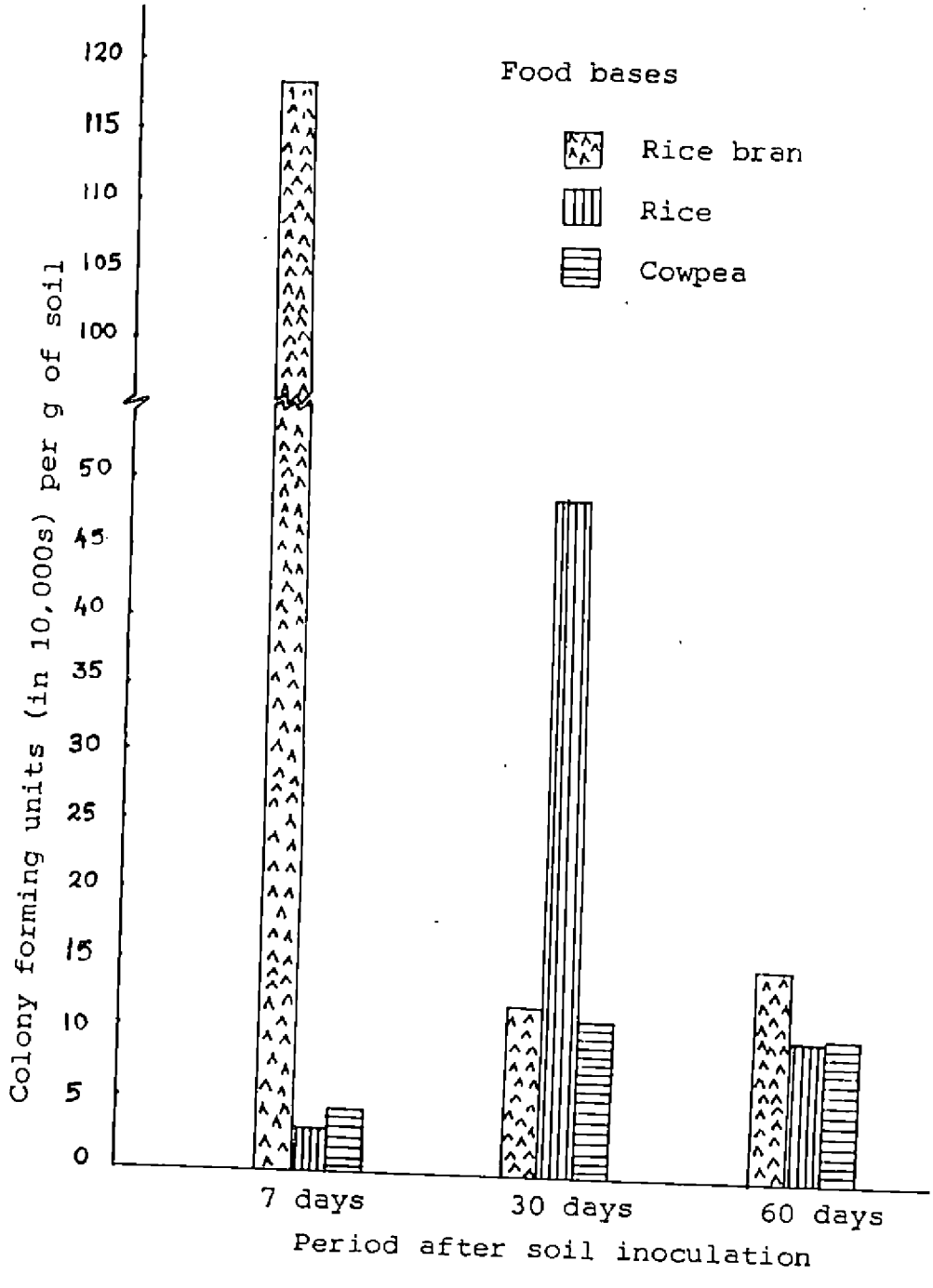


Fig.12. Population of T. longibrachiatum at different intervals in ginger rhizosphere amended with food based antagonist.

were 3.05 x 10^4 and 4.46 x 10^4 cfu per g of soil respectively but an increase in population count was noticed at 30 days (48.72 x 10^4 and 11.17 x 10^4 cfu per g of soil respectively). In the case of rhizosphere soil amended with rice bran-antagonist mixture, the population count declined to 12.18 x 10^4 cfu per g of soil at 30 days but a slight increase to 15.39 x 10^4 cfu per g of soil was observed at 60 days of inoculation. After 30 days of inoculation the rate of decline in the population count of the antagonist in the rhizosphere soil amended with cowpea-antagonist mixture was less when compared to rice-antagonist preparation.

Statistical analysis revealed the superiority of rice bran to rice and cowpea at 7 days of incubation. But at 30 days rice was found to be superior to rice bran and cowpea. There was no significant difference between cowpea and rice at 60 days.

2.2.3. Penicillium simplicissimum

Wheat bran was found to be the best food base for the survival of P. simplicissimum (633.20 x 10^4 cfu per g) in the rhizosphere soil when compared to rice (142.86 x 10^4) and cowpea (2.84 x 10^4) at 7 days of introduction

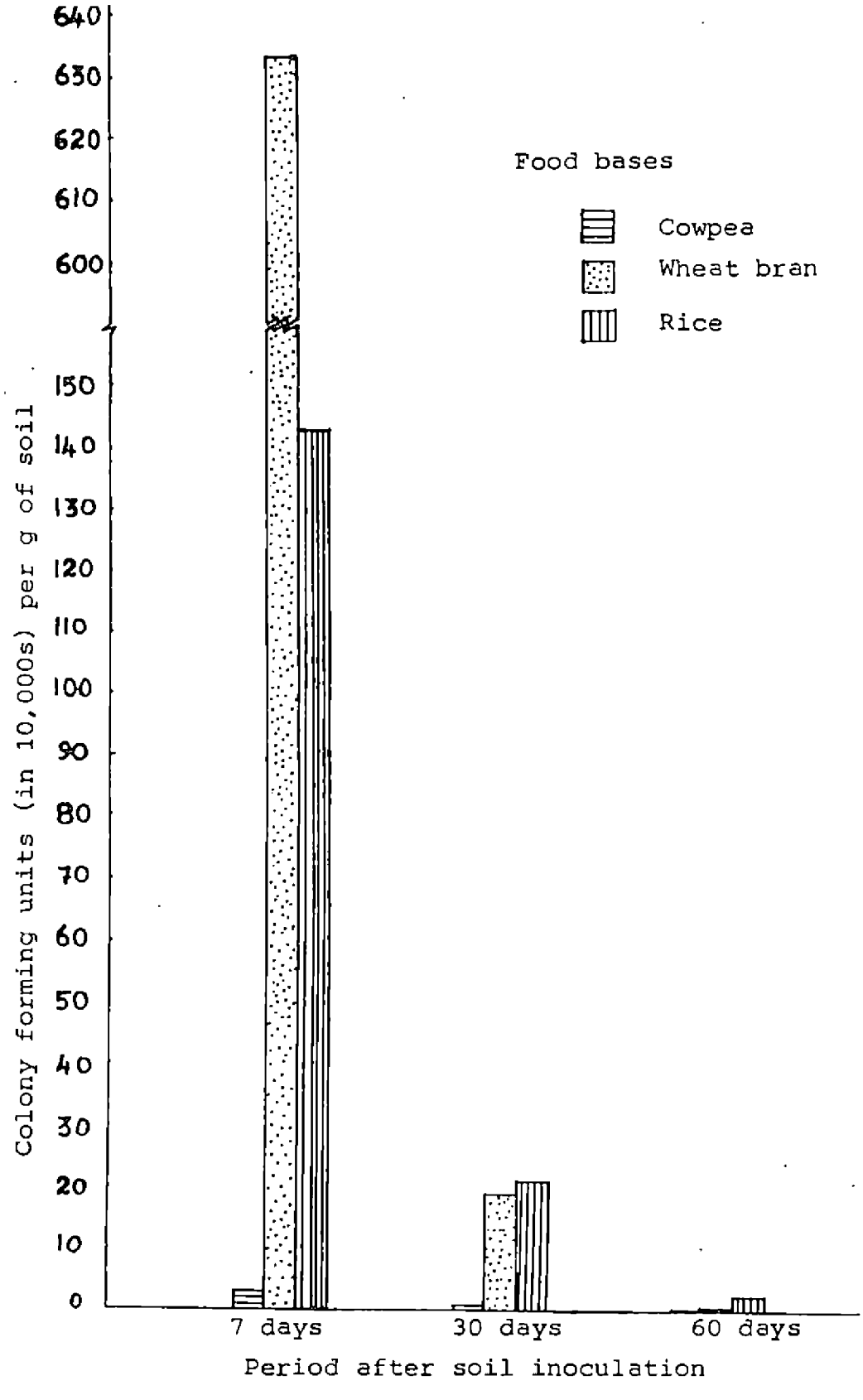


Fig.13. Population of P. simplicissimum at different intervals in ginger rhizosphere amended with food based antagonist.

into soil (Table 9 and Fig.13). A reduction in population count in the rhizosphere soil was observed after 7 days with all the three food based antagonist cultures tried but the reduction was more with wheat bran P. simplicissimum culture. The antagonist grown in cowpea recorded the minimum number of propagules in the rhizosphere soil throughout the period of estimation.

At 7 days of introduction, wheat bran based antagonist was found to be superior to rice and cowpea. But at 30 days of introduction rice based antagonist was found superior to cowpea and was on par with wheat bran. Rice recorded the maximum population count (2.46×10^4 cfu per g of soil) at 60 days after application.

2.2.4. Bacillus subtilis

Rhizosphere of ginger receiving wheat bran based B. subtilis recorded the maximum number of colonies (727.13×10^4 colonies per g of soil) at 7 days of inoculation when compared to rice (421.94×10^4) and rice bran (134.40×10^4) based antagonist cultures (Table 9 and Fig.14). Even though the population count declined with regard to all the three food base antagonist mixtures, the same trend was also noticed at 30 days of inoculation.

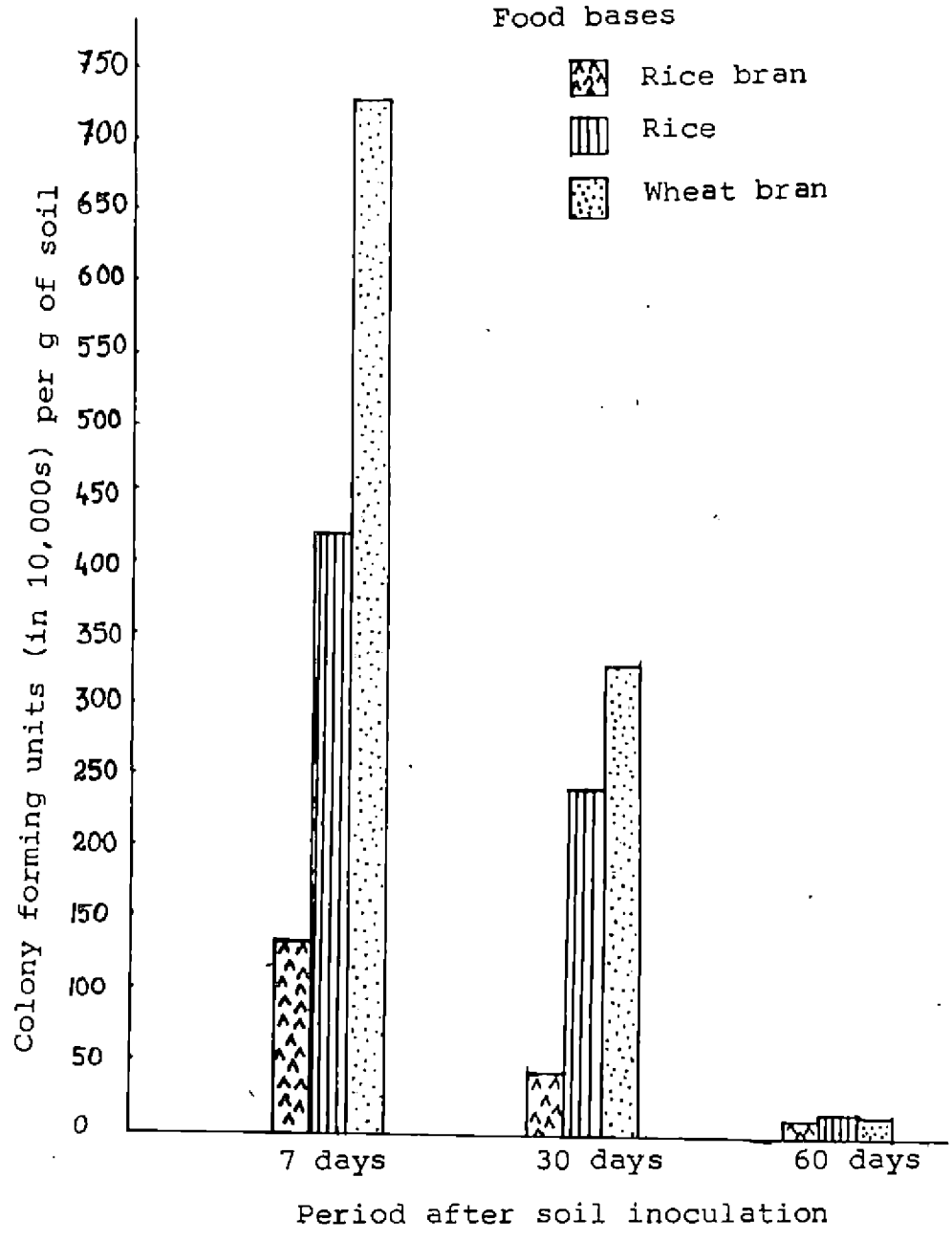


Fig.14. Population of B. subtilis at different intervals in ginger rhizosphere amended with food based antagonist.

Wheat bran was found superior to rice and rice bran based antagonist mixtures up to 30 days of inoculation. But at two months, there was no significant difference between rice and wheat bran-antagonist preparation and rice bran-antagonist preparation recorded the least population count.

2.3. Enumeration of antagonistic microflora in black pepper rhizosphere

2.3.1. *Trichoderma herzianum*

Rhizosphere soil amended with wheat bran based *T. herzianum* harboured maximum number of propagules up to two months of estimation (Table 10 and Fig.15). At 7 days of inoculation population count in the rhizosphere soil amended with wheat bran based antagonist (147.65×10^4) was followed by paddy straw (136.85×10^4 cfu per g of soil) and rice (22×10^4 cfu per g of soil). The population count increased in the rhizosphere soil treated with wheat bran (166.66×10^4 cfu per g of soil) and rice (132.18×10^4 cfu per g of soil) antagonist preparation up to 30 days of inoculation. The rate of increase was more with rice antagonist preparation but the population count declined after 30 days. In the case of paddy straw antagonist preparation, the population count declined after 7 days of introduction. At 30 days and 60 days of applications paddy

Table 10. Colony forming units of *Trichoderma harzianum*, *T. longibrachiatum*, *Penicillium citrinum* and *Bacillus subtilis* in the rhizosphere of black pepper amended with food based antagonist

Antagonists	Food bases	Colony forming units (in 10000s) per g of soil		
		7 days after application	30 days after application	60 days after application
Trichoderma harzianum	Paddy straw	136.85 (4.918)	14.49 (2.673)	4.51 (1.506)
" "	Wheat bran	147.65 (4.994)	166.66 (5.115)	69.36 (4.239)
	Rice	22.10 (3.095)	132.18 (4.884)	63.05 (4.143)
C.D.(0.05)		0.150	0.063	0.097
T. longibrachiatum	Rice bran	22.12 (3.096)	19.95 (2.993)	5.87 (1.769)
"	Rice	25.64 (3.244)	263.08 (5.572)	4.98 (1.605)
	Cowpea	15.56 (2.744)	18.49 (2.917)	1.41 (0.343)
C.D.(0.05)		0.106	0.039	0.064
Penicillium citrinum	Cowpea	220.26 (5.394)	163.14 (5.094)	69.10 (4.235)
" "	Wheat bran	2302.63(7.741)	231.02 (5.442)	89.29 (4.491)
	Rice	656.46 (6.486)	288.21 (5.663)	55.74 (4.026)
C.D.(0.05)		0.038	0.061	0.135
Bacillus subtilis	Rice bran	270.27 (5.599)	114.09 (4.736)	16.25 (2.788)
" "	Rice	542.55 (6.296)	282.05 (5.642)	23.26 (3.146)
	Wheat bran	407.02 (6.008)	166.66 (5.115)	16.95 (2.830)
C.D.(0.05)		0.010	0.030	0.021
Untreated control		0.00	0.00	0.00

Logarithmic transformation was used for analysis. Transformed values are given in parentheses.

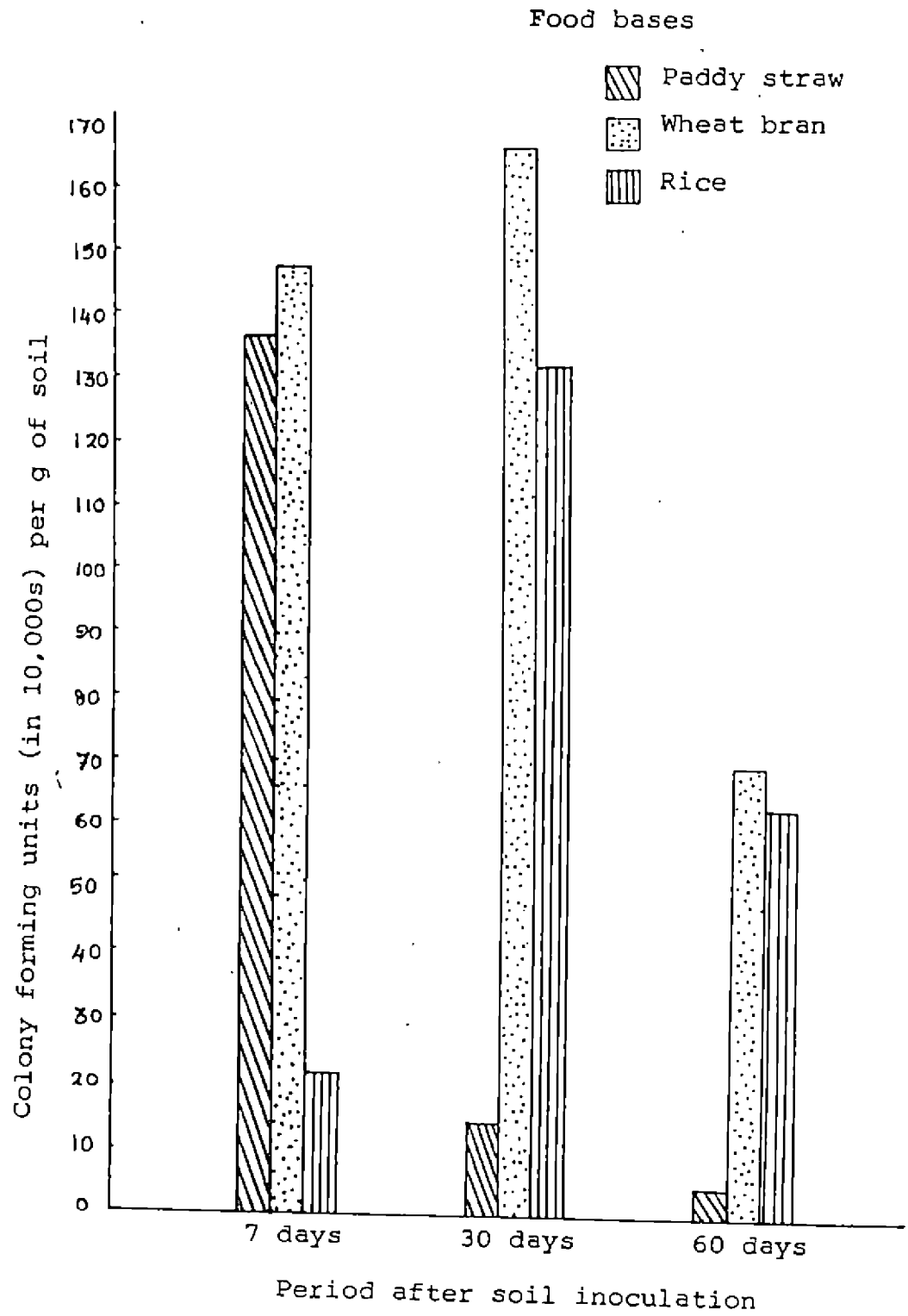


Fig.15. Population of T. harzianum at different intervals in black pepper rhizosphere amended with food based antagonist.

straw based antagonist recorded the minimum population counts. Wheat bran-antagonist preperation was found to be superior to all other food bases tested during the entire period of observation.

2.3.2. Trichoderma longibrachiatum

Rice based antagonist recorded the maximum number of propagules (25.64×10^4 cfu per g) in the rhizosphere soil at 7 days followed by rice bran (22.12×10^4) and cowpea (15.56×10^4). The same trend was also noticed at 30 days of inoculation (Table 10 and Fig.16). In the case of rice, a sudden increase in the population count to 263.08×10^4 cfu per g of soil was observed at 30 days and thereafter the population count declined (4.98×10^4). The population count in the rhizosphere soil amended with cowpea antagonist preparation slightly increased to 30 days of inoculation but declined subsequently. The number of propagules in the rhizosphere soil amended with rice bran based antagonist declined after 7 days but it recorded the maximum population count at 60 days. Minimum count of the population at 60 days was noticed in rhizosphere soil amended with cowpea.

There was significant difference among the treatments. Rice based antagonist was found to be superior during

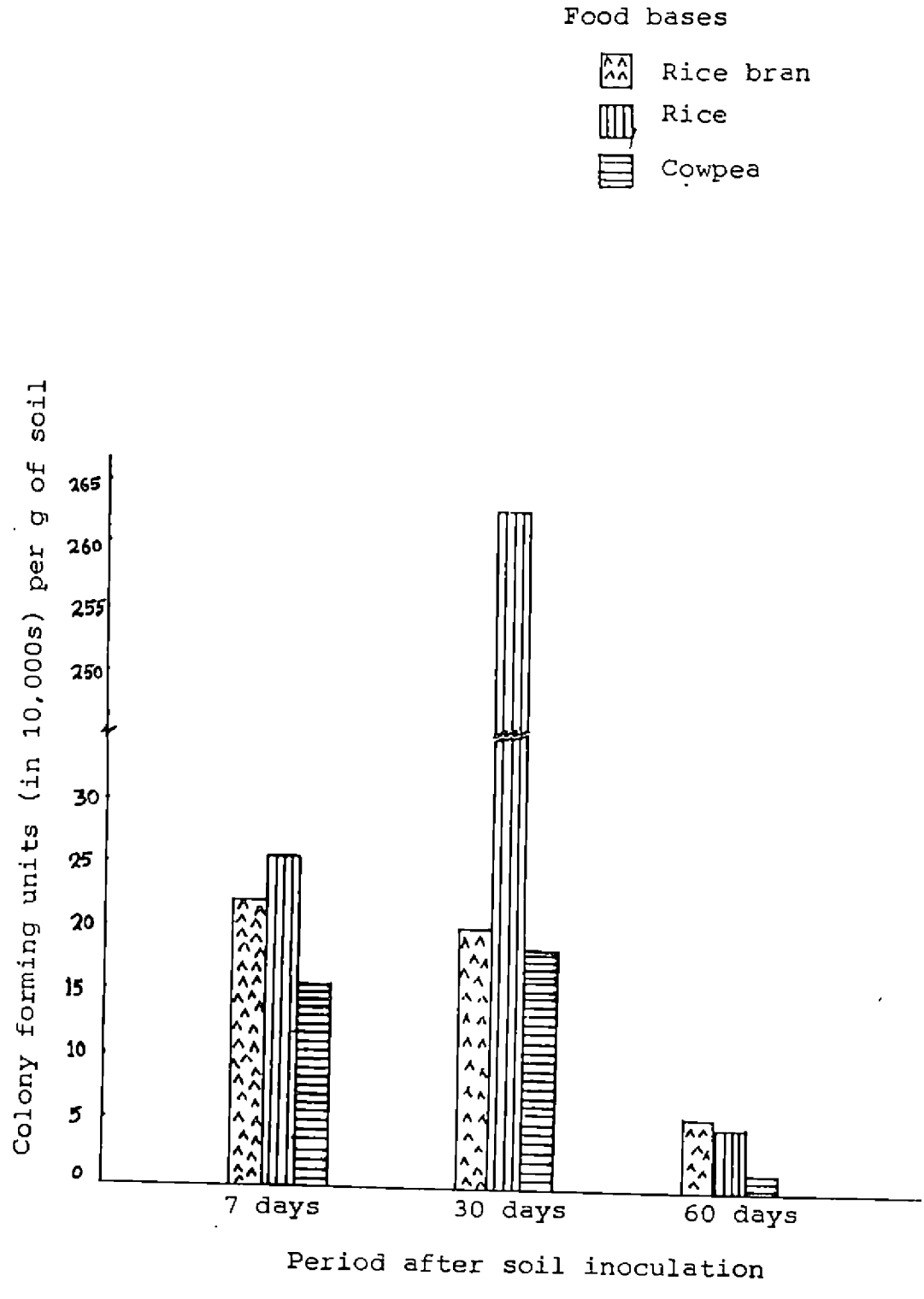


Fig.16. Population of T. longibrachiatum at different intervals in black pepper rhizosphere amended with food based antagonist.

the first two observations though during the last observation rice bran out-yielded to rice and cowpea. Cowpea based antagonist recorded the minimum population count throughout the period of observation.

2.3.3. *Penicillium citrinum*

Among the food bases used for *P. citrinum* wheat bran based antagonist recorded the maximum count at 7 days of inoculation (2302.63 x 10^4 cfu per g of soil) followed by rice (656.64 x 10^4) and cowpea (220.26 x 10^4). The population count in the rhizosphere soil declined with regard to all three food bases during subsequent observations. But at 30 days, rate of decline was more with wheat bran-antagonist preparation. Rice-antagonist preparation recorded the maximum population count at 30 days (Fig. 17).

Wheat bran was found superior to other food bases at 7 days of inoculation but at 30 days rice was found to be the best. At 60 days of inoculation, wheat bran proved to be superior while rice and cowpea were on par.

2.3.4. *Bacillus subtilis*

The maximum population count was recorded in the rhizosphere soil amended with rice *B. subtilis* preparation

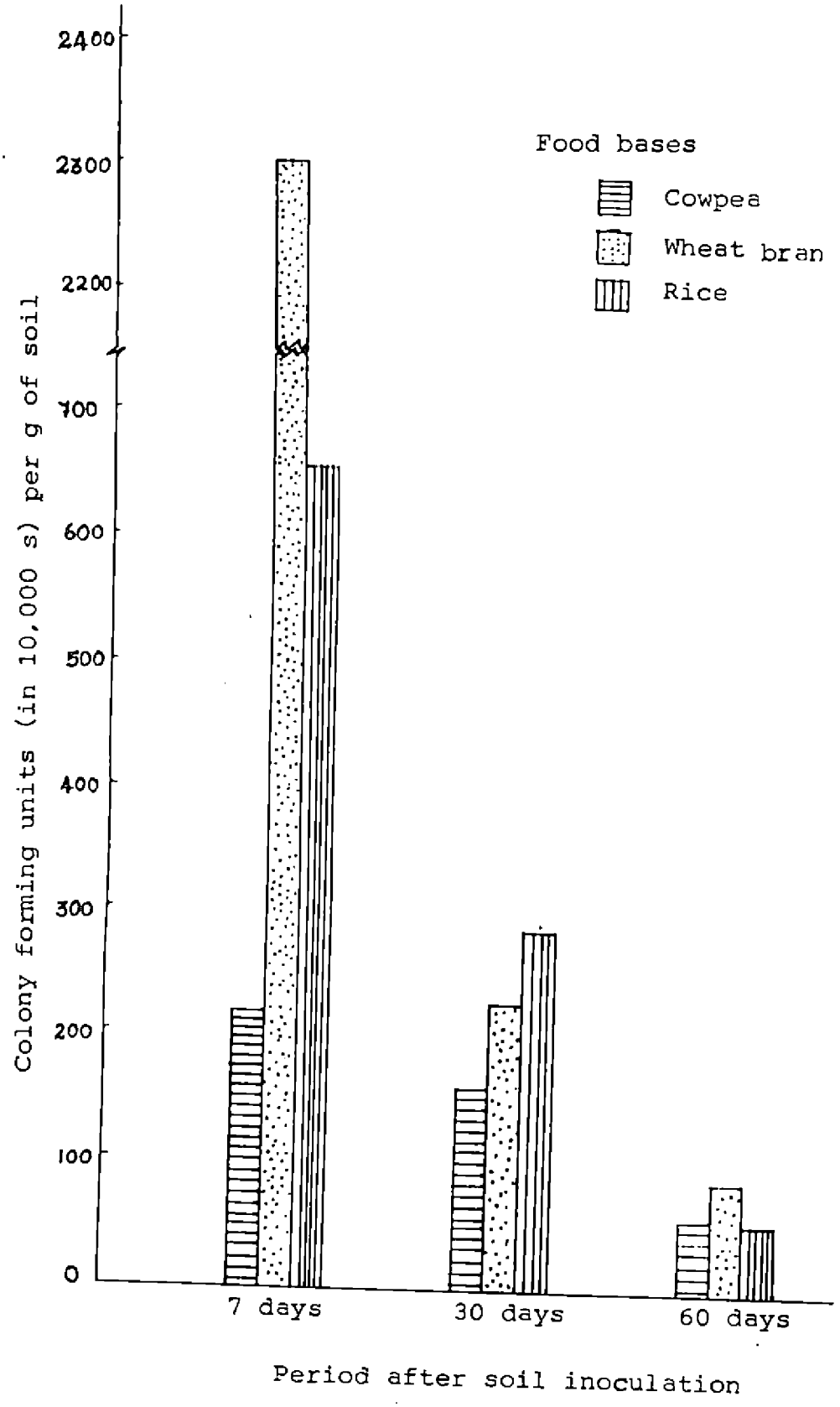


Fig.17. Population of R. citrinum at different intervals in black pepper rhizosphere amended with food based antagonist.

followed by wheat bran and rice bran throughout the period of observation. At 7 days of inoculation, rice-*B. subtilis* preparation yielded 542.55 x 10^4 colonies per g of soil followed by wheat bran (407.02 x 10^4) and rice bran (270.27 x 10^4). The population count declined with regard to all three food bases tried after 7 days of inoculation (Fig.18).

There was significant difference among the treatments and rice based antagonist was found to be superior to wheat bran and rice bran throughout the period of observation.

3. Effect of carrier based antagonists in controlling collar rot and web blight of cowpea caused by *Rhizoctonia solani*, soft rot of ginger caused by *Pythium myriotylum* and quick wilt (foot rot) of black pepper caused by *Phytophthora palmivora*

The four antagonistic microorganisms which were found to be effective in *in vitro* studies against *R. solani* *P. myriotylum* and *P. palmivora* were applied to standing crops of cowpea, ginger and pepper raised in pots as mentioned in Materials and Methods. In all the cases, the plants were inoculated with the concerned pathogen after 7 days of introduction of food based antagonists to soil.

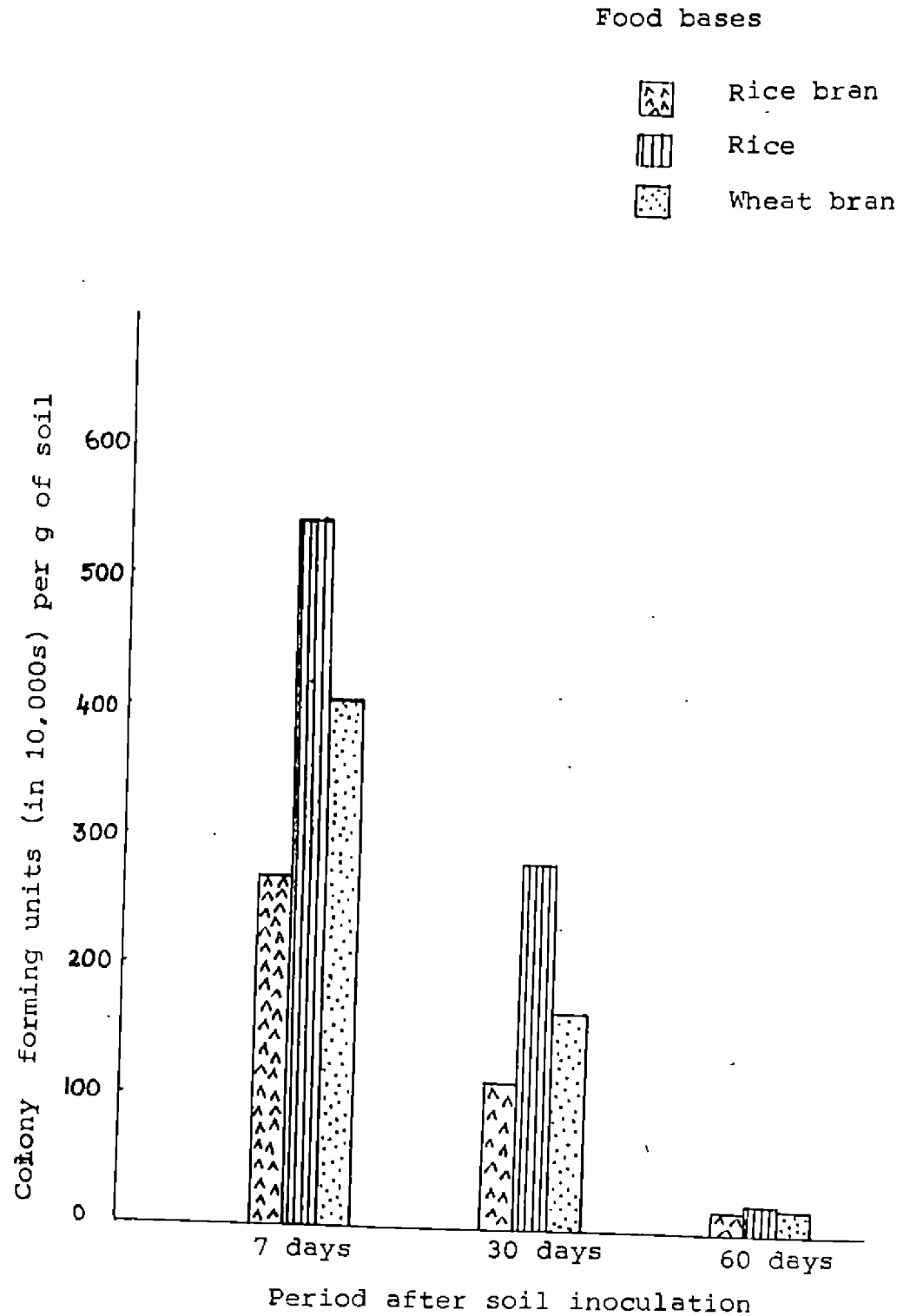


Fig.18. Population of B. subtilis at different intervals in black pepper rhizosphere amended with food based antagonist.

3.1. Collar rot and web blight of cowpea incited by *R. solani*

Four antagonistic microorganisms viz., *T. harzianum* *T. longibrachiatum*, *A. terreus* and *B. subtilis* were applied to standing crop of cowpea. After 7 days of application of antagonist, the plants were inoculated with pure culture of *R. solani*.

The symptom of collar rot first appeared on untreated plants after 7 days of inoculation with the pathogen. Even in control pots no web blight symptoms could be observed during the period of observation. Table 11 summarises the data on the observations.

The disease incidence was 93.33 per cent in control pots which did not receive any antagonistic organism but inoculated with the pathogen. When compared to untreated control, disease incidence was less in plants which received antagonist and on an average 42.78 per cent disease was noticed.

Of the four antagonists tried, *T. longibrachiatum* was found to be most effective in checking down the infection. Overall only 37.75 per cent infection was observed. The lowest infection per cent of 26.67 was observed on plants receiving *T. longibrachiatum* grown in rice.

Table 11. Effect of different antagonists grown in various food bases on disease incidence caused by *Rhizoctonia solani* in cowpea

Treatment	No.of plants	No.of plants infected	Percentage of infected plants
Aspergillus terreus grown in rice	30	20	66.67
" " grown in wheat bran	30	10	33.33
" " grown in cowpea	30	11	36.67
Trichoderma longibrachiatum grown in rice	30	8	26.67
" " grown in rice bran	30	12	40.00
" " grown in cowpea	30	14	46.67
T. harzianum grown in wheat bran	30	11	36.67
" " grown in rice	30	15	50.00
" " grown in paddy straw	30	16	53.33
Bacillus subtilis grown in wheat bran	30	14	46.67
" " grown in rice bran	30	9	30.00
" " grown in rice	30	14	46.67
Untreated control	30	28	93.33

B. subtilis was also found to be effective in checking down the infection. The percentage of infection recorded was 41.11. Among the three food bases tried, rice bran - *B. subtilis* preparation recorded a minimum of 30 per cent of infection.

A. terreus and *T. harzianum* also proved to be effective in checking down disease incidence when compared to control. In *A. terreus* treated plants, average infection was 45.55 per cent and the least infection (33.33 per cent) was noticed in plants treated with wheat bran - *A terreus* preperation. *T. harzianum* also showed the same trend with 46.67 per cent disease incidence. Wheat bran-*T. harzianum* preparation recorded the minimum disease incidence (36.67 per cent).

3.2. Soft rot of ginger incited by *P. myriotylum*

The disease symptom was first observed on untreated plants on the 7th day of inoculation with the pathogen. Subsequently infection was noted in treated plants also. The data on disease incidence in different treatments are presented in Table 12.

Both *P. simplicissimum* and *T. longibrachiatum* were found to be effective in checking down the infection.

Table 12. Effect of different antagonists grown in various food bases on disease incidence caused by *Pythium myriotylum* in ginger

Treatment	No.of plants	No.of plants infected	Percentage of infected plants
Penicillium simplicissimum grown in wheat bran	15	2	13.33
" " grown in rice	15	0	0.00
" " grown in cowpea	15	2	13.33
Trichoderma longibrachiatum grown in cowpea	15	3	20.00
" " grown in rice	15	0	0.00
" " grown in rice bran	15	5	33.33
T. harzianum grown in wheat bran	15	15	100.00
" grown in paddy straw	15	15	100.00
" grown in rice	15	13	86.67
Bacillus subtilis grown in rice bran	15	14	93.33
" grown in wheat bran	15	11	73.34
" grown in rice	15	15	100.00
Untreated control	15	15	100.00

Out of 45 plants treated with food based P. simplicissimum, only 4 plants were found to be infected by the pathogen, i.e., 8.89 per cent. Rice based P. simplicissimum was found to be the best treatment and no disease incidence was noted in this case. In the case of T. longibrachiatum, 17.77 per cent disease incidence was observed and plants receiving T. longibrachiatum grown in rice did not show any symptom of soft rot.

In B. subtilis and T. harzianum treated plants infection per cents were 88.89 and 95.96 respectively. In control, cent per cent infection was noticed.

Of the four antagonists tried against P. myriotylum, P. simplicissimum and T. longibrachiatum were found to be effective in checking the infection. B. subtilis and T. harzianum were not at all effective in suppressing the disease symptoms.

3.3. Quick wilt (foot rot) of black pepper incited by P. palmivora

The attempt to produce symptoms of quick wilt in potted plants by artificial inoculation did not succeed. This may be due to lack of congenial environmental conditions for infection during the period of study. Therefore the effect of the antagonists on the disease were not obtained.

Discussion

DISCUSSION

Biocontrol agents represent a living dynamic system. The development of a formulation containing a viable microorganism is one of the most difficult challenges in biological control. In the attempts to develop effective systems of growth and delivery of antagonists, scientists have proposed the possibility of using different carrier based formulations. The fact that direct soil augmentation with biocontrol agents has a greater impact on soil-borne plant pathogens, when the agent is introduced with a proper food base rather than without, has provided an impetus to search for new food base sources for mass multiplication. A variety of materials such as cereal grains, agricultural bye-products and peat has been put to evaluation in *in vitro* by several workers (Turner and Tribe, 1975; Ahmed and Tribe, 1977; Henis *et al.*, 1978; 1979; Sivan *et al.*, 1984; Padmanaban and Alexander, 1986).

In the present study efficacy of different food bases was determined by inoculating the same with the selected antagonist and estimating the population dynamics at different intervals of incubation. The growth pattern and

survival ability of six antagonists in different food bases are discussed.

1. Population dynamics of antagonist in food bases

1.1. Rice

Rice was found to be the best medium for growth and sporulation of T. harzianum (Table 2 and Fig.1). The growth of T. longibrachiatum in rice was better than in all other food bases except rice bran (Table 3 and Fig.2). For A. terreus and P. citrinum, rice was found to be a promising food base based on the colony count at 15 days of incubation (Table 4). Rice recorded the maximum number of colony forming units of P. simplicissimum (Table 6 and Fig.5) and B. subtilis the minimum number (Table 7 and Fig.6) after two weeks of incubation. For B. subtilis the maximum number of viable colonies was recorded in rice up to 135 days of incubation when compared to other food bases.

Among the six antagonists tried, P. simplicissimum recorded the maximum count and T. longibrachiatum minimum count in rice. The low plate count of T. longibrachiatum may be attributed to its lesser ability of sporulation when compared to the other test fungi. The viable count of all the organisms declined after 15 days of incubation except T. harzianum where it increased up to 75 days and thereafter

it declined. The main organic fraction in rice is starch which is an easily utilisable form of substrate for organisms and this may be the reason for the decline of population noted after prolonged incubation. In general milled rice was found to be a promising growth medium for almost all the isolates tested.

This was the first attempt to find out the suitability of rice as a food base for the antagonists tested in the present study. Milled rice has been successfully used by Turner and Tribe (1975) and Ahmed and Tribe (1977) for mass multiplication of Coniothyrium minitans. This information together with the results of the present study indicates the suitability of milled rice as a food base for a variety of antagonists.

1.2. Wheat bran

The growth and multiplication of all the isolates in the food base wheat bran was quite promising except T. longibrachiatum. Wheat bran was found to be a good medium for the growth of T. harzianum (Table 2 and Fig.1). Many workers reported the use of wheat bran as a growth medium for T. harzianum. Hadar et al. (1979) found that wheat bran was the best medium for growth and sporulation of T. harzianum. Gangadharan and Jeyarajan (1988) obtained good growth of

T. harzianum and T. viride in wheat bran. The population count recorded were 22.0 x 10^6 and 21.9 x 10^6 cfu per g of substrate respectively. In addition to this, reports on the suitability of wheat bran as a major component in growth medium have appeared recently (Elad et al., 1980; Lewis and Papavizas, 1984; Sivan et al., 1984; Chang et al., 1986; Mukhopadyay et al., 1986).

In the case of T. longibrachiatum, growth was slow in the initial steges and profuse mycelial growth was observed after two weeks of incubation (Table 3 and Fig.2). The growth of A. terreus (Table 4 and Fig.3) and P. citrinum (Table 5 and Fig.4) was best in wheat bran based on the colony count obtained at 15 days of incubation. Wheat bran was found to be a good medium for P. simplicissimum (Table 6 and Fig.5) and B. subtilis (Table 7 and Fig.6). The viable counts of all the organisms declined after 15 days of incubation except that of Trichoderma spp. where it increased up to 45 days and then declined. P. citrinum recorded the maximum number of colony forming units in wheat bran and T. longibrachiatum the least after two weeks of incubation. The sporulating ability of T. longibrachiatum is low when compared to other fungal antagonists tried and this may be the reason for low count. High population count

of P. citrinum indicates that wheat bran may be best suited for growth of this fungus.

1.3. Paddy straw

Paddy straw was found to be a good medium for growth of T. harzianum only next to wheat bran and rice (Table 2 and Fig.1). Even though the population count declined after two weeks of incubation, it harboured the maximum number of viable propagules at 105 days of incubation when compared to other food bases. Colony counts recorded at different intervals revealed that the growth of T. longibrachiatum (Table 3 and Fig.2) and P. simplicissimum (Table 6 and Fig.5) was poor in paddy straw when compared to other test fungi. In the case of A. terreus (Table 4 and Fig.3). paddy straw recorded a moderately good number of colony forming units at 15 days and the population count increased up to 45 days and thereafter declined. In the initial stages of incubation, the growth of P. citrinum (Table 5 and Fig.4) and B. subtilis (Table 7 and Fig.6) was slow but thereafter an increase was observed up to 75 days and 45 days respectively. The survival ability of all the organisms tried except P. simplicissimum was better in paddy straw. The major organic fraction in paddy straw is cellulose and this component is comperatively resistant

to microbial degradation. The residual carbon present in the substrate may provide the source for subsequent growth and survival. Among the antagonists tried, *A. terreus* recorded the maximum population count in paddy straw and *T. longibrachiatum* the minimum count.

Gangadharan and Jeyarajan (1988) observed poor growth of *T. viride* and *T. harzianum* in paddy straw when compared to all other substrates tried. They obtained a population count of 10.1×10^6 and 11×10^6 cfu per g of substrate for *T. viride* and *T. harzianum* respectively. Wheat straw and cotton straw were used as substrates for growth of *T. harzianum* by Sivan *et al.* (1984). After 7 days of incubation, they obtained a population count of 490×10^6 and 210×10^6 cfu per g of substrate respectively. In the present study, the population count recorded in paddy straw after two weeks of incubation was 473.43×10^6 cfu per g substrate. Paddy straw was found to be the good food base for the survival of the antagonists for a prolonged period of incubation especially in the case of *T. harzianum*, *A. terreus*, *P. citrinum* and *B. subtilis*.

1.4. Rice bran

Among the seven food bases tried for *T. harzianum* rice bran was found only better than soil + dried cowdung

and forest soil (Table 2 and Fig.1). Rice bran proved to be the best medium for T. longibrachiatum as observed from the population count at 15 days of incubation (Table 3 and Fig.2). Rice bran also recorded maximum viable count of the test fungus throughout the period of observation. For B. subtilis rice bran was found to be a promising growth medium (Table 7 and Fig.6). Rice bran was found to harbour a moderate number of viable propagules of P. citrinum (Table 5 and Fig.4) and A. terreus (Table 4 and Fig.3). With regard to rice bran, B. subtilis recorded the maximum number of colonies and P. simplicissimum (Table 6 and Fig.5) the least. Rice bran was not a good medium for mass multiplication of T. viride and T. harzianum (Gangadharan and Jeyarajan, 1988). This is in agreement with the present study.

1.5. Cowpea

The growth and sporulation of T. harzianum in cowpea was better than in rice bran, forest soil and soil + cowdung at 15 days of incubation (Table 2 and Fig.1). For T. longibrachiatum, rice bran and rice were followed by cowpea in the efficacy as a food base which recorded a population count of 125 x 10^6 cfu per g substrate (Table 3 and Fig.2). Cowpea was found to be a good medium for growth

and sporulation of A. terreus (Table 4 and Fig.3) and P. citrinum (Table 5 and Fig.4) at 15 days of incubation. The viable counts declined after two weeks of incubation with respect to the antagonists T. harzianum, T. longibrachiatum and P. citrinum. No count was obtained from 75th day onwards. In the case of A. terreus, no viable count was recorded from 45th day of incubation. The growth of P. simplicissimum increased up to 45 days and thereafter it declined (Table 6 and Fig.5). The population count of B. subtilis declined after two weeks of incubation (Table 7 and Fig.6). The major organic fraction in cowpea is protein which is an easily utilisable form and since the residual carbon source is negligible, the survival ability of the antagonists might have lost. P. citrinum recorded the maximum number of colony forming units in cowpea and T. longibrachiatum the minimum count.

There are no earlier studies on the use of cowpea as a growth medium for fungal or bacterial antagonists. The present study revealed that cowpea was not a good medium for any of the antagonist tested compared to other food bases under investigation.

1.6. Forest soil

The growth of antagonists in forest soil was poor. Among the antagonists tried, T. harzianum recorded the maximum

count (Table 2 and Fig.1) and B. subtilis the minimum count (Table 7 and Fig.6) at 15 days of incubation. The poor growth may be attributed to the low organic content when compared with other substrates used. This result confirms the earlier findings of Gangadharan and Jeyarajan (1988). They observed poor growth of T. harzianum (11.0×10^6 cfu) and T. viride (10.0×10^6 cfu) in peat soil. The decline of population during prolonged incubation period was not so pronounced in the case of forest soil. Apart from this, the population of B. subtilis was on an increasing trend from the initial period upto 165 days of incubation. Since the organic matter in forest soil is in the form of humus which is domineted by cellulose and lignin, it is presumable that there will be enough source of carbon to support the growth of the microorganism for longer periods. Iswaran et al. (1969) and Rao (1977) have successfully used peat-like material available in India for mass multiplication of Rhizobium, a nitrogen fixing bacterium. Even though forest soil did not yield high colony count throughout the period of incubation, the survival ability of antagonists was better in this medium when compared to other food bases tried.

1.7. Soil + dried cowdung (1:1)

In soil + cowdung also, the growth of antagonists was poor. Gangadharan and Jeyarajan (1988) tested the efficacy of dried farm yard manure as a substrate for T. harzianum and T. viride. They observed poor growth of these organisms in this medium. In the present study soil + cowdung was found to be a poor medium for growth of T. harzianum. The growth of P. citrinum (Table 5 and Fig.4) and B. subtilis (Table 7 and Fig.6) was better in soil + cowdung than in forest soil. This may be due to the added effect of cowdung present in the medium. In addition to carbon, cowdung provide nutrients which are easily utilised by microorganisms. Lakshmi et al. (1977) reported that farm yard manure (FYM) + soil, FYM alone or FYM + charcoal supported the survival of Azospirillum upto 31 weeks.

Based on the growth habits of the different isolates in the various media, three promising food bases were selected for each antagonist for further evaluation. Rice was selected as a common medium for all six antagonists while wheat bran was used for all except T. longibrachiatum. Rice bran was chosen for T. longibrachiatum and B. subtilis. Cowpea was selected for T. longibrachiatum, A. terreus,

P. citrinum and *P. simplicissimum*. Paddy straw was used as a food base only for *T. harzianum*.

2. Pot culture experiment

The three food bases selected for each antagonist based on the growth and survival were tried against the soil borne pathogens viz., *Rhizoctonia*, *Pythium* and *Phytophthora*. The efficacy of food bases were evaluated in pot culture based on the population size of the introduced antagonist in the rhizosphere of crop plants and the percentage of disease incidence.

2.1. Enumeration of antagonists in crop rhizosphere

The population of introduced antagonist in the rhizosphere was estimated at one week, one month and two months of introduction by serial dilution and plate count technique.

Among the three food bases tried for *T. harzianum*, wheat bran preparation was found to harbour the maximum number of propagules in the rhizosphere of all the three crop plants viz., cowpea, ginger and pepper by the first week of inoculation (Table 8, 9 and 10). Though there was a gradual reduction in population of antagonist in the rhizosphere of cowpea and ginger, a slight increase was

noted in case of pepper after one week. A similar trend was also noticed in the pepper rhizosphere amended with rice - T. harzianum preparation. Among the three crops, ginger was found to have a pronounced rhizosphere effect in the proliferation of wheat bran based antagonist in the rhizosphere. But a quantitative estimation attempted by Lewis and Papavizas (1984) in non-rhizosphere soil has revealed the possibility of multiplication of carrier based antagonists introduced into the soil. They have recorded an increase from 10^4 to 5×10^7 cfu per g of soil by the third week of incubation and thereafter the population was found to stabilize followed by a decline in viable count and reaching 10^4 cfu per g of soil.

The growth of T. longibrachiatum was favoured in the rhizosphere of ginger and cowpea amended with rice bran - T. longibrachiatum preparation when compared with other food bases (Table 8, 9 and 10). The antagonist grown in rice was found to perform better in ginger and pepper rhizosphere soil as evidenced by the estimated population counts at one month of introduction.

A. terreus was tried only against R. solani. Based on the estimation, wheat bran was found to be the most suitable food base with regard to survival of antagonist in cowpea rhizosphere (Table 8 and Fig.9). The fact that a

fairly high population of the antagonist recorded at different intervals indicates that the other two food bases can also support the growth of the antagonist in rhizosphere soil of cowpea.

Wheat bran was found to be a better substrate in maintaining the population of P. simplicissimum in the rhizosphere of ginger (Table 9 and Fig.13). This fungus was only tried against Pythium.

The antagonist grown in wheat bran survived better in the rhizosphere of pepper after introduction into soil when compared with other two food bases tried for P. citrinum (Table 10 and Fig.17).

The only bacterial antagonist used in this study was tried against all three pathogens. The food base rice was found to exhibit a pronounced effect on the multiplication of the antagonist in the rhizosphere of cowpea and pepper as indicated by higher population counts recorded up to one month of introduction. But contrary to this, rate of multiplication of B. subtilis in ginger rhizosphere amended with wheat bran - B.subtilis preparation, was more when compared to rice bran - B. subtilis and rice - B. subtilis preparation (Table 8, 9 and 10).

The data recorded on population size of the antegonist in the rhizosphere soil of cowpea, ginger and pepper amended with carrier based antagonist showed a gradual decline in the population. Exceptions are rice - T. longibrachiatum and cowpea - T. longibriachiatum preparation in ginger rhizosphere and wheat bran, rice - T. harzianum, rice-and cowpea - T. longibrachiatum in pepper rhizosphere. The decline in population count indicated that the antagonist failed to proliferate in the rhizosphere. This may be due to many environmental factors such as competition for food by native soil microbes and the antagonistic effect of the microbial flora in the soil.

On a perusal of the data of in vitro evaluation on the growth and survival of antagonists in different food bases and the population size estimated after introduction of antagonist in crop rhizosphere indicated no relation between the population count in the food base and survival ability of antegonist in rhizosphere soil. This variation may be attributed to the prevailing biotic and abiotic environment which may enhance or inhibit the growth of the particular antegonist.

The present study on the estimation of antagonistic population in the rhizosphere of three different crop plants

revealed that there exists difference in stimulation of the antagonist in the rhizosphere not only with regard to carrier material but also crop plants. The influence of different food bases on the growth of the antagonist may be explained in terms of nutrient content of food bases. Wright (1955) and (1956) has already found that appropriate food bases are essential for the production of antibiotics. The presence of nutrients within the preparation probably favours the establishment and activity of the antagonist in the soil giving it some advantages over the soil microorganisms (Mangenot and Diem, 1979). The difference with regard to crop plants may be attributed to the rhizosphere effect which indicates the overall influence of plant roots on soil microorganisms. Several factors such as soil type, pH, moisture, temperature and age and conditions of plants are known to influence the rhizosphere effect. One of the most important factors responsible for rhizosphere effect is a great variety of organic substances available at the root region by way of exudates from roots which directly or indirectly influence the quality and quantity of microorganisms in the root region. The substances exuded by plant roots include amino acids, sugars, organic acids, vitamins, nucleotides and many other unidentified substances. The nature and amount of substances thus exuded

are dependent on the species of the plant, age and environmental conditions under which they grow (Rao, 1977).

2.2. Control of collar rot of cowpea, soft rot of ginger and quickwilt of pepper using carrier based antagonists

Among the four antagonists A. terreus, T. harzianum, T. longibrachiatum and B. subtilis tried against R. solani, T. longibrachiatum was found to be the best antagonist in checking down the collar rot symptoms in cowpea. Several studies have been conducted in India and abroad to assess the potential of different species of Trichoderma against the pathogen R. solani and the results on the successful control of diseases caused in many crop plants are available. Majority of the reports highlight the efficacy of T. harzianum as a successful antagonist against Rhizoctonia (Akthar, 1977; Hadar et al., 1979; Henis et al., 1979; Elad et al., 1980a; 1980b; Lewis and Papavizas, 1980; Lifshitz et al., 1985; Elad et al., 1986; Cole and Zvenyika, 1988). Many workers have used wheat bran as a food base for the introduction of T. harzianum to soil (Hadar et al., 1979; Henis et al. 1978; 1979; Elad et al., 1986). Elad et al. (1980a) obtained successful control of damping-off of beans caused by R.solani by using wheatbran + sawdust mixture as a carrier for T. harzianum. But the present study clearly showed the

superiority of T. longibrachiatum over T. harzianum in checking the disease. Out of the three food bases used for T. longibrachiatum rice based preparation recorded the minimum disease severity indicating the efficacy of rice as a food base for the antagonist T. longibrachiatum.

There was a reduction in disease severity in B. subtilis pots also when compared to other two antagonists viz., A. terreus and T. harzianum. Similar reports on the control of many crop diseases incited by R. solani using B. subtilis are available. Damping off of radish (Olsen and Baker, 1968), damping-off of pepper (Broadbent et al., 1971) and wheat rot disease (Merriman et al., 1974) are some of the Rhizoctonia diseases successfully controlled by the antagonist B. subtilis. Among the three food bases tried in the present study, rice bran based antagonist exhibited the maximum efficacy in checking down the collar rot symptom. A. terreus grown in wheat bran and cowpea also showed a fairly good amount of disease suppression. However the same antagonist multiplied in rice recorded the maximum disease severity among all the treatments.

A comparison of the data recorded on the number of colony forming units with the data on the percentage incidence of disease indicated that a direct correlation

exists only in the case of A. terreus wherein wheat bran based antagonist recorded the maximum population density in the cowpea rhizosphere as well as minimum disease severity. However the antagonists T. harzianum, T. longibrachiatum and B. subtilis did not show any direct correlation.

Among the four antagonists viz., T. harzianum, T. longibrachiatum, P. simplicissimum and B. subtilis tried against Pythium inciting soft rot of ginger, P. simplicissimum and T. longibrachiatum were found to be the most promising antagonists. There are no earlier studies on the use of P. simplicissimum and T. longibrachiatum as a biocontrol agent of Pythium myriotylum. Many workers obtained control of Pythium infection in various crops with Trichoderma spp. (Wright, 1956; Liu and Vaughan, 1965; Fajola and Alasoadura, 1975; Yehia et al., 1981; Padmanaban and Alexander, 1984; 1986; 1987; Sivan et al., 1984; Mukhopadhyay and Chandra, 1986; Lifshitz et al., 1986a and Ahmad and Baker, 1988). Sivan et al. (1984) and Mukhopadhyay and Chandra (1986) obtained control of Pythium damping off when carrier based T. harzianum was added to soil. A comparison of the three food bases used to culture the antagonist inoculum revealed the efficacy of rice as a growth medium over the other food bases tried fof P. simplicissimum (wheat bran and cowpea) and T. longibrachiatum (rice bran and cowpea). While no disease symptoms could be

observed in any of the plants treated with rice based P. simplicissimum inoculum, wheat bran and cowpea based antagonist recorded a same percentage of disease incidence (13.33). T. longibrachiatum was also found to give a reasonable control but T. harzianum and B. subtilis did not show any profound effect in checking down the disease.

The superiority of rice as a growth medium for T. longibrachiatum over the other substrates was also evidenced by the complete suppression of disease symptoms in potted plants. The only bacterial antagonist tried did not show any effect in checking down the pathogen. Though a direct correlation between the number of colony forming units of antagonist present in the rhizosphere and the disease severity could not be observed during the early days of inoculation, the population estimate done one month after application indicated that the rhizosphere amended with rice based antagonists harboured the maximum number of propagules. The population size during the first week of application was more in rice bran based T. longibrachiatum treatments and wheat bran based P. simplicissimum treatments. But a faster rate of decline in population was noted in these treatments compared to treatments which received rice based antagonist.

Efficacy of different food based antegonists in the control of quickwilt disease of pepper was not possible from the present study due to the failure in inducing disease symptoms artificially. This may be due to the prevelence of unfavourable environmental conditions for the development of disease during the period of study. Rhizosphere soil amended with food base - P. citrinum preparation harboured good number of viable propagules at one week after introduction to soil. Among the three food bases tried, wheatbran - P. citrinum preparation yielded meximum number of viable propagules in rhizosphere soil.

Though many workers have reported that reduction in the activity of a pathogen is often correlated with an increase in the population of the antagonist as assessed in rhizosphere soil samples (Papavizas and Davey, 1960; Zentmyer, 1963; Vruggink, 1970; Vojinovic, 1973; Atkinson et al., 1975) no such correlation was observed in the present study. This may be attributed to the inherent drawback of the dilution plate count technique which often gives an over estimation of viable count of profusely sporulating fungus than those which are poor spore producers such as T. longibrachiatum as observed in the present study.

An overall review of the data on the growth of antagonist in different food bases, establishment of introduced antagonist in the rhizosphere of crop plants and the effect of introduced antagonist on disease incidence revealed that the treatment responses are quite variable. The antagonistic activity of microorganisms in soil is influenced by the type of the antagonist, host and existing environmental conditions. T. longibrachiatum grown in milled rice was found to be the best combination in reducing the disease incidence both in the case of collar rot of cowpea caused by R. solani and soft rot of ginger caused by P. myriotylum. Apart from this isolate, P. simplicissimum grown in rice was found to be equally good in controlling soft rot. Besides rice, cowpea and wheat bran were also found promising as a growth medium for P. simplicissimum. B. subtilis - wheat bran preparation and T. harzianum - wheat bran preparation were also found to be effective in reducing disease incidence in cowpea.

Summary

SUMMARY

Techniques for mass multiplication and production of antagonistic microflora recently isolated from the forest soils of Kerala for the biocontrol of soil-borne pathogens viz., Rhizoctonia, Pythium and Phytophthora were investigated. The antagonists used were Trichoderma harzianum, T. longibrachiatum, Aspergillus terreus, Penicillium citrinum, P. simplicissimum and Bacillus subtilis. The food bases tried were rice, wheat bran, paddy straw, rice bran, cowpea, forest soil and soil + dried cowdung. The growth and survival of the antagonist in various food bases were estimated by in vitro evaluation. A pot culture experiment was laid out during the period from March to September, 1989 at the College of Horticulture, Vellanikkara to assess the population dynamics of the introduced antagonist in the rhizosphere of crop plants and to find out the effect of carrier based antagonists in controlling collar rot of cowpea caused by Rhizoctonia solani, soft rot of ginger caused by Pythium myriotylum and quick wilt (foot rot) of black pepper caused by Phytophthora palmivora.

A. Growth of antagonists in various food bases

1. Milled rice was found to be a promising growth medium for all the isolates tested.

2. Wheat bran was also found equally good for all the isolates except for T. longibrachiatum.

3. Rice bran was found to encourage the growth of T. longibrachiatum as well as B. subtilis.

4. Good growth of A. terreus and P. citrinum and moderate growth of T. longibrachiatum and P. simplicissimum were recorded with cowpea as a food base.

5. In general paddy straw, forest soil and soil + cowdung were found to be poor substrates compared to others. However, paddy straw was found to be a good food base for the survival of antagonists for a prolonged period of incubation especially for T. harzianum, A. terreus, P. citrinum and B. subtilis

B. Population dynamics of introduced antagonists in rhizosphere of crop plants

1. Trichoderma harzianum wheat bran preparation was found to harbour the maximum number of propagules in the rhizosphere of ginger, cowpea and pepper up to 30 days of soil inoculation. At 60th day of introduction, rice based antagonist recorded the maximum number of viable propagules in cowpea rhizosphere and wheat bran based antagonist in ginger and pepper rhizospheres.

2. The survival ability of T. longibrachiatum grown in rice bran was better in the rhizosphere of ginger and cowpea after introduction into soil. In pepper rhizosphere, T. longibrachiatum grown in rice survived better when compared to rice bran and cowpea antagonist preparations.

3. Wheat bran was found to be the most suitable food base for the survival of A. terreus in cowpea rhizosphere throughout the period of observation.

4. The rhizosphere of ginger amended with wheat bran based P. simplicissimum harboured the maximum number of viable propagules up to 30 days while rice based P. simplicissimum recorded the maximum number of viable propagules at 60 days of introduction.

5. P. citrinum grown in wheat bran survived better in the rhizosphere of pepper when compared with other food based antagonist.

6. Rice was found to be the best food base for the survival of B. subtilis in the rhizosphere of cowpea and pepper up to 30 days of introduction. But after two months of soil inoculation, rice bran based B. subtilis recorded the maximum number of viable propagules in cowpea rhizosphere.

7. Survival of B. subtilis was better in ginger rhizosphere inoculated with wheat bran B. subtilis preparation.

8. A decline in population count of carrier based antagonist was observed in the rhizosphere of ginger, cowpea and pepper after a week of introduction into soil. But contrary to this ginger rhizosphere amended with rice T. longibrachiatum and cowpea T. longibrachiatum preparation and pepper rhizosphere with wheat bran T. harzianum, rice T. harzianum, rice T. longibrachiatum and cowpea T. longibrachiatum preparation showed an increase in the number of viable propagules.

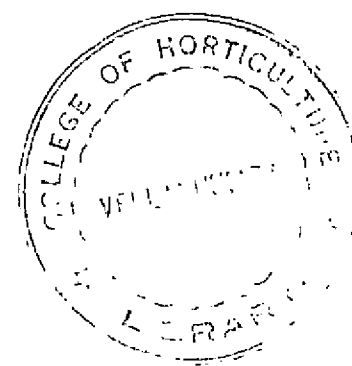
C. Control of collar rot of cowpea, soft rot of ginger and quick wilt of pepper using carrier based antagonists

1. Trichoderma longibrachiatum cultured in rice was found to be the most effective antagonist in checking down collar rot of cowpea followed by rice bran based B. subtilis and wheat bran based Aspergillus terreus.

2. Rice based P. simpliciasimum and T.longibrachiatum were effective in suppressing soft rot symptoms in ginger.

3. Efficacy of antagonists based on the disease suppression could not be assessed in the case of pepper crop since development of symptoms by artificial inoculation was not successful.

4. No correlation between the rhizosphere population of antagonists and disease severity could be noticed in most of the organisms tested except A. terreaus with cowpea and T. longibrachiatum with ginger.



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*Originals not seen

Appendices

Appendix. 1. Analysis of variance for growth and survival of T. harzianum in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	6.315**	12.473**	88.283**	6.184**	10.67**	15.838**
Error	14	0.004	0.002	0.172	0.050	0.015	0.046
Total	20						

Appendix 2. Analysis of variance for growth and survival of T. longibrachiatum in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	8.089**	2.610**	1.796**	3.902**	3.566**	10.328**
Error	14	0.019	0.014	0.024	0.047	0.013	0.080
Total	20						

** Significant at one per cent level

Appendix.3. Analysis of variance for growth and survival of A. terreus in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	10.528**	6.842**	6.598**	7.472**	6.358**	13.524**
Error	14	0.015	0.026	0.007	0.005	0.023	0.035
Total	20						

Appendix 4. Analysis of variance for growth and survival of P. citrinum in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	8.033**	6.242**	7.692**	7.939**	8.58**	12.050**
Error	14	0.011	0.002	0.008	0.005	0.030	0.008
Total	20						

** Significant at one per cent level

Appendix 5. Analysis of variance for growth and survival of *P. simplicissimum* in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	21.399**	20.439**	26.425**	15.506**	13.298**	16.216**
Error	14	0.010	0.002	0.022	0.015	0.037	0.128
Total	20						

Appendix 6. Analysis of variance for growth and survival of *B. subtilis* in different food bases

Source	df	Mean squares					
		15 days	45 days	75 days	105 days	135 days	165 days
Treatment	6	12.205**	10.997**	7.936**	5.016**	3.605**	4.573**
Error	14	0.017	0.010	0.008	0.007	0.023	0.016
Total	20						

** Significant at one per cent level

Appendix 7. Analysis of variance for population of T. harzianum in cowpea rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	14.87**	22.26	4.03**
Error	12	0.002	0.003	0.003
Total	14			

Appendix 8. Analysis of variance for population of T. longibrachiatum in cowpea rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	23.14**	7.74**	2.68**
Error	12	0.005	0.002	0.004
Total	14			

** Significant at one per cent level

Appendix 9. Analysis of variance for population of *A. terreus* in cowpea rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	6.26**	0.135**	1.02**
Error	12	0.001	0.001	0.004
Total	14			

Appendix 10. Analysis of variance for population of *B. subtilis* in cowpea rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	0.60**	0.69**	1.66**
Error	12	0.001	0.0002	0.01
Total	14			

** Significant at one per cent level

Appendix 11. Analysis of variance for the population of *T. harzianum* in ginger rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	17.54**	8.83**	11.73**
Error	12	0.003	0.006	0.002
Total	14			

Appendix 12. Analysis of variance for the population of *T. longibrachiatum* in ginger rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	20.18**	3.41**	45.61**
Error	12	0.001	0.002	0.31
Total	14			

** Significant at one per cent level

Appendix 13. Analysis of variance for the population of *P. simplicissimum* in ginger rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	39.11**	13.17**	28.39**
Error	12	0.006	0.05	0.011
Total	14			

Appendix 14. Analysis of variance for the population of *B. subtilis* in ginger rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	3.72**	5.69**	18.34**
Error	12	0.002	0.02	0.08
Total	14			

** Significant at one per cent level

Appendix 15. Analysis of variance for population of T. harzianum in black pepper rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	5.74**	9.09**	12.01**
Error	12	0.01	0.002	0.005
Total	14			

Appendix 16. Analysis of variance for population of T.longibrachiatum in black pepper rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	0.33**	11.42**	3.04**
Error	12	0.01	0.001	0.002
Total	14			

** Significant at one per cent level

Appendix 17. Analysis of variance for population of *P. citrinum* in black pepper rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	6.89**	0.41**	0.33**
Error	12	0.001	0.002	0.01
Total	14			

Appendix 18. Analysis of variance for population of *B. subtilis* in black pepper rhizosphere

Source	df	Mean squares		
		7 days	30 days	60 days
Treatment	2	0.614**	1.03**	0.19**
Error	12	0.0001	0.001	0.0002
Total	14			

** Significant at one per cent level

STANDARDISATION OF FOOD BASES FOR SELECTED ANTAGONISTIC MICROFLORA AGAINST SOIL-BORNE PATHOGENS

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of
the requirement for the degree

Master of Science in Agriculture

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1990

ABSTRACT

Techniques for mass multiplication and production of antagonistic microflora isolated from the forest soils of Kerala for the biocontrol of soil-borne pathogens viz., Rhizoctonia, Pythium and Phytophthora were investigated. The antagonists used were Trichoderma harzianum, T. longibrachiatum, Aspergillus terreus, Penicillium citrinum, P. simplicissimum and Bacillus subtilis. The food bases tried were rice, wheat bran, paddy straw, rice bran, cowpea, forest soil and soil + dried cowdung. The growth and survival of antagonists in various food bases were estimated in vitro. A pot culture experiment was laid out during the period from March to September 1989 at the College of Horticulture, Vellanikkara to assess the population dynamics of the introduced antagonists in rhizosphere of crop plants and to find out the effect of carrier based antagonist in controlling collar rot of cowpea caused by Rhizoctonia solani, soft rot of ginger caused by Pythium myriotylum and quick wilt of black pepper caused by Phytophthora palmivora.

Milled rice was found to be the most promising food base for all the isolates tested. Wheat bran was also

found good for all the isolates except T. longibrachiatum. T. longibrachiatum as well as B. subtilis were found to grow well in rice bran. A. terreus and P. citrinum exhibited good growth while moderate growth of T. longibrachiatum and P. simplicissimum was observed with cowpea as a food base. In general paddy straw, forest soil and soil + cowdung were found to be poor substrates compared to other food bases. But in peddy strew, T. harzianum, A. terreus, P. citrinum and B. subtilis survived better compared to other food bases.

The maximum number of viable propagules in the rhizospheres of ginger, cowpea and pepper was recorded with wheat bran T. harzianum preparation up to one month of introduction to soil. Rice based antagonist recorded maximum population in cowpea rhizosphere while wheat bran based antagonist recorded the maximum in ginger and pepper rhizospheres after two months of soil inoculation. The survival ability of T. longibrachiatum grown in rice bran was better in ginger and cowpea rhizospheres after introduction to soil while in pepper rhizosphere, rice based T. longibrachiatum recorded maximum population compared to other food based antagonists. Wheat bran was found to be the best food base for the survivel of A. terreus in cowpea rhizosphere. Wheat bran P. simplicissimum preparation recorded the

maximum population count in ginger rhizosphere up to one month while rice based P. simplicissimum was superior to others after two months of introduction. P.citrinum grown in wheat bran survived better in the rhizosphere of pepper than other food based antagonists. Rice B.subtilis preparation recorded the maximum population count in cowpea and pepper rhizospheres up to one month. But after two months of soil inoculation, rice bran based B. subtilis recorded the maximum population. Survival of B. subtilis was better in ginger, rhizosphere inoculated with wheat bran B. subtilis preparation. A decline in population count was observed in ginger cowpea and pepper rhizospheres amended with carrier based antagonist.

T. longibrachiatum grown in rice was found to be the most effective in checking down collar rot of cowpea. Rice based P. simplicissimum and T. longibrachiatum were effective in suppressing soft rot symptoms in ginger. Efficacy of food based antagonists on the control of quick wilt disease could not be assessed due to failure in inducing symptoms artificially.