ROLE OF MICROFLORA ON THE QUALITY OF VERMI-PRODUCTS IN IMPROVING PLANT GROWTH

By

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THESIS

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2007

DECLARATION

I hereby declare that this thesis entitled "**Role of microflora on the quality of vermi-products in improving plant growth**" is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Nisha Jose

Nisha Jose

Dedicated to

my beloved

family members

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Introduction

1. INTRODUCTION

Earthworms are considered as farmer's friend as they contribute in many ways to the richness of the soil. They are literarily known as "ploughs of the earth" because of their ability to burrow through the soil. These creatures eat their way through soil. They ingest bacteria and small particles and enrich the earth with their waste products, called castings. Their burrowing action allows more water and air to enter the soil, thus making it richer. Thus, this deceptively simple creature is largely responsible for all the richness of the soil. Its journey across the soil creates the fertile farm lands all over the world. Movement of worms and worm castings are more active mainly on the top layer of rich soil. Thus, the earthworms form an integral part of sustainable agriculture.

The success of sustainable agriculture very much depends on the availability of good quality organic manures at low price. Vermiculture involving the use of earthworms is a versatile natural technology for effective recycling of non toxic organic wastes in the soil. Earth worms play an important role in enriching the soil with organic matter. This ability is being exploited nowadays for the production of vermicompost. It is the product of combined action of earth worms and various soil microorganisms. On an average vermicompost contains 1.5% N, 0.5% P and 0.8% K and also many micronutrients. This is the single source of many of the nutrients required for the crop. Besides composting, the earthworms enrich the soil through the release of faecal castings which are rich in nutrients and microbes. Vermiwash, liquid organic manure is an aqueous extract of a column of freshly formed vermicompost and surface washings of earthworm containing beneficial microorganisms and readily available plant nutrients.

Organic farming and its significance in India is not of recent origin, but has a long history back to the Neolithic age of 7500-6500 BC. Now, vermiculture technology is an important component of organic farming and is being widely used. In addition to various nutrients, vermiproducts also contain several growth

enhancing substances, beneficial microbes like N-fixers and P-solubilizers and cellulose decomposers. The microbes present in the composted agricultural wastes have been reported to suppress soil borne plant diseases and improve plant growth (Hameeda *et al.*, 2006). Even though the beneficial aspects of earthworms are known to farmers, the vermitechnology is still conventional. Earthworms are being reared in pits with ample supply of organic matter for composting. In the present scenario of commercial agriculture, rearing of worms in a conventional way is cumbersome. In this situation, it is felt that it will be economical and easy, if we could prepare a consortium of microorganisms similar in constitution as that in the vermiproducts for commercial field level application. With this in view, present study on the 'Role of microflora on the quality of vermi-products in improving plant growth' is taken up with the following objectives.

1. Isolation and estimation of microorganisms associated with the vermiproducts.
2. Evaluation and selection of microorganisms based on their efficiency in nitrogen fixation, phosphorus solubilization and antagonistic properties.
3. Preparation of microbial consortium using efficient microorganisms from vermiproducts
4. Evaluation of the prepared microbial consortium in improving plant growth using the test plant amaranth.
5. *In vitro* evaluation of the effect of vermiproducts on pathogenic and beneficial microorganisms.

Review of Literature

2. REVIEW OF LITERATURE

In sustainable agriculture the role of earthworms is well established. Darwin (1881) emphasized the role of earthworms in fragmenting of organic debris and accelerating its decomposition in his book "The Formation of Vegetable Mould through the Action of Worms with Observations of Their Habits". This is one of the earliest mentions about the role of earthworms in decomposition of organic matter. Thereafter studies worth mentioning were done only in later part of 20th century. Edwards and Lofty (1977) found out that earthworms belonged to megascolidae and lumbricidae and are valuable to agriculture. Most commonly used earthworms for culturing is *Eisenia foetida* that survives in a wider range of temperature. It is a tough worm but easy to handle and it out competes other species (Tsukumoto and Watanbe, 1977; Edwards and Bates, 1992). The earthworms are reported to contribute to the beneficial effect of soil through the improvement of soil properties (Edwards and Lofty, 1980).

2.1 Earthworms and soil fertility

The compost produced by using the earth worm is called vermicompost. Edwards (1982) reported that the physical structure of vermicompost produced from the organic waste through worm action 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.

According to Mackay and Kladivko (1985), earthworms play a variety of important roles in agro ecosystems. Their feeding and burrowing activities results in incorporation of organic residues and amendments to the soil, enhanced decomposition of organic matter, increased humus formation, efficient nutrient cycling and improved soil structural development. According to them, earth worms could also contribute to the distribution of surface litter, providing spatial

heterogeneity and microbial activity. Tomati *et al.* (1988) reported that earthworm castings are a pool of concentrated organic nutrients. Total nitrogen, organic matter, nitrate nitrogen, phosphorus, potassium, sodium and magnesium were at a higher level in castings than in the surrounding soil. According to Bhawalker (1989), the earth worms are the natural bioreactors as they effectively harness the beneficial effects of soil microflora.

Ismail (1993) worked out the degradation of organic matter by earthworms and reported that enzymes and gut microorganisms took active part in organic matter digestion. After absorption of nutrients from the organic matter, the earthworm ejects the waste as casting. Earth worms ingest organic matter and eject it as much finer particles after passing through the grinding gizzard they all possesses as reported by Edwards (1995).

According to Ismail (1997), vermiwash is a liquid biofertilizer collected after passage of water through a column of worm action and it is reported as very useful foliar spray. It is a collection of excretory products and mucous secretions of earthworms along with micronutrients from the soil organic molecules. Fragasso *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. According to Singh and Kumar (2000) the earthworms could be considered as biological indicators of soil fertility, since they preferentially support healthy populations of bacteria, fungi and actinomycetes. Earthworms are also scored as the host of other microorganisms that are essential for sustaining a healthy soil.

Vermiculture is the process of mass multiplication of earthworms and it is an excellent tool of organic farming which helps in maintaining the fertility status of the soil for a long time. Bhattacharya *et al.* (2003) considered vermicompost as a preparation of a mixture of worm castings, organic material, humus, living earthworms, cocoons and other organisms.

2.2 Microflora associated with surface and gut of earthworms

Basalik (1913) was the first to document studies on the effects of earthworms on microorganisms. He isolated more than 60 species of microorganisms from the gut contents and found no difference in species composition from the original soil microflora apparently indicating the absence of an exclusive gut flora. Khambata and Bhat (1957) observed specific groups of bacteria in the worm intestine and they could isolate oxalate and cellulose decomposers. Contreas (1980) observed *Vibrio* sp. which accounted for 73% of the total bacteria and *Streptomyces lipmanii* which accounted for 90% of the actinomycetes in the gut of *Eisenia lucens*. These species were low in population in the wood substrates where the earthworms were living. Barois and Lavelle (1986) showed that intestinal mucus produced by the earthworm *Pontoscolex corethrurus*, contained large amount water soluble low molecular weight organic compounds. These were assimilated easily by the rapidly multiplying microbial population in the gut. Sacheu (1987) reported the presence of many microorganisms in the gut of earthworms. The ability of preferentially or randomly ingested organisms to survive the passage through earthworm gut and their resultant capacity to recover and proliferate in the earthworm casts were influenced by many factors including enzymes, mucus and antimicrobial substances as reported by Brown (1995).

Panomareva (1953) found that there was an increase in the number of pigmented and other bacteria of *Bacillus cereus* group after passage through earthworm intestine. Parle (1963) observed that population of bacteria increased rapidly during passage of food through worm gut. Citernesi *et al.* (1977) isolated *Clostridium beijerinckii, C. butyricum, C. paraputrificum* from the gastro enteric cavity of *E. foetida*. Wolter *et al.* (1999) reported that the numbers of bacteria changed in a very uniform pattern during gut passage irrespective of food materials and season. They increased from the crop or gizzard to the foregut and then declined or remained constant in the hindgut.

Hutchinson and Kamel (1956) reported that alimentary canal of *Lumbricus terrestris* contain species of *Verticillium, Torula, Trichoderma viride, Penicillium, Rhizopus nigricans, Pythium, Mucor, Gliocladium roseum, Fusarium culmorum , Cladosporium cladosporioides* and *Cephalosporium*. They also found that spread of the fungal species in a sterile soil was influenced by the presence of earthworms. The population of some fungi increased during the passage through the gut of *Lumbricus rubellus* which indicated that viability of some fungal species was enhanced during passage through the gut (Kristufek *et al.*, 1992). Nair *et al.* (1997) reported that the total number of microorganisms were higher in the gut content of *Eudrillus euginiae* when compared to the surface microflora and they isolated *Bacillus* sp., *Mucor* sp., *Aspergillus flavus*, *A. niger*, *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. from the surface and gut content of two types of earthworms. Alonso *et al.* (1999) reported that species of *Aspergillus, Fusarium, Gliocladium, Paecilomyces, Penicillium* and *Trichoderma* were identified in the gut of *Onychochaeta borinca.* Novakova and Pizl (2003) found that *A. fumigatus* was the most frequent micro fungal species in the intestine of *Eisenia andrei.*

Parle (1963) observed that there was a rapid increase in the population of actinomycetes during passage of food through the earth worm intestine and this was dominated by *Streptomyces* sp. Krisstufek *et al.*(1993) reported that *Streptomyces* sp. and *Micromonospora* sp. appeared to be the important colonizers of the gut of earthworms *L. rubellus* and *Octolasion montanum.* Actinomycetes isolated from the intestinal tract of the earthworms *E. foetida* and *Pachyiyulus flavipes* showed increased antibiotic activity towards other bacteria and yeast (Nguyen Duc *et al.,* 1996). It was shown that intestinal tracts of earthworms fed with vermicompost, actinomycetes were partly digested and the rest were activated and accumulated in the excreta, where 55.8% of the actinomycetes complex represented by *Micromonospora* sp. (Zenova *et al.*, 1996).

The nitrogen fixation activity in the gastro enteric cavity of many soil animals including earthworms were reported by Citernesi *et al.* (1977). The earthworm gut provided a better habitat for the growth and life of microorganisms and had larger population of microorganisms including nitrogen fixing and denitrifying bacteria than in soil (Karsten and Drake, 1997).

2.3 Microflora associated with vermi-products

Harinikumar *et al.* (1988) observed that VAM propagules may survive and remain infective in air dried casts of the earthworm *E. euginiae* for several months. Joy *et al.* (1992) obtained 35 fungal species from the earth worm casts and *A. flavus* was predominant in the casts followed by *A. niger*. Mba (1994) found that earthworm casts of *P. corethrurus* were found to contain tolerant actinomycetes and efficient rock phosphate solubilizers. Mba (1997) also reported rock phosphate solubizing microbes from casts of *E. euginiae*. Devliegher and Verstraete (1997) reported that count of total bacteria, siderophore producing bacteria, and fluorescent pseudomonads were greater in the casts and burrowing walls of the earthworm. Earthworm *Lampito mauritii* failed to digest *Aspergillus nidulans, A. niger, A. flavus, A. fumigatus, T. koningii, Fusarium monoliformae, Mucor plumbeus,* and *Rhizopus* sp. and there was a marked increase in the count of this fungal species in the worm casts (Parthasarathy *et al.*, 1997). Karmegham and Daniel (1999) found that the fresh casts had a higher population of bacteria, fungi and actinomycetes compared with the surrounding soil and the increased amount of inorganic P released during cast deposition was related to and preceded by increased microbial and phosphatase activity. Microbes like *Pseudomonas* sp., *Bacillus* sp., and *Aspergillus* sp. from the earth worm casts were known to mineralize phosphate (Vinotha *et al.*, 2000). Alauzet *et al.* (2001) found that the main filamentous fungal genera found in the earth worm casts were *Aspergillus, Trichoderma, Fusarium* and *Penicillium*. Piekarz and Lipiec (2001) reported that the population of bacteria, *Streptomyces* and fungi in the earthworm casts increased with ageing of the cast. Orazova *et al.* (2003) observed that the casts

harboured the most diverse community comprising both soil and litter associated fungi and it was dominated by *Trichosporiella hyalina, Humicola grisea* and *Monilia* sp.

Indira *et al.* (1996) reported that examination of vermicompost revealed the presence of 10^6 bacteria, 10^5 actinomycetes and 10^5 fungi by dilution plate technique and also found that population of beneficial microorganisms like P solubilizing bacteria, nitrogen fixing organisms and entomophagus fungi were in the range of 10^5 to 10^6. Among the P solubilizing microbes, *Bacillus* and *Aspergillus* were found to be prominent while species belonging to *Azotobacter*, *Azospirillum* and *Rhizobium* were in N_2 fixing group. Meera (1998) found that vermicompost contained about $67x10^6$ bacteria, $8.3x10^5$ actinomycetes and $1.3x10^5$ fungi per gram of the vermicompost. According to Sailajakumari (1999), microbial population of the enriched compost was bacteria ($64x10^4$), fungi ($1.8x10^5$) and actinomycetes ($25x10^4$). Romero *et al.* (2001) reported that the population of bacteria decreased during the process of composting and had a positive association with microbial respiration. Pizl and Novakova (2003) reported that *A. fumigatus, A. flavus, Geotrichum candidum, P. expansum, P. roquefortii and R. stolonifer* were most frequently found in vermicompost. Anastasi *et al.* (2005) isolated and identified fungal entities from green and vermicompost. They obtained a total of 194 entities, 118 from green compost and 142 from vermicompost and 66 were common to both. The genera with highest load and number of species in both composts were that of *Penicillium* and *Aspergillus*. Thankamony (2005) made quantitative estimation of microbial population in vermicompost and recorded bacteria ($104.3x\ 10^5$), fungi ($44.33x\ 10^4$), actinomycetes($48.48x\ 10^5$), nitrogen fixing bacteria ($19.02x\ 10^3$) and phosphate solubilizing bacteria ($5.51x\ 10^3$) per g of compost.

Sunitha and Subramanian (2005) reported that biowash contained *Pseudomonas* ($4.3x10^3$), *Azotobacter* ($2.2x10^3$), and *Azospirillum* ($2.4x10^1$) in sufficient quantity.

Piontelli *et al.* (1981) reported that common coprophilus fungi occurring in the horse dung were *Pilobolus kleinii, Mucor hiemalis, Lasiobolus ciliatus, Acremonium* sp. and *Chaetomium mucorum*. Nirmalnath *et al.*(2001) observed that vermicompost obtained from the cow dung harboured the microbial load in the order bacteria ($73x10^5$), fungi ($18x10^3$), actinomycetes ($100x10^4$), *Azotobacter* ($40.67x10^4$)and phosphate solubilizing bacteria ($29x10^4$). Yongabi *et al.* (2003) observed that cow dung microflora includes *Salmonella* sp., *E. coli*, *Campylobacter* sp., and *Listeria monocytogenes* and the predominant mycoflora includes *A. niger* and *Rhizopus* sp. Paul *et al.* (2005) reported that using municipal solid waste and cow dung (1:1) mixture, the microbial count in the worm worked substrate was $152.67x10^6$, $35.46x10^4$ and $96.86x10^4$ for bacteria, fungi and actinomycetes respectively and these were double than the microbial count in the worm unworked substrates.

2.4 Vermi-products for growth promotion

The vermi-products play an important role in increasing the soil nutrient availability and in plant growth stimulation. Bano *et al.* (1987) compared the nutrient status of vermicompost with organic manures and found that the percentage of nitrogen in vermicompost was same as that in other organic manures. They also reported that high P_2O_5 content in earth worm casts improved phosphate availability. Wormcast when used as manure in place of FYM significantly influenced both vegetative and flowering characters and also increased mycorrhizal root colonization (Kale *et al.*, 1987). The nutrient content of the vermicompost could differ greatly depending on the material used for composting (Edwards and Burrows, 1988). Lui *et al.* (1988) reported that the application of earth worm casts increased the dry weight of soybean by 40 to 70% and the nitrogen absorbed by the plants from the soil increased to 30 to 50%. They also found that phosphorus and potassium content in the plant were twice as that in control. Tomati *et al.* (1988) found that the fertilizing effect of earthworm casts depend on microbial metabolites, mainly growth regulators. They reported that

when used in plant propagation earthworm cast promoted root initiation and root biomass. Ismail *et al.* (1991) obtained increased number of flowers and fruits in watermelon using vermicompost as organic manure. The vermicompost application enhanced the activity of beneficial microbes like nitrogen fixing bacteria and mycorrhizal fungi and they were found to play a significant role in nitrogen fixation and phosphate mobilization leading to higher nutrient uptake by the plant as reported by Kale *et al.* (1992). Prabhakumari *et al.* (1995) found that vermicompost contained about three times more nutrients than FYM. Reddy and Mahesh (1995) observed a significant increase in grain yield of green gram due to the application of vermicompost compared to farm yard manure.

Jiji (1996) found that the requirement of chemical fertilizers in cowpea and bitter gourd was significantly reduced when recommended dose of FYM was substituted by an equal quantity of vermicompost. Sagayalfred and Gunthilagaraj (1996) obtained a more germination percentage of amaranth seeds and subsequent increase in yield with the incorporation of earthworms into the seed bed. Meera (1998) observed that the use of vermicompost coated seeds produced the maximum uptake of N, P and K at peak flowering stage and also at harvest. Highest marketable yield was recorded for vermicompost application in amaranth. Kale (1998) found that vermiwash foliar spray was effective in increasing the growth and yield response of anthurium and it was better than 0.5% urea spray. Niranjana (1998) reported that vermicompost application gave higher biomass yield till 45 days after transplanting in amaranth.

Buckerfield *et al.* (1999) reported that vermicompost continued to promote plant growth following pasteurization but when the vermicompost was sterilized growth of radishes was better than in the sand control without vermicompost. Subsequent weekly applications of the diluted liquid improved plant growth and significant increases of upto 20% in radish yields were recorded. The application of vermiwash as soil drench and foliar spray along with inorganic fertilizers produced marked increase in fruit yield of tomato (Ranijasmin, 1999). Arunkumar

(2000) found that FYM and vermicompost application were superior to POP recommendations with respect to yield and growth characters in amaranth. According to Atiejeh *et al.* (2002) the hormone like activity of humic acid from the vermicompost increased the growth of tomato and cucumber seedlings..

Thangavel *et al.* (2003) reported that application of vermiwash and vermicast extract at fortnightly intervals after transplantation of the paddy seedlings increased the growth and yield of paddy and the vermicast extract performed better compared to vermiwash. According to Preetha *et al.* (2005) five tonnes of vermicompost together with 50:50:50 N, P_2O_5, K_2O gave the highest yield and nutrient uptake in amaranth production. Hameeda *et al.* (2007) reported that rice straw vermicompost applied at 2.5 t/ha showed significant improvement in shoot length, leaf area, plant biomass, root volume and mycorrhizal colonization.

2.5 Vermi-products for plant disease management

The use of composts to suppress soil-borne plant pathogens has been extensively reviewed in recent decades, and several microorganisms have been identified as biocontrol agents in compost-amended substrates. Nelson *et al.* (1983) observed that in mature composts, *Rhizoctonia solani* sclerotia were killed by *Trichoderma* spp. and found that *T. hamatum* and *T. harzianum* isolated from the compost were found to suppress *Rhizoctonia* damping off. Hotink and Fahi (1986) found that composts and water extracts of compost were highly effective in many cases for the control of plant diseases. Chung and Hotink (1990) identified *Bacillus* sp., *Enterobacter* sp., *Flavobacterium balustinum*, *Pseuedomonas* sp. and *Streptomyces* sp. as biocontrol agents in compost. Alvarez *et al.* (1995) reported that addition of some compost to soil increased the incidence of tomato rhizosphere bacteria exhibiting antagonism towards *Fusarium oxysporum* f.sp. *radicis lycopersici, Pyrenochaeta lycopersici, Pythium ultimum* and *R. solani*. The

antagonistic effects observed were associated with marked increase in the percentage of siderophore producers.

According to Hardy and Sivasithamparam (1995) most of the fungal and actinomycete isolates obtained from the composted eucalyptus bark suppressed the growth of *Phytophthora drechsleri* in a steamed composted eucalyptus bark medium. Craft and Nelson (1996) found that suppression of *Pythium* diseases of creeping bent grass using brewery sludge compost is directly related the microbial activities in the compost. The application of vermicompost extract as a substratum drench immediately after the planting of carnation decreased the spread of fusarium wilt caused by *F. o.* f.sp. *dianthi* (Orilikowski *et al.*, 1998). Zhang *et al.* (1998) also reported that a biocontrol agent fortified compost mix suppressive to several diseases by soil borne pathogens, induced systemic acquired resistance in cucumber against anthracnose caused by *Colletotrichum orbiculare.*

Szczech (1999) found that vermicompost when added to various container media significantly inhibited the infection of tomato plants by *F. o.* f.sp. *lycopersici* and the protective effect increased in proportion to the rate of application of vermicompost. Clapperton *et al.* (2001) reported that the mechanisms reduced the severity of take all disease and increased growth of white spring wheat by the application of compost appeared to be microbially mediated. This indicated that, there was a larger and more active microbial population when earthworms were present. Chaoui et *al.* (2002) found that there was a reduction in severity of *P. ultimum* and suppression of *R. solani* with increasing rate of substitution of vermicompost. They also reported that the incidence of verticillium wilt in strawberries was reduced by the application of 5 and 10 tonnes of commercial paper and food waste vermicompost.

Mun and Kirienko (2002) reported that brewing residue was processed by the earth worm to make compost having a high degree of humification and large population of Bacilli and Ascomycetes. They also found that, planting cucumber

plants on a mix of green house soil and vermicompost improved the structure of the microbial community of the green house soil and suppressed the root rot pathogens. Punja *et al.* (2002) observed that root and stem rot of green house cucumber caused by *F. o.* f.sp. *radicis cucumerianum* was reduced by the application of different types of compost and found that autoclaved compost had no effect on disease suppression, suggesting that microbial antagonism was involved. Wiekzorek *et al.* (2002) revealed that the soil application of biocompost based on earthworm *E. foetida* protected tomato plants against *F.o.* f.sp. *lycopersici*. It also limited the infection of cucumber seedlings with *P. ultimum.*

According to Singh *et al.* (2003) aqueous extract of vermicompost inhibited spore germination of several fungi and they inhibited the development of powdery mildew on balsam and pea caused by *Erysiphe cichoracearum* and *E. pisi* respectively on the field at very low concentrations(0.1-0.5%). Rivera *et al.* (2004) isolated 36 microorganisms from the vermicompost and 13 of which were antagonistic to *R. solani* (damping off of Tomato) in an *in vitro* study. They also reported that addition of 25 to 50% of vermicompost promoted seedling growth and prevented damping off caused by *R. solani.* According to Scheurell and Mahaffe (2004) aerated compost tea produced with kelp and humic acid additives reduced the damping off of cucumber caused by *P. ultimum.*

Suarez-Estrella *et al.* (2007) investigated on the *in vitro* and *in vivo* inhibition and suppression of antagonistic bacteria and fungi from several compost classes against *F. o.* f.sp. *melonis.* They found that strains with the highest biological control activity isolated from mature compost samples were mostly identified as *Aspergillus* sp. Dianez *et al.* (2007) reported that suppression of soil borne diseases of horticultural crops by compost attributed to the activities of antagonistic microorganisms and found that a great diversity of biological control agents naturally colonize compost.

2.6 Beneficial use of microorganisms in improving the plant growth and biocontrol of diseases

Plant growth promoting microorganisms are naturally occurring soil microorganisms that are able to aggressively colonize plant roots and improve plant growth when applied to roots, tubers or seeds. Several fungi belonged to the genus *Trichoderma*, *Aspergillus*, and bacteria like *Pseudomonas, Bacillus, Azotobacter* has been widely used in plant growth promotion and as potential biocontrol agent.

2.6.1 Growth promoting effects of fungi

Baker *et al.* (1984) first reported the growth improvement of radish by application of *T. harzianum* and *T.viride* in soil. According to Windham *et al.* (1986) diffusible growth regulating factors produced by *Trichoderma* spp. induced growth enhancement in plants. The fungus *T. harzianum* which was applied to pathogen free soil increased the emergence of seedlings, plant height, leaf area, and dry weight and the fungus was applied to the soil by three different methods *viz.*, conidial suspension, wheat bran/peat preparation and seed coating as reported by Kleifeld and Chet (1992). Inbar *et al.* (1994) reported that *T. harzianum* treated cucumber and pepper seedlings were much more developed and vigorous and had higher chlorophyll content, and they were more resistant to damping off disease.

Cruz and Cisterna (1998) reported that in *Capsicum annum* the application of *T. harzianum* significantly increased the seed germination, root length, root dry weight, plant height, and leaf number. According to Harman (2000) *Trichoderma* spp. could increase the rate of plant growth and development and also produce more robust roots. Karpagavally and Ramabhadran (2001) observed that application of *T. harzianum* and *T. viride* by seed treatment reduced the damping off incidence and improved the growth of root and shoot length and also

dry matter production in tomato seedlings. According to Phuwiwat *et al.* (2001) *T. harzianum* strain PC01 at concentrations of $5x10^9$ and $10x10^9$ conidia/ml gave significantly better growth and yield of the Chinese radish. Significant increase in root length and number of root nodules and grain yield in green gram over control by foliar spray of *T. virens* and *T. viride* was reported by Dubey (2002). According to Mukhopadhayay (2005), root coloniszation by *Trichoderma* spp. frequently enhanced root growth and development and the uptake and use of nutrients. They found that *Trichoderma* spp. added directly to the rhizosphere or as seed treatment protected the plant against numerous classes of plant pathogens.

2.6.2 Fungi as potential biocontrol agent

Weindling (1932) first demonstrated that *T. viride* was parasitic on and antagonistic to *R.solani.* Naim and El esavy (1965) found that, out of many species of fungi isolated from the rhizosphere of cotton seedlings, only *Aspergillums terreus* and *A. flavus* were proved to be antagonistic to *R. solani* on glucose peptone agar. Wells *et al.* (1972) first reported under field conditions that an isolate of *T. harzianum* had significantly high potential as biocontrol agent against *Sclerotium rolfsii.* Bora (1977) reported that when the antagonistic property of the different soil fungi was estimated against *R. solani* isolated from egg plant the greatest antagonism was showed by *A. niger.* According to Marshall (1982) there was a reduction in the incidence of damping off of snap bean caused by *R. solani* by seed treatment with *T. harzianum.*

Gokulapalan and Nair (1984) reported that *T. harzianum, T. viride, A. niger* and *A. flavus* exhibited inhibitory action against *R. solani* infecting rice. *A. niger* isolated from the rhizosphere of coffee seedlings was antagonistic to the collar rot pathogen (*R .solani*) *in vitro* and hyper parasitized the pathogen completely in dual culture as observed by Venkatasubbiah and Safeeulla (1984). Gokulapalan and Nair (1991) observed inhibition zones when *R. solani* was grown with *T. harzianum, T. viride and Chaetomium globosum.* They noticed that

after seven days *Trichoderma* spp. completely over grown and parasitized *R. solani*. Sen *et al.* (1993) found that *A. niger*, isolated from the rhizosphere of a healthy musk melon adjacent to wilted areas, proved to be an effective biocontrol agent against *R. solani* by way of antibiosis, overgrowth, and hyper-parasitism. Gogoi and Roy (1996) reported that *A. terreus* reduced the incidence of sheath blight pathogen *R. solani* on cv. IR 50 in pot tests.

According to Neelamegham and Govindarajulu (2002), *T. viride* alone or in combination with farm yard manure were effective in damping off of tomato with concomitant increase in seedling length and biomass of tomato. Meena *et al.* (2003) found that *T. harzianum* was more effective in controlling the banded leaf and sheath blight pathogen *R. solani* in maize. Howel (2003) reported that the enzymes chitinase, or glucanase produced by the biocontrol agents were responsible for suppression of pathogen by breaking down the polysacharides chitan , β glucans that were responsible for the rigidity of fungal cell wall, there by destroying the cell wall integrity. According to Priyadharsini (2003), the fungal antagonist *T. harzianum* was successful in managing the diseases and enhancing the growth of amaranth. Ray and Kumar (2007) studied the efficacy of *T. harzianum* and *T. viride* against *R. solani,* the incitant of aerial blight of soybean under *in vitro* condition and found that they were effective.

2.6.3 Growth promoting effects of bacteria

Plant growth promoting rhizobacteria (PGPR) were originally defined as root colonizing bacteria (rhizobacteria) which either promote plant growth or control plant diseases (Kloepper and Schroth, 1981). Some plant growth promoting rhizobacteria like *Pseudomonas fluorescens* and *P. aeruginosa* may promote the plant growth by secreting hormones such as gibberlic acid (Katznelson and Cole, 1965; Weller, 1998)). Kloepper *et al.* (1980) attributed the enhancement of plant growth to siderophores produced by fluorescent pseudomonads. The growth promoting activity of *Bacillus subtilis* had been

demonstrated by Podile and Dube (1988) on cotton, cucumber, pigeon pea and eggplant. Hoftee *et al.* (1991) reported that the plant growth promoting strains of *P. fluorescens* ANP 15 and *P. aeruginosa* 7NSK2 increased the germination of maize seeds.

Gupta *et al.* (1995) observed that inoculation of *P. fluorescens* increased the rate of seedling emergence, total root dry weight, and shoot weight in tomato plants. Bochow (1999) studied the mode of action of root colonizing *B. subtilis* and showed an increased level of phytohormones and increased activity of beta 1, 3-glucanase. Sood and Sharma (2001) found that the application of *B. subtilis* increased the potato tuber yield from 115 to 268 quintals/h and this was at par with 100 per cent NPK treatment. Seed bacterisation with talc based formulation of *P. fluorescens* enhanced the root length and height of rice plants (Raji and Lekha, 2003). Jeon *et al.* (2003) found three strains of *P. fluorescens* which produced several plant growth promoting phytohormones, including indole 3-acetic acid (auxin).

2.6.4 Bacteria as potential biocontrol agent

According to Sakthivel *et al.* (1986) *P. fluorescens* produced siderophores and it was antagonistic to *R. solani.* Devi *et al.* (1989) observed that efficient strains of fluorescent and nonfluorescent *Pseudomonas* inhibited the mycelial growth and affected sclerotial viability of *R. solani* in rice. Gnanamanickam *et al.* (1992) reported that *P. fluorescens, Bacillus* sp., and *Enterobacter* sp. showed antibiosis towards *R. solani* in rice. Rosales *et al.* (1993) reported that *Bacillus laterosporus, B. subtilis, B. pumilus* and *P. aeruginosa* were inhibitory to *R. solani* under *in vitro* conditions. *Pseudomonas* application on seed, root, soil and foliage of rice increased crop yield and controlled the sheath blight incidence (Vidhyasekharan and Muthamilan, 1999). Singh *et al.* (2002) reported that among the various antagonists tried maximum protection against chilly due to *R. solani* was observed with the application of fluorescent *Pseudomonas*. Kloepper *et al.*

(2004) reported that specific strains of the species *Bacillus amyloliquefaciens, B. subtilis, B. pastuerii, B. pumilus, B. mycoides* and *B. sphaericus* elicit significant reductions in the disease or severity in several hosts. They also reported that the *Bacillus* spp. that elicit induced systemic resistance also elicit plant growth promotion. Gandhi and Kumar (2006) found that *Pseudomonas maltophila* showed maximum growth inhibition against the potato black scurf pathogen *R. solani* in dual culture method.

2.6.5 Role of *Azotobacter* and phosphate solubilizing microbes in enhancing plant growth and controlling disease

Azotobacter is a Gram negative bacterium belonging to the family Azotobacteraceae, a coherent group of aerobic free living diazotrophs able to fix atmospheric nitrogen in nitrogen free or nitrogen poor media with organic C compounds as an energy source. Knosp *et al.* (1984) found that *A. chroococcum* is known to produce siderophores which acts as growth factors and as phytopathogenic suppressive agents. Narula and Tauro (1986) reported that the beneficial effects of *Azotobacter* were attributed to their ability to produce ammonia, vitamins, growth substances like IAA and auxins, gibberlins and cytokinins. Arunkumar (1997) reported maximum yield increase in amaranth with *Azotobacter* treatment along with FYM and 75% fertilizer nitrogen. Kumar *et al.* (2001) found that seed inoculation of wheat varieties with phosphorus solubilizing and phytohormone producing *A. chroococcum* showed better response compared to control. According to Kurkeja *et al.* (2004) the rhizosphere colonizing bacteria including *A. chroococcum,* that posses the ability to enhance plant growth when applied to seeds, roots, or tubers are called plant growth promoting rhizobacteria. Ahmad *et al.* (2005) found that *A. paspali* secreted IAA into the culture media and significantly increased the dry weight of leaves and roots of several plant species following root treatment. Behl *et al.* (2006) demonstrated the contribution of *A. chroococcum* on the solubilization of calcium phosphates. *Azotobacter* like other soil microorganisms played a significant role in mobilizing phosphorus from the

native soil pool, as well as from the added insoluble phosphates such as rock phosphates for plants to use.

Suneja and Lekshminarayana (1993) reported production of siderophores by *A. chroococcum* which solubilize Fe^{3+} and suppress plant pathogens through iron deprivation. Beniwal *et al.* (1996) conducted extensive field experiments to evaluate the effect of *A. chroococcum* strains or mutants on the incidence of flag smut. The results revealed that flag smut incidence on wheat was significantly less under bioinoculation compared to the control. Verma *et al.* (2001) reported that *Azotobacter* inhibited phytopathogenic fungi through antifungal substances.

Phosphate solubilizing microorganisms as a group forms an important part of the microorganisms, which benefit plant growth and development. Gerretson (1948) obtained increased yield and phosphorus uptake of oat plants inoculated with soil containing phosphate dissolving microorganisms as compared to the control. According to Kundu and Gaur (1980), combined inoculation of *Bacillus polymixa* and *Pseudomons striata* increased the yield and P uptake by potato tubers. Dubey and Billore (1992) found that addition of rock PO_4 and inoculation of phosphate solubilizing microorganisms such as *B. megaterium, P. striata, Penicillium* sp. and *Aspergillus awamori*, increased the yield of potatoes. Vassileva *et al.* (1998) observed that a citric acid producing strain of *A. niger* grown on olive cake based medium was able to solubilize rock phosphate, which when introduced in to a calcareous P deficient soil, improved the growth of *Trifolium repens* in a green house experiment. Egamberdiyeva *et al.* (1999) reported that phosphorus solubilizing bacteria were able to mobilize more phosphorus in plants and improved plant growth in cotton, wheat and maize. Rodriguez and Fraga (1999) found that inoculation with *B. subtilis* significantly increased the biomass and also nitrogen and phosphorous accumulation in plant tissues. According to Vassilev *et al.* (2006) microbially mediated solubilization of insoluble phosphates through release of organic acids were often combined

with the production of other metabolites, which took part in biological control against soil borne pathogens.

Gaur and Sadasivam(1993) observed that manurial value of vermicompost could be improved by inoculation with nitrogen fixing and phosphate solubilizing microorganisms. Singh *et al.* (2000) reported that inoculation of composting material with *E. foetida* and *A. chroococcum* and phosphate solubilizing fungi *A. awamori* helped to increase the rate of composting and they caused a marked decrease in the C: N ratio and an increase in P content of vermicompost. Kumar and Singh (2001) reported that inoculation of N_2 fixing bacteria *A. chroococcum* in to vermicompost increased the contents of N. Enriching vermicompost with rock phosphate also improved significantly the available P when inoculated with *P. striata.*

2.7 Effect of PGPM consortia on plant growth and disease management

Potential benefits of application of a single biocontrol agent has been demonstrated in many studies, inconsistent performance may occur when only a single organism was used because a single biocontrol agent is not likely to be active in all kinds of soil environment and agricultural ecosystems. The possibility of introduction of a consortium of inoculants may provide an ever wider spectrum of disease suppression. The use of combined inoculation of fungal and bacterial antagonists has already been reported as an effective disease management method (Pierson and Weller, 1994).

Duffy *et al.*(1996) demonstrated in field trials that the combinations of *T. koningii* and *P. fluorescens* Q29Z-80 increased the yield compared to *P. fluorescens* Q29Z-80 alone, but not different from *T. koningii* alone. Compatibility of *B. subtilis* with *Azospirillum* under *in vitro* and *in vivo* had been proved by Sankar and Jayarajan (1996). Siderenko *et al.* (1996) found that combined inoculation of *Azotobacter* sp., *Bacillus*. sp. and *Pseudomonas* sp.

resulted in an increased plant height, biomass and tuber yield in potato. Jubina and Girija (1998) found that fungal antagonists like *Aspergillus* sp. and *Penicillium* sp. and several rhizosphere bacteria like fluorescent pseudomonas delayed the foliar infection and provided prolonged protection to black pepper against *Phytophthora capsici*.

Soil application of talc based formulation of *T. viride* and *P. fluorescens* either individually or in combination for reducing the pre and post emergent damping off of tomato was recommended by Manoranjitham *et al.* (2000). Anith and Das (2001) found that *T. viride* and *Alcaligenes* applied alone or in combination significantly reduced the mortality of rooted cuttings due to *P. capsici* in black pepper nursery. According to Jetiyanon and Kloepper (2002) the mixtures of PGPR provided greater disease suppression than individual strains against multiple plant diseases, and they found that *T. harzianum* (IISR1369) and *P. fluorescens* (IISR6) treatment combination gave maximum disease suppression in black pepper and cardamom. Jisha *et al.* (2002) studied the mutual compatibility of *T. harzianum* and fluorescent *Pseudomonas* and found that they were compatible to each other. According to Mishra *et al.* (2002) the use of mixtures of biocontrol agents would be a reliable means for management of plant diseases.

Bohra and Mathur (2002) obtained maximum disease control with combination of *Pseudomonas* and *Trichoderma* against *Fusarium solani*. Chaube and Sharma (2002) reported that *T. harzianum* or *T.viride* and *P. fluorescens* were compatible and improved plant growth and suppressed seedling disease of cabbage, brinjal and tomato significantly when treatments were combined and integrated with solarisation. Karunakaran *et al.* (2003) observed that under glass house condition, the treatment combinations *P. fluorescens* and *B. subtilis*, *P. fluorescens* and *T. viride* were found to be best for the growth of crop plants. Srinath *et al.* (2003) studied the influence of arbscular mycorrhizal fungus *Glomus mossae* and the plant growth promoting microorganisms *Bacillus coagulans* and

T. harzianum on the growth and nutrition of micro propagated *Ficus benjamina* plantlets. They reported that the plants showed maximum plant height, biomass, P content and mycorrhizal root colonization when all the three organisms were co inoculated together.

The combined inoculation of chickpea plants with N- fixing *Rhizobium*, a phosphate solubilizing *B. megaterium* subsp. *phosphaticum* strain PB and a biocontrol fungus *Trichoderma* sp. recorded increased germination, nutrient uptake, plant height, number of branches, nodulation and yield and total biomass of chickpea compared to either individual inoculations or uninoculated control (Rudresh *et al.,* 2004). Chinnusamy *et al.* (2006) reported that the combined application of microbial inoculants like *P. striata*, vesicular arbuscicular mycorrhizal fungus and *Azospirillum* resulted in positive interaction with each other and significant improvement in yield and nutritional parameters in wet land rice. Rini and Sulochana (2006) reported that the combination *T. harzianum* (TR20) and *P. fluorescens* (P28) was most effective in reducing the seedling rot of chilly caused by *R. solani* and recorded the highest per plant yield. Shahida (2007) found that *Trichoderma* sp. and *P. fluorescens* were compatible with each other. Dhanya (2007) reported that *P. fluorescens*, *Bacillus* sp. and *Trichoderma* sp. isolated from vanilla rhizosphere were mutually compatible.

2.8 Effect of vermi-products on beneficial and pathogenic organisms.

As this is a new area of research, no detailed research reports are available on the effects of vermiproduct extracts on different microorganisms. But similar work has been done by many workers using phytoextracts and essential oils and their effects on pathogenic microorganisms are well established.

Szczech (1999) reported that vermicompost lost its activity after heating. When sterilized extracts of vermicompost was added to the PDA media, it stimulated the growth of *F. oxysporum*. The extracted body fluid of earth worm

called vermiwash has shown the formation of inhibitory zones for some plant pathogens in Petri plates at the point of its application (Shobha, 2005).

Sundarraj *et al.* (1996) reported that plant extracts of *Allium sativum, Prosopsis julifera, Gynandropsis pentaphylla, Leucas aspera, Vitex negundo* and gingey oil cake showed inhibition of mycelial growth of *R. solani*, the sheath blight pathogen. The essential oils from foliar source of lemon grass possessed markedly high efficacy against *R. solani* in inhibiting mycelial growth according to Rayaz *et al.* (2000). Satya *et al.* (2005) evaluated the *in vitro* fungitoxic effect of various medicinal plants against *R. solani*, the rice sheath blight pathogen. They found that leaf extract of zimmu (*A. cepa* x *A. sativum*) showed the maximum antifungal activity against *R. solani*.

Obi (1998), reported that hot water and oil extracts of *Azadirachta indica* and *Xylopia ethiopica* significantly reduced spore germination and growth of *Colletotrichum lindemuthianum*. Ogbebor et *al.* (2007) found that extracts of *Ocimum bacilicum* and *Allium sativum* exhibited total inhibitory effects on the mycelial growth of *Colletotrichum gloeosporiodes*.

Pernaz *et al.* (1999) found that purified chestnut cystatin inhibited the growth of the phytopathogenic fungi *Botrytis cinerea, Colletotrichum graminicola*, and *Septorium nodorum*, but not that of the saprophyte *T. viride*. Mittal *et al.* (2004) found under *in vitro* evaluation, benzene extracted leaf extract of *Ipomea fistulosa* showed fungicidal properties against the plant pathogens (*C. gloeosporiodes, Fusarium solani, F. o.* f.sp. *cumini*) and also stimulatory effects on *T. harzianum*.

Materials and Methods

3. MATERIALS AND METHODS

An investigation on the "Role of microflora on the quality of vermi-products in improving plant growth" was conducted at Department of Plant Pathology, College of Horticulture, Vellanikkara during 2005-2007. The study was carried using the products of the earthworm *Eisenia foetida*. Vermi-products *viz.*, vermicompost, vermicasting and two types of vermiwash were used. Red amaranth variety Arun was selected as the test plant, since it quickly responds to various nutrients and growth regulators. The materials used and methods adopted in the experiment are presented below.

3.1 Collection of study materials

The samples of earthworm and vermi-products were collected from the ABARD (Agro Biotechnological Agency for Rural Employment and Development) vermicompost unit, College of Horticulture, Vellanikkara. These vermi-products were collected from three tanks of uniform size and maintenance. The details of the collected samples are given below.

1. Vermicompost- It is the preparation of a mixture of worm castings, organic wastes, bedding materials, humus, live earth worms and cocoons. Three random samples of 100 g each were collected from the selected tanks. The samples were mixed well, sieved and dried in shade for one day.
2. Vermicasting - This is the faecal castings of the earth worms. It is granular in appearance and seen on the upper layers of the bedding materials. The samples were collected as described above.
3. Vermiwash – It is a liquid biofertilizer collected by passing water through a column of worm action. Two types of vermiwash used were-

Vermiwash 1 - Vermiwash from worms fed on banana psuedostem and cowdung at 8:1 proportion.

Vermiwash 2 - Vermiwash from worms fed on banana psuedostem and cowdung at 1:1 proportion.

The vermiwash was collected through a tap provided at the bottom of each tank. From each tank, 100 ml sample was collected and pooled together. These collected samples were used for further laboratory studies.

4. Fresh cow dung- Samples of fresh cow dung were also collected from ABARD vermicompost unit. This was used to serve as control.

3.2 Quantitative estimation of microorganisms

The population of native microorganisms such as fungi, bacteria, nitrogen fixing bacteria, phosphorus solubilizing bacteria and actinomycetes present on the surface and gut of earthworms and also in the vermi-products were estimated by serial dilution and plating method (Johnson and Curl, 1972).

3.2.1 Estimation of surface and gut microflora of *E. foetida*

The earthworm samples were collected from each of the selected tank. These worms were made free of adhering particles of organic matter. Three earth worms of uniform size were used for the estimation of surface and gut microflora. The surface microflora of each earth worm was estimated by gently immersing the earth worm in five ml sterile water for five minutes. One ml of the resulting supernatant was serially diluted under aseptic condition to get a final dilution of 10^{-4}.

Gut microflora of the above earthworms were also estimated. The worms were initially transferred to a sterile Petridish and were killed by brief exposure to 95 per cent ethanol. The hindgut of each earthworm was dissected aseptically and the gut

contents were carefully transferred to 9 ml of sterile water blanks in test tubes and serially diluted to get a final dilution of 10^{-4}.

One ml of the appropriate dilution was plated for the enumeration of different microorganisms. Martin Rose Bengal Agar, Nutrient Agar, Jensen's nitrogen free Agar, Pikovskaya's Agar and Kenknights Agar (Appendix I) were used for estimating fungi, bacteria, nitrogen fixing bacteria, phosphorus solubilizing bacteria and actinomycetes at dilutions 10^{-3}, 10^{-4}, 10^{-2}, 10^{-2} and 10^{-3} respectively. The plates were incubated at room temperature for varying duration ranging from two days for bacteria, four days for fungi and phosphorus solubilizing bacteria and seven days for actinomycetes and nitrogen fixing bacteria. After the incubation period, the number of colonies formed in each media was counted. The final data were expressed as the mean of three replications in terms of number of colonies present per each earthworm.

3.2.2 Quantitative estimation of microflora from vermi-products

Serial dilution and plating method was employed for the estimation of microflora. One gram of the collected sample was added to 9 ml sterile water blank to give 10^{-1} dilution and was shaken for 15 minutes in orbital shaker. One ml of this dilution was then transferred to a test tube containing 9 ml of sterile distilled water to get 10^{-2} dilution. The dilution was serially repeated to get 10^{-8} dilution. One ml of the vermiwash was taken for serial dilution. One ml from the appropriate dilution was plated for the enumeration of fungi (10^{-4}), bacteria (10^{-8}), nitrogen fixing bacteria (10^{-3}), phosphorus solubilizing bacteria (10^{-3}) and actinomycetes (10^{-4}) on specific medium. Five replications of each dilution used for the isolation of representative organisms were kept and Petridishes containing medium mixed with sterile water were kept as control.

The Petridishes were then incubated for 2-7 days for the development of microbial colonies. After incubation, the number of colonies formed was counted. The final data were expressed as the mean of five replications in terms of number of colonies per gram of vermi-products.

3.3 Qualitative estimation of microorganisms

The representative typical colonies of different fungi, bacteria and actinomycetes developed in the dilution plates were picked up and transferred to their specific media. The isolates were then purified and preserved for further studies.

3.3.1 Fungi

The fungal isolates were purified by hyphal tip method. Preliminary identification of the fungal isolates was attempted based on cultural and morphological characters. Cultural characters of the isolates such as colour, shape, and texture and growth rate in the PDA were observed. For measuring the growth rate, 10 mm disc from the actively growing zone of each fungal isolate was transferred to the centre of the medium in Petridish. The plates were incubated at room temperature and diameter of the fungal growth was measured at an interval of 24 h until the fungal growth covered the entire Petridish or attained the maximum growth for a period of ten days. Morphological characters of the fungal structures were studied by preparing slides. The slides were then observed under microsope and compared the mycelial and spore characters with original descriptions of the fungi.

3.3.2 Bacteria

The representative colonies of the different bacteria developed in the dilution

plates in NA medium were picked up with the help of a sterilized loop and the isolates were purified by streak plate method. The Petridishes were poured with molten NA medium and waited for cooling. A series of parallel non-overlapping streaks were made on the surface of the medium with sterilized loop containing bacterial isolate. The single colonies developed at the end of the streaks were again picked and transferred to NA slants.

3.3.3 Nitrogen fixing bacteria

The raised mucoid colonies of the nitrogen fixing bacteria developed in the Jensen's nitrogen free agar medium were picked up and purified by streak plate method. The purified colonies were transferred to slants.

3.3.4 Phosphorus solubilizing bacteria

The bacterial isolates showed solubilization zone in the Pikovskaya's agar medium in dilution plates were picked up and purified by streak plate method and maintained in Pikovskaya's agar slants.

3.3.5 Actinomycetes

The single colonies of the actinomycete developed on Kenknights agar were transferred to test tube slants of the same medium and maintained for further use.

The isolated microorganisms from different vermi-products were tested for their efficiency in nitrogen fixation, phosphorus solubilization and antagonistic properties.

3.4 Screening for efficiency in nitrogen fixation

The bacterial isolates developed on the Jensen's nitrogen free agar medium were tested for their efficiency in nitrogen fixation. The nitrogen fixing ability of different isolates was estimated by modified Lawry's method of protein estimation (Lawry, 1951).

The different isolates of nitrogen fixing bacteria were initially grown in 250 ml conical flasks containing 100 ml of sterile Jensen's nitrogen free broth at 28°C for seven days. Three replications were maintained for each isolate. One ml of this broth culture was used for the estimation of protein by Lawry'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 up to 4.5 ml with distilled water. To this five ml of alkaline $CuSO_4$ solution (Appendix II) was added. After ten 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. The protein content was estimated from a standard graph prepared with 20,40,60,80 and 100 µg of bovine serum albumin in distilled water. The amount of nitrogen fixed per gram of sucrose utilized was calculated from the nitrogen protein ratio 1:6.25.

3.5 Screening for efficiency in phosphorus solubilization

The bacterial isolates which showed solubilization zone in the Pikovskaya's agar medium were tested for efficiency in phosphorus solubiliszation.

The bacterial isolates were grown on Pikovskaya's agar slants for two days. A loopful of the culture was taken and spotted at the centre of the Pikovskaya's agar

plates containing tricalcium phosphate. The plates were incubated at 30°C for four days and diameter of the colony and halo zones were measured. The phosphorus solubilizing efficiency (Srivastav *et al.*, 2004) was estimated as follows-

$$\text{Phosphorus solubilisation efficiency} = \frac{Z-C}{C} \times 100$$ where,

Z- Solubilisation zone (mm)

C – Colony diameter (mm)

3.6 *In vitro* screening of the microflora for antagonistic properties against *Rhizoctonia solani*

The microflora obtained from the vermi-products was screened against *R. solani*, which is the causal organism of leaf blight disease in amaranth. The cultures of *R. solani* were procured from the Department of Plant Pathology.

3.6.1 Screening of fungal isolates

The fungal isolates were tested for their antagonistic effect against *R. solani* by dual culture method outlined by Skidimore and Dikinson(1976). Mycelial disc (10 mm) of the pathogen from seven day old culture grown on PDA agar was inoculated asceptically on one side of a Petridish. After this 10 mm mycelial disc of the fungal isolate was inoculated on the same PDA plate on the opposite half of the Petridish towards the periphery on the same day. Three replications were maintained for each isolate. The pathogen grown in monoculture served as control. The plates were examined for the antagonistic activity and the growth measurements of pathogen and fungal isolates were taken daily till the pathogen grown in monoculture reached full growth. The nature of reaction of antagonists on the pathogen was also recorded. The

per cent inhibition of mycelial growth of the pathogen was calculated using the formula suggested by Vincent (1947).

$$PI = \frac{C - T}{C} \times 100 \quad \text{where}$$

PI = Per cent Inhibition.

C = Growth of the pathogen in control plates (mm)

T = Growth of the pathogen in dual culture (mm)

The nature of antagonistic reaction of the fungal isolate tested against *R.solani* was assessed by following the method (Purkayastha and Battacharya, 1982) and assigned to four categories.

Type of reaction.

A. Homogenous	: Free intermingling of hyphae.
B. Overgrowth	: Pathogen over grown by antagonists.
C. Cessation of growth	: Cessation of growth at line of contact.
D. Aversion	: Development of clear zone of inhibition.

For selecting the most efficient fungal isolates, which showed antagonism against *R.solani,* a modified Antagonistic Index (AI) suggested by Kasinathan (1998) was employed, which is the product of PI, CB, SOOP, and IZ.

AI = PI x CB x SOOP x IZ where,

PI	-Per cent inhibition of the growth
CB	- Colonization behaviour of the antagonist on pathogen
SOOP	- Speed of overgrowth on pathogen
IZ	- Width of inhibition zone

3.6.2 Screening of bacterial isolates

The bacterial isolates were screened for their antagonistic efficiency against the pathogen. A preliminary screening of all the bacterial isolates was carried out. Mycelial disc of 10 mm size taken from seven day old culture of pathogen was placed at the centre of a Petridish containing PDA, 24 h prior to the inoculation of the bacteria. Four bacterial isolates were streaked on four sides of the same Petridish at equidistant points from the disc. The pathogen grown in monoculture served as control. The dishes were visually examined for the inhibition of growth of the pathogen by bacterial isolates.

The bacterial isolates selected after the initial screening were tested again to confirm their antagonistic effect against *R. solani by* dual culture method (Utkhede and Rahe, 1983). For this mycelial disc of 10 mm size of the pathogen was placed at the centre of Petridish with PDA medium, 24 h prior to the inoculation of the bacteria. The bacterial isolate was inoculated as a line of streak on either side of the pathogen leaving 2.25 cm from the edge of Petridish. Three replications were maintained for each isolate. The Petridishes were incubated at room temperature and observations on the growth of the pathogen were recorded up to when growth in the control plate fully covered the medium. The per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.6.1.

3.6.3 Screening of actinomycetes isolates

The actinomycetes were tested for their antagonistic effect by dual culture method as explained in 3.6.2. The isolates were inoculated four days prior to inoculation of the pathogen. The growth of the actinomycetes and pathogen was recorded at regular intervals up to five days.

3.7 Identification of selected isolates

Based on the above three parameters *viz.,* efficiency in nitrogen fixation, efficiency in phosphorus solubilization, and antagonistic properties against *R.solani,* the most efficient isolates were selected and an attempt was made to identify them.

3.7.1 Fungi

The fungal isolates which showed the promising AI were selected and an attempt was made to identify them up to species level, based on cultural and morphological characters. The cultural characters like shape, colour, growth and texture of the colonies on PDA medium were studied. The morphological characters like shape, and size of the hyphae, conidiophore, and conidia were recorded by slide culture technique (Riddle, 1950) and compared with the standard characters of the fungi. Photomicrographs of the organisms were also taken. For further confirmation the cultures were sent to "National Centre of Fungal Taxonomy (NCFT)", New Delhi.

3.7.2 Bacteria

The bacterial isolates were selected based on their efficiency in nitrogen fixation, phosphorus solubilization and antagonistic properties. Characterization of different bacterial isolates was done based on cultural, morphological and biochemical characters. For each test 24-48 h old cultures were used.

3.7.2.1 Cultural characters

The bacterial isolates were streaked on nutrient agar medium in Petridishes.

After an incubation period of 24 h, the colonies were observed for its shape, elevation and margin.

3.7.2.2 Morphological characters

For morphological studies, 24 h old culture of the bacteria was used. Gram staining was employed to study the Gram reaction. The shape of the bacteria was identified under oil immersion objective of the microscope. To know the ability of bacterial isolates to produce endospores, endospore staining was done.

3.7.2.2.1 Gram's staining

Gram's staining was done as per the procedure described by Hucker and Conn (1923). The bacterial smear was prepared on a clean glass slide and it was heat fixed by passing over a flame. The smear was then flooded with Hucker's ammonium crystal violet solution (Appendix II) for one minute and then washed in a gentle stream of running tap water. It was then flooded with Gram's iodine solution (Appendix II) for one minute and again washed. Later, the smear was decolorized with 95 per cent ethyl alcohol. After washing, the smear was again stained with saffranin for one minute and the excess stain was washed off in running water. The smear was then blot dried and examined under light microscope for Gram reaction.

3.7.2.2.2 KOH Test

A loopful of bacterial isolate was spread on a clear glass slide. Two drops of three per cent KOH solution was spread over it and thoroughly mixed with the help of a needle and lifted up and down to know the solubility in KOH.

3.7.2.2.3 Endospore staining

Smears of 24 h old bacterial isolates were prepared on clean glass slide and heat fixed. Then a few drops of 1.5 % amidoblack (Appendix II) were added and allowed to stay for 70 sec. Then the slide was washed under a gentle stream of running water, stained for 20 sec with 1% carbol fuchsin and washed thoroughly under tap water. The slide was blot dried and observed under microscope for endospores.

3.7.2.2.4 Pigment production

The bacterial cultures were streaked on King's A and King's B medium (Appendix I) and incubated at room temperature for 48 h. The dishes were observed under ultraviolet light for fluorescent pigment production around the colonies.

3.7.2.3 Biochemical characters

The following tests were done for the biochemical characterization of the selected isolates.

3.7.2.3.1 Catalase test

A few drops of three percent hydrogen peroxide were placed at the centre of a sterile glass slide and a loopful of bacterial isolate was agitated in the solution. Formation of effervescence indicated the positive reaction. (Cappucino and Sherman, 1992).

3.7.2.3.2 Starch hydrolysis test

A loopful of bacterial isolate was spot inoculated on Petridish containing NA medium with 0.2 per cent soluble starch (Appendix I). Starch hydrolysis was tested after 48 h of incubation by flooding the agar surface with Lugol's iodine solution. A colourless zone in contrast to the blue background indicated positive starch hydrolysis (Cappucino and Sherman, 1992).

3.7.2.3.3 Arginine dihydrolase reaction

The bacterial isolates were stab inoculated into the semi-solid medium of Thornley (1960) (Appendix I). The surface of the medium was sealed with three ml of one per cent molten agar. The tubes were incubated for seven days at room temperature and observed for colour change.

3.7.2.3.4 Levan production from sucrose

Peptone beef extract medium containing five per cent sucrose (Appendix I) was used for this test. The bacterial isolates were streaked over the medium in sterilized Petridish and the growth characters were observed after 48 h. Presence of large, white domed and mucoid colonies indicated the production of levan from sucrose (Hayward, 1964)..

3.7.2.3.5 Lipase Test

Bacterial isolates were streaked on Sierra's medium containing 10 per cent tween 80 (Appendix I) and incubated for three days. Petridishes were examined daily

for the presence of a dense precipitate around the bacterial growth, which is indicated the lipid hydrolysis (Sierra, 1957).

3.7.2.3.6 Nitrate reduction test

Nitrate agar medium (Appendix I) was dispensed in test tubes, autoclaved and inoculated with 24 h old cultures of bacteria. The test tubes were then incubated at room temperature and tested for the reduction of nitrate at regular intervals up to 15 days. The test was performed by adding a few drops of Griess Llosvay's reagent consisting of sulphanilic acid (0.8% in 5 M acetic acid) and dimethyl alpha-naphthyl amine (0.5%in 5 M acetic acid) to the nitrate broth culture. Absence of pink or red colour development indicated the presence of nitrate as such or reduced to ammonia and free nitrogen.

3.7.2.3.7 Lecithin Test

Lecithin test was done by spot inoculating bacterial cultures in Petridishes containing nutrient agar medium with egg yolk (2 egg /400 ml). Then inoculated plates were incubated under room temperature from 24 to 48 h. After 48 h incubation plates were examined for the development of opaque zone around the bacterial growth.

3.7.2.3.8 Oxidase test

Ready to use oxidase disc from Himedia, were used for the test. A disc was placed at the centre of glass slide. A loopful of inoculum was taken and rubbed on the disc. Time taken for development of colour, if any, was noted.

3.7.2.3.9 Citrate utilization test

One day old bacterial cultures were streaked on the surface of the slants of Simmon's Citrate agar (Appendix I) and observed for any colour change of the medium (Schaad, 1992).

3.8 Production of Indole Acetic Acid (IAA) by the selected isolates

The selected isolates were tested for their ability to produce Indole Acetic Acid in culture broth. A loopful of 24 h old bacterial isolates and mycelial disc of 10 mm diameter fungal isolates were inoculated into LB (Luria Bertani) medium and incubated for 24 h on rotary shaker at room temperature. After centrifugation at 10,000 rpm for 15 minutes, two ml of the supernatant was taken and three drops of Ortho-Phosphoric acid were added to the aliquot. It was mixed and four ml of reagent (one ml of 0.5 M $FeCl_3$ in 50 ml of 35 per cent $HClO_4$) was added and incubated at room temperature for 25 minutes. Then, the absorbance was measured using spectrophotometer at 530 nm against a reagent blank. Standard curve was prepared using different concentrations of IAA (Gordon and Weber,1951).

3.9 *In vitro* evaluation of mutual compatibility of the selected fungal and bacterial isolates.

The mutual compatibility between the selected fungal and bacterial isolates was tested before preparing the microbial consortium.

3.9.1 *In vitro* evaluation of mutual compatibility among fungi

Compatibility among fungi was tested by employing dual culture technique as mentioned in 3.6.1.

3.9.2 *In vitro* evaluation of mutual compatibility among bacteria

The mutual compatibility among bacteria was tested by cross streaking technique. Bacterial isolates of 48 h old were streaked perpendicular to each other on the Petridishes containing nutrient agar. The plates were then incubated at room temperature and observed daily for the lysis of growth at the juncture point of two isolates for 72 h. Lysis at the juncture point indicated incompatibility.

3.9.3 *In vitro* evaluation of mutual compatibility between fungi and bacteria.

A mycelial disc of 10 mm size of the fungal isolate was inoculated at the centre of the Petridish plated with PDA medium. On the next day bacterial isolates were inoculated as a line of streak on either side of the disc, leaving 2.25 cm from the periphery of Petridish. The plates were then incubated at room temperature and observed daily for any type of inhibition. The absence of inhibition indicated compatibility.

3.10 Preparation of microbial consortium

Based on the results on the beneficial aspects of the selected microorganisms, an attempt was made to prepare a consortium of microorganisms similar in nature that present in vermi-products. Three types of consortia were prepared using the selected isolates. Fungal and bacterial consortium was prepared separately to study

their effects alone in plant growth. The fungal consortium was prepared using five day old cultures of the selected fungi grown separately on PDA medium. The fungal mycelia on PDA scrapped out and were mixed in sterile water. These fungal mycelia in sterile water were blended using a mixer grinder. The total volume was made up to two litres by adding sterile water. A bacterial consortium was prepared using 48 h old culture of the selected bacteria grown in NA medium. Bacterial cells were harvested by scrapping the surface growth with a sterile microscopic slide, and then mixed in sterile water. The total volume was made up to two litres with sterile water.

A combined consortium of selected fungal and bacterial isolates was prepared in the same manner as mentioned above. The total volume was made up to two litres using sterile water. The cell count of the each isolate was made adjusted to 10^6 spores per ml for fungi and 10^8 cfu per ml for bacteria.

3.11 In vivo evaluation of microbial consortium in improving plant growth

A pot culture experiment was conducted to evaluate the efficacy of the prepared microbial consortium and to compare its efficiency with vermi-products in improving the plant growth of amaranth

The details of the pot culture experiment

Design: CRD
Treatments: 14
Replication: 10
Variety: Arun

Treatments

T_1: Vermicompost

T_2: Sterilized vermicompost

T_3: Vermiwash 1

T_4: Sterilized vermiwash 1

T_5: Vermiwash 2

T_6: Sterilized vermiwash 2

T_7: Vermicasting

T_8: Sterilized vermicasting

T_9: Fresh cow dung

T_{10}: Sterilized fresh cow dung

T_{11}: Fungal consortium

T_{12}: Bacterial consortium

T_{13}: Fungal+bacterial consortium

T_{14}: Control

3.11.1 Preparation of potting mixture and planting

Earthern pots of size 20 cm x 20 cm were filled with potting mixture containing soil and sand in the ratio 10:3. Cow dung was not added since it was given as a treatment. The potting mixture was sterilized by fumigating with five per cent formalin and the pots were kept covered with polythene sheet for 10 days. After that the polythene sheet was removed and loosened the soil thoroughly and the pots were kept open for 5 days. Then 25 day old amaranth seedlings were transplanted in each pot. Two plants were maintained in each pot. The FYM (both sterilized and unsterilized) was applied as per package of practices recommendations of KAU for amaranth. The vermiproducts were applied based on their nutrient content. The

treatments were given as seed treatment, and soil application thrice (first at 2 DAT and then at 15 days interval).

I:Application of the consortium

A: seed treatment -- seeds of amaranth were treated with suspension of the consortium for 30 minutes. Then kept for drying in shade and sown in the nursery.

B: soil application- 200 ml of suspension of the already prepared consortium was added to the pots and thoroughly mixed with soil.

II: Application of vermi-products and fresh cow dung

A: seed treatment- the seeds were treated with slurry of vermicompost, vermicasting and fresh cowdung for 30 minutes and sown in the nursery. The slurry was prepared by mixing the vermi-products with sterile water. The vermiwash 1&2 were diluted with water (1:1) and the seeds were kept immersed in that solution for 30 minutes. The excess solution was drained out and seeds were sown in the nursery.

B: soil application- the vermi-products (both sterilized and unsterilized) were applied to the soil directly and incorporated by mixing with soil.

3.12 Observations

The observations were taken at regular intervals on the following parameters.

3.12.1 Plant height

Height of the plant was recorded at four stages of plant growth *viz.,* 15, 30, 45 and 60th days after transplanting from the base of the plant to the top most leaf.

3.12.2 Number of leaves

Total number of leaves in each plant was counted and the average recorded for each treatment at the above four growth stages.

3.12.3 Number of branches

Total number of branches in each plant was counted and the average recorded for each treatment at 30, 45 and 60th days after transplanting.

3.12.4 Stem girth

The girth of the main stem at the collar region was taken using a twine and the average recorded for each treatment at 30, 45 and 60th days after transplanting.

3.12.5. Yield per harvest

Three cuttings were taken from each plant at 30, 45 and 60th days after transplanting. The yield obtained per harvest was taken and the average was expressed in grams per plant for each treatment. The leaf weight and stem weight were taken separately for each cutting in each plant and the average was worked out.

3.12.6 Leaf to stem ratio

The leaf to stem ratio was obtained by dividing the weight of leaves by weight of stem. Leaf to stem ratio was worked out for three cuttings.

3.12.7 Fresh and dry weight of shoot

Fresh weight of shoots were taken separately and immediately after uprooting the plants. Dry weight of shoot was taken after drying the sample under sun and later in a drying oven at 60^0C to a constant weight.

3.12.8 Fresh and dry weight of root

Fresh weight of roots were taken separately after uprooting and removing soil particles adhered to root system by gentle wash with water. Dry weight was taken after drying the sample till a constant weight was obtained.

3.12.9 Assessment of disease incidence

The incidence of leaf blight disease caused by *R. solani* in amaranth was recorded at 45 and 60 days after transplanting and the per cent disease incidence was calculated as follows-

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

3.12.10 Assessment of disease severity

Disease severity was calculated by randomly selecting hundred leaves from each treatment and was scored using 0-5 scale. Score chart for the assessment of disease severity was given below.

Grade	Description
0	No symptoms
1	1- 10% leaf area infected
2	10-25% leaf area infected
3	25-50% leaf area infected
4	50-75% leaf area infected
5	> 75% leaf area infected

Per cent disease severity was calculated using the formula suggested by Wheeler (1969).

Percent disease index

$$= \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed x Maximum disease grade}} \times 100$$

3.12.11 Estimation of soil microflora

Rhizosphere soil samples were collected from different treatments at the time of final harvest. Populations of fungi, bacteria and actinomycetes were estimated by serial dilution and plating technique.

3.12.12 Nutrient Analysis

Nutrients N, P and K in the plant samples were analysed after the harvest. For this, the plants were harvested, washed off the soil particles and dried under sun for 2-3 days and there after dried in a hot air oven at 60^0 C. After drying, samples were powdered and the fine powder was used for estimation of nutrient elements. The methods used were given below.

Nutrient	Digestion Procedure	Method of Estimation	Reference
N	H_2SO_4 single acid Digestion	Micro kjeldhals Estimation	Jackson (1973)
P	2:1 HNO_3: $HClO_4$ Diacid digestion	Vanadomolybdate yellow colour method using spectrophotometer	Jackson (1973)
K	2:1HNO_3: $HClO_4$ Diacid digestion	Direct reading using Flame Photometer	Jackson (1973)

3.12.13 Crude protein content

The total nitrogen content of the oven-dried sample was estimated by Microkjeldahl method. The values obtained were multiplied by a factor 6.25 to get the crude protein content, and expressed as per cent on dry weight of leaves.

3.13 *In vitro* evaluation on the effect of vermi-products and cow dung extracts on pathogenic and beneficial microorganisms

An *in vitro* study was carried out to find the effect of extracts of the vermi-products and cow dung on both beneficial and pathogenic microorganisms using poisoned food technique, growth on enriched media, and filter paper disc method.

Freshly collected vermiwash 1 & 2 were used to study their effect on the growth of microorganisms. Worm cast was collected and dried in shade for one day. Hundred gram of the dried cast was kept in a muslin cloth bag and immersed in 250 ml water for 24 hours. The extract thus obtained was used for the *in vitro* studies. The

cow dung and vermicompost extract was prepared in the same manner as mentioned above. Two fungal pathogens *viz., Rhizoctonia* sp., *Colletotrichum* sp. and two beneficial microorganisms *viz., Trichoderma harzianum, Pseudomonas fluorescens* were used as test organisms in this study. The following vermi-products at these different concentrations were used for the evaluation.

Extract	Concentration (%)
Vermicompost	5,10,15
Vermicasting	5,10,15
Vermiwash 1	5,10,15
Vermiwash 2	5,10,15
Fresh cow dung	5,10,15

3.13.1 Poisoned food technique

The effect of different extracts on test fungi was studied by poisoned food technique. The extract was passed through Millipore filter reduce the microbial load and subjected to steam sterilization for 30 minutes for three consecutive days. Sufficient quantity of the standard extract to get the required concentration was added to molten agar medium at 45° C and then poured in to sterile Petridishes. Mycelial disc of 10 mm diameter was taken separately from actively growing cultures of the above mentioned fungi and placed on the centre of the medium. Three replications were maintained for each test fungi. The diameter of the fungal colony was recorded up to and when the growth in the control plates were fully covered the medium. The per cent inhibition was calculated using the formula as given in 3.6.1.

3.13.2 Growth on enriched media

The different extracts were sterilized by autoclaving at 121° C for 20 minutes. The PDA medium was then enriched by adding 5, 10 and 15 per cent of these extracts and sterilized. Their effects were then tested as in poisoned food technique and the per cent inhibition was calculated.

3.13.3 Filter paper disc method

This technique was employed to study the effect of different extracts on *Pseudomonas fluorescens*. The required concentrations of the extracts were prepared in sterile distilled water. Sterile filter paper discs of 5 mm diameter were dipped in the prepared solutions for 20 minutes. The King's B medium was seeded with 48 h old culture of the test bacteria, and poured in sterile Petridishes. Then three discs were placed on these sterile medium seeded with bacterial culture. Sterile filter paper discs dipped in sterile water served as control. Three replications were maintained for each concentration. The plates were incubated at room temperature for three days and observed for any type of inhibition.

3.14 Statistical analysis

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986). Multiple comparisons among treatment means were done using DMRT.

Results

4. RESULTS

The results of the study on the "Role of microflora on the quality of vermi-products in improving plant growth' are presented in this chapter.

4.1 Quantitative estimation of surface and gut microflora of earth worm

The results of the estimation of surface and gut microflora of the earth worm *Eisenia foetida* are presented in the Table 1.

From the data it was evident that, the bacterial population was predominant in the surface microflora which recorded 7 x 10^4 cfu per earthworm, this was followed by actinomycetes (6.33 x 10^3). The fungal population was in the order of 2.33 x 10^3 cfu per earthworm. The population of nitrogen fixing and phosphorus solubilizing bacteria was found to be 3.67 x 10^2 and 0.33 x 10^2 cfu respectively.

Table 1: Quantitative estimation of surface and gut microflora of earthworms

Organism	Surface microflora per earthworm*	Gut microflora per earthworm*
Fungi	2.33 x10^3	4.67 x10^3
Bacteria	7.00 x10^4	11.33 x10^4
Nitrogen fixing bacteria	3.67 x10^2	3.00 x10^2
Phosphurus solubilizing bacteria	0.33 x10^2	-
Actinomycetes	6.33 x10^3	8.33 x10^3

* Mean of three replications

Estimation of gut microflora was also done. The population of fungi, bacteria and actinomycetes were found to be more in the gut content than in the surface. The microbial population followed the same trend and dominated by bacteria (11.33x 10^4cfu per earthworm), then actinomycetes (8.33 x 10^3) fungi (4.67 x 10^3). The population of nitrogen fixing bacteria was less compared to surface flora and recorded about 3 x10^2 cfu per earth worm. The phosphorus solubilizing bacteria was absent in the gut content.

4.2 Quantitative estimation of microflora of vermi-products

The results on the estimation of microflora in vermi-products are presented in the Table 2, and it was found that the population of different microflora varied with vermi-products. In general, the bacterial population was found to be higher followed by actinomycetes and fungi. The highest count of fungi 43 x 10^4 cfu g^{-1} was observed in the samples of vermicast. The lowest fungal count was observed in fresh cow dung (11.5 x 10^4 cfu g^{-1}). Among vermi-products, the fungal count was minimum in vermiwash 1 (13 x 10^4 cfu ml^{-1}). The bacterial population was found to be more in fresh cow dung, which recorded 127 x 10^8 cfu g^{-1} of sample followed by vermicast (97 x 10^8 cfu g^{-1}). The least count was recorded with vermiwash 1 (66 x 10^8 cfu ml^{-1}). The population of nitrogen fixing bacteria was found to be more in vermicompost and recorded about 31 x 10^3 cfu g^{-1}. The phosphorus solubilizing bacteria recorded the highest count of 4.5 x 10^3 cfu ml^{-1} in vermiwash 2.

The population of actinomycetes varied with products with a maximum count of 56.5 x 10^4 cfu g^{-1} in vermicompost, and the minimum count of 19.5x 10^4 cfu g^{-1} in fresh cow dung and this was followed by vermiwash 2 (21.5 x 10^4 cfu ml^{-1}).

Table 2: Quantitative estimation of microflora of vermiproducts

Product	Fungi 10^4	Bacteria 10^8	Nitrogen fixing bacteria 10^3	Phosphorus solubilizing bacteria 10^3	Actinomycetes 10^4
Vermicompost	29.50 (5.48)	83.50 (9.19)	31.00 (5.61)	4.00 (2.11)	56.50 (7.55)
Vermicasting	43.00 (6.59)	97.00 (9.87)	24.50 (4.99)	4.00 (2.11)	37.00 (6.12)
Vermiwash 1	13.00 (3.58)	66.00 (8.15)	23.00 (4.84)	3.50 (1.99)	26.50 (5.19)
Vermiwash 2	15.00 (3.90)	90.50 (9.52	29.50 (5.45)	4.50 (2.23)	21.50 (4.69)
Fresh cowdung	11.50 (3.46)	127.00 (11.28)	22.50 (4.80)	1.50 (1.41)	19.50 (4.47)

The colonies of different microorganisms developed on the specific media showing visual morphological differences were picked up from the dilution plates, and purified. From this, the most commonly and predominantly occurring isolates were selected for further studies. Altogether thirty two isolates of fungi, thirty nine isolates of bacteria (including five isolates of nitrogen fixing and three isolates of phosphorus solubilizing bacteria), and four isolates of actinomycetes were obtained from the vermi-products.

4.3 Identification of fung

Preliminary identification of the fungal isolates from the vermi-products was done. The data is presented in the Table 3. Attempts to identify the fungi upto generic level based on cultural and morphological characters revealed that, out of the different fungal isolates, five belonged to the genus *Trichoderma*, nine to the genus *Aspergillus*, five to the genus *Penicillium*, and one each to *Fusarium, Mucor, Rhizopus, Syncephalastrum*, and rest of the isolates remained unidentified.

The different isolates of fungi from cow dung did not show much difference from those of vermi-products. The main genera observed were *Trichoderma, Aspergillus* and *Penicillium*.

4.3.1 Growth of fungal isolates

The growth rate of thirty two fungal isolates on PDA medium was studied. From the data furnished in the Table 4, it was found that the fungal growth varied widely. Among the different isolates, those belonged to the genera *Trichoderma* (VF5, VF8, VF16, VF22, and VF25) were very fast growing and covered the entire Petri dish with in a period of three days. The isolate of *Syncephalastrum* (VF10),

Table 3: Identification of fungal isolates from vermiproducts

Sl. No.	Fungal isolates	Tentative identification
1	VF1	*Penicillium* sp.
2	VF2	*Aspergillus* sp.
3	VF3	Unidentified
4	VF4	*Aspergillus* sp.
5	VF5	*Trichoderma* sp.
6	VF6	*Aspergillus* sp.
7	VF7	Unidentified
8	VF8	*Trichoderma* sp.
9	VF9	Unidentified
10	VF10	*Syncephalastrum* sp.
11	VF11	*Aspergillus* sp.
12	VF12	Unidentified
13	VF13	*Aspergillus* sp.
14	VF14	*Penicillium* sp.
15	VF15	*Aspergillus* sp.
16	VF16	*Trichoderma* sp.
17	VF17	*Penicillium* sp.
18	VF18	*Aspergillus* sp.
19	VF19	*Aspergillus* sp.
20	VF20	*Fusarium* sp.
21	VF21	*Aspergillus* sp.
22	VF22	*Trichoderma* sp.
23	VF23	Unidentified
24	VF24	*Penicillium* sp.
25	VF25	*Trichoderma* sp.
26	VF26	*Penicillium* sp.
27	VF27	Unidentified
28	VF28	Unidentified
29	VF29	*Rhizopus* sp.
30	VF30	Unidentified
31	VF31	*Mucor* sp.
32	VF32	Unidentified

Table 4: Growth rate of fungal isolates from vermiproducts

Sl.No.	Fungal isolates	Mean growth in diameter (mm)*									
		Days after incubation (DAI)									
		1	2	3	4	5	6	7	8	9	10
1	VF1	10	13	19	25	31.5	38	45	51	58	64
2	VF2	23	50	64	78	90					
3	VF3	15	31	51	62	73	82	90			
4	VF4	21	37	65	76	80	90				
5	VF5	28	53	90							
6	VF6	10	15	23	29	37	40	46	53	59	66
7	VF7	10	11	18	22	27.5	31	34	39	43	57
8	VF8	33	76	90							
9	VF9	14	29	56	70	90					
10	VF10	20	39	71	90						
11	VF11	14	22	26	32	40	45	51	58	65	71
12	VF12	10	16	22	31	36	43	49	55	63	68
13	VF13	17	24	33.5	41	49	56	63	72	80	90
14	VF14	19	23	47	53	80	90				
15	VF15	21	37	55	66	74.5	90				
16	VF16	31	66	90							
17	VF17	11	20	29	37	45	54	69	79.5	90	
18	VF18	23	51	81	90						
19	VF19	19	37	56	70	81	87	90			
20	VF20	15	35	61.5	75	85	90				
21	VF21	20	35	42	51	63	75	82	90		
22	VF22	35	79	90							
23	VF23	22	30	44	59	68	79	90			
24	VF24	10	15	22	29	31	35	40	46	51	57
25	VF25	31	72	90							
26	VF26	10	22	31	45	51	63	75	86	90	
27	VF27	13	26	37	46	55	63	76	84.5	90	
28	VF28	15	27	39	48	59	75	90			
29	VF29	23	47	86	90						
30	VF30	16	45	78	90						
31	VF31	21	41	63	90						
32	VF32	12	17	21	26	31	34	39	44	49	53

* Mean of three replications

Fusarium (VF20), *Rhizopus* (VF29) and *Mucor* (VF31) completed the growth within a period of four to six days.

The growth rate of different species of *Aspergillus* (VF2, VF4, VF6, VF11, VF13, VF15, VF18, VF19, and VF21) was found highly varying. Most of the isolates covered the entire Petridish with in a period of five to seven days. However, the *Penicillium* isolates (VF1, VF14, VF17, VF24, and VF26) were slow growing and only two isolates *viz.*, VF17 and VF26 attained full growth in nine days. The unidentified isolates (VF3, VF7, VF9, VF12, VF23, VF27, VF28, VF30, and VF32) showed varying growth rate in Petridishes.

4.4 Selection of efficient organisms for microbial consortia

The microbial consortium was prepared based on the performance in following aspects.

4.4.1 Efficiency in nitrogen fixation

Five isolates of nitrogen fixing bacteria were screened for their efficiency in nitrogen fixation and the results are given in the Table 5.

There were differences in the nitrogen fixing ability of the selected isolates. It varied from 2.054 to 5.53 mg per gram of sucrose consumed. The isolate NB2 recorded the maximum nitrogen fixing ability of 5.53 mg. Thus, the isolate NB2 was selected for further work.

Table 5: Nitrogen fixing ability of the bacterial isolates

Sl. No.	Bacterial isolate	Rate of nitrogen fixation mg/gram of the sucrose
1	NB1	2.054
2	NB2	5.530
3	NB3	3.107
4	NB4	3.914
5	NB5	4.412

4.4.2 Efficiency in phosphorus solubilization

Three isolates of phosphorus solubilizing bacteria obtained from the vermi-products were tested for their efficiency in phosphorus solubilization. As evidenced from the Table 6, the phosphorus solubilizing capacity of the isolates showed differences. The isolate PB1 showed maximum efficiency (49.41 per cent) followed by PB2 (33.6 per cent) and the least was observed in PB3. Based on this result, the isolate PB1 was selected.

Table 6: Phosphorus solublization capacity of the bacterial isolates

Sl. No.	Isolate	P solubilization(%)
1	PB1	49.41
2	PB2	33.6
3	PB3	12.8

4.4.3 Antagonism of the microflora against *Rhizoctonia solani*

4.4.3.1 Fungi

The thirty two fungal isolates were screened for their antagonistic effect against *R. solani*. The antagonistic reaction of the organism in dual culture and per

cent inhibition of the pathogen over control were recorded.

From the data on the above aspect presented in Table 7, it was observed that out of the thirty two isolates tested, five isolates (VF5, VF8, VF16, VF22 and VF25) showed 100 per cent inhibition by complete overgrowth on *R. solani*. Among the other fungal isolates, one fungus VF7 was completely overgrown by the pathogen. The rest of the isolates showed a per cent inhibition ranging from 30 to 76.11 per cent on the seventh day of incubation.

It was noticed that among fungal isolates, which showed 100 per cent inhibition, all the isolates of *Trichoderma* overgrew the pathogen. The isolates VF2 and VF15 showed a slight aversion initially as they left a clear zone of inhibition between paired organisms. But after four days of incubation, they started to overgrow the pathogen. The isolates VF6 and VF23 showed a slight aversion towards the pathogen. The rest of the isolates led to cessation of growth at the point of contact with the pathogen. The monoculture of the pathogen kept as control attained full growth after four days of incubation.

4.4.3.1.1 Selection of efficient fungal antagonists

From among the different isolates, the most efficient isolates were selected by employing the method of Kasinathan (1998), as presented in the Table 8. It was revealed from the results that, the fungal isolates showed an antagonistic index (AI) ranging from 120 to 1500. All the *Trichoderma* spp. (VF5, VF8, VF16, VF22, and VF25) recorded an AI of 1500. This was followed by VF2 (*Aspergillus* sp.), with an AI of 1369.98 and then VF15 (*Aspergillus* sp.) which showed an AI of 1308.6.

Table 7: Antagonism of fungal isolates against *R. solani*

Sl. No.	Isolates	Days after incubation(growth in mm)*														Per cent inhibition (PI)	Type of reaction
		1		2		3		4		5		6		7			
		A	P	A	P	A	P	A	P	A	P	A	P	A	P		
1	VF1	12	21	19	35	25	42	30	54	32	58					35.56	C
2	VF2	19	19	33	28	49	30	63	26	65	25	66.5	23.5	68.5	21.5	76.11	B&D
3	VF3	25.5	22	34	31	49	35	57	33							58.89	C
4	VF4	18	21	28	31	32	40	36	48	39	51					43.33	C
5	VF5	28	22	49	34	75	15	90	0							100.00	B
6	VF6	11	21	19	29	26.5	36	31	45	38	47	39	48	41	48	45.56	D
7	VF7	11	23	15	30	26	41	28	49	27	63	13	77	0	90	0	C
8	VF8	30	25	49	41	78	12	90	0							100.00	B
9	VF9	16	23	22	29.5	29	40	33	47	39	51					43.33	C
10	VF10	24	24	39	37	53	37									42.22	C
11	VF11	12	23	16	44	23	57	25	58	29	61					32.22	C
12	VF12	10	21	19	42	23	55	27	58	29	60					33.33	C
13	VF13	14	23	16	39	24	58	28	62							31.11	C
14	VF14	17	21.5	26	40	33	53	35	55							38.89	C
15	VF15	24	22	36	33	43	35	49.5	38	59.5	29.5	62.5	27.5	65.5	24.5	72.70	B&D
16	VF16	30	24	51	39	79	11	90	0							100	B

17	VF17	10	22	18	36	23	52	29	55	31	59					34.44	C
18	VF18	23	21	43.5	27	47	31	58	32							64.44	C
19	VF19	17	23	29	38	35	49	37	53							41.11	C
20	VF20	13	22	25	34	30	46	36	54							40.00	C
21	VF21	26	22	39	31.5	53	35	55	35							61.11	C
22	VF22	29	21	50	37	75	14.5	90	0							100.00	B
23	VF23	14	25	23	42	29	54	32	55	34	55					38.33	D
24	VF24	10	21	13	39	19	46	23	5725	62	27	63				30.00	C
25	VF25	28	21	51	38	76	14	90	0							100.00	B
26	VF26	11	22	24	38	31	47	38	50	39	51					43.33	C
27	VF27	15	19	25	37	36	50	38	52							42.22	C
28	VF28	16	21	29.5	32	40	36	43	44	45	45					50.00	C
29	VF29	21	18	40	41	44	46									48.88	C
30	VF30	23	22	41	37	48	40	51	39							56.67	C
31	VF31	18	23	24	36	31	48	34	56							37.78	C
32	VF32	11	19	22	39	34	46	37	52.5							41.67	C
	Monoculture of pathogen																
	R. solani	-	2.2	-	4.1	-	6.45	-	90								

** Mean of three replications*

P- Pathogen *A- Fungal isolate (Antagonist)*

B. Overgrowth *C. Cessation of growth* *D. Aversion*

Table 8: Antagonistic index of the fungal isolates against *R. solani*

Sl. No.	Isolate no.	PI	CB	SOOP	IZ	AI
1	VF1	35.56	2	2	1	142.24
2	VF2	76.11	3	3	2	1369.98
3	VF3	58.89	3	2	1	353.34
4	VF4	43.33	2	2	1	173.32
5	VF5	100.00	5	3	1	1500.00
6	VF6	45.56	2	2	2	364.48
7	VF7	0	1	1	1	0
8	VF8	100.00	5	3	1	1500.00
9	VF9	43.33	2	2	1	173.32
10	VF10	42.22	3	2	1	253.32
11	VF11	32.22	2	2	1	128.88
12	VF12	33.33	2	2	1	133.32
13	VF13	31.11	2	2	1	124.44
14	VF14	38.89	2	2	1	155.56
15	VF15	72.70	3	3	2	1308.60
16	VF16	100.00	5	3	1	1500.00
17	VF17	34.44	2	2	1	137.76
18	VF18	64.44	3	2	1	386.64
19	VF19	41.11	2	2	1	164.44
20	VF20	40.00	2	2	1	160.00
21	VF21	61.11	2	2	1	244.44
22	VF22	100.00	5	3	1	1500.00
23	VF23	38.33	2	2	2	306.64
24	VF24	30.00	2	2	1	120.00
25	VF25	100.00	5	3	1	1500.00
26	VF26	43.33	2	2	1	173.32
27	VF27	42.22	2	2	1	168.88
28	VF28	50.00	2	2	1	200.00
29	VF29	48.88	2	2	1	195.52
30	VF30	56.67	3	2	1	340.02
31	VF31	37.78	2	2	1	151.12
32	VF32	41.67	2	2	1	166.68

PI- Per cent inhibition

SOOP- speed of overgrowth on pathogen

IZ- Inhibition zone

CB-Colonization behaviour of antagonists on pathogen

4.4.3.2 Bacteria

Among the thirty one bacterial isolates screened, only two showed inhibition of the pathogen and the data is presented in Table 9.

Table 9: *In vitro* screening of the bacterial isolates against *R. solani*

Sl. No.	Isolates	Days after incubation(growth in mm)*					
		2		Per cent inhibition	4		Per cent inhibition
		M	D		M	D	
1	VB4	40	36	10	90	43	52.22
2	VB26	40	34	15	90	42	53.33

* Mean of three replications
M- Monoculture of *R. solani*
D- Dual culture of *R. solani* and bacterial isolates

The isolates VB4 and VB26 showed a clear zone of inhibition demarcating the growth of pathogen and bacteria. After four days of incubation, the maximum inhibition of mycelial growth of the pathogen was recorded by the isolate VB26 (53.33 per cent) which was followed by VB4 (52.22 per cent).

4.4.3.3 **Actinomycetes**

The actinomycetes were screened for their antagonistic properties using the dual culture technique. Four isolate of actinomycetes were inoculated in the Petridishes, four days prior to the pathogen due to their slow growth. The results revealed that none of the isolate was antagonistic to the pathogen, as the pathogen showed full growth in the Petridishes.

4.5 Identification of the efficient fungal isolates

The seven fungal isolates (Table 10) from different vermi-products, which showed the promising AI, were selected and were identified based on cultural and morphological characters (Plate 1).

Table 10: Selected fungal isolates

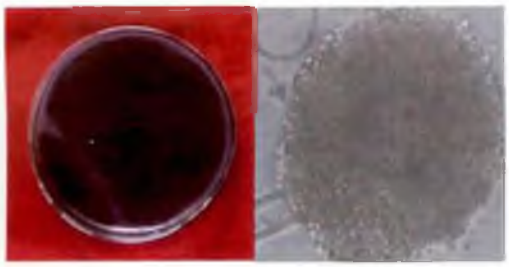
Sl. No.	Isolate	Product
1	VF2	Vermiwash 2
2	VF5	Vermicast
3	VF8	Vermiwash 1
4	VF15	Vermicast
5	VF16	Vermicompost
6	VF22	Vermicompost
7	VF25	Vermiwash 1

The measurements of various structures of these fungal isolates were taken using micrometry. The results are given in the Table 11 and 12. Photomicrographs of the isolates (400x) were also taken. The cultures were sent to National Centre of Fungal Taxonomy (NCFT), New Delhi and the identification got confirmed.

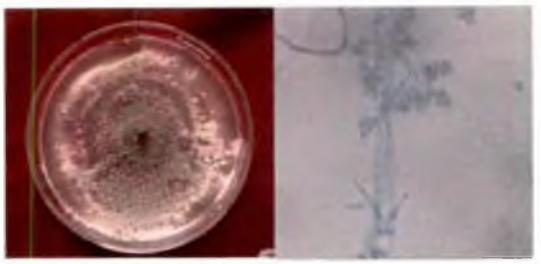
4.5.1 *Trichoderma* spp. (VF5, VF22 and VF25)

Colonies were found to grow rapidly; it was initially white later turned to whitish green to bright green. The reverse side of the colony remained uncoloured. The mycelium was hyaline, smooth walled, septate, branched and having 2.1- 3.2 µm width (Table 11). Conidiophore much branched and form continuous ring like zones. Philiades are short skittle shaped, narrow at the base attenuated abruptly with sharp

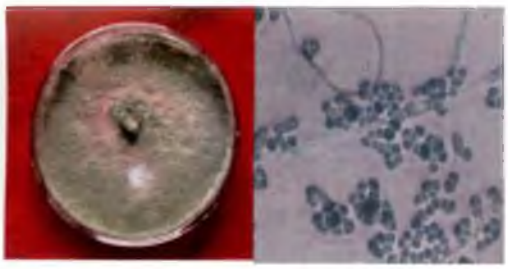
Plate 1: Identification of selected fungal isolates



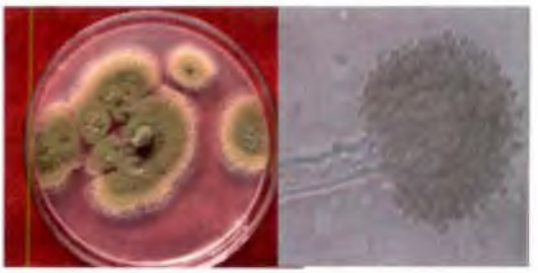
VF2- *Aspergillus niger*



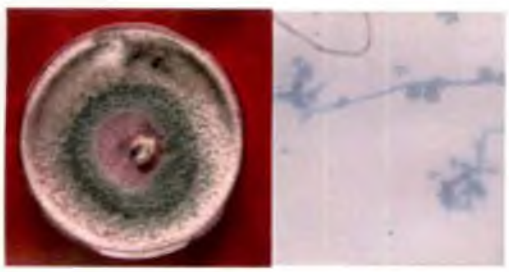
VF5-*Trichoderma harzianum*



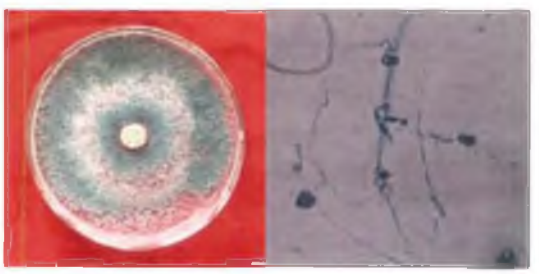
VF8 - *Trichoderma viride*



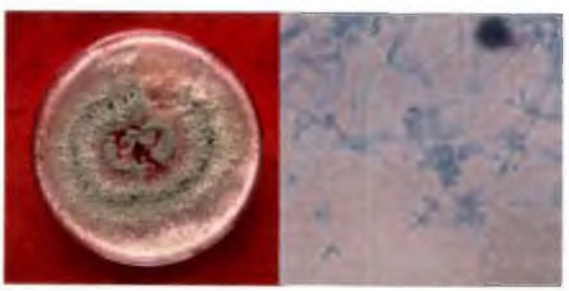
VF15 - *Aspergillus flavus*



VF16 -*Trichoderma viride*



VF22-*Trichoderma harzianum*



VF25 - *Trichoderma harzianum*

pointed neck with a length of 5.9- 9.8µm. Phialospores accumulated at the tip of phialides and are subglobose or short obovoid with broad truncate base and 2.8-3.3µm in diameter . They are smooth walled, pale green and much darker in mass. Based on these characters, these isolates (VF5, VF22 and VF25) were identified as *Trichoderma harzianum*. This identification got confirmed from NCFT with their identification no. 1367.07 (VF5), 1368.07 (VF22) and 1366.07 (VF25).

4.5.2 *Trichoderma* spp. (VF8 and VF16)

Mycelium was watery white and became hairy from the formation of loose scanty aerial mycelium, which made the colonies floccose to somewhat whitish. Colonies turned green to dark green with maturity. The reverse of the colony remained uncoloured. Mycelium was hyaline, septate, smooth walled, much branched and measuring 2.8-3.5µm wide (Table 11). Conidiophore showed dendroid branching system. Philiades are slender, not crowded with a length ranging from 7.6- 11.5µm. Phialospores are globose and rough walled with 3.7µm and 3.8 µm in diameter. Based on these characters, these isolates were identified as *Trichoderma viride* Rifai. This identification got confirmed from NCFT with their identification no.1369.07 (VF8), 1370.07 (VF16).

Table 11: Morphological characters of *Trichoderma* spp.

Isolate	Mycelium width(µm)	Phialides length(µm)	Phialospores (µm)	Organism
VF5	2.1-3.2	7.7-9.8	2.8	*T.harzianum*
VF8	2.8-3.1	7.6-11.5	3.7	*T.viride*
VF16	3.3-3.5	7.9-10.6	3.8	*T.viride*
VF22	3-3.2	5.9-9.8	3.1	*T.harzianum*
VF25	2.8-3.2	6.2-9.8	3.3	*T.harzianum*

4.5.3 *Aspergillus* sp. (VF2)

Colonies were fast growing with white mycelium and heavily sporulating in deep brownish black colour. Conidial heads are larger and globose to radiate in well defined columns. They showed thick walled, colourless to brownish conidiophore having 310-375 µm length and 15.8 µm width (Table 12). Vesicle is globose 45.4-54 µm and fertile all over. Sterigmata are biseriate. Conidia are globose, brown in colour, irregularly roughened and measure 3.9-5.9 µm diameter. Based on these characters, this isolate was identified as *Aspergillus niger*. This identification got confirmed from NCFT with their identification no.1372.07.

4.5.4 *Aspergillus* sp. (VF15)

Colonies were fast growing. The mycelium was mostly submerged and heavily sporulating. The conidial heads were yellow to green initially and became brownish with age. They are radiate and splitting to several columns. The reverse of the colony remained yellowish. Conidiophore was heavy walled and colourless having 320-474 µm length and 10.8 µm width. Vesicle is globose to subglobose 36.5-45 µm diameter and the fertile area is half to $3/4^{th}$. The conidia are globose to subglobose, echinulate and measure 3.5-4.6 µm. Based on these characters, this isolate was identified as *Aspergillus flavus*. This identification got confirmed from NCFT with the identification no.1371.07.

Table 12: Morphological characters of *Aspergillus* spp.

Isolate	Conidiophore		Vescicle (µm)	Conidia (µm)	Organism
	Length (µm)	Width (µm)			
VF2	310-375	15.8	45.4-54	3.9-5.9	*A. niger*
VF15	320-474	10.8	36.5-45	3.5-4.6	*A. flavus*

4.6 Identification of the efficient bacterial isolates

Cultural, morphological and biochemical characterization of the selected isolates (Table 13) from different vermi-products were done.

Table 13: Selected bacterial isolates

Sl.No.	Isolate	Product
1	VB4	Vermicast
2	VB26	Vermicompost
3	NB2	Vermiwash 2
4	PB1	Vermicompost

4.6.1 Cultural characters

The colony characters of the isolates in NA medium were studied and result is given in the Table 14.

Table 14: Colony characters of the bacterial isolates

Sl. No.	Isolates	Size	Form	Elevation	Margin
1	VB4	Punctiform	Circular	Convex	Entire
2	NB2	1.5 mm	Circular	Convex	Entire
3	VB26	1.1mm	Irregular	Flat	Undulate
4	PB1	Punctiform	Circular	Flat	Entire

The isolates VB4 and PB1 showed punctiform colonies, whereas NB2 and VB26 showed a colony size of 1.5 mm and 1.1 mm respectively. The isolates VB4, NB2 and PB1 produced colonies of circular form and VB26 produced irregular

colonies. The isolates VB4 and NB2 produced colonies with convex elevation, whereas VB26 and PB1 formed flat colonies. Colonies with entire margin was observed in all isolates except VB26 which showed undulate margin.

4.6.2 Morphological characters

The results of the gram staining (Table 15) revealed that the isolates VB26, NB2 and PB1 were Gram negative. These isolates showed positive reaction to KOH test by forming thick thread. The isolate VB4 showed Gram positive reaction.

Table 15: Morphological characters of the selected bacterial isolates

Sl. No.	Isolates	Gram's reaction	Endospore staining	KOH test	Pigment production	Shape of cells
1	VB4	+	+	-	-	Rod
2	NB2	-	-	+	-	Rod
3	VB26	-	-	+	-	Rod
4	PB1	-	-	+	-	Rod

All the isolates were found to be rod shaped. Endospore staining was also done and it was found that, among the four isolates, only VB4 showed the formation of endospores. Pigment production of bacterial isolates were studied by streaking them on King's A and King's B medium. None of the isolates produced fluorescent pigments in King's A and King's B medium.

4.6.3 Biochemical characters

Biochemical characters of the four bacterial isolates were studied and presented in Table 16.

Table 16: Biochemical characterization of the selected bacterial isolates

Sl. No.	Test name	Bacterial isolates			
		VB4	NB2	VB26	PB1
1	Catalase test	+	+	+	+
2	Starch hydrolysis	-	-	-	-
3	Arginine dihydrolase reaction	+	+	+	+
4	Levan production from sucrose	-	-	-	-
5	Lipase test	-	-	+	+
6	Nitrate reduction test	+	+	+	-
7	Lecithin test	+	-	-	-
8	Oxidase test	v	+	+	+
9	Citrate utilization	+	+	-	-

+ : Positive
- : Negative
v : Variable or doubtful

4.6.3.1 Catalase test

All the isolates produced effervescence when hydrogen peroxide was added to smear of bacteria indicating the presence of catalase.

4.6.3.2 Starch hydrolysis

None of the isolates were able to hydrolyse starch as evidenced by the absence of a colourless zone around the bacterial growth in contrast to the blue background.

4.6.3.3 Arginine dihydrolase reaction

All the isolates gave pink colour to the medium indicated their ability to hydrolyse arginine.

4.6.3.4 Levan formation from sucrose

All the isolates failed to produce levan from sucrose as indicated by the absence of raised convex colonies on the media supplemented with five per cent sucrose.

4.6.3.5 Lipase test

The isolate VB26 and PB1 showed positive lipase activity by forming a dense precipitate around the bacterial growth after 24 h of incubation.

4.6.3.6 Nitrate reduction test

All the bacterial isolates except PB1 showed the ability to reduce nitrate

4.6.3.7 Lecithin test

The isolates VB4 showed positive reaction to lecithin test by forming opaque zone around the bacterial growth.

4.6.3.8 Oxidase test

A loopful of each isolate was taken and rubbed on oxidase disc. All isolates except VB4 showed purple colour development, proving that the isolates were positive for oxidase test.

4.6.3.9 Citrate utilization

The isolates VB4 and NB2 were able to change the colour of the Simmon's citrate agar from green to blue, indicating positive reaction.

Based on the cultural, morphological and biochemical characters the bacterial isolates were tentatively identified as *Azotobacter* sp. (NB2), *Pseudomonas* sp.(PB1), *Bacillus* sp.(VB4) and non fluorescent *Pseudomonas* (VB26).

4.7 Production of Indole Acetic Acid (IAA) by the selected isolates

The result of the tests conducted on production of IAA by the selected isolates is presented in the Table 17.

Table17: IAA production by the selected fungal and bacterial isolates

Sl. No.	Isolate	IAA production mg/ml
1	VB4	0.106
2	VB26	0.081
3	NB2	0.119
4	PB1	0.141
5	VF2	0.00
6	VF5	0.094
7	VF8	0.128
8	VF15	0.00
9	VF16	0.096
10	VF22	0.089
11	VF25	0.085

From the data it was clear that among the eleven isolates tested nine were able to produce IAA in the culture broth. The fungal isolates VF2 and VF15 did not produce IAA. Among the bacterial isolates, the highest IAA production was observed in PB1 (0.141 mg/g) and the lowest was recorded in VB26 (0.081 mg/g). Among the fungal isolates the maximum IAA production was noticed in VF8 (0.128 mg/g) and the minimum was observed with VF25 (0.085mg/g).

4.8 Mutual compatibility of the selected isolates

Based on the three aspects studied *viz.*, efficiency in nitrogen fixation, phosphorus solubilization and antagonistic action against *R. solani,* eleven microorganisms were selected. Out of these seven fungi and two bacteria were selected for their antagonistic properties against the pathogen and the remaining two bacterial isolates were selected based on their nitrogen fixing and phosphorus solubilizing capacity.

Plate 2: Effect of microbial consortia on plant growth

T_{14}-Control
T_{11}- Fungal consortium

T_{14}- Control
T_{12}- Bacterial consortium

T_{14}- Control
T_{13}- Fungal + bacterial consortium

Compatibility among the seven selected fungi having antagonistic property was studied. No inhibition on the growth of any antagonistic fungi was observed, which indicated the mutual compatibility among the antagonists. The results on the mutual compatibility among the bacterial isolates revealed that, there was no lysis at the juncture point of any of the two bacterial isolate for 72 h, indicated that they were compatible to each other. The compatibility between the selected fungal and bacterial isolates was studied by dual culture method. The results showed that both organisms had growth on the Petridishes. The fungal antagonist overgrew the bacteria indicating compatibility.

4.9 Evaluation of microbial consortium in improving plant growth

A pot culture experiment was conducted to evaluate the efficacy of the prepared microbial consortium in improving the plant growth in amaranth (Plate 2). The treatments were given as seed treatment and three soil applications. Observations on growth parameters, per cent disease incidence and disease severity and total soil microflora were recorded. The uptake of nutrients N, P, K was estimated in each treatment. The results are given in Table 18 to 30.

4.9.1 Plant height

The observations recorded at different intervals showed significant variation among the treatments. From the data (Table 18), it was observed that at 15 DAT the maximum shoot length (14.73 cm) was recorded by T_8 which was on par with T_2, T_7 T_{11}, T_{12} and T_{13}. The minimum shoot length was observed with T_3 (6.97 cm). At 30 DAT, treatment T_2 recorded the maximum shoot length (31.92 cm) which was on par with T_1, T_8, T_{11}, T_{12} and T_{13}. Among all the treatments minimum shoot length was observed with T_3 (17.61 cm).

Table 18: Effect of various treatments on plant height at different intervals

Sl. No.	Treatments	Plant height (cm)			
		15 DAT	30 DAT	45 DAT	60DAT
1	T_1	12.40^{bc}	30.49^{ab}	52.47^{a}	88.07^{a}
2	T_2	13.83^{ab}	31.92^{a}	57.60^{a}	90.27^{a}
3	T_3	6.97^{d}	17.61^{e}	33.23^{bc}	58.80^{bc}
4	T_4	10.08^{d}	21.71^{de}	30.50^{bc}	55.10^{bc}
5	T_5	9.48^{d}	20.21^{de}	33.30^{bc}	55.00^{bc}
6	T_6	10.67^{cd}	23.95^{cd}	37.05^{b}	58.77^{bc}
7	T_7	12.93^{ab}	27.19^{bc}	52.27^{b}	85.82^{a}
8	T_8	14.73^{a}	30.88^{ab}	57.75^{a}	85.73^{a}
9	T_9	8.97^{d}	17.93^{e}	29.63^{bc}	58.63^{bc}
10	T_{10}	10.17^{d}	21.71^{de}	27.25^{c}	60.50^{bc}
11	T_{11}	13.92^{ab}	25.89^{ab}	56.23^{a}	83.77^{a}
12	T_{12}	14.38^{ab}	28.14^{ab}	50.90^{a}	68.43^{b}
13	T_{13}	13.17^{ab}	29.25^{ab}	56.37^{a}	88.27^{a}
14	T_{14}	9.02^{d}	17.69^{e}	30.60^{bc}	47.00^{c}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Maximum plant height was recorded by T_8 (57.75 cm) at 45 DAT, which was on par with T_1, T_2, T_{11}, T_{12} and T_{13}. The minimum shoot length was observed with T_{10} (27.25 cm). At 60 DAT, treatment T_2 (90.27 cm) was found to be most effective in increasing the plant height which was significantly superior to the control (T_{14}) which recorded 47 cm height.

4.9.2 Number of leaves

The data (Table19) on the number of leaves at different intervals showed significant difference among the treatments.

At 15 DAT, the treatment T_8 (7.67) was found to be more effective in improving the foliage growth of amaranth and was on par with T_1, T_2, T_4, T_7, T_{11}, $T_{12}$and T_{13}. The lowest number of leaves was recorded in treatment T_5 (3.67). At 30 DAT, the maximum leaf number of 37.17 was recorded by T_2 which was on par with T_1, T_7, T_8 and T_{13}. The minimum number of leaves was observed in T_3 (12.17).

The maximum number of leaves was recorded by T_8 (69.56) at 45 DAT and was on par with all other treatments except T_5, T_{10} and T_{14}. The lowest number of leaves was recorded by T_{14} (35.50). At 60 DAT, the treatment T_7 (96.83) recorded the maximum number of leaves, which was on par with T_1, T_2, T_8, T_{11} and T_{13}. The treatment T_{14} (54.83) recorded the lowest number of leaves.

4.9.3 Number of branches and girth of the main stem

The statistical analysis of the data (Table 20) revealed that there was significant difference among treatments on number of branches and girth of the main stem at different intervals.

Table 19: Effect of various treatments on number of leaves at different intervals

Sl. No.	Treatments	Number of leaves			
		15 DAT	30 DAT	45 DAT	60DAT
1	T_1	7.00^{abc}	34.67^{ab}	62.00^{abc}	88.50^{ab}
2	T_2	7.50^{ab}	37.17^{a}	65.33^{ab}	93.83^{a}
3	T_3	5.00^{e}	12.17^{g}	57.33^{abcd}	56.83^{de}
4	T_4	6.50^{abcd}	17.5^{efg}	58.00^{abcd}	68.17^{cde}
5	T_5	3.67^{f}	14.83^{fg}	37.67^{cd}	70.83^{cde}
6	T_6	6.00^{cde}	25^{cde}	46.00^{abcd}	61.17^{de}
7	T_7	7.17^{abc}	32.33^{abc}	63.83^{ab}	96.83^{a}
8	T_8	7.67^{a}	33.67^{ab}	69.56^{a}	83.83^{abc}
9	T_9	5.5^{de}	14.83^{fg}	54.67^{abcd}	69.10^{cde}
10	T_{10}	5.5^{de}	21.5^{def}	40.67^{bcd}	73.67^{bcd}
11	T_{11}	7.50^{ab}	25.33^{cde}	63.56^{ab}	86.17^{abc}
12	T_{12}	6.5^{abcd}	27.83^{bcd}	53.83^{abcd}	68.67^{cde}
13	T_{13}	7.00^{abc}	32.83^{abc}	66.83^{a}	91.5^{a}
14	T_{14}	6.17^{bcde}	16.00^{fg}	35.50^{d}	54.83^{e}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Table 20: Effect of various treatments on number of branches and stem girth at different intervals

Sl. No.	Treatments	30 DAT		45 DAT		60 DAT	
		Branches	Girth (cm)	Branches	Girth (cm)	Branches	Girth (cm)
1	T_1	8.00^{a}	2.17^{bcd}	14.67^{ab}	3.23^{a}	19.33^{a}	4.13^{bc}
2	T_2	8.33^{a}	2.73^{a}	15.50^{ab}	3.53^{a}	19.17^{a}	4.40^{ab}
3	T_3	5.00^{cd}	1.30^{ef}	9.33^{c}	2.00^{ef}	16.33^{def}	3.27^{fg}
4	T_4	5.67^{bc}	1.30^{ef}	9.17^{c}	2.38^{cde}	13.83^{gh}	3.58^{cdef}
5	T_5	3.33^{d}	1.10^{f}	10.83^{c}	1.93^{ef}	16.33^{def}	3.33^{ef}
6	T_6	4.83^{cd}	1.83^{cd}	10.83^{c}	2.13^{de}	16.00^{ef}	3.50^{def}
7	T_7	7.83^{a}	1.72^{de}	14.67^{ab}	3.12^{ab}	19.00^{bc}	3.87^{bcde}
8	T_8	8.50^{a}	2.43^{ab}	16.33^{a}	3.32^{a}	21.00^{a}	4.77^{a}
9	T_9	4.17^{cd}	1.28^{ef}	10.17^{c}	2.32^{cde}	16.67^{de}	3.32^{ef}
10	T_{10}	5.67^{bc}	2.02^{bcd}	8.33^{c}	2.32^{cd}	15.00^{fg}	3.58^{cdef}
11	T_{11}	7.00^{ab}	2.05^{bcd}	14.88^{ab}	2.30^{cde}	17.67^{cd}	4.07^{bcd}
12	T_{12}	7.12^{ab}	2.26^{abc}	13.67^{b}	2.67^{bc}	16.50^{def}	3.50^{def}
13	T_{13}	7.17^{ab}	2.27^{abc}	15.17^{ab}	2.60^{cd}	18.50^{bc}	3.67^{cdef}
14	T_{14}	3.50^{d}	1.15^{f}	8.33^{c}	1.60^{f}	13.28^{h}	2.77^{g}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

At 30 DAT, the maximum number of branches was observed in T_8 (8.5) and maximum girth was showed by T_2 (2.73 cm). The number of branches (3.33) and girth (1.1 cm) was minimum for T_5. At 45 DAT, the treatment T_8 showed the maximum number of branches (16.33) and was on par with T_1, T_2, T_7, T_{11} and T_{13}. The stem girth was maximum for T_2 (3.53 cm). The minimum number of branches (8.33) was recorded by T_{10} and T_{14}. The stem girth was minimum for T_{14} (1.60 cm). The treatment T_8 recorded the highest number of branches (21) and stem girth (4.77 cm) at 60 DAT. The lowest number of branches (13.28) and stem girth (2.77 cm) was recorded by T_{14}. It was observed that all stages treatment T_8 showed the maximum number of branches.

4.9.4 Yield per harvest

Observations on three cuttings were taken and results are presented in the Table 21, 22 and 23.

The statistical analysis of the data showed significant difference among the treatments in yield. During the first harvest (30 DAT), treatment T_2 recorded the maximum yield of 94.79 g/plant and was on par with T_8 and T_{13}. The yield was minimum for T_{14} (39.25 g/plant). The maximum leaf weight was recorded by T_{13} (64.84 g/plant) and it was on par with T_2 and T_8. The lowest leaf weight was recorded by T_{14} (29.96 g/plant). The stem weight was maximum for T_7 (37.78 g/plant) and minimum for T_9 (8.89 g//plant). The leaf to stem ratio was maximum for T_9 (3.92) and was on par with T_{14} and minimum for T_7 (1.05).

The second cutting was taken at 45 DAT, the highest yield was recorded by T_2 (120.82 g/plant) and it was on par with T_1, T_7, T_8, T_{11} and T_{13}. The treatment T_{14} showed the lowest yield of 41.78 g /plant. The leaf weight was maximum for T_{13}

Table 21: Effect of various treatments on the fresh yield of amaranth during first harvest

Sl. No.	Treatments	Average yield (g/plant)	Leaf weight (g/plant)	Stem weight (g/plant)	Leaves/stem ratio
1	T_1	77.27^{b}	47.18^{b}	30.09^{b}	1.57^{de}
2	T_2	94.79^{a}	58.05^{a}	36.74^{a}	1.58^{de}
3	T_3	47.27^{de}	34.79^{de}	12.48^{efg}	2.79^{bc}
4	T_4	56.21^{cd}	41.67^{bcd}	14.54^{efg}	2.86^{bc}
5	T_5	54.24^{de}	36.09^{cde}	16.15^{ef}	2.23^{bcd}
6	T_6	58.05^{cd}	39.74^{bcde}	18.31^{de}	2.17^{cde}
7	T_7	77.26^{b}	39.48^{bcde}	37.78^{ab}	1.05^{e}
8	T_8	90.83^{a}	63.53^{a}	27.30^{bc}	2.33^{bcd}
9	T_9	43.77^{de}	34.88^{de}	8.89^{g}	3.92^{a}
10	T_{10}	51.00^{de}	37.48^{bcde}	13.52^{efg}	2.77^{bc}
11	T_{11}	72.28^{b}	42.89^{bcd}	29.38^{bc}	1.45^{de}
12	T_{12}	69.29^{bc}	46.14^{bc}	23.15^{cd}	1.99^{cde}
13	T_{13}	94.32^{a}	64.84^{a}	29.48^{bc}	2.19^{cde}
14	T_{14}	39.25^{e}	29.96^{e}	9.29^{fg}	3.22^{ab}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4 - Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10} - Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Table 22: Effect of various treatments on the fresh yield of amaranth during second harvest

Sl. No.	Treatments	Average yield (g/plant)	Leaf weight (g/plant)	Stem weight (g/plant)	Leaves/stem ratio
1	T_1	99.29^{abcd}	62.75^{bc}	36.54^{bc}	1.72^{efg}
2	T_2	120.82^{a}	71.36^{ab}	49.46^{a}	1.44^{g}
3	T_3	61.69^{ef}	45.47^{bc}	16.22^{ef}	2.81^{abcd}
4	T_4	72.69^{de}	56.24^{bc}	16.45^{ef}	3.41^{a}
5	T_5	63.71^{ef}	45.58^{bc}	18.13^{def}	2.51^{bcdef}
6	T_6	77.44^{cde}	53.55^{bc}	23.89^{de}	2.24^{cdefg}
7	T_7	105.72^{abc}	70.67^{ab}	35.05^{bc}	2.02^{defg}
8	T_8	117.68^{a}	74.08^{ab}	43.60^{ab}	1.70^{fg}
9	T_9	78.38^{cde}	59.99^{bc}	18.39^{def}	3.26^{ab}
10	T_{10}	83.85^{bcde}	60.16^{bc}	23.69^{de}	2.53^{bcdef}
11	T_{11}	111.68^{ab}	76.01^{ab}	35.67^{bc}	2.13^{defg}
12	T_{12}	87.47^{bcde}	63.35^{bc}	24.12^{de}	2.62^{abcde}
13	T_{13}	116.44^{a}	88.48^{a}	27.96^{cd}	3.06^{abc}
14	T_{14}	41.78^{f}	31.36^{c}	10.42^{f}	3.01^{abc}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Table 23: Effect of various treatments on the fresh yield of amaranth during third harvest

Sl. No.	Treatments	Average yield (g/plant)	Leaf weight (g/plant)	Stem weight (g/plant)	Leaves/stem ratio
1	T_1	68.33^{ab}	41.33^{abcde}	27.00^{abcde}	1.53^{ab}
2	T_2	79.66^{a}	49.13^{ab}	30.54^{ab}	1.61^{ab}
3	T_3	46.95^{bc}	31.60^{bcdef}	15.35^{cdefg}	2.05^{ab}
4	T_4	39.93^{bc}	27.58^{cdef}	12.35^{efg}	2.23^{a}
5	T_5	36.68^{c}	23.20^{ef}	13.48^{defg}	1.72^{ab}
6	T_6	25.36^{c}	18.08^{f}	7.28^{g}	2.48^{a}
7	T_7	79.10^{a}	42.59^{abcd}	36.51^{a}	1.17^{b}
8	T_8	80.72^{a}	52.41^{a}	28.31^{abcd}	1.85^{ab}
9	T_9	42.64^{bc}	27.33^{cdef}	15.31^{cdefg}	1.79^{ab}
10	T_{10}	53.46^{abc}	32.92^{bcdef}	20.54^{bcdefg}	1.60^{ab}
11	T_{11}	69.18^{ab}	46.08^{abc}	23.10^{abcdef}	1.99^{ab}
12	T_{12}	68.29^{ab}	44.74^{abc}	23.55^{abcdef}	1.89^{ab}
13	T_{13}	76.64^{a}	47.60^{ab}	29.04^{abc}	1.64^{ab}
14	T_{14}	35.26^{c}	24.98^{def}	10.28^{fg}	2.42^{a}

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4 - Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10} - Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

(88.48 g/plnt) and minimum for T_{14} (31.36 g/plant). The highest stem weight was recorded by T_2 (49.46 g/plant) and was on par with T_8. The stem weight was minimum for T_{14} (10.42 g/plant). From the leaf to stem ratio it was found that maximum leaf to stem ratio of 3.41 was recorded by T_4 and minimum with T_2 (1.44).

The third cutting was taken at 60 DAT, the maximum yield was observed with T_8 (80.72 g/plant) which was on par with all other treatments except T_3, T_4, T_5, T_6, T_9 and T_{14}. The minimum yield was recorded by T_6 (25.36 g/plant). The leaf weight was maximum for T_8 (52.41 g/plant) and minimum for T_6 (18.08 g/plant). The highest stem weight was showed by T_7 (36.51 g/plant) and the lowest was recorded with T_6 (7.28 g/plant). The calculation of leaf to stem ratio revealed that it was maximum for T_6 (2.48) and minimum for T_7 (1.17).

4.9.5 Fresh and dry weight of shoots

The shoot fresh weight and dry weight in each treatment was taken at the time of depotting. From the Table 24, it was evident that the treatments showed significant difference among the treatments. The shoot fresh weight was maximum for T_8 (173.75 g/plant) which showed 210.49 per cent increase over control and was on par with T_1, T_2, T_7 and T_{13}. The minimum shoot fresh weight was seen in T_4 (45.72 g/plant). The shoot dry weight was also maximum for T_8 (24.29 g/plant) which showed an increase of 309.61 per cent over control, and were on par with T_1, T_2 and T_{13}. The minimum shoot dry weight was recorded by T_{14} (5.93 g/plant).

4.9.6 Fresh and dry weight of roots

From the data presented in the Table 25, it was found that the root fresh weight and dry weight vary significantly among treatments. The maximum root fresh

Table 24: Effect of various treatments on fresh and dry weight of shoots

Sl. No.	Treatments	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Per cent increase over control	
				Shoot fresh weight	Shoot dry weight
1	T_1	171.76^{a}	24.27^{a}	206.93	309.27
2	T_2	147.55^{ab}	19.81^{abc}	163.67	234.06
3	T_3	51.34^{ef}	7.95^{efg}	-8.25	34.06
4	T_4	45.72^{f}	7.95^{efg}	-18.29	34.06
5	T_5	60.42^{ef}	6.92^{fg}	7.97	16.69
6	T_6	76.66^{def}	12.57^{defg}	36.99	111.97
7	T_7	131.75^{abc}	17.25^{bcd}	135.44	190.89
8	T_8	173.75^{a}	24.29^{a}	210.49	309.61
9	T_9	88.99^{cde}	13.41^{cdef}	59.02	126.14
10	T_{10}	83.68^{de}	12.15^{defg}	49.54	104.89
11	T_{11}	113.99^{bcd}	15.56^{bcd}	103.7	162.39
12	T_{12}	91.28^{cde}	14.25^{bcde}	63.12	140.30
13	T_{13}	132.01^{abc}	20.61^{ab}	135.90	247.55
14	T_{14}	55.95^{ef}	5.93^{g}	0	0

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Table 25: Effect of various treatments on fresh and dry weight of roots

Sl. No.	Treatments	Root fresh weight (g/plant)	Root dry weight (g/plant)	Per cent increase over control	
				Root fresh weight	Root dry weight
1	T_1	24.98^{a}	5.39^{ab}	204.63	118.21
2	T_2	18.02^{abcd}	4.10^{bcd}	119.76	65.99
3	T_3	7.83^{e}	1.80^{e}	-4.51	-27.13
4	T_4	8.26^{e}	2.76^{de}	0.73	11.74
5	T_5	11.61^{cde}	2.71^{de}	41.59	9.71
6	T_6	13.31^{cde}	3.42^{cde}	62.32	38.46
7	T_7	15.36^{bcde}	3.46^{cde}	87.32	40.08
8	T_8	22.50^{ab}	5.78^{a}	174.39	134.00
9	T_9	14.17^{bcde}	3.94^{bcd}	72.80	59.51
10	T_{10}	14.11^{bcde}	3.31^{cde}	72.07	34.00
11	T_{11}	16.91^{abcde}	3.86^{bcd}	106.22	56.27
12	T_{12}	9.95^{de}	2.81^{de}	21.34	13.76
13	T_{13}	20.09^{abc}	4.56^{abc}	145.51	84.61
14	T_{14}	8.20^{e}	2.47^{de}	0	0

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2 – Sterilized vermicompost
T_3- Vermiwash 1
T_4 - Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10} - Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

weight was recorded with T_1 (24.98 g/plant) and was on par with T_2, T_8, T_{11} and T_{13}. The treatment T_1 showed 204.63 per cent increase over control. The minimum root fresh weight was recorded by T_3 (7.83 g/plant). The root dry weight was maximum for T_8 (5.78 g/plant) which showed 134.00 per cent increase over control. Minimum dry weight was recorded by the treatment T_3 (1.80 g/plant).

4.9.7 Per cent disease incidence and severity of leaf blight

Data on the effectiveness of various treatments on controlling the incidence and severity of *Rhizoctonia* leaf blight in amaranth under natural conditions was presented in the Table 26 and 27. Generally the incidence and severity of leaf blight disease was less during the cropping period. Two observations were taken on the disease incidence and severity at 45 and 60 DAT.

Data on disease incidence at 45 DAT revealed that there was a significant difference among the treatments. The minimum incidence of 15 per cent was noticed in T_{11} and T_{13} which showed 74.99 per cent reduction over control. The maximum incidence was recorded in T_{14} (60 per cent) followed by T_{10} (50 per cent). At 60 DAT, the least disease incidence was recorded by T_7 (30 per cent) which showed 64.39 per cent reduction over control. It was followed by T_2 and T_{13} (35 per cent). The disease incidence was maximum for plants in T_4 (89.47 per cent).

Statistical analysis of the data on disease severity also showed significant difference among the treatments. The observations made on 45 DAT revealed that treatment T_{13} recorded the minimum disease severity of 3.33 per cent and maximum per cent reduction (77.47 per cent) over control, which was followed by T_{12} and was on par with T_7 and T_{11}. The maximum disease severity was noticed in T_{14} (14.78 per cent). At 60 DAT, the minimum disease severity was recorded by T_{11} (7.25 per cent)

Table 26: Effect of various treatments on the incidence of leaf blight in amaranth

Sl. No.	Treatments	Per cent disease incidence		Per cent reduction over control	
		45 DAT	60 DAT	45 DAT	60 DAT
1	T_1	20.00[b]	40.00[c]	66.66	52.52
2	T_2	25.00[c]	35.00[b]	58.33	58.46
3	T_3	30.00[d]	60.00[e]	49.99	28.78
4	T_4	35.00[e]	89.47[i]	41.66	-6.20
5	T_5	35.00[e]	65.00[f]	41.66	22.85
6	T_6	30.00[d]	55.00[d]	49.99	34.72
7	T_7	25.00[c]	30.00[a]	58.33	64.39
8	T_8	30.00[d]	40.00[c]	49.99	52.52
9	T_9	40.00[f]	60.00[e]	33.32	28.78
10	T_{10}	50.00[g]	70.00[g]	16.65	16.91
11	T_{11}	15.00[a]	40.00[c]	74.99	52.52
12	T_{12}	30.00[d]	55.00[d]	49.99	34.72
13	T_{13}	15.00[a]	35.00[b]	74.99	58.46
14	T_{14}	60.00[h]	84.21[h]	0	0

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2– Sterilized vermicompost
T_3- Vermiwash 1
T_4- Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10}- Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

Table 27: Effect of various treatments on the severity of leaf blight in amaranth

Sl. No.	Treatments	Per cent disease severity		Per cent reduction over control	
		45 DAT	60 DAT	45 DAT	60 DAT
1	T_1	5.22^{d}	8.44^{bc}	64.69	55.06
2	T_2	6.56^{f}	10.11^{de}	55.62	46.16
3	T_3	10.22^{i}	12.33^{f}	30.86	34.34
4	T_4	11.89^{j}	16.22^{i}	19.56	13.63
5	T_5	9.44^{h}	14.78^{gh}	36.13	21.29
6	T_6	12.00^{j}	14.40^{g}	18.82	23.32
7	T_7	4.33^{bc}	10.89^{e}	70.71	42.01
8	T_8	4.56^{c}	9.22^{cd}	69.15	50.90
9	T_9	6.00^{e}	15.44^{ih}	59.41	17.78
10	T_{10}	7.89^{g}	17.33^{j}	46.62	7.72
11	T_{11}	4.18^{b}	7.25^{a}	71.72	61.39
12	T_{12}	4.11^{b}	12.11^{f}	72.19	35.51
13	T_{13}	3.33^{a}	8.22^{b}	77.47	56.23
14	T_{14}	14.78^{k}	18.78^{k}	0	0

In each column figures followed by same letters do not differ significantly according to DMRT

T_1- Vermicompost
T_2 – Sterilized vermicompost
T_3- Vermiwash 1
T_4 - Sterilized vermiwash 1
T_5- Vermiwash 2
T_6- Sterilized vermiwash 2
T_7- Vermicasting
T_8- Sterilized vermicasting
T_9- Fresh cow dung
T_{10} - Sterilized fresh cow dung
T_{11}- Fungal consortium
T_{12}- Bacterial consortium
T_{13}- Fungal+ bacterial consortium
T_{14}- Control

and showed 61.39 per cent reduction over control. The severity was maximum for plants in T_{14} (18.78 per cent).

4.9.8 Effect of various treatments on population of soil microflora

The results of the effect of various treatments on the population of soil microflora are given in the Table 28. The statistical analysis of the data showed significant difference among the treatments on the population of various microflora except phosphorus solubilizing bacteria.

Considerable variation in the population of fungi was noticed among the treatments. The highest fungal count of 24.67x 10^3 cfu g^{-1} was noticed in T_7 followed by T_{13} (22.33 x10^3 cfu g^{-1}) and the lowest was recorded in T_6 (5x10^3 cfu g^{-1}). The bacterial population was maximum in T_1 (75 x 10^6 cfu g^{-1} of soil) and closely followed by T_6. The least count of 41 x 10^6 cfu g^{-1} was observed in T_{14}. The observations on the population of nitrogen fixing bacteria showed that maximum number was in T_7 (15.67 x 10^3 cfu g^{-1}) and minimum number was in T_5 (3.33 x 10^3 cfu g^{-1}). It was observed that there was no significant difference among treatments in the population of phosphorus solubilizing microorganisms. The highest count of 1.67x10^3 cfu g^{-1} was noticed in T_{12}. The lowest count (0.33x10^3cfu g^{-1}) was observed in T_6, T_7, T_8 and T_{13}. The population of actinomycetes also showed a significant fluctuation among treatments. The highest population of 40.33x 10^4 cfu g^{-1} was recorded with T_8 which was on par with T_1 and T_3. The least count was observed with T_5 (17x 10^4 cfu g^{-1}).

4.9.9 Nutrient analysis

The analysis for major nutrients in each treatment was carried out and the