INFLUENCE OF MICROFLORA ASSOCIATED WITH EARTHWORM *(EUDRILUS EUGENIAE* KINBERG) AND VERMICOMPOST ON GROWTH AND PERFORMANCE OF CHILLI *(CAPSICUM ANNUUM* L.)

By

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DEDICATED

To my Acha and Amma ...

DECLARATION

I hereby declare that this thesis entitled **"Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli *(Capsicum annuum L.)*"** 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 any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Vellayani,

12. 11. 1999.

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CERTIFICATE

Certified that this thesis entitled **"Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli (*Capsicum annuum L.*)"** is a record of research work done independently by Ms. SUJA P.R. (97-11-20) 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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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

INTRODUCTION

The concept of organic farming is gaining much importance now a days. The main aim is to reduce the consumption of chemical fertilizers and harmful plant protection chemicals. In developed countries, while the emphasis is on total organic farming, it is doubtful whether this can be adapted as such in developing countries like India. Here, the problems are too complex to satisfy the food demands of the evergrowing population. In such a situation, the ideal technology will be the one based on integrated nutrient and pest management practices for crop production. In integrated nutrient management practices, the primary objective is to reduce the use of chemical fertilizers by a judicious combination of organic manures and biofertilizers.

The management of soil fertility through biological means is a vital component of sustainable agriculture. However, the transition from chemical to sustainable agriculture will take at least three to six years. This is the time normally taken to enhance soil biological activity to sustain crop yield. But, this time can be reduced to some extent by adopting the vermiculture technology.

The present investigation on "Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli, (*Capsicum annuum* L.)" was done to understand certain basic and applied aspects of this ecofriendly technology. The important objectives were :

1. To isolate and characterise the surface and gut microflora of the composting species of earthworm, *Eudrilus eugeniae.*

2. To enumerate the total microflora of vermicompost produced by *Eudrilus eugeniae.*

3. To screen and select efficient cultures of nitrogen fixing and phosphate solubilizing bacteria for enrichment of vermicompost.

4. To compare the efficacy of ordinary and enriched vermicompost in inducing better plant growth and yield in chilli.

5. To study the effect of combined application of earthworm and *Pythium aphanidermatum* on the incidence of damping off in chilli.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Earthworm and soil fertility

The importance of earthworms in maintaining soil fertility was first stressed by Charles Darwin (1881) in his book 'The Formation of Vegetable Mould through the Action of Worms'. Teotia *et al. (1950)* observed that the porosity of soil was significantly improved due to the burrowing activity of earthworms.

Lavelle (1988) reported that earthworms build up soil fertility through a nutrient recycling process by selectively activating both mineralization and humification processes. Fragaso *et al.* (1997) observed that earthworms could affect nutrient and organic matter dynamics through their mutualistic interaction with microflora and selective ingestion of soil particles.

Microflora associated with earthworms

Khambata and Bhat (1953, 1955, 1957) studied specific groups of bacteria in the worm intestine and isolated oxalate and cellulose decomposers. Ponomareva (1953) found that there was an increase in the number of pigmented and other bacteria of *Bacillus cereus* group after passage through earthworm intestine. Barois and Lavelle (1986)

proposed a mutualistic relationship between microflora and earthworms for the exploitation of complex organic matter of tropical soils. Wolters and Joergensen (1992) showed that the earthworm, *Aporrectodea caliginosa* altered the biomass and metabolic activity of the edaphic microflora over a wide range of soils.

Gut microflora of earthworms

Bassalik (1913) isolated more than 50 species of bacteria from the alimentary canal of *Lumbricus terrestris*. He found that none of these species differed from those present in the soil from which the worms were collected. Parle (1959) reported that the total number of bacteria and actinomycetes increased exponentially from the anterior to the posterior portion of the worm gut. Further, certain groups of microorganisms were stimulated selectively during passage through the earthworm gut. Contreras (1980) found that in the gut of *Eisenia lucens*, *Vibrio* spp. accounted for 73 per cent of the total bacteria and *Streptomyces lipmanii* accounted for 90 per cent of the actinomycetes. These species were of relatively low abundance in the wood substrate where the earthworms were living.

Many factors including enzymes, mucus and antimicrobial substances influenced the ability of preferentially or randomly ingested

organisms to survive the passage through earthworm gut and their resultant capacity to recover and proliferate in earthworm casts (Brown, 1995). Barois and Lavelle (1986) showed that the intestinal mucus produced by the earthworm, *Pontoscolex corethrurus*, contained large amounts of water-soluble low molecular weight organic compounds. These were assimilated easily by the rapidly multiplying microbial population in the gut. Nair *et al.* (1997) reported that the total number of different microorganisms were higher in the gut content of *Eudrilus eugeniae* when compared to surface microflora. Most of the organisms isolated either from the surface or the gut contents were of common occurrence in the soil.

Parle (1963) observed that the population of bacteria increased rapidly during passage of food through worm gut. Rouelle (1983) introduced the bacterium, *Rhizobium japonicum* into the gut of *Lumbricus terrestris* and *Eisenia foetida*. He found that the bacteria survived in the worm gut. Kristufek *et al.* (1995) also found that the bacterial counts increased in number during the passage of food materials through the gut of earthworms. The difference between bacterial counts in the foregut and hind gut were significantly higher in *Lumbricus rubellus* (4.2×10^9 vs 8.8×10^9) than in *Aporrectodea caliginosa* (10.3×10^9 vs 13.4×10^9). This sort of differences in the number of bacteria

were due to the different chemical and microbiological composition of material consumed and also due to the different feeding habits of both the species of earthworms.

Hutchinson and Kamel (1956) found that some fungal species survived the passage through the alimentary canal of earthworms. Dash *et al.* (1979) and Stringanova *et al.* (1989) reported that fungal spores with thick-walled or wrinkled coats and the spores and mycelia of certain dark coloured fungi were resistant to breakdown by the intestinal enzymes of earthworms. Kristufek *et al.* (1992) found that the population of some fungi increased during passage through the gut of *Lumbricus rubellus*, which indicated that viability of some fungal species was enhanced during passage through the gut.

Parle (1963) observed that during passage of food through the intestine of earthworms, the population of actinomycetes increased rapidly. This accounted for 17-79 per cent of the total gut flora and was dominated by *Streptomyces* spp. Ravasz *et al.* (1986) and Kristufek *et al.* (1993) reported that the number of actinomycetes was higher in the gut contents than in the soil. Further, a higher proportion of *Streptomyces* spp. isolated from earthworm gut produced antibiotics which were active against *Bacillus subtilis* and *Saccharomyces cerevisiae* or both.

Citernesi *et al.* (1977) reported nitrogen fixation activity in the gastroenteric cavity of many soil animals including earthworms. Simek *et al.* (1991) showed that nitrogenase activity was associated not only with the gut content and body surface of worms, but also in their cast.

Vermicompost

Use of earthworms for decomposition of organic matter was reported by Graff (1981) and Tomati *et al.* (1983). They found that the earthworm, *Eudrilus eugeniae* was best suited for organic waste degradation. Bano *et al.* (1987) also reported that *Eudrilus eugeniae* was superior to other earthworm species for vermicomposting technology. The successful use of this earthworm for vermicompost production in Kerala had also been reported by Jiji *et al.* (1995) and Prabhakumari *et al.* (1995). Anina (1995) found that *Eudrilus eugeniae* reduced the time taken for compositing from 56 to 41 days when compared to local worms.

Viljoen and Reinecke (1992) observed that *Eudrilus eugeniae* was best suited for vermiculture in regions with a tropical or moderate climate as it exhibited a high degree of intolerance for temperature below 16 °C.

Madhukeshwara *et al.* (1996a) suggested that the growth and fecundity of earthworms and rate of compost production showed variation with respect to the substrate used in the feed mix. Singh and Rai (1996) found that *Eudrilus eugeniae* was good for vermicomposting garbage, especially kitchen wastes mixed with cattle dung.

Edwards (1982) reported that the physical structure of vermicompost produced from organic waste depended on the original material from which they were produced. However, the final product was usually a finely powdered, peat-like material with excellent structure, porosity, aeration, drainage and moisture holding capacity.

Rouelle and Randriamamonjizaka (1983) found that vermicompost could supply the full requirement of trace elements and P and to some extent the initial requirement of K for plant growth. Bano *et al.* (1987) compared the nutrient status of vermicompost with organic manures. They found that the percentage of N in vermicompost was same as that of other organic manures. However, the high P_2O_5 content in earthworm casts improved the phosphate availability.

Edwards and Burrows (1988) reported that the nutrient content of vermicompost could differ greatly depending on the parent material used

for composting. However, when the nutrient status was compared with that of commercial plant growth medium to which inorganic nutrients were added, it usually contained more of mineral elements. But there was deficiency for magnesium. Further, during the processing of wastes by earthworms, many of the nutrients were converted to forms which were more readily taken up by plants, such as nitrate or ammoniacal nitrogen, exchangeable phosphorus and soluble potassium, calcium and magnesium. Bijulal (1997) found that the major effect of vermicompost application was a reduction in P fixation and thus increasing P availability in acidic soils.

Ismail *et al.* (1991) obtained increased number of flowers and fruits in water melon using vermicompost as an organic manure. Santos *et al.* (1993) evaluated the production of two cultivars of lettuce using two different organic composts. The organic composts and chemical fertilizers resulted in similar yields, but with vermicompost an additional yield of 3.4 t per ha was obtained. Increased yields of potato, tomato, cabbage and silage maize were also obtained by using vermicompost. Enhanced respiratory and photosynthetic activity in tomato and increased ascorbic oxidase, diphenol oxidase and catalase activities in cabbage were reported by Gorodni *et al.* (1994) due to vermicompost

application. Dharmalingam (1995) got 16 per cent increase in yield in soyabean due to vermicompost pelleting of seeds.

Jiji *et al.* (1996) found that the requirement for chemical fertilizers in cowpea var. 'Malika' and bitter gourd var. 'Preethi' was significantly reduced when the recommended dose of farm yard manure was substituted by an equal quantity of vermicompost. Madhukeshwara *et al.* (1996b) observed that vermicompost mixed with sand in the ratio of 1:4 was an efficient organic substrate for raising healthy tomato nurseries. Pushpa (1996) reported that vermicompost could be used to increase fruit yield and quality of tomato. Similarly, Rajalekshmi (1996) got an yield increase of 12 per cent in chilli due to the application of vermicompost. Ushakumari *et al.* (1996) also reported that the application of 12 t of vermicompost along with full and 75 per cent of the recommended dose of inorganic fertilizers yielded 43 and 26 per cent more yield in bhindi than the normal package of practices recommendations.

Rani Jasmin (1999) observed that the application of vermiwash along with inorganic fertilizers produced marked increase in fruit yield of tomato.

Microflora of earthworm cast and vermicompost

Stockli (1928) observed that the total microbial count in earthworm cast doubled in the first week after they were deposited. Thereafter, for a further period of three weeks, their number did not increase eventhough it fluctuated considerably during this period. Ghilarov and Mamajev (1963) reported that 3940 x 10^3 microorganisms per gram of earthworm casts as against 2000 x 10^3 microorganisms per gram of soil in grass fields. This was due to the fact that the vermicasts were generally rich in ammonia and partially digested organic matter which provided a good subtrate for the growth of microorganisms. Barois and Lavelle (1986) and Scheu (1991) also observed that some of the intestinal mucus secreted during passage through the earthworm gut and egested with the casts, continued to stimulate microbial activity and growth. Nair *et al.* (1997) reported that vermicompost produced by *Eudrilus eugeniae* had a higher population of microorganisms when compared to that of ordinary compost.

Teotia *et al.* (1950) reported that worm casts had a higher bacterial count of 32.0 million per gram compared with 6.0 – 9.0 million per gram in the surrounding soil. Bhatnagar (1975) and Loquet *et al.* (1977) observed that earthworm casts contained greater number of

cellulolytic, hemicellulolytic, nitrifying and denitrifying bacteria than the surrounding soil.

The number of actinomycetes was reported to be low in wormcast by Teotia *et al.* (1950) . However, Dkhar and Mishra (1986) could isolate higher population of actinomycetes in worm casts compared to surrounding soil.

Stockli (1928) reported that the number of *Azotobacter* was more in earthworm casts. The presence of *Azotobacter* in worm casts was also reported by Teotia *et al.* (1950). Mba (1987) observed an increase in nitrogen fixation during composting of ground Dallas grass *(Paspalum dilatatum)* by the earthworm, *Eudrilus eugeniae.* The total nitrogen content of the substrate increased in the presence of earthworms. The nitrogenase activity also increased ten fold in earthworm casts when compared to grass substrate.

Indira *et al.* (1996) reported that the population of nitrogen fixing bacteria was in the range of 10^5 to 10^6 in earthworm casts. *Azotobacter*, *Azospirillum* and *Rhizobium* were predominant among the nitrogen fixing organisms. Similarly, Nair *et al.* (1997) found that higher number

of *Azotobacter* sp. was present in vermicompost produced by *Eudrilus eugeniae* when compared to surface and gut microflora.

Mba (1994, 1997) found that the earthworm casts of *Pontoscolex corethrurus* and *Eudrilus eugeniae* contained efficient rockphosphate solubilizers. Indira *et al.* (1996) also detected phosphate solubilizers like *Bacillus* and *Aspergillus* spp. in the vermicompost of *Eudrilus eugeniae*.

Earthworms and plant productivity

Aldag and Graff (1974) compared the growth of oat seedlings in brown podzol soil with and without the application of the earthworm, *Eisenia foetida*. They reported an increase of 8.7 per cent in dry matter yield and 21 per cent in total protein yield in oat seedlings grown in soil with earthworms. In a series of pot experiments Atlavinyte and her co-workers (Atlavinyte *et al.*, 1968; Atlavinyte, 1971; 1974 and Atlavinyte and Vanagas, 1982) clearly showed that there was a strong correlation between the number of earthworms (*Allolobophora caliginosa*) present in the soil and the growth of barley. The addition of 400 to 500 numbers of *Allolobophora caliginosa* per m^2 increased the biological productivity of barley by 78 to 96 per cent. This increase in yield was proportional to the number of earthworms applied to the soil.

The effect of earthworm on growth of roots of apple trees was demonstrated by van Rhee (1977). He reported an increase of 70 per cent in aggregate stability and up to 140 per cent in density of roots of less than 0.05 mm diameter, when worm-free polders were inoculated with *Allolobophora caliginosa* and *Lumbricus terrestris* at the rate of 500 per m^2. These changes also resulted in an average yield increase of 2.5 per cent.

Haimi *et al.* (1992) studied the effect of the earthworm, *Lumbricus rubellus,* on net production and nitrogen content of birch seedlings in laboratory microcosms. They found that the leaf and stem biomass of birch in treatments with earthworms increased by 33 and 24 per cent respectively when compared to the control. Similarly, Mba (1996) observed that in cowpea, aerial biomass was increased significantly by *Eudrilus eugeniae.*

Alfred and Gunthilagaraj (1996) reported significant differences in the establishment of *Amaranthus dubius* between soils with and without earthworms. Seed germination, plant height and yield were more in bed with earthworms. Ushakumari *et al.* (1996) observed that when the earthworms were introduced into the field along with waste-manure

mixture and reduced inorganic fertilizer, an yield increase of 51.3 per cent was obtained in bhindi.

Callaham and Hendrix (1998) found that increased earthworm abundance resulted in significantly increased nitrogen content in plant tissues such as that of *Aristida stricta* shoots and *Pinus palustris* stem and leaves. The dry matter accumulation also increased with the number of earthworms.

Spain *et al.* (1992) found that the growth of *Panicum maximum* increased significantly with earthworms, but varied with the species of earthworms. Thus, when the plant growth was improved by *Chuniodrilus zielae* and *Stuhlmannia porofera,* it remained unaffected with *Millsonia anomala.* Doube *et al.* (1995) also reported that the influence of earthworms on plant growth could be affected by soil type. This was based on a series of green house experiments in which they added *Aporrectodea trapezoides* or *A.rosea* to three different soil types such as sandy loam, loam and clay. In one of the experiments, both the earthworm species produced significant increases in the growth of wheat seedlings in sandy loam soil, but had no effect in the loam or clay soils. Similarly, these earthworm species increased the growth and yield of

barley in sandy loam soil, with no effect in loam soil and decreased the growth and grain yield in clay soil.

***Pythium* spp.**

The association of *Pythium* spp. with plant diseases was established as early as 1900 and it soon became apparent that these fungi were important plant pathogens. Subsequent work by Chi and Hanson (1962), Filer (1967), Laviolette and Athow (1971) and Roncardori and Mc Cartier (1972) had shown that *Pythium* spp. infected mainly juvenile or succulent tissues restricting infection either to seedlings or feeder roots or root tips of older plants.

Jenkins and Averre (1983) isolated *Pythium aphanidermatum, P. myriotylum, P. debaryanum* and *P. ultimum* from roots of diseased tomato, cucumber and lettuce in hydroponic culture systems in green houses. Bates and Stanghellini (1984) observed that root rot was the limiting factor for commercial production of spinach in greenhouse. Infected plants either died or were severely stunted. Symptoms occurred within six days after the plants were inoculated with *Pythium.*

Lim and See (1983) reported that the development of post emergence damping off in *Brassica* spp. was favoured by high seedling

density, high relative humidity and soil moisture content and found that the disease was soilborne. Naiki and Gonda (1986) observed that young seedlings were most sensitive to the disease, and that damping off was most severe within two weeks after sowing.

The earthworms could also enhance the dispersal of micro organisms by ingesting them at one location from a particular food source and egesting them elsewhere. Hutchinson and Kamel (1956) inoculated sterilized soil with different species of fungi and reported that the rate of spread of fungi through the soil was much greater when the worms were present. Hoffman and Purdy (1964) showed that the spread of dwarf bunt *(Tilletia controversa)* disease by earthworms occurred mainly due to the ingestion of teliospores produced by this pathogen without loss of viability during passage through the worm gut. Rao (1979) concluded that *Megascolex insignis* fed upon decaying roots of papaya could spread viable spores of *Pythium aphanidermatum*. Similar reports were made earlier by Baweja (1939) in the case of *Pythium* and Khambata and Bhat (1957) in the case of *Fusarium*. Toyota and Kimura (1994) also found that earthworms disseminated the soilborne plant pathogen, *Fusarium oxysporum* f.sp. *raphani*.

Stephens *et al.* (1993) and Doube *et al.* (1994) made an interesting observation that the application of *Aporrectodea trapezoides* earthworms significantly reduced the symptoms caused by *Rhizoctonia solani* in wheat seedlings.

Jiji (1997) reported that the local species of earthworm, *Megascolex* sp., could not survive under restricted soil environmental conditions in earthen pots.

Use of *Azotobacter* and phosphate solubilizing bacteria on plant growth

The first attempt to test *Azotobacter* as a crop inoculant was reported from the former USSR in 1902, using oats grown in pot culture. Rubenchik (1963) got an average yield increase of 13.7 per cent in spring wheat, 15.3 per cent in winter wheat, 12 per cent in barley, 15.1 per cent in oats, 18.7 per cent in rye, 22.3 per cent in millet and 14 per cent in corn due to *Azotobacter* inoculation.

Mehrotra and Lehri (1971) studied the effect of *Azotobacter* inoculation in brinjal, tomato and cabbage at varying levels of NPK and organic manure application. A slurry of lignite-based inoculant was used for root dip at transplanting. The yield increases were 42 per cent, 29 per

cent and 45 per cent for brinjal, tomato and cabbage respectively. Lehri and Mehrotra (1972) also reported significant yield increases of 15 to 62 per cent and 25 to 50 per cent respectively in brinjal and cabbage with *Azotobacter* inoculation.

Dibut *et al.* (1995) and Gupta *et al.* (1995) found that in tomato, soil inoculation with *Azotobacter chroococcum* increased seed germination by 33 to 46 per cent besides reducing the period between sowing and transplanting by 5 to 7 days. Soil inoculation also increased the number of flowers and fruits.

Arunkumar (1997) reported maximum yield increase in amaranthus with *Azotobacter* treatment along with farm yard manure and 75 per cent fertilizer nitrogen. In brinjal, the keeping quality was significantly improved in *Azotobacter* treatment along with full dose of vermicompost and 75 per cent fertilizer nitrogen.

Luchnik (1975) reported that the use of organic manures resulted in high sugar and vitamin C content which resulted in better keeping quality of cabbage. Meir-plo gen and Lehri (1981) studied the quality of different food plants grown with composts from biogenic waste. They

found that storage quality and contents of desirable nutrients such as vitamin C and sugar were improved in compost treatments.

Gerretson (1948) reported increased yield and phosphorus uptake of oat plants inoculated with soil containing phosphate dissolving microorganisms as compared to uninoculated control. Zenkova (1955) and Smierzchalska (1962) also reported significant increase in yield varying from 5 to 10 per cent in lucerne, cabbage and cucumber as a result of inoculation with phosphate solubilizing bacteria.

Kundu and Gaur (1980) observed that combined inoculation of *Bacillus polymyxa* and *Pseudomonas striata* increased the yield and P uptake by potato tubers. Maximum increase of 35.2 per cent was obtained when both the bacteria were inoculated.

Dubey and Billore (1992) found that addition of rock phosphate and inoculation of phosphate solublizing microorganisms such as *Bacillus megaterium, Pseudomonas striata, Penicillium* sp. and *Aspergillus awamori,* increased the yield of potatoes. Shehana (1997) observed that the available phosphorus content of soil, plant P content, P uptake, yield attributes and dry matter production in banana were

favourably influenced by the use of phosphate solubilizing bacteria along with a lesser amount of P than the recommended level.

Gaur *et al.* (1978) reported that the inoculation with nitrogen fixing and phosphate solubilizing microorganisms improved the manurial value of compost. Sadasivam *et al.* (1981) also found that inoculating rock phosphate amended compost with cultures of *Azotobacter* and phosphate solubilizing strains of *Aspergillus* sp. increased the nitrogen and humus content. Kapoor *et al.* (1983) observed that inoculation of *Azotobacter* into already decomposed material increased the nitrogen content. He also found that composting significantly increased the availability of soluble P and this was further increased on inoculation with *Aspergillus* sp.

Bharadwaj and Gaur (1985) and Rasal *et al.* (1988) reported that due to increased microbial population and nitrogen fixation, phosphate solubilization processes got accelerated in the compost and it became ready for use within four months with low C : N ratio and high N and P contents. Banik and Dey (1985) found that the inoculation of efficient rockphosphate solubilizers with farm yard manure and rock phosphate either alone or together significantly increased the uptake of phosphorus. Hajra (1988) observed that *Azotobacter chroococcum* inoculation

increased the nitrogen content of final compost by 78 per cent over the control. An increase of 64 per cent in citrate soluble P was also observed when the compost was treated with a phosphate solubilizer, *Aspergillus awamori.*

Anina (1995) found that enrichment of vermicompost with nitrogen fixing bacteria, *Azospirillum* and phosphate solubilizing microorganism along with one per cent rock phosphate had a significant effect on nutrient contents. *Azospirillum* treatments had a higher nitrogen content. Use of this vermicompost increased plant height and number of leaves, better shoot: root ratio and maximum yield in chilli.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation on 'Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli, (*Capsicum annuum* L.) was done at College of Agriculture, Vellayani during 1997-'99 as part of a Science, Technology and Environment Department (Government of Kerala) funded research project.

3.1 Collection of earthworm and vermicompost samples.

Samples of earthworm and vermicompost were collected from the already existing vermicompost pits at College of Agriculture, Vellayani. Three pits of uniform size and age were selected for this purpose.

3.2 Isolation of microorganisms

3.2.1 Isolation of surface and gut microflora of *Eudrilus eugeniae*

The earthworm samples were collected from each of the selected pit at 15 day interval, for a total period of 60 days. Five earthworms of uniform size were used for the estimation of surface and gut microflora. These worms were transferred to 100 ml Erlenmeyer flasks containing 50 ml of sterile water blank and gently shaken for 10 minutes. One ml of the resulting suspension was serially diluted under aseptic conditions to get a final dilution of 10^{-4}.

The above earthworms were also used for the isolation of gut microflora. For this, the worms were initially transferred to a sterile petriplate and killed by brief exposure to 95 per cent ethanol. The hind gut of each worm was cut with the help of a sterile scalpel and the gut contents were squeezed out. These were then aseptically transferred to 50 ml of sterile water blank and serially diluted to get a final dilution of 10^{-6}.

One ml of appropriate dilution was used for the isolation of bacteria, fungi, actinomycetes, nitrogen fixing bacteria, phosphate solubilizing bacteria and soilborne plant pathogens such as *Pseudomonas solanacearum*, *Pythium* spp. and *Phytophthora* spp. on suitable media (Appendix I) as per the details given below.

Organism isolated	Dilution used		Medium used
	Surface flora	Gut flora	
Bacteria	10^{-4}	10^{-6}	Vermicompost extract agar
Fungi	10^{-4}	10^{-4}	Martin's rose bengal agar
Actinomycetes	10^{-4}	10^{-6}	Kauster's agar
Nitrogen fixing bacteria	10^{-2}	10^{-4}	Jensen's nitrogen free agar
Phosphate solubilizing bacteria	10^{-4}	10^{-4}	Pikovskaya's modified agar
Pythium spp. and *Phytophthora* spp.	10^{-4}	10^{-4}	Martin's peptone dextrose agar
Pseudomonas solanacearum	10^{-4}	10^{-6}	Triphenyl tetrazolium chloride agar

3.2.2 Enumeration of total microflora of vermicompost

Vermicompost samples were also collected from the same pits from where the earthworms were collected, at 15 day interval, for a total period of 60 days. Three random samples of 100 g each were collected from each pit and mixed well. One g of vermicompost from each composite sample was aseptically transferred to 99 ml of sterile water blank and serially diluted to get a final dilution of 10^{-6}. One ml of appropriate dilution was plated for the enumeration of bacteria (10^{-6}), fungi (10^{-4}), actinomycetes (10^{-6}), nitrogen fixing bacteria (10^{-4}), phosphate solubilizing microorganisms (10^{-6}), *Pseudomonas_solanacearum* (10^{-6}), *Pythium* spp. and *Phytophthora* spp. (10^{-4}) on specific medium mentioned above.

The plates were incubated at 28 $\pm$ 2°C for varying duration ranging from two days (bacteria and *Pseudomonas solanacearum*), four days (fungi and soilborne pathogens such as *Pythium* spp. and *Phytophthora* spp.) and seven days (actinomycetes, nitrogen fixing bacteria and phosphate solubilizing microorganisms). The final data were expressed as the mean of three replications in terms of the number of microorganisms present per earthworm for surface and gut microflora and on per gram basis for the microflora of vermicompost.

3.3 Characterisation of nitrogen fixing and phosphate solubilizing bacteria.

3.3.1 Nitrogen fixing bacteria.

3.3.1.1 Colony characters

Colony characters such as size, shape, elevation and pigment production, if any, were studied by aseptic culturing of different isolates of nitrogen fixing bacteria on Jensen's nitrogen free agar medium by pour plate technique. The various observations were taken after incubation for 72 h at 28°C in a BOD incubator.

3.3.1.2 Motility

The test for motility was done by observing a hanging drop preparation of 48 h old broth culture of each isolate under a light microscope (450 x) with reduced illumination.

3.3.1.3 Gram staining

The heat fixed smear of each isolate was initially stained with 0.2 per cent crystal violet solution (Appendix II) for one minute. The slides were then gently washed with tap water and treated with Gram's iodine solution (Appendix II) for 30 seconds. After decanting the excess of iodine solution, the smear was decolorised with 95 per cent ethyl alcohol for 30 seconds with gentle agitation. The slides were washed in tap water and counterstained with

safranin (Appendix II) for 30 seconds. After washing again in tap water, the slides were blot-dried and examined for Gram reaction under oil immersion objective of a microscope.

3.3.1.4 Spore staining

The heat fixed smear of each isolate was stained with five per cent aqueous solution of malachite green by steaming for five minutes. The slides were then washed in tap water and counter stained with safranin for 30 seconds. After washing in tap water, the slides were blot dried and examined for the presence or absence of endospores under oil immersion objective of a microscope.

3.3.1.5 Capsule staining

Air-dried smear of each isolate was initially stained with crystal violet solution (Appendix II) for five minutes and then washed with 20 per cent aqueous $CuSO_4$ solution. The slides were air dried and examined for the presence or absence of capsule under oil immersion objective of a microscope.

3.3.1.6 Starch hydrolysis

The ability of different isolates of nitrogen fixing bacteria to hydrolyse starch was tested using the medium containing 0.2 per cent soluble starch

(Appendix I). 48 h old culture of each isolate was aseptically spotted on the surface of starch agar medium in petriplates. They were incubated for four days at 28°C in a BOD incubator. Each plate was then flooded with Lugol's iodine solution (Appendix II). A colourless zone around bacterial growth in contrast to a blue background of the medium indicated positive starch hydrolysis.

3.3.1.7 Casein hydrolysis

The ability of different isolates to hydrolyse casein was tested using the medium containing 0.1 per cent casein (Appendix I). 48 h old culture of each isolate was aseptically spotted on the surface of casein agar medium in petriplates. They were incubated for four days at 28°C in a BOD incubator. The presence of a clear zone around bacterial growth indicated positive casein hydrolysis.

3.3.1.8 Growth in different carbon sources

Jensen's nitrogen free broth supplemented with two per cent glucose, sucrose, lactose or mannitol was used to study the ability of each isolate of nitrogen fixing bacteria to utilize different carbon sources. Five ml sterile broth in test tubes (in triplicate) was inoculated with 0.1 ml of 48 h old broth culture of each isolate and then incubated for 72 h at 28°C in a BOD

incubator. The data were recorded in terms of presence or absence of growth in each inoculated tube.

3.3.1.9 pH tolerance

The ability of different isolates of nitrogen fixing bacteria to grow at pH ranging from 3.0 to 7.0 was tested by inoculating aseptically 0.1 ml of 48 h old broth culture of each isolate into five ml of sterile Jensen's nitrogen free broth (in triplicate) initially adjusted to different levels of pH such as 3.0, 5.0 and 7.0 using either 0.1 N HCl or 0.1 N NaOH. The tubes were incubated at 28°C for 72 h in a BOD incubator. The data were recorded in terms of presence or absence of growth in each inoculated tube.

3.3.1.10 Growth rate of different isolates of nitrogen fixing bacteria.

The growth rate of different isolates of nitrogen fixing bacteria was estimated in terms of optical density by using a spectrophotometer (Systronics UV-VIS Spectrophotometer 118). For this, 0.1 ml of 48 h old broth culture of each isolate was aseptically inoculated into five ml of sterile Jensen's nitrogen free broth (in triplicate) without $CaCO_3$ and then incubated for a maximum period of six days at 28 °C in a BOD incubator. The extent of growth at different time intervals of 24, 48, 72, 96, 120 and 144 h was measured in terms of optical density at 600 nm in the spectrophotometer.

3.3.2 Phosphate solubilizing bacteria

The characterisation of phosphate solubilizing bacteria was also done by different tests mentioned above. However, here specific broth such as modified Pikovskaya's broth (for utilization of different carbon sources and pH tolerance), nutrient agar (Appendix I) (for colony characters, motility, Gram staining, spore staining and capsule staining) and King's B medium (for pigment production) (Appendix I) were used.

3.4 Screening for efficient cultures of nitrogen fixing and phosphate solubilizing bacteria.

3.4.1 Selection of efficient nitrogen fixing bacteria.

The nitrogen fixing ability of different isolates were estimated by a modified technique based on Lowry's method of protein estimation (Lowry, 1951).

3.4.1.1 Lowry's method of protein estimation.

The different isolates of nitrogen fixing bacteria were initially grown in 250 ml Erlenmeyer flasks containing 100 ml of sterile Jensen's nitrogen free broth at 28°C for seven days in a BOD incubator. Three replications were maintained for each isolate. One ml of this broth culture was used for protein estimation by Lowry's method . The sample was initially digested with four ml of 1N KOH solution at 60°C in a water bath for 10 minutes. After cooling

to room temperature, 0.5 ml aliquot was taken and the volume was made upto 4.5 ml with distilled water. To this five ml of alkaline $CuSO_4$ solution (prepared by mixing 50 ml of two per cent Na_2CO_3 solution in 0.1 N NaOH and 10 ml of freshly prepared 0.5 per cent $CuSO_4$ in one per cent sodium potassium tartarate solution) was added. After 10 minutes, 0.5 ml of Folin ciocalteau reagent was added rapidly with immediate mixing. The intensity of blue colour developed was measured after 30 minutes, at 660 nm in a spectrophotometer (Systronics UV-VIS Spectrophotometer 118). The protein content was estimated from a standard graph prepared with 20, 40, 60 and 80 µg of bovine serum albumin (BSA) in distilled water. The amount of nitrogen fixed per gram of sucrose utilized was calculated from the nitrogen : protein ratio of 1:6.25.

3.4.2 Selection of efficient phosphate solubilizing bacteria.

The different isolates of phosphate solubilizing bacteria were screened for their phosphate solubilizing ability by estimating the extent of solbilization of tricalcium phosphate under *in vitro* conditions.

3.4.2.1 Quantitative estimation of solubilization of tricalcium phosphate

The different isolates of phosphate solubilizing bacteria were initially grown in 250 ml Erlenmeyer flasks containing 100 ml of sterile modified

Pikovskaya's broth. The flasks were incubated at 28 °C for seven days in a BOD incubator. The broth culture was centrifuged at 5000 rpm for 10 minutes in a centrifuge (Hettich Zentrifugen EBA 12R) and the supernatant was used for the estimation of soluble phosphorus by Olson's method (Olson *et al.*, 1954). To 10 ml of the supernatant, 3.0 g of activated charcoal and 100 ml of 0.5 M sodium bicarbonate solution were added. The mixture was shaken for 30 minutes in a shaker and then filtered through Whatman No. 40 filter paper. Ten ml of the filtrate was then carefully transferred to 50 ml volumetric flasks. To this, 10 ml of ammonium molybdate hydrogen chloride solution (Appendix II) was added and after washing down the neck of the flask with distilled water, two ml of dilute stannous chloride solution (Appendix II) was added and the volume was made up to 50 ml. The contents were mixed immediately and the intensity of the blue colour developed was measured after 10 minutes at 660 nm in a spectrophotometer (Systronics UV/VIS Spectrophotometer 118). The amount of available phosphorus in the culture filtrate of each isolate was calculated from a standard graph prepared by the same procedure with known quantities (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ppm) of analar potassium dihydrogen ortho phosphate. The phosphate solubilizing ability of above isolates was compared with a known culture of phosphate solubilizing bacterium, *Bacillus megaterium* var. *phosphaticum*.

3.5 Enrichment of vermicompost with nitrogen fixing and phosphate solubilizing bacteria.

One isolate each of the nitrogen fixing and phosphate solubilizing bacteria was used for enrichment of vermicompost.

3.5.1 Enrichment with nitrogen fixing bacteria

The efficient isolate of nitrogen fixing bacteria which was selected on the basis of its nitrogen fixing ability was grown in 500 ml Erlenmeyer flasks containing 250 ml of Jensen's nitrogen free broth. These were incubated at 28°C in BOD incubator for seven days. The resulting broth culture was mixed with finely powdered (150 μ) and sterilized charcoal powder at the rate of 40 per cent of its water holding capacity. After curing for 48 h at room temperature, the carrier based inoculum was used for enrichment of vermicompost at the rate of one kg per 100 kg of vermicompost. The enriched vermicompost was packed in plastic bags and incubated for two weeks at room temperature before further use.

3.5.2 Enrichment with phosphate solubilizing bacteria

The procedure mentioned above was used for enrichment of vermicompost with phosphate solubilizing bacteria. Here, however, instead of Jensen's nitrogen free broth, a nutrient broth was used for mass production of phosphate solubilizing bacteria.

3.6 Use of enriched vermicompost for plant growth in chilli.

A pot experiment was conducted with the following treatment combinations.

Crop : Chilli

Variety : Jwalamukhi

Design : CRD

Replication : 3

Organic manures

1. Farm yard manure - F
2. Vermicompost - V

Nitrogen levels

1. 100 per cent of recommended dose - N_1
2. 75 per cent of recommended dose - N_2
3. 50 per cent of recommended dose - N_3

Phosphorus levels

1. 100 per cent of recommended dose - P_1
2. 75 per cent of recommended dose - P_2
3. 50 per cent of recommended dose - P_3

Potassium level

1. 100 per cent of recommended dose - K

Treatment combinations

T_1 - Control as per Package of Practices (POP) recommendations (N_1P_1K+F)

T_2 - POP with farm yard manure substituted by ordinary vermicompost (N_1P_1K+V)

T_3 - POP + vermicompost enriched with nitrogen fixing bacteria (N_1P_1K + VN)

T_4 - POP (75 per cent N) + vermicompost enriched with nitrogen fixing bacteria (N_2P_1K + VN)

T_5 - POP (50 per cent N) + vermicompost enriched with nitrogen fixing bacteria (N_3P_1K + VN)

T_6 - POP with farm yard manure substituted by vermicompost enriched with phosphate solubilizing bacteria (N_1P_1K + VP)

T_7 - POP (75 per cent P_2O_5) + vermicompost enriched with phosphate solubilizing bacteria (N_1P_2K + VP)

T_8 - POP (50 per cent P_2O_5) + vermicompost enriched with phosphate solubilizing bacteria (N_1P_3K + VP)

The treatment effects were compared with the existing package of practices recommendations (POP) of Kerala Agricultural University (1996) for NPK application (75:40:25 kg/ha) for chilli.

The potting mixture was prepared by mixing soil and sand in the ratio of 2:1. Earthen pots of 12 inch diameter were filled with this mixture at the rate of nine kg per pot. Organic manure in the form of farm yard manure,

vermicompost or enriched vermicompost was added at the rate of one kg per pot. Chemical fertilizers were applied in the form of urea, mussoriphos and muriate of potash. Half the dose of nitrogen, potassium and the full dose of phosphorus were applied as basal dose at the time of transplanting. One fourth of nitrogen and the remaining half of potassium were applied as top dressing one month after transplanting. Rest of the nitrogen was applied two months after transplanting.

The seeds of chilli, variety, Jwalamukhi were obtained from the Department of Plant Breeding, College of Agriculture, Vellayani. Dry seed treatment with Carbendazim at the rate of 2.0 g per kg seed was done before sowing. After 45 days, healthy seedlings were selected and transplanted to experimental pots at the rate of two seedlings per pot. The plants were irrigated regularly. The attack of chilli thrips and mites was controlled by spraying with 0.05 per cent Monocrotophos as and when required. The fruits were harvested at weekly intervals for a total period of seven weeks. After taking fresh weight, these fruits were stored in paper bags to ascertain their keeping quality at room temperature. Rest of the observations on plant height, number of branches, fresh and dry weight of shoot and root were taken at the time of harvest. The onset of flowering was recorded as the number of days taken from transplanting to the first appearance of flowers.

The dry weights of shoot and root were recorded after drying the samples to a constant weight at 60°C.

3.7 Interaction between earthworm, pathogen and host

A pot experiment was conducted with the following treatment combinations.

Crop : Chilli

Variety : Jwalamukhi

Design : CRD

Replication : 5

Earthworms

1. *Eudrilus eugeniae* - E
2. *Megascolex* sp. - M

Pathogen

1. *Pythium aphanidermatum* - P

Organic manures

1. Farm yard manure - F
2. Vermicompost - V

Treatment combinations

T_1 - Control as per POP recommendations - POP (F)

T_2 - POP + *Pythium aphanidermatum* – POP (F) + P

T_3 - POP + *Eudrilus eugeniae* – POP (F) + E

T_4 - POP + *Pythium aphanidermatum* + *Eudrilus eugeniae* – POP(F) + P + E

T_5 - POP + *Megascolex* sp. - POP (F) + M

T_6 - POP + *Pythium aphanidermatum* + *Megascolex* sp. – POP(F) + P+M

T_7 - POP with farm yard manure substituted by vermicompost – POP (V)

T_8 - POP with vermicompost + *Pythium aphanidermatum* - POP(V) + P

T_9 - POP with vermicompost + *Eudrilus eugeniae* - POP(V) + E

T_{10} - POP with vermicompost + *Pythium aphanidermatum* + *Eudrilus eugeniae* – POP(V) + P+E

T_{11} - POP with vermicompost + *Megascolex* sp. – POP (V) + M

T_{12} - POP with vermicompost + *Pythium aphanidermatum* + *Megascolex* sp. - POP(V) + P + M.

3.7.1 Isolation of pathogen

The pathogen causing damping off in vegetables was isolated from naturally infected chilli plants, collected from the Instructional Farm of College of Agriculture, Vellayani. Infected stem bits (0.5 cm long) from the

collar region were initially surface sterilized with 0.1 per cent $HgCl_2$ solution for one minute, followed by repeated washing with sterile water. These bits were transferred aseptically to potato dextrose agar medium (Appendix I) in sterile petriplates and incubated at room temperature for 48 hours. When fungal growth was visible, mycelial bits were transferred to fresh potato dextrose agar slants and purified by frequent transfer by hyphal tip method to the same medium. The pathogen was identified based on colony characters and spore morphology.

The pathogenicity of the isolate was tested by re-infecting healthy seedlings of chilli (variety Jwalamukhi) raised in soil pre-inoculated with 72-hour-old culture of the pathogen. The development of typical water soaked lesions in the collar region along with wilting of infected seedlings was taken as the criteria for virulence of the pathogen.

3.7.2 Mass production of pathogen

Sand-oat meal medium was used for mass production of the pathogen. The substrate was prepared by mixing sand and oat meal in the ratio of 9:1 (v/v). After moistening with water, the mixture was taken in 1000 ml Erlenmeyer flasks and autoclaved for one hour at 1.05 kg per cm^2. These were inoculated with mycelial bits of the pathogen and incubated at room temperature for 10 days. The resulting inoculum was used for seedling

infection by incorporating at the rate of 5 g per kg of potting mixture both at the time of transplanting and after one month of transplanting.

3.7.3 Earthworms

Two species of earthworms, *Eudrilus eugeniae* (exotic species) and *Megascolex* sp. (local species) were used for this study. *Eudrilus eugeniae* was procured from the Instructional farm of College of Agriculture, Vellayani, while *Megascolex* sp. was collected from the banana plots of the same farm. The earthworms were added at the rate of five worms per kg of potting mixture. All the pots were tightly covered with fine mesh black nylon nets to prevent the escape of earthworms.

3.7.3 Pot experiment

Healthy seedlings of chilli variety, Jwalamukhi were transplanted to 12 inch earthen pots filled with 10 kg of potting mixture in which either farm yard manure (treatments T_1 to T_6) or vermicompost (treatments T_7 to T_{12}) at the rate of one kg per pot was used as a source of organic manure. NPK fertilizers were applied as per the package of practices recommendations of Kerala Agricultural University (1996) for chilli (75:40:25 kg./ha) in the form of urea, mussoriphos and muriate of potash. Half the dose of nitrogen and potassium and the full dose of phosphorus were applied as basal dose at the time of transplanting. One fourth of nitrogen and the remaining half of

potassium were applied as top dressing one month after transplanting. Rest of the nitrogen was applied two months after transplanting. The plants were irrigated regularly.

The data on the percentage of disease incidence due to infection by the pathogen either with or without an induced injury in the collar region were taken both within a fortnight and after one month of transplanting. The observations on plant height, number of branches, onset of flowering, number of fruits per plant, yield per plant, fresh and dry weights of shoot and root were taken as described in the previous experiment. The number of earthworms were counted at the time of harvest by carefully depotting each pot and counting the number of worms present.

3.8 Statistical analysis

The data on various biometric observations were analysed by the methods described by Snedecor and Cochran (1967) for the analysis of variance of completely randomised design.

RESULTS

RESULTS

4.1 Isolation of microorganisms

The observations on the relative population of different types of microorganisms associated with *Eudrilus eugeniae* (Plate 1) and the vermicompost produced by this earthworm were taken on 15, 30, 45, and 60 day of composting.

4.1.1 Surface microflora of *Eudrilus eugeniae*

The total number of bacteria (4.3×10^4), fungi (6.0×10^3) and actinomycetes (42.5×10^3) were maximum on 45 day of compost formation (Table 1; Fig. 1) However, the number of nitrogen fixing bacteria (3.0×10^1) was more on 60 day. Phosphate solubilizing bacteria and soilborne pathogens like *Pseudomonas solanacearum, Pythium* spp. and *Phytophthora* spp. were absent.

4.1.2 Gut microflora of *Eudrilus eugeniae*

As in the case of surface microflora, the total number of bacteria (11.3×10^6) and fungi (19.1×10^3) were maximum on 45 day of composting (Table 2; Fig.2). However, the number of actinomycetes (19.6×10^5) was more on 30 day. Nitrogen fixing bacteria (1.3×10^3) could be isolated only on 15 day. Phosphate solubilizing bacteria, *Pseudomonas solanacearum, Pythium* spp. and

Plate 1

Adult worms of *Eudrilus eugeniae* Kinberg

Table 1. Surface microflora of *Eudrilus eugeniae*

Microorganism	Dilution factor	Number of microorganisms per earthworm* Day of observation			
		15	30	45	60
Bacteria	10^4	2.7	2.2	4.3	3.0
Fungi	10^3	1.1	0.7	6.0	2.2
Actinomycetes	10^3	11.1	5.7	42.5	17.2
Nitrogen fixing bacteria	10^1	-	1.1	-	3.0
Phosphate solubilizing bacteria	10^2	-	-	-	-
Pseudomonas solanacearum	10^2	-	-	-	-
Pythium spp.	10^2	-	-	-	-
Phytophthora spp.	10^2	-	-	-	-

* Mean of three replications

Fig. 1 Surface microflora of *Eudrilus eugeniae*

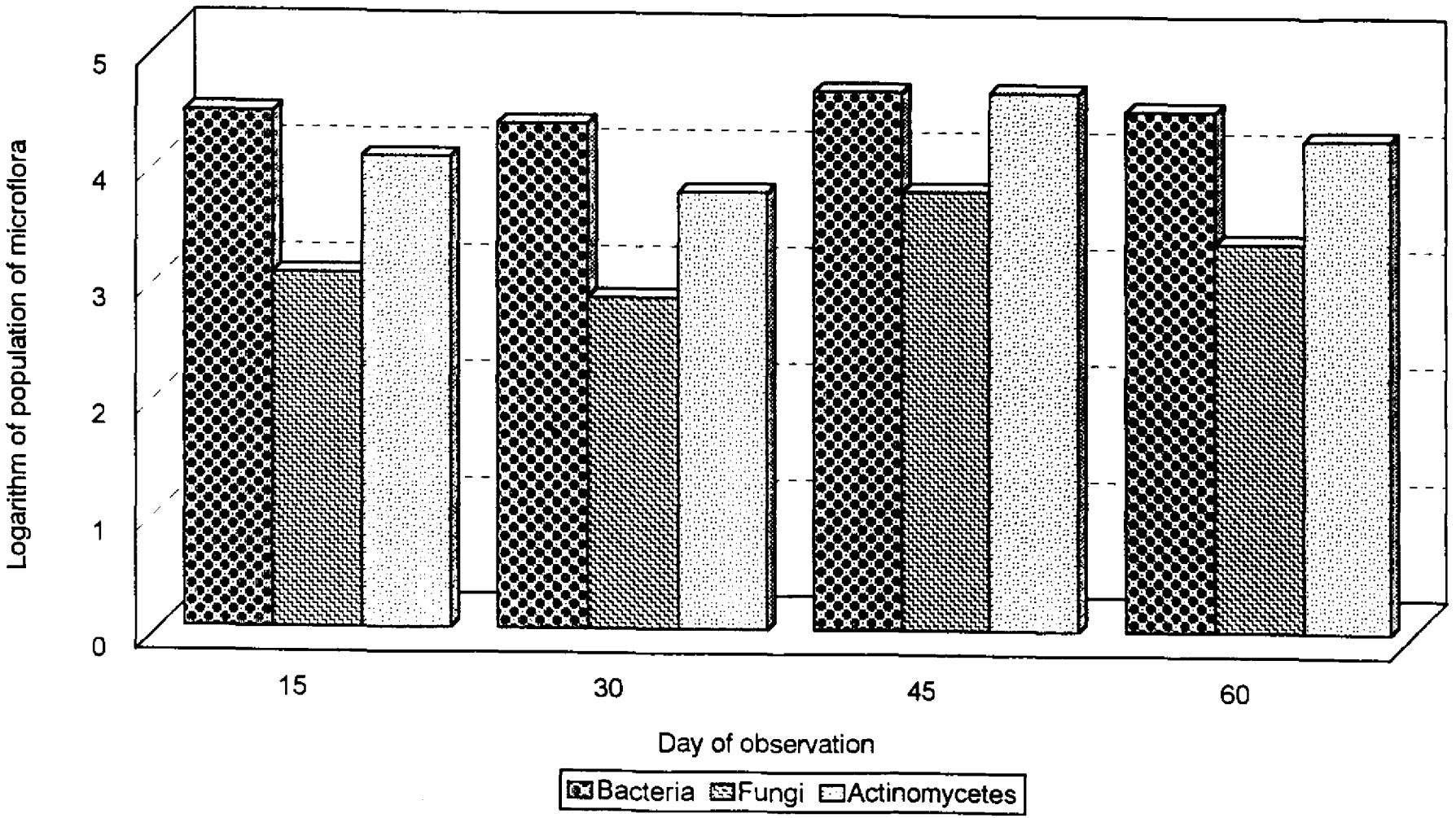
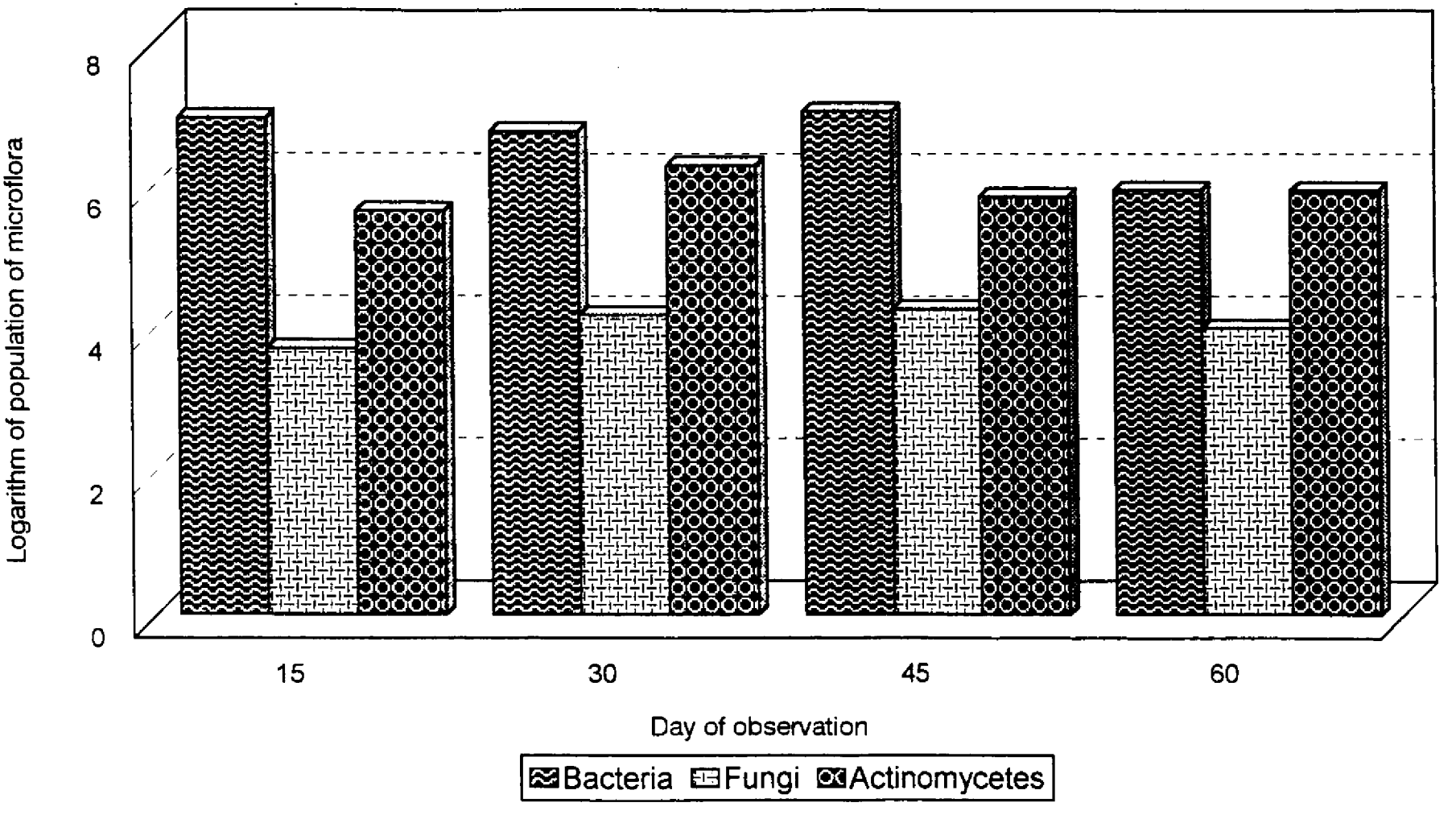


Table 2. Gut microflora of *Eudrilus eugeniae*

Microorganism	Dilution factor	Number of microorganisms per earthworm* Day of observation			
		15	30	45	60
Bacteria	10^6	9.0	5.9	11.3	0.9
Fungi	10^3	5.3	15.5	19.1	10.2
Actinomycetes	10^5	4.5	19.6	7.2	8.8
Nitrogen fixing bacteria	10^3	1.3	-	-	-
Phosphate solubilizing bacteria	10^4	-	-	-	-
Pseudomonas solanacearum	10^6	-	-	-	-
Pythium spp.	10^4	-	-	-	-
Phytophthora spp.	10^4	-	-	-	-

* Mean of three replications

Fig. 2 Gut microflora of *Eudrilus eugeniae*



Phytophthora spp. were absent in the gut content of various earthworm samples.

4.1.3 Total microflora of vermicompost

The total number of bacteria (11.9 x 10^6), fungi (11.2 x 10^4) and actinomycetes (49.7 x 10^4) were maximum on 30, 45 and 60 day respectively (Table 3; Fig. 3). The number of phosphate solubilizing bacteria (3.1 x 10^6) and nitrogen fixing bacteria (8.9 x 10^3) were more on 15 and 30 day of sampling. However, soilborne pathogens such as *Pseudomonas solanacearum, Pythium* spp. and *Phytophthora* spp. were absent in all vermicompost samples collected at different time intervals.

4.2 Characterisation of nitrogen fixing bacteria

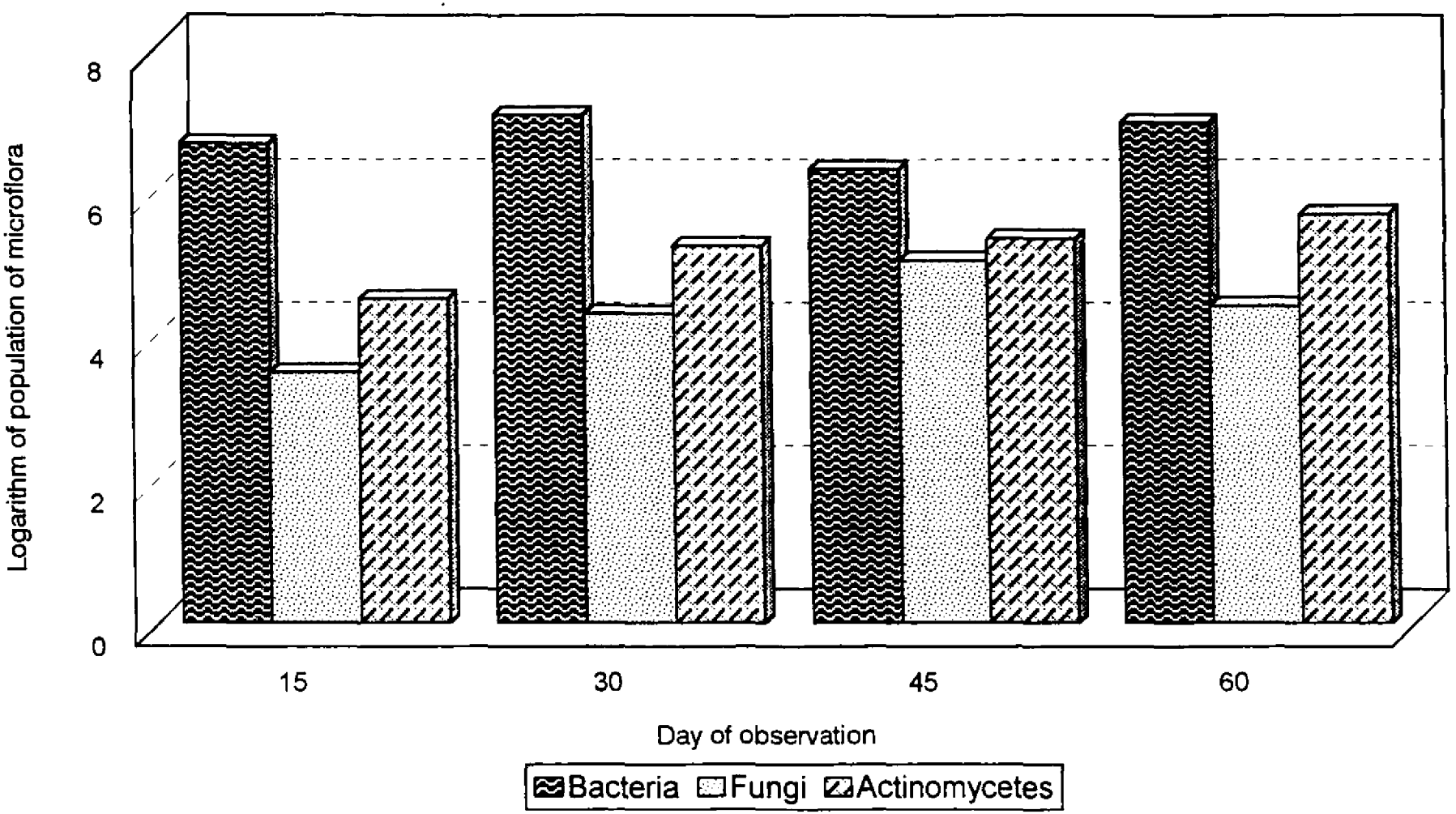
The comparative data on various colony characters such as size, shape, elevation and pigment production, motility, gram reaction, presence or absence of endospores and capsule, ability to hydrolyse starch and casein, ability to utilize different carbon sources such as glucose, sucrose, lactose and mannitol and pH tolerance of the four isolates of nitrogen fixing bacteria are given in table 4.

Table 3. Microflora of vermicompost produced by *Eudrilus eugeniae*

Microorganism	Dilution factor	Number of microorganisms per gram of vermicompost* Day of observation			
		15	30	45	60
Bacteria	10^6	4.7	11.9	2.1	9.4
Fungi	10^4	0.3	2.0	11.2	2.6
Actinomycetes	10^4	3.2	17.9	22.7	49.7
Nitrogen fixing bacteria	10^3	6.7	8.9	-	2.2
Phosphate solubilizing bacteria	10^6	3.1	0.4	0.2	-
Pseudomonas solanacearum	10^6	-	-	-	-
Pythium spp.	10^4	-	-	-	-
Phytophthora spp.	10^4	-	-	-	-

* Mean of three replications

Fig. 3 Microflora of vermicompost produced by *Eudrilus eugeniae*



4.2.1 Growth rate and nitrogen fixing ability of different isolates

There were significant differences in the growth rate of various isolates of nitrogen fixing bacteria in Jensen's nitrogen free broth. The mean optical density of 0.589 for isolate 3 was significantly higher than rest of isolates (Table 4; Fig. 4). The nitrogen fixing ability of these diazotrophs varied from 0.8 to 6.7 mg per g of sucrose consumed (Fig. 5). Here also, this was maximum for isolate 3 (6.7 mg) followed by isolate 4 (6.4 mg). The isolate 3 (Plate 2) was selected for enrichment of vermicompost. Based on various morphological and physiological characters (Table 5), this isolate was tentatively identified as *Azotobacter* sp.

4.3 Characterisation of phosphate solubilizing bacteria

The comparative data on various colony characters such as size, shape, elevation and pigment production, motility, gram reaction, presence or absence of endospores and capsule, ability to hydrolyse starch and casein, ability to utilize different carbon sources such as glucose, sucrose, lactose and mannitol and pH tolerance of the two isolates of phosphate solubilizing bacteria are given in table 6.

4.3.1 Selection of efficient phosphate solubilizing bacteria

The percentage of phosphate solubilized in modified Pikovskaya's broth varied from 26.4 per cent for *Bacillus megaterium* var. *phosphaticum* to 32.7 per

Table 4. Growth rate of different isolates of nitrogen fixing bacteria under *in vitro* conditions

Isolates	Hours of Incubation							Mean
	0	24	48	72	96	120	144	
Isolate 1	0.009	0.010	0.163	0.315	0.327	0.337	0.352	0.229
Isolate 2	0.034	0.100	0.166	0.238	0.283	0.293	0.298	0.202
Isolate 3	0.062	0.145	0.378	0.525	0.863	1.088	1.064	0.589
Isolate 4	0.058	0.125	0.244	0.392	0.547	0.797	1.123	0.469
CD (0.05)								0.096

Fig. 4 Growth rate (OD 600) of different isolates of nitrogen fixing bacteria under *in vitro* conditions

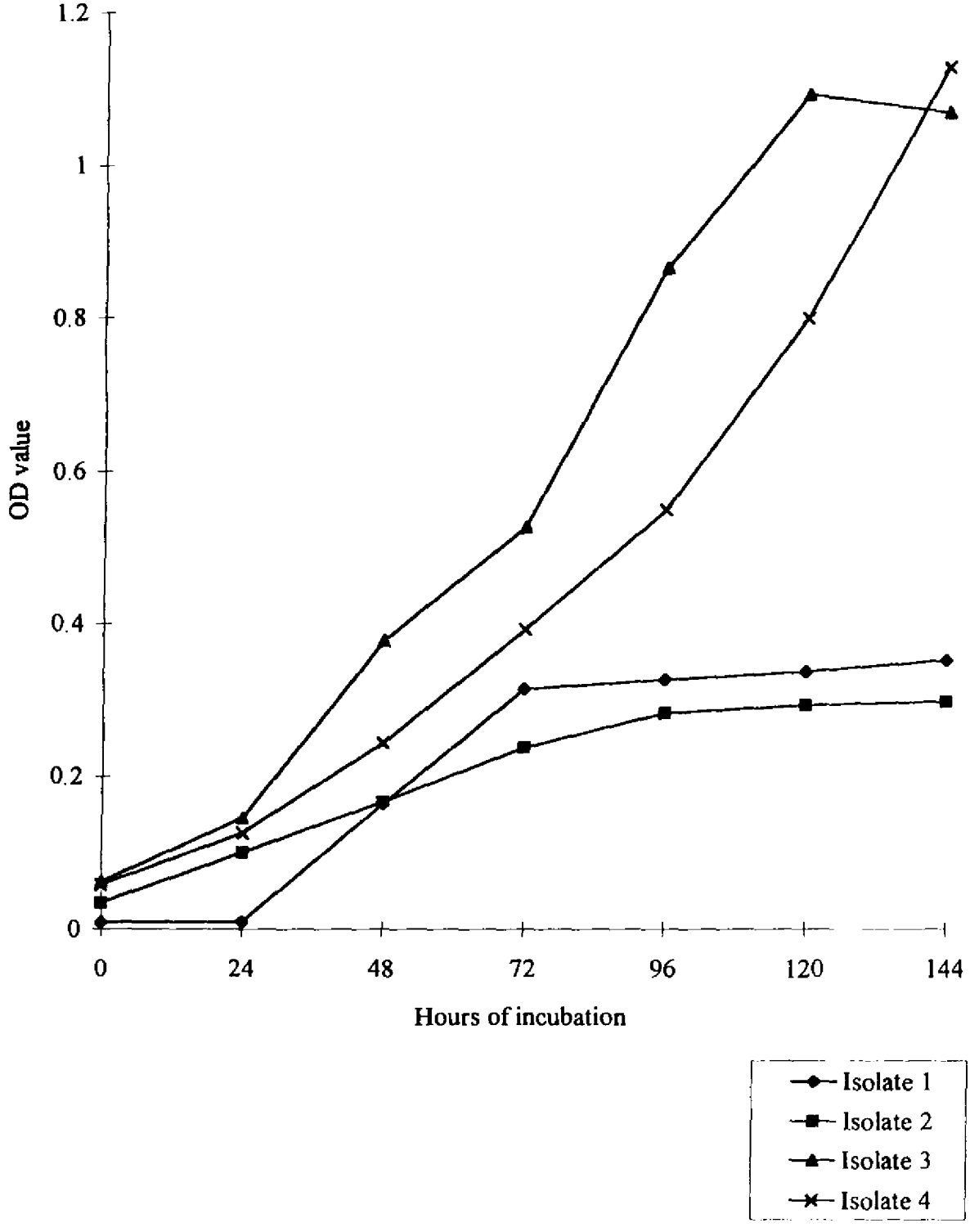


Table 5. Characterisation of different isolates of nitrogen fixing bacteria.

Characters	Isolates			
	1	2	3	4
a. Colony characters*				
size	1-2 mm	1-2 mm	2-4 mm	2-4 mm
shape	round	round	round	round
elevation	low convex	convex	convex	low convex
pigment production	-	-	yellowish brown	yellow
b. Motility	-	-	+	-
c. Gram staining	-	-	-	-
d. Spore staining	-	-	-	-
e. Capsule staining	-	+	+	+
f. Starch hydrolysis	-	-	-	-
g. Casein hydrolysis	-	-	-	-
h. Growth in different carbon sources				
Sucrose	+	+	+	+
Glucose	+	+	+	+
Lactose	+	+	+	+
Mannitol	+	+	+	+
i. pH tolerance				
3.0	-	-	-	-
5.0	+	+	+	+
7.0	+	+	+	+

* After 48 hours

Fig. 5 Nitrogen fixing ability of different isolates of diazotrophs under *in vitro* conditions

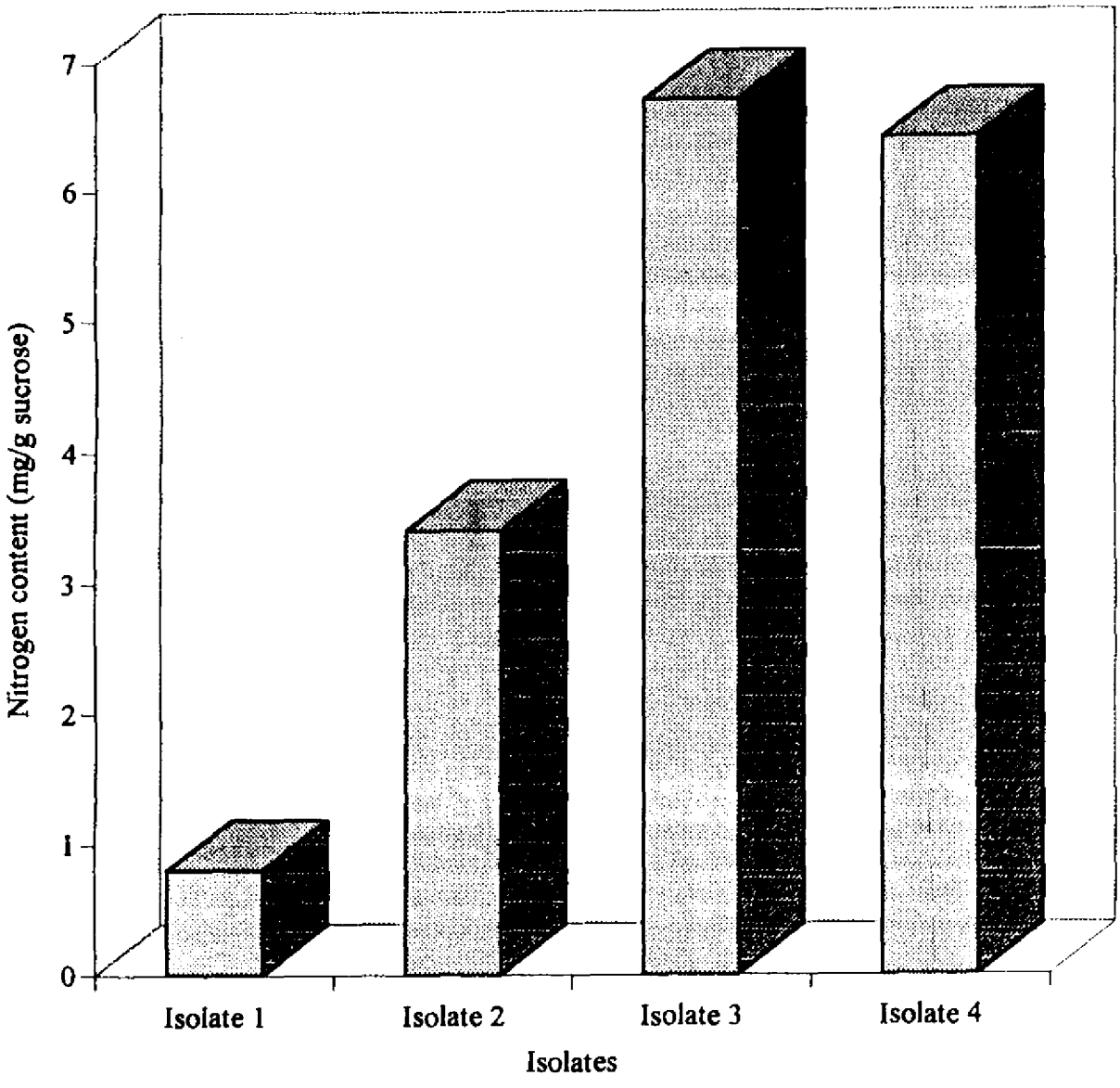


Plate 2

Growth of *Azotobacter* sp. on Jensen's nitrogen free agar


N3

Table 6. Characterisation of different isolates of phosphate solubilizing bacteria.

Characters	Isolates	
	1	2
a. Colony characters*		
size	2-4 mm	2-4 mm
shape	circular	circular
elevation	flat	flat
pigment production	-	-
b. Motility	+	+
c. Gram staining	-	-
d. Spore staining	-	-
e. Capsule staining	-	-
f. Starch hydrolysis	-	-
g. Casein hydrolysis	-	-
h. Growth in different carbon sources		
Sucrose	+	+
Glucose	+	+
Lactose	+	+
Mannitol	+	+
i. pH tolerance		
3.0	-	-
5.0	+	+
7.0	+	+

* After 48 hours

cent (Fig. 6) for isolate 1. The isolate 1 was selected for enrichment of vermicompost. Based on the various morphological and physiological characters the isolate 1 was tentatively identified as *Pseudomonas* sp.

4.4 Enrichment of vermicompost with nitrogen fixing bacteria

4.4.1 Influence of enriched vermicompost on growth parameters of chilli

There were significant differences between treatments in the fresh and dry weights of shoot (Table 7). The fresh and dry weights of 43.7 and 22. 3 g respectively, were significantly high in the treatment combination of T_5 when compared to the control (T_1) treatment. The increase in the fresh and dry weights of shoot in treatments such as T_4, T_2 and T_3 were statistically on par with that of T_5. But there were no significant differences between treatments in plant height, number of branches formed and in the fresh and dry weights of roots. The plant height of 51.7 cm and the fresh and dry weights of roots, 9.3 and 3.4 g respectively, were also maximum in the T_5 treatment (Fig.7). However, the number of branches formed (17.7) was more in the T_2 treatment.

4.4.2 Influence of enriched vermicompost on flowering and yield of chilli

There were significant differences between treatments in the yield of chilli. The per plant yield of 103.0 g was maximum in the treatment

Fig. 6 Percentage of phosphate solubilized by different isolates of bacteria

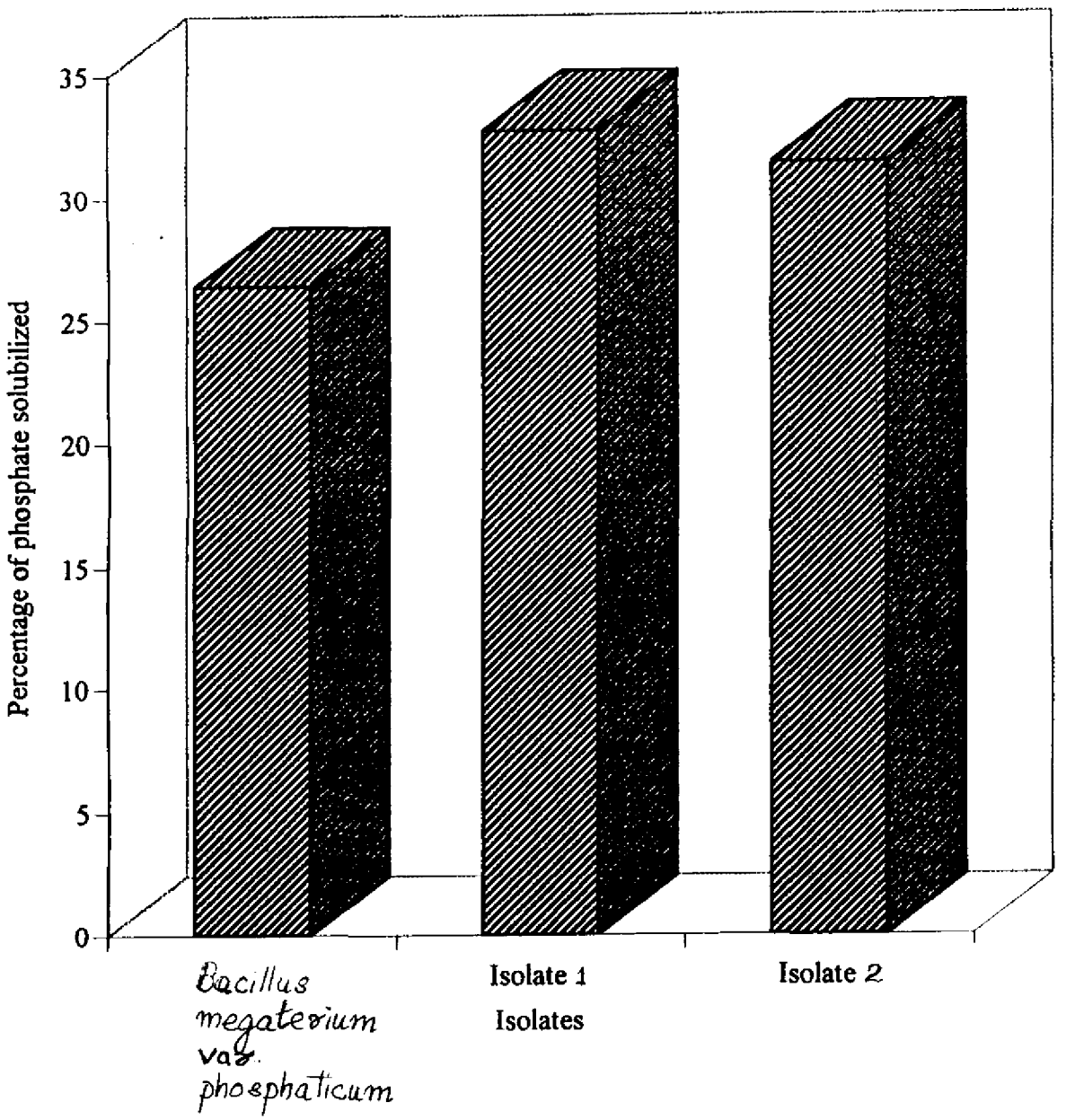
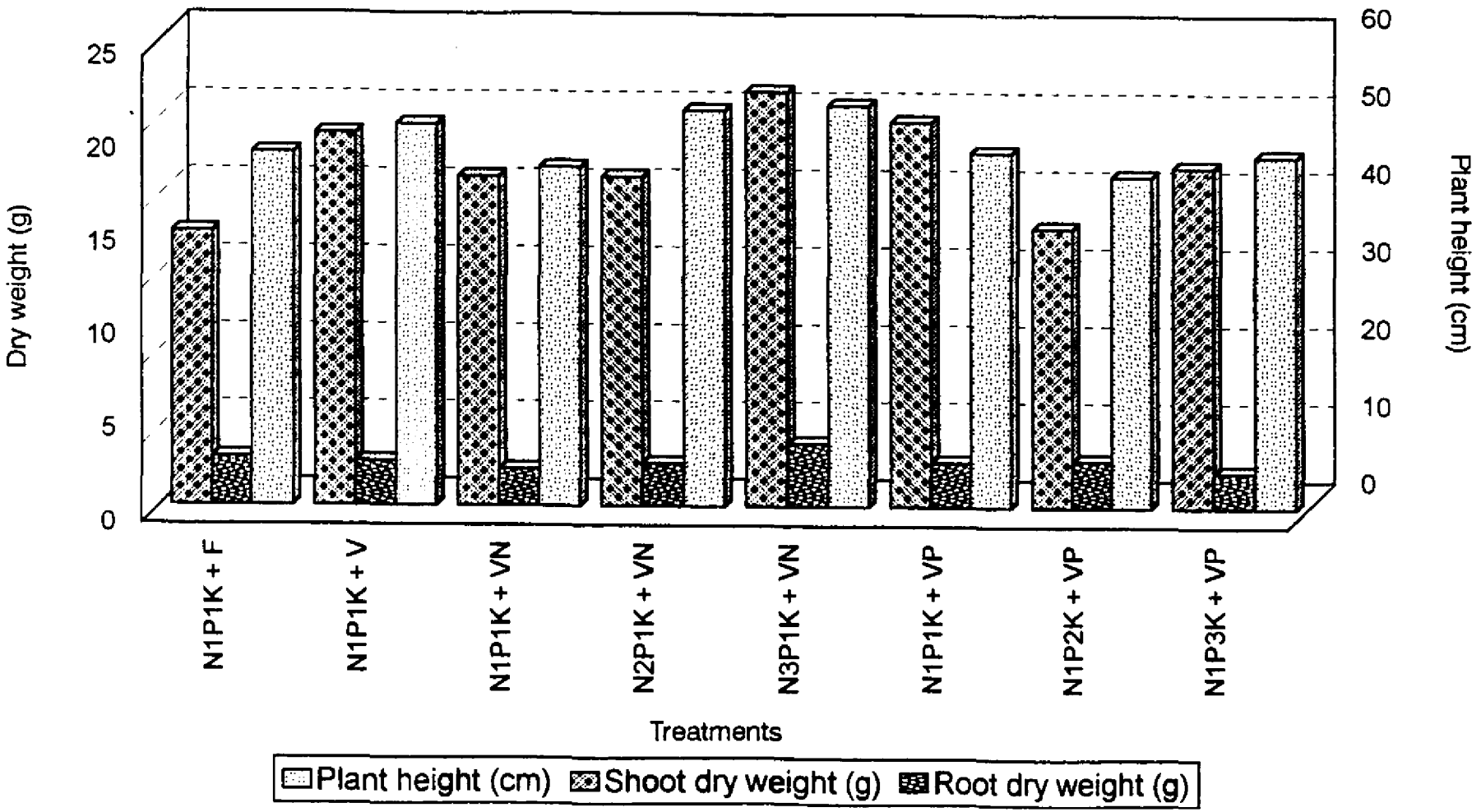


Table 7. Influence of vermicompost enriched with nitrogen fixing bacteria on growth parameters of chilli

Treatment number	Treatment combinations	Height (cm)	Number of branches	Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root
T_1	N_1P_1K+F	45.5	14.3	28.3	7.0	14.7	2.6
T_2	N_1P_1K + V	49.2	17.7	38.0	6.7	20.0	2.4
T_3	N_1P_1K + VN	43.7	14.0	35.3	5.7	17.7	2.0
T_4	N_2P_1K + VN	51.0	16.3	38.3	6.3	17.7	2.3
T_5	N_3P_1K + VN	51.7	15.7	43.7	9.3	22.3	3.4
CD (0.05)		NS	NS	14.7	NS	7.2	NS

Fig. 7 Influence of enriched vermicompost on growth parameters of chilli



combination of T_3 followed by 97.3 g in T_4 treatment (Table 8; Fig. 8). These were significantly high and showed an yield increase of 234.1 and 221.1 per cent respectively over the control treatment. The yield increase in treatments such as T_5 and T_2 was statistically on par with that of the best treatment combination of T_3. But there were no significant differences between treatments in the onset of flowering and number of fruits formed per plant. The earliest onset of flowering was observed on 40 day in T_2 treatment. However, the number of fruits formed per plant (20.0) was more in the T_4 treatment.

4.5 Enrichment of vermicompost with phosphate solubilizing bacteria

4.5.1 Influence of enriched vermicompost on growth parameters of chilli

There were no significant differences between treatments in plant height, number of branches formed and in the fresh and dry weights of shoot and root. The plant height of 49.2 cm and the number of branches formed, 17.7, were maximum in the treatment combination of T_2. The fresh and dry weights of shoot and the fresh weight of roots were more in the T_6 treatment. These were 41.0, 20.7 and 7.5 g respectively (Table 9). However, the dry weight of roots (2.6 g) was marginally higher in the control treatment.

Table 8. Influence of vermicompost enriched with nitrogen fixing bacteria on flowering and yield of chilli.

Treatment number	Treatment combinations	On set of flowering (DAT)	Number of fruits per plant	Yield per plant (g)	Percentage yield increase over control
T_1	N_1P_1K + F	45.0	10.3	44.0	-
T_2	N_1P_1K + V	40.0	12.3	57.3	130.2
T_3	N_1P_1K + VN	43.7	17.0	103.0	234.1
T_4	N_2P_1K + VN	49.0	20.0	97.3	221.1
T_5	N_3P_1K + VN	47.0	16.0	75.7	172.0
CD (0.05)		NS	NS	52.2	

DAT - Days after transplanting

Fig. 8 Influence of enriched vermicompost on yield components of chilli

Table 9. Influence of vermicompost enriched with phosphate solubilizing bacteria on growth parameters of chilli

Treatment number	Treatment combinations	Height (cm)	Number of branches	Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root
T_1	$N_1P_1K + F$	45.5	14.3	28.3	7.0	14.7	2.6
T_2	$N_1P_1K + V$	49.2	17.7	38.0	6.7	20.0	2.4
T_6	$N_1P_1K + VP$	45.7	14.3	41.0	7.5	20.7	2.4
T_7	$N_1P_2K + VP$	42.7	13.3	28.3	6.3	15.0	2.5
T_8	$N_1P_3K + VP$	45.3	15.3	35.0	5.2	18.3	1.9
CD (0.05)		NS	NS	NS	NS	NS	NS

4.5.2 Influence of enriched vermicompost on flowering and yield of chilli

There were significant differences between treatments in the onset of flowering and in the number of fruits formed per plant. The earliest onset of flowering (38.3 days) was observed in the treatment combination of T_6 (Table 10). In this treatment, the number of fruits formed per plant, 22.0, was also significantly higher than the control treatment (Fig. 8). The number of fruits formed in T_8 (17.0), T_7 (16.0) and T_2 (12.31) treatments were statistically on par with T_6 treatment.

There was no significant difference between treatments in the yield of chilli. The per plant yield of 76.7 g was maximum in T_6 treatment followed by that of 71.7 g, in the T_8 treatment (Table 10). This showed an yield increase of 174.3 and 163.0 per cent respectively over that of the control treatment where the per plant yield was only 44.0 g.

4.6 Influence of enriched vermicompost on keeping quality of chilli

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with farm yard manure, normal vermicompost or vermicompost enriched with either nitrogen fixing or phosphate solubilizing bacteria, as a source of organic manure. The keeping quality of harvested fruits at room temperature was maximum for 8.0 days in the

Table 10. Influence of vermicompost enriched with phosphate solubilizing bacteria on flowering and yield of chilli.

Treatment number	Treatment combinations	On set of flowering (DAT)	Number of fruits per plant	Yield per plant (g)	Percentage yield increase over control
T_1	$N_1P_1K + F$	45.0	10.3	44.0	-
T_2	$N_1P_1K + V$	40.0	12.3	57.3	130.2
T_6	$N_1P_1K + VP$	38.3	22.0	76.7	174.3
T_7	$N_1P_2K + VP$	48.3	16.0	66.0	150.0
T_8	$N_1P_3K + VP$	46.3	17.0	71.7	163.0
CD (0.05)		9.6	11.2	NS	

DAT - Days after transplanting

T_4 treatment followed by 7.7 days in the T_3 treatment (Table 11). In the control treatment this was on an average of only 6.3 days.

4.7 Interaction between earthworm, pathogen and host

4.7.1 Pathogen

Based on the colony characters (fast growth rate, white coloured and aseptate mycelia) and spore morphology (lobe-shaped sporangia), the pathogen was identified as *Pythium aphanidermatum*.

4.7.2 Interaction between *Eudrilus eugeniae* and *Pythium aphanidermatum* on the incidence of damping off in chilli

The damping off of chilli caused by *Pythium aphanidermatum* was observed only in seedlings of T_2 and T_8 treatments (Table 12, Plate 3) with induced injury in the collar region at the time of transplanting.

4.7.3 Interaction between *Megascolex* sp. and *Pythium aphanidermatum* on the incidence of damping off in chilli

The damping off of chilli caused by *Pythium aphanidermatum* was observed only in seedlings of T_2 and T_8 treatments (Table 13) with induced injury in the collar region at the time of transplanting.

Table 11. Influence of vermicompost enriched with biofertilizers on keeping quality of chilli

Treatment number	Treatment combinations	Keeping quality (DAH)
T_1	N_1P_1K + F	6.3
T_2	N_1P_1K + V	7.0
T_3	N_1P_1K + VN	7.7
T_4	N_2P_1K + VN	8.0
T_5	N_3P_1K + VN	7.0
T_6	N_1P_1K + VP	6.7
T_7	N_1P_2K + VP	6.4
T_8	N_1P_3K + VP	7.0
CD (0.05)		NS

DAH – Days after harvest

Table 12. Interaction between *Eudrilus eugeniae* and *Pythium aphanidermatum* on the incidence of damping off in chilli

Treatment number	Treatment combinations	Pathogen inoculated	
		At the time of transplanting	One month after transplanting
T_1	POP (F)	-	-
T_2	POP (F) + P	+	-
T_3	POP (F) + E	-	-
T_4	POP (F) + P + E	-	-
T_7	POP (V)	-	-
T_8	POP (V) + P	+	-
T_9	POP (V) + E	-	-
T_{10}	POP (V) + P + E	-	-

\+ disease incidence

\- no disease incidence

Plate 3

Damping off of chilli due to *Pythium aphanidermatum*

A – Infected seedling

B – Healthy seedling

A – Infection at the collar region

B – Healthy seedling

B
A
camlin


A
B

Table 13. Interaction between *Megascolex* sp. and *Pythium aphanidermatum* on the incidence of damping off in chilli

Treatment number	Treatment combinations	Pathogen inoculated	
		At the time of transplanting	One month after transplanting
T_1	POP (F)	-	-
T_2	POP (F) + P	+	-
T_5	POP (F) + M	-	-
T_6	POP (F) + P + M	-	-
T_7	POP (V)	-	-
T_8	POP (V) + P	+	-
T_{11}	POP (V) + M	-	-
T_{12}	POP (V) + P + M	-	-

\+ disease incidence

\- no disease incidence

4.7.4 Effect of combined application of *Eudrilus eugeniae* and *Pythium aphanidermatum* on growth parameters of chilli

There was 100 per cent seedling mortality in treatments such as T_2 and T_8 where the application of *Pythium aphanidermatum* was coupled with surface injury in the collar region of seedlings (Table 14; Fig. 9). But in the absence of such an induced injury, there was no incidence of damping off. This was observed in T_4 and T_{10} treatments, where there was a combined application of *Eudrilus eugeniae* and *Pythium aphanidermatim* . However, in these treatments, the plant growth was generally poor. (Plate 4 and 5). The plant height of 44 and 48 cm and the fresh and dry weights of roots of 7.3 and 3.0 g and 7.1 and 2.7 g respectively were significantly low in the T_4 and T_{10} treatments (Table 14; Fig. 9) when compared to that of 71.0 cm and 17.3 and 6.6 g respectively in the control treatment.

The plant growth was better in treatments such as T_3, T_7 and T_9 without pathogen inoculation. The number of branches formed was significantly high in T_3 (21.7) and T_9 (22.3) treatments when compared to that of 15.0 in the control treatment (Table 14). The number of branches in T_7 treatment (19.7) was statistically on par with that of T_9 treatment.

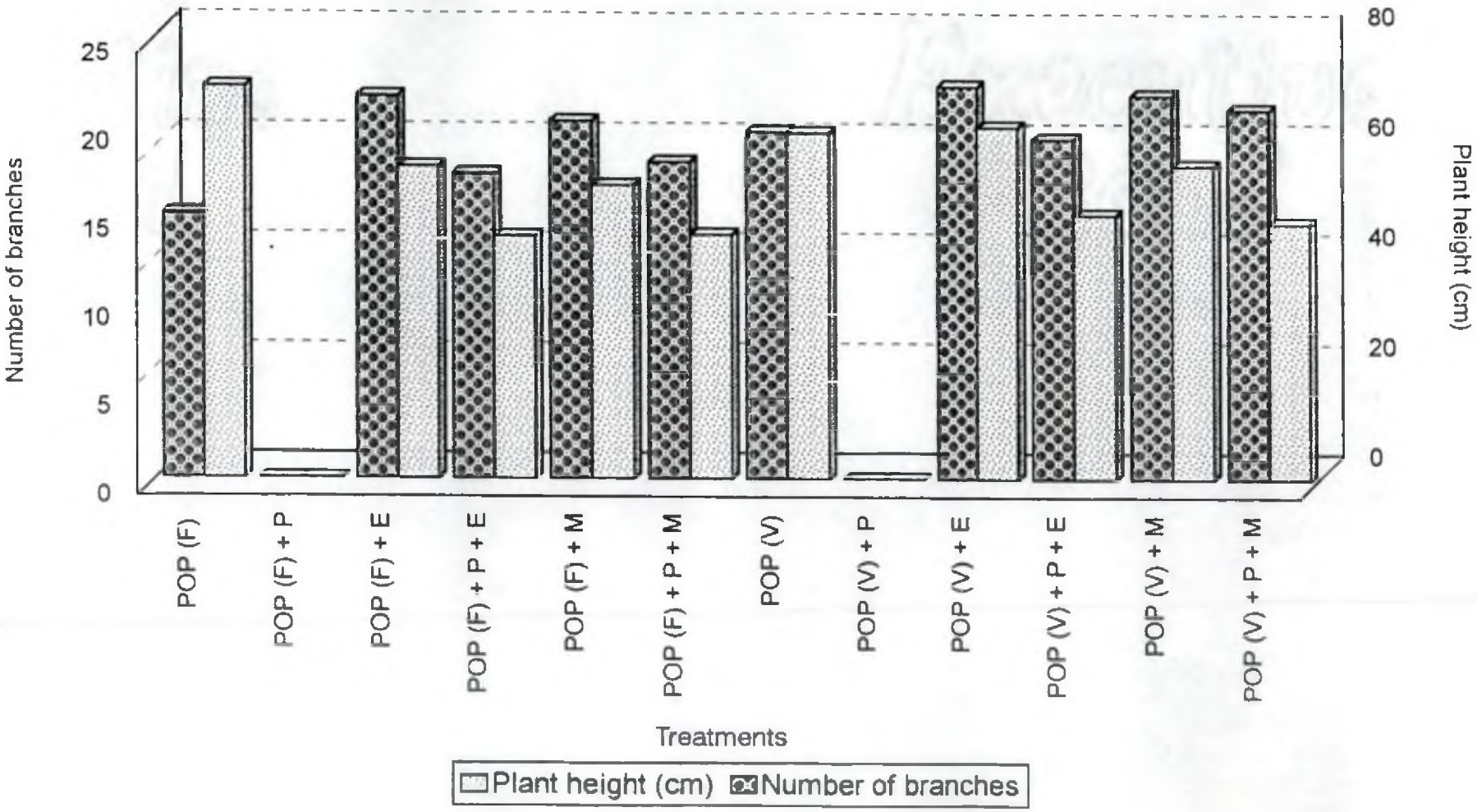
The plant height of 71.0 cm and root fresh weight of 17.3 g were maximum in the control treatment. However, the plant height of 62.8 and 64.0

Table 14. Effect of combined application of *Eudrilus eugeniae* and *Pythium aphanidermatum* on growth parameters of chilli

Treatment number	Treatment combinations	Height (cm)	Number of branches	Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root
T_1	POP (F)	71.0	15.0	56.8	17.3	31.5	6.6
T_2	POP (F) + P*	-	-	-	-	-	-
T_3	POP(F) + E	56.7	21.7	61.7	17.0	32.7	6.8
T_4	POP(F)+P+E	44.0	17.3	55.7	7.3	29.0	3.0
T_7	POP (V)	62.8	19.7	62.7	17.0	33.7	5.6
T_8	POP(V) + P*	-	-	-	-	-	-
T_9	POP (V) + E	64.0	22.3	63.7	16.1	32.9	5.4
T_{10}	POP(V)+P+E	48.0	19.3	53.7	7.1	27.9	2.7
CD (0.05)		11.0	6.4	NS	10	NS	3.3

* Incidence of damping off

Fig. 9 Effect of combined application of earthworms and *Pythium aphanidermatum* on plant height and number of branches of chilli




T3
T4

Plate 5

Effect of combined application of *Eudrilus eugeniae* and *Pythium aphanidermatum* on growth and yield of chilli

Treatments with vermicompost application

T_9 - POP with vermicompost + *Eudrilus eugeniae*

T_{10} - POP with vermicompost + *Pythium aphanidermatum* + *Eudrilus eugeniae*


T9
T10

cm respectively in the T_7 and T_9 treatments and root fresh weight of 17.0 and 16.1 g respectively in T_3, T_7 and T_9 treatments were statistically on par with the control treatment. The dry weight of roots, 6.8 g was more in the T_3 treatment (Fig. 10). The root dry weights of 5.6 and 5.4 g respectively in the T_7 and T_9 treatments were statistically on par with the T_3 treatment.

4.7.5 Effect of combined application of *Eudrilus eugeniae* and *Pythium aphanidermatum* on flowering and yield of chilli

There were significant differences between treatments in the onset of flowering, number of fruits formed and in the per plant yield of chilli. The earliest onset of flowering was observed on 42 day in the T_7 treatment (Table 15; Fig. 11). This was significant when compared to that of 52 days taken for onset of flowering in the control treatment. Such an effect was also observed in almost all the treatments except the T_4 treatment with pathogen inoculation. In T_4 and T_{10} treatments, the average number of fruits formed per plant, 10.7 and 15.3 respectively, were also less than that of the control treatment (Table 15). The number of fruits formed, 44.0 was maximum in the T_3 treatment. This was significantly higher than the control (16.0) treatment. The number of fruits formed in treatments such as T_9 (31.3) and T_7 (28,3) were statistically on par with the T_3 treatment.

Fig. 10 Effect of combined application of earthworms and *Pythium aphanidermatum* on shoot and root dry weights of chilli

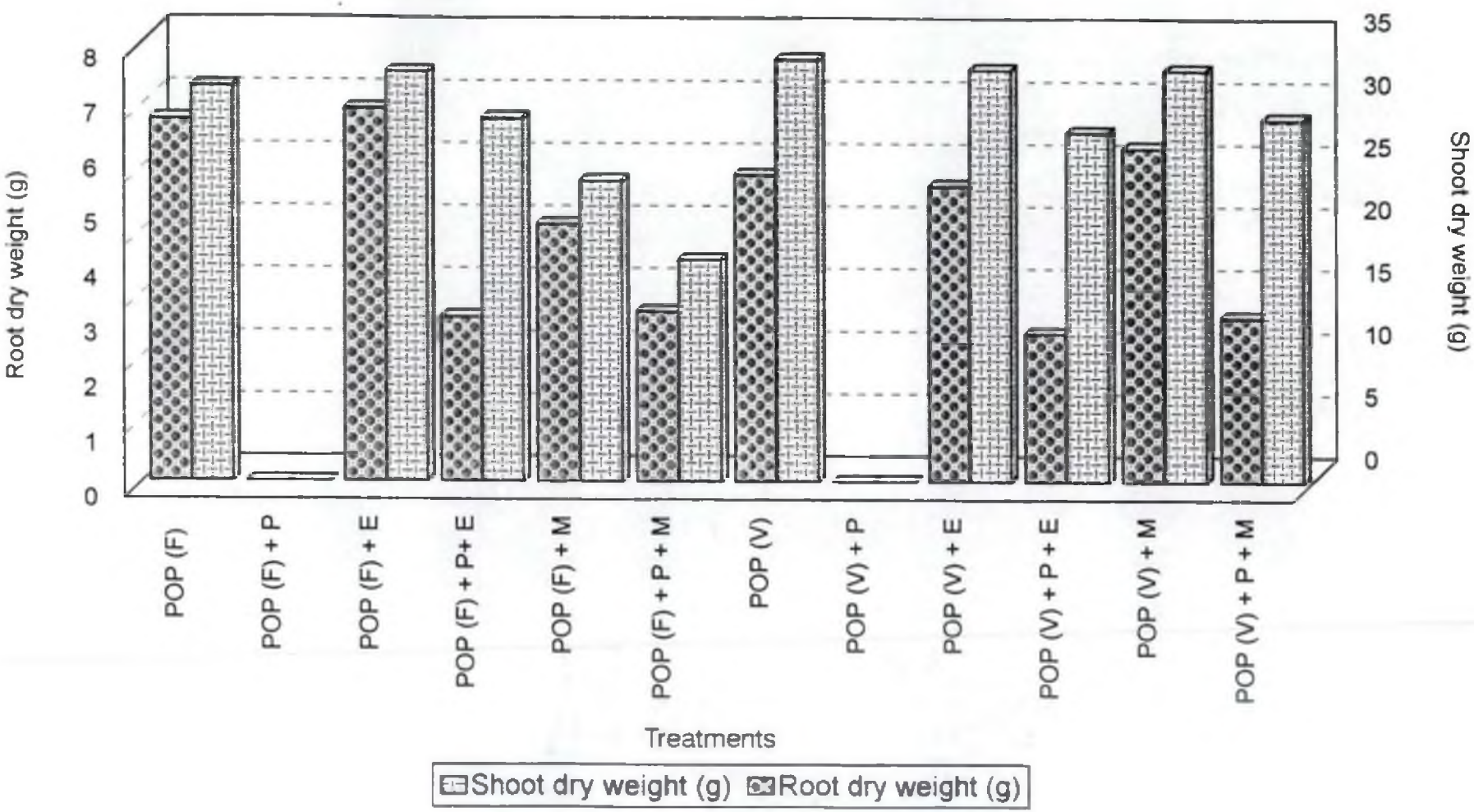


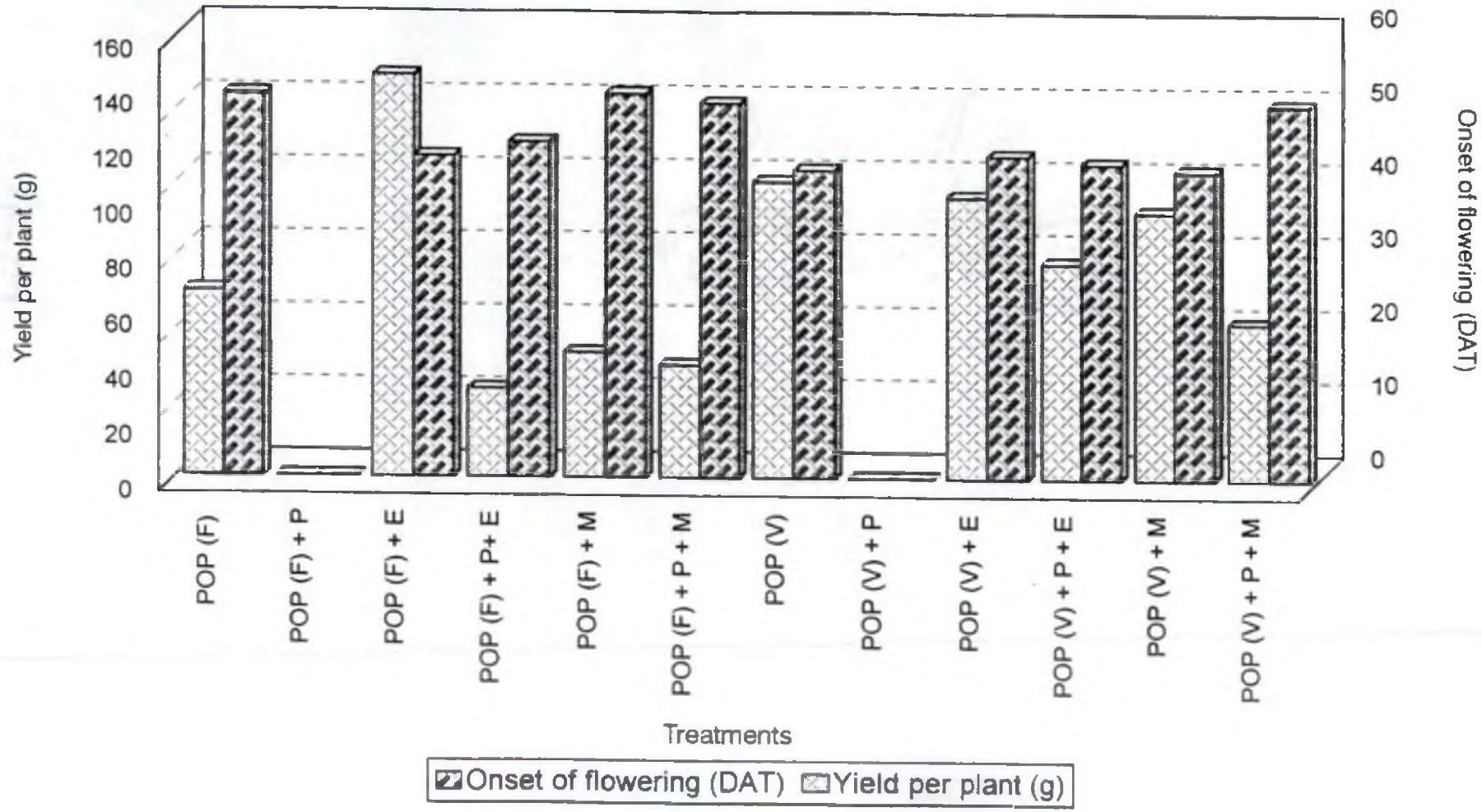
Table 15. Effect of combined application of *Eudrilus eugeniae* and *Pythium aphanidermatum* on flowering and yield of chilli.

Treatment number	Treatment combinations	Onset of flowering (DAT)	Number of fruits per plant	Yield per plant (g)
T_1	POP (F)	52.0	16.0	67.0
T_2	POP (F) + P*	-	-	-
T_3	POP (F) + E	43.7	44.0	146.7
T_4	POP (F) + P + E	45.7	10.7	32.0
T_7	POP (V)	42·0	28.3	107.7
T_8	POP (V) + P*	-	-	-
T_9	POP (V) + E	44.0	31.3	102.3
T_{10}	POP (V) + P + E	43.0	15.3	78.3
CD (0.05)		6.9	23.2	71

* Incidence of damping off

DAT - Days after transplanting

Fig. 11 Effect of combined application of earthworms and *Pythium aphanidermatum* on yield components of chilli



The per plant yield of chilli, 32.0 g was low in the T_4 treatment with pathogen inoculation (Table 15; Plate 4; Fig. 11). However, such a reduction was not observed in the T_{10} treatment. The yield increase was significant in T_3 treatment. Here, the per plant yield was 146.7 g when compared to only 67.0 g in the control treatment. The net yield of 107.7, 102.3 and 78.3 g respectively in the T_7, T_9 and T_{10} treatments were statistically on par with T_3 treatment.

4.7.6 Effect of combined application of *Megascolex* sp. and *Pythium aphanidermatum* on growth parameters of chilli

There was 100 per cent seedling mortality in treatments such as T_2 and T_8 where the application of *Pythium aphanidermatum* was coupled with surface injury in the collar region of seedlings (Table 16). But in the absence of such an induced injury, there was no incidence of damping off. This was observed in T_6 and T_{12} treatments, where there was a combined application of *Megascolex* sp. and *Pythium aphanidermatum*. However, in these treatments, the plant growth was relatively poor with significant reduction in the fresh and dry weights of roots. These were 9.3 and 3.1g and 9.0 and 3.0 g respectively, when compared to 17.3 and 6.6 g in the control treatment (Table 16; Fig. 9). The fresh and dry weights of shoot were also less in these treatments. Such an effect was also observed in the T_5 treatment. The plant height was significantly low in all treatments, except the T_7 treatment, where the plant height of 62.8 cm, was statistically on par with that of 71.0 cm in the control treatment. There were

Table 16. Effect of combined application of *Megascolex* sp and *Pythium aphanidermatum* on growth parameters of chilli

Treatment number	Treatment combinations	Height (cm)	Number of branches	Fresh weight (g)		Dry weight (g)	
				Shoot	Root	Shoot	Root
T_1	POP (F)	71.0	15.0	56.8	17.3	31.5	6.6
T_2	POP (F) + P*	-	-	-	-	-	-
T_5	POP(F) + M	53.3	20.3	45.7	14.5	24.0	4.7
T_6	POP(F)+P+M	44.3	18.0	34.0	9.3	17.7	3.1
T_7	POP (V)	62.8	19.7	62.7	17.0	33.7	5.6
T_8	POP(V) + P*	-	-	-	-	-	-
T_{11}	POP (V) + M	57.0	21.8	63.7	15.0	32.9	6.1
T_{12}	POP(V)+P+M	46.5	21.0	54.3	9.0	29.0	3.0
CD (0.05)		8.4	NS	NS	7.9	NS	3.4

* Incidence of damping off

no significant differences between treatments in the number of branches formed. Maximum number of branches were formed in T_{11} (21.8) and T_{12} (21.0) treatments when compared to that of 15.0 in the control treatment.

4.7.7 Effect of combined application of *Megascolex* sp. and *Pythium aphanidermatum* on flowering and yield of chilli

Significant differences between treatments were observed in the onset of flowering, number of fruits formed and in the per plant yield of chilli. Flower initiation was earliest in the T_7 and T_{11} treatments (42 day) when compared to 52 days taken in the control treatment (Table 17; Fig. 11). The number of fruits formed was also significantly high in T_7 and T_{11} treatments where on an average 28.3 fruits were formed as compared to 16.0 in the control treatment. The per plant yield of chilli, 107.7 g, was also significantly high in T_7 treatment. In the T_6 treatment with pathogen inoculation, the yield was only 40.7 grams. All other treatments including the control were on par with the T_7 treatment.

4.7.8 Combined application of earthworms and *Pythium aphanidermatum* on keeping quality of chilli

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with or without the application of either *Eudrilus eugeniae* or *Megascolex* sp. and *Pythium aphanidermatum*. The keeping quality of harvested fruits at room temperature was maximum for 9.3

Table 17. Effect of combined application of *Megascolex* sp. and *Pythium aphanidermatum* on flowering and yield of chilli.

Treatment number	Treatment combinations	Onset of flowering (DAT)	Number of fruits per plant	Yield per plant (g)
T_1	POP (F)	52.0	16.0	67.0
T_2	POP (F) + P*	-	-	-
T_5	POP (F) + M	52.3	15.0	45.3
T_6	POP (F) + P + M	51.0	12.7	40.7
T_7	POP (V)	42.0	28.3	107.7
T_8	POP (V) + P*	-	-	-
T_{11}	POP (V) + M	42.0	28.3	97.0
T_{12}	POP (V) + P + M	51.0	14.7	56.7
CD (0.05)		8.6	9.6	63.9

* Incidence of damping off

DAT - Days after transplanting

Table 18. Combined application of earthworms and *Pythium aphanidermatum* on keeping quality of chilli.

Treatment number	Treatment combinations	Keeping quality (DAH)
T_1	POP (F)	7.7
T_2	POP (F) + P*	-
T_3	POP (F) + E	9.0
T_4	POP (F) + P + E	8.7
T_5	POP (F) + M	8.7
T_6	POP (F) + P + M	8.7
T_7	POP (V)	9.3
T_8	POP (V) + P*	-
T_9	POP (V) + E	9.3
T_{10}	POP (V) + P + E	9.3
T_{11}	POP (V) + M	9.0
T_{12}	POP (V) + M + E	8.3
CD (0.05)		NS

* - Treatments with pathogen application

DAH - Days after harvest

Table 19. Relative population of *Eudrilus eugeniae* in the interaction study with *Pythium aphanidermatum*

Treatment number	Treatment combinations	Number of earthworms	
		Before the experiment	After the experiment
T_1	POP (F)*	-	-
T_2	POP (F) + P*	-	-
T_3	POP (F) + E	50	48.0
T_4	POP (F) + P + E	50	60.0
T_7	POP (V)*	-	-
T_8	POP (V) + P*	-	-
T_9	POP (V) + E	50	41.7
T_{10}	POP (V) + P + E	50	49.0
CD (0.05)		NS	NS

* Treatments without earthworm application

days in the T_7 and T_9 treatments where vermicompost was used as a source of organic manure (Table 18). Such an effect was also observed in the T_{10} treatment with pathogen inoculation. In treatments with farm yard manure as a source of organic manure, this varied from 8.7 days in T_4, T_5 and T_6 treatments to 9.0 days in the T_3 treatment (Table 18) as compared to an average of only 7.7 days in the control treatment.

4.7.9 Relative population of *Eudrilus eugeniae* in the interaction study with *Pythium aphanidermatum*

Fifty adult worms of *Eudrilus eugeniae* were added in T_3, T_4, T_9 and T_{10} treatments with earthworm application. Two of these treatments such as T_4 and T_{10} were also with pathogen inoculation. An increase in the number of adult worms was obtained only in the T_4 treatment (Table 19). But there was reduction in the number of worms at the time of harvest in the remaining three treatments. The average number of earthworms present varied from 49.0, 48.0 and 41.7 respectively in the T_{10}, T_3 and T_9 treatments. In most of these treatments, the earthworms were found in very close association with the plant root system (Plate 6). However, in the other experiment with *Megascolex* sp., no adult worms could be isolated at the end of the experiment.

DISCUSSION

DISCUSSION

Vermicompost is an important source of organic manure. It not only helps to reduce environmental pollution from garbage accumulation, but also provides an easy-to-adopt technology to the farmers to meet their requirements of organic manures to a great extent. Similarly, a number of biofertilizers are also available for application to different crops. Two of these biofertilizers such as *Azotobacter* and phosphate solubilizing bacteria were used during this investigation for enrichment of vermicompost to test their efficacy to improve plant growth and yield in chilli. These bacteria were initially isolated as part of the surface or gut microflora of an efficient composting species of earthworm, *Eudrilus eugeniae* or from the vermicompost produced by this earthworm. Another important objective was to study the occurrence, if any, of some of the commonly occurring soilborne plant pathogens such as *Pseudomonas solanacearum*, *Pythium* spp. and *Phytophthora* spp. in association with either the earthworm or vermicompost and to know whether the earthworm as such or the vermicompost would act as a carrier for such pathogens.

The observations on the surface and gut microflora of *Eudrilus eugeniae* and the vermicompost produced by this exotic species of earthworm were taken at 15 day interval on 15, 30, 45 and 60 day of composting. These

time intervals were selected because they corresponded approximately with the different phases of composting in an earthworm-active vermicompost pit.

The total number of bacteria and fungi isolated from the surface and gut content of *Eudrilus eugeniae* was maximum on 45 day. This corresponded with the peak composting activity of the earthworm. The number of bacteria and fungi isolated as surface flora were to the extent of 4.3×10^4 and 6.0×10^3 respectively (Table 1; Fig.1). In the gut content, the population of these microorganisms were 11.3×10^6 and 19.1×10^3 respectively (Table 2; Fig.2). The occurrence of higher number of bacteria and fungi in the gut content of earthworms had been reported earlier by Parle (1963), Contreras (1980) and Kristufek *et al.* (1993 and 1995). This may be due to the enzymes and mucus with water soluble organic compounds produced in the gut of the earth worms which stimulate the multiplication of microorganisms (Barois and Lavelle, 1986, and Brown, 1995). The number of actinomycetes isolated (42.5×10^3) as surface flora was also more on 45 day of composting. However, their number (19.6×10^5) was more in the gut content (Table 2; Fig.2). Such enhanced number of actinomycetes in the gut content of earthworm had been reported earlier also by Parle (1963), Ravasz *et al.* (1986) and Kristufek *et al.* (1993).

The population of beneficial microorganisms such as nitrogen fixing bacteria and phosphate solubilizing bacteria showed considerable variations in

their relative number during the different phases of vermicomposting. While, from the surface flora, more number of nitrogen fixing bacteria (3.0×10^1) were isolated on 60 day (Table 1), in the gut content their number (1.3×10^3) was more on 15 day of composting. The presence of nitrogen fixing bacteria as part of the surface and more important, the gut microflora of earthworms had been reported earlier by Citernesi *et al.* (1977), Simek *et al.* (1991) and Nair *et al.* (1997). As regards phosphate solubilizers, none could be isolated as part of the earthworm flora during this investigation. Such microorganisms are more frequently observed either in vermicast or vermicompost.

Soilborne pathogens such as *Pseudomonas solanacearum, Pythium* spp. and *Phytophthora* spp. were absent either as the surface or gut microflora of *Eudrilus eugeniae*. This indicated that such organisms did not form a part of the normal microflora of earthworms. The occasional occurrence of soilborne pathogens, especially of fungal origin might be due to a chance ingestion of fungal spores. (Baweja, 1939; Khambata and Bhat, 1957; Hoffman and Purdy, 1964; Rao, 1979 and Toyota and Kimura, 1994). However, such organisms may be present as part of vermicompost as a result of their chance occurrence either in the soil or in the infected plant materials used for composting.

Different types of bacteria, fungi and actinomycetes, beneficial microorganisms such as nitrogen fixing bacteria and phosphate solubilizers were isolated from vermicompost samples collected at different time intervals. The total number of bacteria (11.9×10^6), fungi (11.2×10^4) and actinomycetes (49.7×10^4) were more on 30, 45 and 60 day of composting (Table 3). It was also observed that the number of bacteria and fungi was more in vermicompost when compared to the surface or gut microflora of *Eudrilus eugeniae*. But, the number of actinomycetes (49.7×10^4) was less than that of the gut content (19.6×10^5). The differential increase in the population of bacteria, fungi and actinomycetes in vermicompost on 30, 45 and 60 day might be due to a sequential succession of microorganisms during compost formation. Usually, the first group of microorganisms to become active upon addition of fresh raw material to a compost pit are bacteria, followed by fungi and actinomycetes. However, a definite correlation between the microflora of vermicompost and that of the surface and gut content of *Eudrilus eugeniae* was lacking.

More number of nitrogen fixing bacteria (8.9×10^3) and phosphate solubilizing bacteria (3.1×10^6) were isolated from vermicompost. Such an occurrence of beneficial microorganisms either in vermicast or vermicompost had been reported earlier by Stockli (1928), Teotia *et al.* (1950); Mba (1987), Indira *et al.* (1996), Mba (1994, 1997) and Nair *et al.* (1997). In all, four isolates of nitrogen fixing bacteria and two isolates of phosphate solubilizing

bacteria were obtained. Based on their nitrogen fixing and phosphate solubilizing ability one isolate each was selected for enrichment of vermicompost. The selected culture of isolate 3 in the case of diazotroph had a nitrogen fixing ability of 6.7 mg per gram of sucrose consumed (Fig. 5). It also had a higher growth rate (OD_{600} = 0.589) in Jensen's nitrogen free broth under *in vitro* conditions (Table 5). This isolate was tentatively identified as *Azotobacter* sp. based on its morphological and physiological characters. In the case of phosphate solubilizing microorganisms, the bacterial isolate 1 was selected for enrichment of vermicompost. This culture had the maximum capacity for solubilizing the insoluble form of tricalcium phosphate to the extent of 32.7 per cent when compared to that of 26.4 percent for a known standard culture of *Bacillus megaterium* var. *phosphaticum*. This isolate was tentatively identified as *Pseudomonas* sp. on the basis of its morphological and physiological characters. Eventhough some of the fungal isolates like *Aspergillus niger* were also found capable of solubilizing tricalcium phosphate, they were not selected for enrichment of vermicompost, because of the potential plant pathogenic nature of certain strains of this fungus.

The enrichment of vermicompost with *Azotobacter* and *Pseudomonas* sp. was done with a carrier based inoculum of the selected culture of bacteria. These were incorporated in vermicompost at the rate of one kg per 100 kg vermicompost. The enriched vermicompost was used along with farm yard

manure and ordinary vermicompost to study separately its effect on plant growth and yield in chilli under pot culture conditions. In these experiments, three levels of nitrogen in the form of urea (100, 75 and 50 per cent of the recommended level of 75 kg per ha), three levels of phosphorus in the form of mussoriphos (100, 75, and 50 per cent of the recommended level of 40 kg per ha) and a uniform level of potassium (100 per cent of the recommended level of 25 kg per ha) were also applied.

In the pot experiment, using vermicompost enriched with nitrogen fixing bacteria, significant differences between treatments were obtained only in the fresh and dry weights of shoot and in the per plant yield of chilli. The fresh and dry weights of shoot, 43.7 and 22.3 g respectively were significantly high in the T_5 treatment combination of N_3P_1K + VN (Table 7). In this treatment, the quantity of nitrogen fertilizer applied was only 50 per cent of the recommended level. This showed that vermicompost enriched with an efficient nitrogen fixing bacterium such as *Azotobacter* sp. can be used for nitrogen economy and also to induce better plant growth in the cultivation of vegetables.

The per plant yield of chilli, 103.0 g, was however, maximum in T_3 treatment (N_1P_1K + VN) with 100 per cent fertilizer application. This was significantly higher than the control treatment (44.0g) of T_1 (N_1P_1K + F) under

normal package of practices recommendations with farm yard manure as a source of organic manure. In the T_2 treatment where ordinary vermicompost was used, the yield was only 57.3 grams. These data indicated that vermicompost enriched with *Azotobacter* was superior to farm yard manure and ordinary vermicompost as a source of organic manure. This was further supported by the fact that in the remaining two treatments also, T_4 and T_5, where enriched vermicompost was used, the per plant yield was higher to the extent of 97.3 g and 75.7 g respectively (Table 8; Fig.8). This was more than that of both the control and T_2 treatments. Such yield increases due to biofertilizer application had been reported earlier in brinjal, cabbage and tomato by Mehrotra and Lehri (1971), in brinjal and cabbage by Lehri and Mehrotra (1972), in chilli by Anina (1995) and in amaranthus by Arunkumar (1995).

Better plant growth and yield responses were not obtained with vermicompost enriched with phosphate solubilizing bacteria. Here, significant differences between treatments were obtained only in the onset of flowering and in the number of fruits formed per plant. The onset of flowering was earliest (38.3 days) in the T_6 treatment combination of N_1P_1K + VP, where NPK application was as per the package of practices recommendations (Table 10). This could be due to the better availability of phosphorus from the insoluble form of mussoriphos as a result of the activity of phosphate solubilizing bacteria, incorporated in vermicompost. But, when the quantity of

P was redued to 75 and 50 per cent of the recommended level as in T_7 and T_8 treatments, such an effect was not observed even with phosphate solubilizing bacteria. This showed that better treatment effect can be achieved only with a normal level of P fertilizer application.

The number of fruits formed per plant was also significantly high in the T_6 treatment. Here, 22 fruits were formed when compared to only 10.3 in the control treatment with farm yard manure as a source of organic manure. The above yield was also higher than that of the T_2 treatment where ordinary vermicompost was used. Eventhough, there were no significant differences between treatments in the net yield of chilli, here also it was maximum (76.7 g) in the T_6 tretment (Table 10). As in the case of vermicompost enriched with *Azotobacter*, the per plant yield of chilli was also better in T_7 and T_8 treatments where enriched vermicompost was used. The yield increase in these treatments were to the extent of 174.3, 150.0 and 163.0 per cent respectively when compared to the control treatment. The beneficial effect of phosphate solubilizing bacteria in enhancing the availability of phosphorus to crop plants and thereby the yield had been reported earlier also by Gerretson (1948), Zenkova (1955), Smierzchalska (1962), Kundu and Gaur (1980), Dubey and Billore (1992) and Shehana (1997).

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with farm yard manure, vermicompost or enriched vermicompost. The keeping quality of chilli was better (8.0 days) in the T_4 treatment (Table 11), where only 75 percent of the recommended level of nitrogen was applied. The beneficial effect of reduced level of nitrogen fertilizer application on the keeping quality of harvested vegetables is a well-established fact. Besides, the application of organic manures could significantly improve the vitamin C content in harvested fruits and there by its keeping quality (Luchnik, 1975 and Mier-plo gen and Lehri, 1981).

The third part of the present investigation was done to find out the interaction, if any, between earthworms and a soilborne pathogen, *Pythium aphanidermatum* in causing the damping off in chilli. Both an exotic species of earthworm, *Eudrilus eugeniae*, and a local species *Megascolex* sp., were used. This study was undertaken, because of certain observations made earlier that the presence of earthworms in soil could become a pre-disposing factor for the incidence of damping off caused by *Pythium aphanidermatum* in vegetables. Two separate pot trials were conducted for each species of earthworm with the chilli variety, Jwalamukhi as the test plant. The pathogen was initially isolated from infected chilli plants and identified as *Pythium aphanidermatum* based on its colony characters and spore morphology.

In the pot trials conducted, it was found that there was no incidence of damping off in chilli due to *Pythium aphanidermatum* in the presence of either the exotic or local species of earthworm. This was observed in treatments such as T_4 and T_{10} with *Eudrilus eugeniae* and T_6 and T_{12} with *Megascolex* sp. (Table 12 and 13). A similar result was also obtained even when the pathogen was introduced one month after transplanting during which period, any injury caused by the earthworms to seedlings, could have become a pre-disposing factor for the incidence of damping off in chilli. But, the fact that there was no disease incidence in either situation clearly indicated that earthworms were not actually involved in causing damping off in chilli. This was further confirmed by the fact that when the application of the pathogen was coupled with an induced surface injury in the collar region of seedlings at the time of transplanting, there was a severe incidence of damping off. This was observed in the T_2 and T_8 treatments (Plate 3).

The contention that the earthworms are responsible for disease incidence might have arisen from the common observation that in fields with significant population of earthworms, the worms are often seen in close association with the root system of plants. This however is due to the thigmotactic instinct of earthworms to coil around solid objects such as the root system of plants without feeding on live roots (Plate 6). In the present investigation also, such a response was observed both in healthy as well as

disease affected plants. The ability of *Pythium aphanidermatum* alone to cause damping off in vegetables had been reported earlier by several workers such as Chi and Hanson (1962), Filer (1967), Laviolette and Athow (1971), Roncardori and Me Carter (1972), Jenkins and Averre (1983) and Bates and Stranghellini (1984).

Once the fact that the earthworms were not responsible for inducing the damping off in chilli was established, the pot trials were continued to find out whether the presence of earthworms in the soil had any direct influence on plant growth and yield especially with the inoculation of the pathogen, *Pythium aphanidermatum*. Significant differences were obtained between treatments in plant height, number of branches formed and in the fresh and dry weights of shoot in various treatment combinations with *Eudrilus eugeniae* (Table 14) and in plant height and fresh and dry weights of root with *Megascolex* sp. (Table 16). Similarly in yield parameters, there were significant differences between treatments in the onset of flowering, number of fruits formed and in the per plant yield of chilli with both the species of earthworms (Table 15 and 17). However, here, only the data on the interaction between *Eudrilus eugeniae* and *Pythium aphanidermatum* is interpreted mainly due to the lack of survival of *Megascolex* sp. towards the end of the experimental period. This type of mortality for *Megascolex* sp. had been reported earlier by Jiji (1997) and might be due to the inability of this

species of earthworm to survive in a restricted soil environmental condition like that occurring in earthen pots.

The plant growth was comparatively better in T_3, T_7 and T_9 treatments without pathogen inoculation. The number of branches formed were significantly high in T_9 (22.3) and T_3 (21.7) treatments when compared to that of 15.0 in the control treatment (Table 14). These treatments, POP(V) + E and POP (F) + E, were with *Eudrilus eugeniae*, but without the application of *Pythium aphanidermatum*. The positive influence of *Eudrilus eugeniae* on plant growth had been reported earlier by van Rhee (1977), Haimi *et al.* (1992), Mba (1996) and Alfred and Gunthilagaraj (1996). However, such a response was not obtained in plant height and fresh weight of roots which were maximum in the control treatment. An interesting observation was that in treatments with pathogen inoculation, the growth response was less even in the presence of earthworms. Thus in T_4 (POP (F) + P+E) and T_{10} (POP (V) + P+E) treatments, the plant heights were only 44.0 and 48.0 cm respectively which were significantly less than the control (71 cm) treatment (Table 14; Fig.9; Plate 4 and 5). In both these treatments, the number of branches formed, 17.3 and 19.3 respectively, fresh and dry weights of shoot, 55.7 and 29.0 g and 53.7 and 27.9 g respectively and the fresh and dry weights of roots, 7.3 and 3.0 g and 7.1 and 2.7 g respectively were less than that of the treatments with *Eudrilus eugeniae* alone. This showed that the occurrence of a

virulent strain of pathogen could adversely affect plant growth even in the presence of earthworms which were otherwise found beneficial for plant growth.

As regard to yield parameters, there were significant differences between treatments not only in the onset of flowering, but also in the number of fruits formed and in the per plant yield of chilli (Table 15). However, the earliest onset of flowering (42.0 days) was observed in the T_7 treatment POP (V) without *Eudrilus eugeniae*. But the number of fruits formed per plant was maximum in the T_3 treatment followed by that of T_9 treatment. In these two treatments, POP(F)+E and POP(V) +E, the number of fruits formed were 44.0 and 31.3 respectively when compared to only 16.0 and 28.3 in the control and T_7 treatments (Table 15; Fig.11). As observed earlier, when these treatments w'.ere combined with pathogen inoculation, the number of fruits formed were less than that of the control treatment. These were only 10.7 and 15.3 respectively in the T_4 and T_{10} treatments (Table 15). Such an effect was also observed in the per plant yield of chilli. The yield of 146.7 g was significantly high in the T_3 treatment followed by that of 107.7 g in the T_7 and 102.3 g in the T_9 treatments. In one of the treatments, T_4 (POP (F) + P+E) the yield of only 32.0 g and it was much less than that of the control treatment where the per plant yield was on an average of 67.0 g. These results again showed that the direct application of *Eudrilus eugeniae* was indeed beneficial for plant

growth and yield. However, such a crop response could be drastically reduced in the presence of a virulent strain of soilborne pathogen.

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with or without the application of either *Eudrilus eugeniae* or *Pythium aphanidermatum* (Table 18). The keeping quality was maximum for 9.3 days in the T_7 and T_9 treatments without pathogen application. However such a response was also observed in the T_{10} treatment with pathogen application. This indicated that soil factors except probably the reduced application of nitrogenous fertilizers did not influence much the keeping quality of harvested chilli.

There were no significant changes in the population pattern of *Eudrilus eugeniae* in treated pots at the end of the experiment. In three of these treatments such as T_3, T_8 and T_{10}, the number of adult worms were less than the initial number of fifty per pot. These were 48.0, 41.7 and 49.0 respectively (Table 19). Only in one treatment, T_4, there was a marginal increase in the population of earthworm by 10 numbers. The reduction in the number of earthworms might be due to the night crawling habit of *Eudrilus eugeniae.*

SUMMARY

SUMMARY

The present investigation on 'Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli (*Capsicum annuum* L.) was done at College of Agriculture, Vellayani during 1997-99 as part of a Science, Technology and Environment Department (Government of Kerala) funded research project.

Among the surface microflora of *Eudrilus eugeniae*, the total number of bacteria (4.3×10^4), fungi (6.0×10^3) and actinomycetes (42.5×10^3) were maximum on 45 day of compost formation. However, the number of nitrogen fixing bacteria (3.0×10^1) was more on 60 day. Similarly, in the case of gut microflora, the total number of bacteria (11.3×10^6) and fungi (19.1×10^3) were maximum on 45 day of composting. However, the number of actinomycetes (19.6×10^5) was more on 30 day. Nitrogen fixing bacteria (1.3×10^3) could be isolated only on 15 day.

In the vermicompost, the total number of bacteria (11.9×10^6), fungi (11.2×10^4) and actinomycetes (49.7×10^4) were maximum on 30, 45 and 60 day respectively. The number of phosphate solubilizing bacteria (3.1×10^6) and nitrogen fixing bacteria (8.9×10^3) were more on 15 and 30 day of sampling. However, soilborne pathogens such as *Pseudomonas*

solanacearum, *Pythium* spp. and *Phytophthora* spp. were absent both as part of earthworm as well as the vermicompost microflora.

There were significant differences in the growth rate of various isolates of nitrogen fixing bacteria in Jensen's nitrogen free broth. The mean optical density of 0.589 for isolate 3 was significantly higher than rest of isolates. The nitrogen fixing ability of these diazotrophs also varied from 0.8 to 6.7 mg per gram of sucrose consumed. The isolate 3 was selected for enrichment of vermicompost. Based on various morphological and physiological characters, this isolate was tentatively identified as *Azotobacter* sp.

Among the different isolates of phosphate solubilizing bacteria, the percentage of phosphate solubilized in modified Pikovskaya's broth varied from 26.4 per cent for *Bacillus megaterium* var. *phosphaticum* to 32.7 per cent for isolate 1. The isolate 1 was selected for enrichment of vermicompost. Based on the various morphological and physiological characters, the isolate 1 was tentatively identified as *Pseudomonas* sp.

In the study on the enrichment of vermicompost with nitrogen fixing bacteria on growth of chilli, significant differences between treatments were observed in the fresh and dry weights of shoot. the fresh and dry weights of 43.7 and 22.3 g respectively, were significantly high in the treatment

combination of T_5 (N_3P_1K+VN) when compared to the control (N_1P_1K+F) treatment. There were significant differences between treatments in the yield of chilli. The per plant yield of 103.0 g was maximum in the treatment combination of T_3 (N_1P_1K+VN) followed by 97.3 g in the T_3 (N_2P_1K+VN) treatment. These were significantly high and showed an yield increase of 234.1 and 221.1 per cent respectively over the control treatment.

In the study on the use of vermicompost enriched with phosphate solubilizing bacteria, there were no significant differences between treatments in plant height, number of branches formed and in the fresh and dry weights of shoot and root. However, there were significant differences between treatments in the onset of flowering and in the number of fruits formed per plant. The earliest onset of flowering (38.3 days) was observed in the treatment combination of T_6 (N_1P_1K+VP). In this treatment, the number of fruits formed per plant, 22.0, was also significantly higher than the control treatment. There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with enriched vermicompost.

In the interaction study between *Eudrilus eugeniae* or *Megascolex* sp. and *Pythium aphanidermatum*, there was 100 per cent seedling mortality in treatments such as T_2 (POP(F)+P) and T_8 (POP(V)+P) where the application

of *Pythium aphanidermatum* was coupled with surface injury in the collar region of seedlings. But in the absence of such an induced injury, there was no incidence of damping off. However, in these treatments the plant growth was generally poor in height, fresh and dry weight of roots. At the same time, the plant growth was better in treatments without pathogen inoculation. The number of branches formed was significantly high in T_3 (POP(F)+E) (21.7) and T_9 (POP(V)+E) (22.3) treatments when compared to that of 15.0 in the control (POP(F)) treatment.

There were significant differences between treatments in the onset of flowering, number of fruits formed and in the per plant yield of chilli, due to combined application of *Eudrilus eugeniae* and *Pythium aphanidermatu m*. The earliest onset of flowering was observed on 42 day in the T_7 (POP(V)) treatment which was significantly earlier when compared to that of the control treatment. The number of fruits formed, (44.0) and yield (146.7 g) were maximum in the T_3 (POP(F)+E) treatment. These were significantly higher than the control treatment.

Significant differences between treatments were observed in the onset of flowering, number of fruits formed and in the per plant yield of chilli due to the combined application of *Megascolex* sp. and *Pythium aphanidermatum*. Flower initiation was earliest in the T_7 (POP(V)) and T_{11} (POP(V)+M)

treatments (42 day). The number of fruits formed was also significantly high in T_7 and T_{11} treatments (28.3). The per plant yield of chilli (107.7 g) was also significantly high in T_7 treatment.

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with or without the application of either *Eudrilus* or *Megascolex* sp. and *Pythium aphanidermatum*.

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* Originals not seen

APPENDICES

APPENDIX - I

Composition of different media

1. Casein nutrient agar

Casein	-	10.0 g
Peptone	-	5.0 g
Beef extract	-	3.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0

2. Jensen's nitrogen free agar (Jensen, 1942)

Sucrose	-	20.0 g
K_2HPO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.5 g
NaCl	-	0.5 g
$FeSO_4$	-	0.1 g
$CaCO_3$	-	2.0 g
Na_2MoO_4	-	0.005 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.2

3. Kauster's agar

Glycerol	-	10.0 g
Casein	-	0.3 g
$MgSO_4.7H_2O$	-	0.5 g
$FeSO_4$	-	0.1 g
KNO_3	-	2.0 g
NaCl	-	2.0 g
K_2HPO_4	-	0.5 g
$CaCO_3$	-	0.2 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0

4. King's B medium (King *et al.*, 1954)

Peptone	-	20.0 g
K_2HPO_4	-	1.5 g
$MgSO_4.7H_2O$	-	1.5 g
Glycerol	-	10.0 ml
Agar	-	15.0 g
Distilled water	-	1000 ml

5. Martin's peptone dextrose agar

Dextrose	-	10.0 g
Peptone	-	5.0 g
KH_2PO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.5 g
Rose Bengal	-	33.0 mg
Mycostatin	-	100 ppm
Bavistin / Benlate	-	0.5 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	6.8

6. Martin's rose bengal agar (Martin, 1950)

Dextrose	-	10.0 g
Peptone	-	5.0 g
KH_2PO_4	-	1.0 g
$MgSO_4.7H_2O$	-	0.5 g
Rose Bengal	-	33.0 mg
Agar	-	15.0 g
Distilled water	-	1000 ml
Streptomycin solution (1%)	-	3.0 ml
pH	-	7.0

7. Nutrient agar

Peptone	-	5.0 g
Beef extract	-	3.0 g
Distilled water	-	1000 ml
pH	-	7.0

8. Pikovskaya's modified agar

Glucose	-	10.0 g
$Ca_3(PO_4)_2$	-	5.0 g
$(NH_4)_2.SO_4$	-	5.0 g
KCl	-	0.2 g
$MgSO_4.7H_2O$	-	0.1 g
$MnSO_4$	-	trace
$FeSO_4.7H_2O$	-	trace
Yeast extract	-	0.5 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH		~~7.0~~
~~Glucose~~	-	20.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml

The potato extract was prepared by boiling 200 g sliced potatoes in one litre of water. The supernatant was filtered and the volume was made up to one litre.

10. Starch agar

Peptone	-	10.0 g
Beef extract	-	5.0 g
Starch soluble	-	2.0 g
Agar	-	2.0 g
Distilled water	-	1000 ml
pH	-	7.0

11. Triphenyl Tetrazolium Chloride medium (Kelman, 1954)

Peptone	-	10.0 g
Casamino acid	-	1.0 g
Glucose	-	5.0 g
Agar	-	20.0 g
Distilled water	-	1000 ml
pH	-	6.8

One per cent solution of tetrazolium chloride was prepared in distilled water, sterilized by autoclaving for eight minutes and stored in the dark. 0.5 ml

of this solution was aseptically added to 100 ml of sterilized medium at the time of plating to get a final concentration of 0.005 per cent.

12. Vermicompost extract agar (Nair *et al.*, 1997)

Glucose	-	1.0 g
K_2HPO_4	-	0.5 g
Agar	-	15.0 g
Vermicompost extract	-	1000 ml
pH	-	6.8

The vermicompost extract was prepared by steaming one kg of vermicompost in two litres of water for 30 minutes. The supernatant was filtered and the previously weighed nutrient components were added to 1000 ml of this extract.

APPENDIX-II

Reagents and stains

1. Crystal violet

Crystal violet	-	0.2 g
Ethyl alcohol (95 per cent)	-	20.0 ml
Distilled water	-	80.0 ml

2. Gram's iodine solution

Iodine	-	1.0 g
KI	-	2.0 g
Distilled water	-	300 ml

3. Counter stain

Safranin (2.5 % solution in 95 % ethanol)	-	10.0 ml
Distilled water	-	100 ml

4. Crystal violet solution

Crystal violet	-	0.10 g
Glacial acetic acid	-	0.25 ml
Distilled water	-	100 ml

5. Lugol's iodine solution

Iodine	-	5.0 g
KI	-	10.0 g
Distilled water	-	100.0 ml

6. Ammonium molybdate solution

Ammonium molybdate	-	15.0 g
Warm distilled water	-	300 ml
HCl (10 N)	-	350 ml

The final volume was made up to 1000 ml with distilled water.

7. Stannous chloride solution

$SnCl_2$	-	10.0 g
conc.HCl	-	25.0 ml

To prepare a dilute $SnCl_2$ solution, 66.0 ml of distilled water was added to 0.5 ml of $SnCl_2$ solution.

INFLUENCE OF MICROFLORA ASSOCIATED WITH EARTHWORM *(EUDRILUS EUGENIAE* KINBERG) AND VERMICOMPOST ON GROWTH AND PERFORMANCE OF CHILLI *(CAPSICUM ANNUUM* L.)

By

SUJA P. R.

ABSTRACT OF THESIS
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ABSTRACT

ABSTRACT

The present investigation on 'Influence of microflora associated with earthworm (*Eudrilus eugeniae* Kinberg) and vermicompost on growth and performance of chilli (*Capsicum annuum* L.) was done at College of Agriculture, Vellayani during 1997-99 as part of a Science, Technology and Environment Department (Government of Kerala) funded research project.

Among the surface microflora of *Eudrilus eugeniae*, the total number of bacteria, fungi and actinomycetes were maximum on 45 day of compost formation. However, the number of nitrogen fixing bacteria was more on 60 day. Similarly, in the case of gut microflora, the total number of bacteria and fungi were maximum on 45 day of composting. However, the number of actinomycetes was more on 30 day. Nitrogen fixing bacteria could be isolated only on 15 day.

In the vermicompost, the total number of bacteria fungi and actinomycetes were maximum on 30, 45 and 60 day respectively. The number of phosphate solubilizing bacteria and nitrogen fixing bacteria were more on 15 and 30 day of sampling. However, soilborne pathogens such as *Pseudomonas solanacearum, Pythium* spp. and *Phytophthora* spp. were absent.

There were significant differences in the growth rate of various isolates of nitrogen fixing bacteria in Jensen's nitrogen free broth. The mean optical density for isolate 3 was significantly higher than rest of isolates. The nitrogen fixing ability of this isolate was also higher. The isolate 3 was selected for enrichment of vermicompost. Based on various morphological and physiological characters, this isolate was tentatively identified as *Azotobacter* sp.

Among the different isolates of phosphate solubilizing bacteria, the isolate 1 which showed maximum solubilization of tricalcium phosphate in modified Pikovskaya's broth was selected for enrichment of vermicompost. Based on the various morphological and physiological characters, the isolate 1 was tentatively identified as *Pseudomonas* sp.

In the study on the enrichment of vermicompost with nitrogen fixing bacteria on growth of chilli, the shoot fresh and dry weights were significantly high in the treatment combination receiving vermicompost enriched with *Azotobacter* sp. along with 50 per cent nitrogen (N_3P_1K+VN) when compared to the control treatment. The per plant yield was significantly high in the treatment combination with 100 per cent nitrogen and vermicompost enriched with *Azotobacter* sp. (N_1P_1K+VN) followed by that receiving 75 per cent nitrogen and vermicompost enriched with *Azotobacter* sp. (N_2P_1K+VN).

In the study on the use of vermicompost enriched with phosphate solubilizing bacteria, there were significantly earlier onset of flowering and significantly higher number of fruits formed per plant in the treatment combination of 100 per cent phosphorus along with vermicompost enriched with *Pseudomonas* sp. There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with enriched vermicompost.

In the interaction study between *Eudrilus eugeniae* or *Megascolex* sp. and *Pythium aphanidermatum*, there was 100 per cent seedling mortality in treatments such as POP(F)+P and POP(V)+P where the application of *Pythium aphanidermatum* was coupled with surface injury in the collar region of seedlings. But in the absence of such an induced injury, there was no incidence of damping off. However, in these treatments the plant growth was generally poor in height, fresh and dry weight of roots. At the same time, the plant growth was better in treatments without pathogen inoculation. The number of branches formed was significantly high in POP(F)+E and POP(V)+E treatments where *Eudrilus eugeniae* was applied when compared to that of the control treatment.

The treatment with vermicompost alone (POP (V)) recorded significantly earlier onset of flowering. The number of fruits formed per plant

and per plant yield were significantly higher in the treatment with farm yard manure and *Eudrilus eugeniae* (POP (F)+E) compared to the control.

Significant differences between treatments were observed in the onset of flowering, number of fruits formed and in the per plant yield of chilli due to the combined application of *Megascolex* sp. and *Pythium aphanidermatum*. Flower initiation was earlier as well as the number of fruits formed were also significantly high in the treatments with vermicompost either alone (POP (V)) or in combination with *Megascolex* sp. (POP(V)+M). The treatment with vermicompost alone recorded the highest per plant yield.

There were no significant differences between treatments in the keeping quality of chilli harvested from treatments with or without the application of either *Eudrilus* or *Megascolex* sp. and *Pythium aphanidermatum*.