CHARACTERIZATION OF RHIZOSPHERE AND ENDOPHYTIC MICROFLORA FROM ORGANICALLY GROWN AMARANTH FOR MANAGEMENT OF LEAF SPOT DISEASES

By

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(2012-11-143)

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COLLEGE OF HORTICULTURE

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Characterization of rhizosphere and endophytic microflora from organically grown amaranth for management of leaf spot diseases"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other university or society.

Vellanikkara **Aparna V. S.**

27.01.2015 **(2012-11-143)**

CERTIFICATE

Certified that this thesis, entitled **"Characterization of rhizosphere and endophytic microflora from organically grown amaranth for management of leaf spot diseases"** is a record of research work done independently by **Ms. Aparna V. S. (2012-11-143)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Aparna V. S. (2012-11-143)** a candidate for the **Master of Science in Agriculture**, with major field in **Plant Pathology**, agree that the thesis entitled **"Characterization of rhizosphere and endophytic microflora from organically grown amaranth for management of leaf spot diseases"** may be submitted by **Ms. Aparna V. S.** in partial fulfilment of the requirement for the degree.

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APARNA V.S.

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Introduction

1. INTRODUCTION

Amaranth is one of the major tropical leafy vegetables grown during summer and rainy season in India. Rapid growth, quick rejuvenation after each harvest, higher yield per unit area and easiness in cultivation make it the cheapest leafy vegetable, which is known as 'poor man's spinach'. Amaranth belongs to the genus *Amaranthus* of the family Amaranthaceae. Genus *Amaranthus* has many species which are used as leafy vegetables, e.g. *A. tricolour*, *A. tristis,* and *A. viridis*. Among the various production constraints, leaf blight caused by soil borne pathogen *Rhizoctonia solani* is a major threat to the growers. The disease is very severe during rainy season under warm and humid conditions and the symptoms include appearance of white, irregular spots on leaf lamina making the produce unmarketable (Nayar *et al*., 1996).

Nowadays, efforts to meet food demand from limited arable area have lead to the use of fertilizers and pesticides in large quantities. To maintain the quality and abundance of agricultural produce plant diseases need to be controlled. There are different methods to control plant diseases and growers mainly depend on fungicides due to ease of application (Pal and McSpadden, 2006). However, environmental pollution and increased human health problems due to the excessive use of agrochemicals has led to considerable change in people's attitude towards their use. Hence, the question arises whether we want more cancer centers or avoid the main cause of the disease. Accordingly, organic farming is being promoted by policy makers and governments. Organic agriculture is the holistic production management system which is supportive to environment, health and sustainability. Biological control is getting attention throughout the world which is essential in organic farming system. This is particularly true in the case of vegetables since the residue toxicity due to pesticides is very heavy in these. In the case of leafy vegetables, use of pesticides is mostly avoided as the residue will be more on the foliage. Hence these are to be cultivated primarily in organic system.

At present, the methods available for controlling the leaf blight disease are prophylactic spraying of baking soda with turmeric powder in the ratio of 1:4 and foliar spray with mancozeb 0.4 per cent in cow dung supernatant at fortnightly intervals (Peethambaran *et al.,* 2008). These are not much effective and more over fungicides are not safe to use on leafy vegetables. Therefore sustainable biological methods are needed for disease management under organic farming. Soil ecosystem consists of many beneficial microflora, possessing antagonistic activity against plant pathogens. But the application of fertilizers and fungicides causes depletion of soil microflora. However substitution of fertilizers with organic sources of nutrients leads to restoration of microbial population which in turn will improve the plant health thereby reducing disease problems. The rhizosphere and endosphere of plants are rich sources of beneficial micro organisms which can act as potential biocontrol agents. The population of these microorganisms can be enhanced by organic cultivation. Hence the present study aims at evaluation of different organic sources of nutrients against leaf spot diseases of amaranth by enhancing the population of beneficial microbes in rhizosphere and endosphere and also to study the antagonistic potential of such microbes against *Rhizoctonia solani* causing leaf blight.

Review of Literature

2. REVIEW OF LITERATURE

Amaranth is a herbaceous annual with upright growth habit, belonging to the family *Amaranthaceae*. It is cultivated for both leaves (vegetable) and seeds (grain). Both leaves and seeds contain protein of an unusually high quality. Genus *Amaranthus* consists of 60-70 species and over 400 varieties within these species which are found throughout the world in both temperate and tropical climates. Of the various species of amaranth, three have been selected over the years for human and animal consumption. *A. hypochondriacus* (prince's feather) and *A. cruentus* (purple amaranth) are commonly grown for grain and *A. tricolor* (tampala) is grown primarily for the leaves (Makus, 1984).

Amaranth (*Amaranthus tricolor* L.) is considered to be the cheapest leafy vegetable in the market and it could rightly be described as the 'poor man's spinach'. It is a short duration crop and fits well with the crop rotations of Kerala but it is affected by many diseases. Fungal pathogens of amaranth have been documented by Weber and Kauffman (1990) who reported that Alternaria leaf spot is the most severe one. In addition to that, seedling damping off caused by *Pythium* sp*, Rhizoctonia* sp*, Aphanomyces* sp. and stem cankers caused by either *Phoma* sp or *Rhizoctonia* sp. had also been reported. Teri and Mlasani (1994) reported leaf blight incited by *Choanephora cucurbitarum* and leaf spot caused by *Alternaria amaranthi* on cultivated amaranth. White rust caused by *Albugo bliti* is yet another serious disease.

In Kerala, Nayar *et al*. (1996) first reported foliar blight of amaranth by *R. solani* which is a serious threat to amaranth growers. Severe leaf blight symptoms are observed during rainy season under warm and humid conditions, which lead to economic losses. All stages of crop are susceptible to the pathogen. Gokulapalan *et al.* (2000) reported that leaf blight of amaranth *(Amaranthus tricolor)* in Kerala, occurs after the heavy south-west monsoon showers of June-September.

Celine *et al*. (2013) also studied on leaf blight of amaranth and the study showed that yield of amaranth was reduced by leaf blight incited by *R. solani* Kuhn.

2.1 SYMPTOMATOLOGY

Nayar *et al*. (1996) who first reported leaf blight of amaranth noticed severe infection during the post monsoon period of August- September. They mentioned the symptoms of disease as light cream coloured spots on the foliage which rapidly spread causing extensive crop loss. Gokulapalan *et al*. (2000) reported the leaf blight symptoms to begin with small irregular whitish cream spots on leaves, which enlarged under humid conditions to form extensive translucent and light green patches and shot hole symptoms. Priyadarshini (2003) reported symptoms of leaf blight of amaranth as small irregular whitish cream spots on the leaves which enlarged in later stages to become translucent irregular patches with brown margins. Severely infected plants defoliated, under high humidity. Uppala (2007) and Peethambaran *et al.* (2008) reported that symptoms of leaf blight on red amaranth appeared as small irregular white or straw coloured spots on the foliage, which enlarged under high relative humidity. Gradually the spots became translucent green with irregular margins. In the later stages, spots turned grey and spread rapidly. Severely infected leaves showed shot hole symptoms and finally led to defoliation.

2.2 CAUSAL ORGANISM - *Rhizoctonia solani* Kuhn

Rhizoctonia solani Kuhn was reported for the first time by Julius Kuhn in 1858 from diseased potato tubers. The vegetative mycelium of *R. solani* is colourless when young but become yellowish or light brown colored as they grow and mature. The mycelium consists of hyphae partitioned into individual cells by a septum containing a dough-nut shaped pore. The hyphae often branch at a right angles and usually possess more than three nuclei per hyphal cell. Under certain conditions the fungus produces brown to black coloured sclerotia (Brown and McCarter, 1976;

Anderson, 1982). *R. solani* is the imperfect stage of *Thanatephorus cucumeris* (Carling and Leiner, 1990).

R. solani consists of several strains. The strains are distinguished from one another by anastomosis (fusion of touching hyphae). Anastomosis groups (AG) are categorized based on their mycelial compatibilities for hyphal fusion. Anastomosis occurs between fungal isolates of the same AG but not between isolates of different AG's. Each AG is genetically independent from all others (Parmeter *et al*., 1970; Ogoshi, 1985; Burpee and Martin, 1992). Anastomosis groups are host plant specific. AG-3 occurs commonly on *Solanaceae* and AG-4 is regularly associated with *Pinaceae*, *Chenopodiaceae*, *Cruciferae, Leguminosae*, *Malvaceae*, and *Solanaceae* (Butler, 1993). Subgroup 2 of AG-2 has been consistently associated with Rhizoctonia blight of turfgrasses (Green *et al.,* 1993; Zhang and Dernoeden, 1995). Four anastomosis groups of *R. solani*, AG-1, AG-2, AG-4, and AG-5, have been isolated from turfgrasses (Aoyagi *et al.,* 1998). The pathogen causes sheath blight which is a serious disease of rice. (Vidhyasekaran *et al.,* 1997), it causes high yield losses, up to 50 per cent in sugar beet (Kiewnick *et al*., 2001), 70 per cent in field-grown lettuce, and about 20 per cent less in potato (Grosch *et al.,* 2005). It is one of the most important soil borne pathogen in cultivated soils (Woodhall *et al*., 2007). Guleria *et al.* (2007) reported that the pathogen is multifaceted in nature and attack almost all parts of crop plants. They cause losses on almost all vegetables and flowers, several field crops, turfgrasses, and even perennial ornamentals, shrubs and trees. The most common symptoms are damping-off of seedlings, root rot, stem rot, fruit and seed decay, foliar blight, stem canker and crown rot in various crops.

2.3 MANAGEMENT OF THE PATHOGEN

The diseases caused by *R. solani* is a major problem which can be controlled by different methods like chemical control, biological control and use of resistant varieties (Celine *et al.,* 2002).

2.3.1 Chemical control

Broad spectrum fungicides as well as specific compounds are used against *R. solani*. However, most of them are not registered for many crops, especially in horticulture and organic farming. *R. solani* was primarily noticed in potato tubers as a plant pathogen and its control gained worldwide attention. In earlier years different methods of chemical control *viz.,* steam formalin treatment (Winston, 1913), formaldehyde dip treatment (Gilman and Melhus, 1923) were employed against black scurf of potato.

The combination of fungicides with the biocontrol fungus *Verticillium biguttatum* showed additive effects on black scurf control in potato (Van de Boogert and Luttikholt, 2004). Foliar spray with mancozeb 0.4 per cent in cow dung supernatant at fortnightly intervals controls leaf blight of amaranth (Peethambaran *et al.,* 2008). Nair and Anith (2009) reported that combined application of PGPR (Plant Growth Promoting Rhizobacteria) and ASM (Acibenzolar-S-Methyl) was effective with reduction in disease incidence and disease severity of 42 and 21 per cent respectively.

2.3.2 Biological control

Biological control, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. Numerous studies have shown that biological control offers an environmentally friendly alternative to protect plants from soil-borne pathogens (Emmert and Handelsman, 1999; Whipps, 2001; Weller *et al*., 2002). Mechanisms of biological control are diverse. One effective mechanism is

the use of antagonist microorganisms such as bacteria, yeast and fungi to control plant disease.

In recent years, both bacterial and fungal antagonists against *R. solani* have been described (Howell, 2003; Faltin *et al*., 2004). However, many of these showed i*n vitro* effects and only very few antagonists were analysed under field conditions (Grosch *et al*., 2005). Although the number of biocontrol products is increasing, these products still represent only a very small proportion of fungicides (Fravel, 2005).

2.3.2.1 Bacterial biocontrol agents

A strain of *Pseudomonas fluorescens* antagonistic to *Rhizoctonia solani* was isolated from the rhizosphere of cotton seedlings. An antibiotic strongly inhibitory to *R. solani* was isolated from *P. fluorescens* cultures and identified as pyrrolnitrin (3-chloro-4-[2'-nitro-3'-chlorophenyl]- pyrrole). Treating of cotton seeds with *P. fluorescens* at time of planting in *R. solani* infested soil increased seedling survival from 30 to 79 per cent and from 13 to 70 per cent, respectively (Howell and Stipanovic, 1979). Wolk and Sarkar (1993) identified ninety nine isolates of fluorescent pseudomonads from roots of bean, which showed antagonism against *R. solani*. Asaka and Shoda (1996) has reported that *Bacillus subtilis* RB14, showed antagonistic activities against several phytopathogens including *R. solani* under *in vitro* conditions to suppress damping-off of tomato seedlings. Montealegre *et al*. (2003) isolated bacteria from the rhizoplane and surrounding soil of healthy and *R. solani* infected tomato plants. The best bacterial strains against *R. solani* were identified as *B. subtilis* and *B. lentimorbus*. A plant growth-promoting bacterium *Delftia tsuruhatensis*, strain HR4 from the rhizoplane of rice effectively suppressed the growth of *R. solani in vitro* (Han *et al*., 2005). Nair and Anith (2009) reported efficacy of the PGPR strain (*Pseudomonas fluorescens*) PN026R in reducing incidence and severity of leaf blight of amaranth by 67 and 35 per cent respectively.

According to Rosales *et al.* (1993), twenty three endophytic bacterial strains isolated from rice controlled rice sheath blight caused by *R. solani*. Chen *et al*. (1995) showed that, of 170 bacterial strains isolated from the internal tissues of cotton, 40 possessed biological control activities against *R. solani* in cotton, and 25 induced systemic resistance to *Colletotrichum orbiculare* in cucumber. Rangeshwaran *et al.* (2002) isolated twenty five endophytic bacteria from root and stem portions of chickpea, sunflower and chilli plants, of these ten isolates inhibited the growth of pathogens *viz., Fusarium oxysporum* f. sp. *ciceris, R. solani* and *Sclerotium rolfsii.* Endophytes from potato plants showed antagonistic activity against fungi (Sessitsch *et al*., 2004). Several endophytes showed antagonism against *R. solani* (Parmeela and Johri, 2004). Endophytic actinobacteria are effective antagonists of the pathogenic fungus *Gaeumannomyces graminis* in wheat (Coombs *et al*., 2004).

Grosch *et al*. (2005) first reported high potential of endophytes as biological control agents against *R. solani* under field conditions in potato and in lettuce. Lahlali *et al.* (2007) isolated 220 bacterial strains from different organs of healthy potato plants and rhizospheric soils, out of which 25 isolates showed antagonism against *R. solani* under *in vitro* condition and among these severed fungal strains showed a high mycelial growth inhibition.

Uppala (2007) observed that, endophytic microbes isolated from amaranth plants suppressed leaf blight of amaranth both under *in vitro* and *in vivo* conditions Rajendran and Samiyappan (2008) isolated 103 endophytic bacterial strains from cotton out of which only two strains of *Bacillus* spp. suppressed *R. solani* inhibiting mycelial growth.

2.3.2.2 Fungal biocontrol agents

Beginning from the first report of *Trichoderma* sp. as biocontrol agent against *R. solani* and *Sclerotinia americana* by Weindling (1934) antibiotic production is suggested as one of the mechanisms involved in the control of fungal pathogens by

Trichoderma spp. Howell (1998) reported that antibiotic production by fungi exhibiting antagonistic activity is most commonly seen in the genera *Trichoderma*

Hadar *et al*. (1979) reported that in green house condition, *T. harzianum* applied in the form of wheat bran culture effectively controlled damping-off of bean, tomato and egg plant seedlings caused by *R. solani.* Elad *et al.* (1981) reported that *Trichoderma harzianum* Rifai treated strawberry plants showed reduction in severity of black root rot up to 18-46per cent in nursery and yield increased by 21-31 per cent. Yedidia *et al*. (1999) demonstrated that inoculating roots of 7 day old cucumber seedlings in an aseptic hydroponic system with *T. harzianum* (T-203) spores to a final concentration of 10^5 per ml initiated plant defense responses both in the roots and leaves of treated plants. There are a variety of fungal species and isolates that have been reported as biocontrol agents but *Trichoderma* species clearly dominate, owing to their ease of growth and wide host range (Whipps and Lumsden, 2001).

Significant reduction in the incidence of *Rhizoctonia* infection was noticed by treatment of potato sprouts with *Trichoderma* isolates (Grosch *et al.*, 2006). Dubey *et al.* (2012) found that seed dressing and soil application formulations developed from the isolates of *Trichoderma viride* (IARI P1; MTCC 5369), *T. virens* (IARI P3; MTCC 5370) and *T. harzianum* (IARI P4; MTCC 5371) gave reduction in wet root rot caused by *R. solani* and improvement in the yield of chickpea.

Gao *et al*. (2005) reported that growth of *R. solani* was inhibited by *Chaetomium spirale* ND35, the mechanism of inhibition was overgrowth on the colony of *R. solani* under *in vitro*. Lahlali and Hijri (2010) reported that six fungal endophytes effectively suppressed the growth of *R. solani in vitro* and in greenhouse. They observed that *Trichoderma atroviride* and *Epicoccum nigrum* have significant *in vitro* inhibitory effect on mycelial growth of *R. solani*, with the greatest inhibition zone observed for *E. nigrum*

2.4 ORGANIC FARMING IN LEAFY VEGETABLES

Being a leafy vegetable, management of foliar diseases using fungicides is not possible in amaranth. Hence researchers, all over the world, study the possibility of organic cultivation and disease management in this crop. Organic farming relies on the management of soil organic matter to enhance the chemical, biological, and physical properties of the soil, in order to optimize crop production. Conventional agriculture frequently relies on targeted short-term solutions to solve nutritional problems e.g. application of a soluble fertilizer. In contrast, organic systems use a strategically different approach, which relies on longer-term solutions (preventative rather than reactive) at the systems level.

The overuse of chemical pesticides has caused soil pollution and harmful effects on human beings. Accordingly, biological control of soil borne diseases has been attracting attention. Crops under organic farming vary from 50per cent to more than 95per cent of those in conventional agriculture. An investigation by Xu (2000) revealed that vegetables in organic fertilization treatments grew better and resulted in a final higher total yield than those in chemical fertilization treatments.

2.5 ORGANIC SOURCES OF NUTRIENTS

Organic manures are valuable sources of nutrients. It has beneficial effects on the improvement of soil organic matter, soil structure and the biological life of the soil. Many studies have demonstrated that application of manures will produce crop yields equivalent or superior to those obtained with chemical fertilizers (Motavalli *et al.,* 1989). Crop quality has also been improved by manure application (Eck *et al.,* 1990; Pimpini *et al.,* 1992). According to Lazarovits (2001) opinion, addition of organic manures increased soil organic matter content and also provided other benefits such as improved plant health and reduction of pathogens**.** Sudhakaran *et al.* (2013) reported that soil respiration and microbial population (bacteria, fungi,

actinimycetes, *Beijerinckia, Azotobacter, Rhizobium, Bacillus* and phosphobacteria) were higher in soils from organic farming than sustainable and conventional farms.

Organic manures improves soil fertility, an experiment conducted by Maerere *et al*. (2001) indicated that applications of cow, goat and poultry manures significantly ($P < 0.01$) increased shoot dry matter yield, taproot length and root dry weight of amaranth (*Amaranthus cruentus* L.). Akaparobi (2009) studied the effect of different levels of farmyard manures (0 t/ha, 15 t/ha and 35 t/ha) on the growth and yield of *Amaranthus cruentus*. They reported that plant height, number of leaves, leaf area, fresh and dry weight increased with increasing farmyard manure levels.

According to Geethakumari *et al*. (2005) poultry manure treated amaranth plants gave higher yield (43.90 t/ha) compared to vermicompost (35.63t/ha), farmyard manure (35.47 t/ha) or neem cake (31.02 t/ha) treated plants. Poultry manure contains high amount of nutrients especially nitrogen that are easily taken up by plants for fast growth (Ewulo, 2005; Awodun, 2007). Agbede *et al*. (2008) reported that poultry manure application significantly increased the plant height, leaf area, stem girth and weight of roots, shoot and grain yield in sorghum plants. Poultry manure application in amaranth lead to increased shoot length, number of branches, number of leaves, stem diameter and fresh shoot weight (Okokoh and Bisong, 2011).

Kumaraswamy (2002) reported that complete substitution of inorganic fertilizers by organic fertilizers like vermicompost is not advisable. That is because the nutrient concentrations of organic manures are generally low compared to inorganic fertilizers. In addition to that very large quantities of organic amendments are required to meet the crop nutrient requirements.

Many studies have been conducted revealing the effectiveness of organic cultivation in increasing the population of soil microbes. Gunapala and Scow (1998) reported that the amount of soil nitrogen in fields under conventional production systems has been negatively correlated with soil microbial components, whereas soil

nitrogen in fields under organic production was positively correlated with the same. The experiments by Bulluck *et al.* (2002) revealed that propagule densities of *Trichoderma* species, thermophilic microorganisms, and enteric bacteria were more in soils amended with organic amendments than fertilizer, whereas propagule densities of *Phytophthora* and *Pythium* species were lower in soils amended with alternative amendments than synthetic fertility amendments. Alternative fertility amendments enhanced beneficial soil microorganisms and reduced pathogen populations. It has been reported that, organic farming practices resulted in higher soil microbial activity (Araujo *et al*., 2009).

2.6 RHIZOSPHERE MICROORGANISMS

In 1904, Hiltner defined rhizosphere as the specific region of soil affected by plant roots. The word, "rhizosphere" comes from ‘rhizo’ or ‘rhiza’ which is a Greek word for root, and ‘sphere’ which denotes an environment or area of influence. Rhizoplane is defined as the surface of the plant root itself along with the tightly adhering soil particles. The rhizosphere is an environment that the plant itself helps to create and where pathogenic and beneficial microorganisms constitute a major influential force on plant growth and health (Lynch, 1990). The rhizosphere is a hot spot of microbial interactions as exudates released by plant roots are a main food source for microorganisms and a driving force of their population density and activities. Plant microbe interaction in rhizosphere may be beneficial or detrimental to the plant. Those detrimental can be of plant pathogen or deleterious microbes. Plant-beneficial microbial interactions can be roughly divided into three categories. First, those microorganisms that, in association with plants, is responsible for its nutrition (*i.e.*, microorganisms that can increase the supply of mineral nutrients to the plant). In this case, while most may not directly interact with the plant, their effects on soil biotic and abiotic parameters certainly have an impact on plant growth. Second, there is a group of microorganisms that stimulate plant growth indirectly by preventing the growth or activity of pathogens. Such microorganisms are referred to

as biocontrol agents. A third group involves those microorganisms responsible for direct growth promotion, for example, by production of phytohormones (Welbaum *et al.*, 2004). Many microorganisms living in the rhizosphere and benefiting from root exudates can have positive effects on plant growth and health. The relationship between these plant-beneficial microorganisms and the plant host corresponds to a symbiosis or an associative symbiosis (cooperation). (Moenne-Loccoz *et al*., 1999).

Rhizosphere microorganisms are indirectly involved in suppression of phytopathogenic bacteria or fungi, and/or phytoparasitic nematodes (Cronin *et al*., 1997; Walsh *et al*., 2001). In certain cases, the biocontrol effect mediated by indigenous free-living plant beneficial microorganisms results in the suppression of plant diseases (Moenne-Loccoz and Defago, 2004). Somers *et al.* (2004) reported that the number and diversity of deleterious and beneficial microorganisms are related to the quantity and quality of the rhizodeposits and to the outcome of the microbial interactions that occur in the rhizosphere. Many pathogenic organisms, bacteria as well as fungi, have coevolved with plants and show a high degree of host specificity (Raaijmakers *et al.,* 2009).

2.6.1 Diversity

Grayston *et al.* (1998) reported that root exudates of different plant species varies and this influences the microorganisms that colonize the rhizosphere of specific plant species. Kloepper *et al.* (1999) found that some rhizobacteria enhances plant defences, leading to systemic protection against foliar pathogens upon seed or root-treatments with the rhizobacteria. Microbial groups and other agents found in the rhizosphere include bacteria, fungi, nematodes, protozoa, algae and microarthropods (Raaijmakers, 2001). Singh *et al.* (2006) suggested that the Proteobacteria and Actinobacteria form the most dominant populations reside in the rhizosphere of different plant species. According to Broeckling *et al.* (2008) plant species, plant

development stage and soil type are the major factors influencing the composition of rhizosphere microbial communities.

Foster (1988) reported that the bacterial populations residing in the rhizosphere are greater than those residing in bulk soils. Population of bacteria in rhizosphere may range from 10^{10} to 10^{12} cells per gram of soil. Vijayaraghavan (2003) isolated rhizosphere microflora from black pepper and reported that population of bacteria was more than fungi and actinomycetes. Rhizosphere harbors many organisms that have a neutral effect on the plant, but also attracts organisms that exert deleterious or beneficial effects on the plant (Nihorimbere *et al.,* 2011).

2.7 ENDOPHYTIC MICROORGANISMS

Anton de Bary coined the term endophyte in 1886 to describe microorganisms that colonize internal tissues of stems and leaves. Endophyte is derived from the Greek word 'endon'(within) and 'phyte' (plant). The term endophyte refers to interior colonization of plants by bacterial or fungal microorganisms. Petrini (1991) first defined endophyte as microorganism living in the plant organization for a certain stage of its life and would not cause disease. Perotti (1926); Hallmann *et al*. (1997) and Azevedo *et al*. (2000) reported that bacteria on roots and in the rhizosphere benefit from root exudates, but some bacteria and fungi are capable of entering the plant as endophytes that do not cause harm and could establish a mutualistic association. Wagenaar and Clardy (2001) identified endophytes as microorganisms growing in the intercellular spaces of higher plants and they are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential.

James and Olivares (1997) stated that all bacteria that colonize the interior of plants, including active and latent pathogens, can be considered to be as endophytes. Kado (1992) and Quispel (1992) suggested that, those bacteria establish

endosymbiosis with the plant, whereby the plant receives an ecological benefit from the presence of the symbiont. Zhang *et al.* (2006) and Aly *et al.* (2010) reported the great potential of endophytes as a major source of biologically active compounds with promising medicinal or agricultural applications. It is now commonly accepted that each of the nearly 300,000 existing plant species host at least one or even several hundred strains of endophytes (Strobel and Daisy, 2003).

2.7.1 Colonization of endophytes

In 1974 De Boer and Copeman found potato stem tissues colonized by greater numbers of endophytic bacteria than the tubers. Fisher *et al*. (1992) reported that endophytic bacteria appear to be preferentially located in the lower part of the stems of corn, with a declining gradient running from the base to the top of the plant. Roots and other below ground tissues tend to yield the highest bacterial population compared with above-ground tissues (McInroy and Kloepper, 1995; Sturz *et al*., 1997). Several studies suggest that endophyte communities inhabiting leaf and wood share some species, but leaves show higher diversity including an additional suite of distinctive organisms (Arnold, 2007; González and Tello, 2011).

Presence of bacterial endophytes is consistently reported consistently reported present in the root, stem, leaf, fruit, and tuber tissues of a wide range of agricultural, horticultural, and forest species. The extensive and frequent occurrence of such populations seems to indicate that healthy plants carry populations of endophytic bacteria, and the plant kingdom represents a vast and relatively unexplored ecological niche for these organisms (Chanway, 1995). Endophytic fungi also exhibit organ and tissue specificity as a result of their adaptation to different physiological conditions in plants (Rodrigues and Samuels, 1990). The set of selective pressures occurring in a certain plant tissue type affects its endophytic inhabitants which differ from that present in other tissues. Hence, different fungi are found to dominate in distinctive

above-ground organs forming characteristic communities' specific to each tissue type (Arnold, 2007).

McInroy and Kloepper, (1995) reported the genotypic diversity of indigenous bacterial endophytes within stems and roots of sweet corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.). There were 14 taxonomic groups present in cotton roots that were not in cotton stems. A study conducted on potato-associated bacterial communities, showed that species richness and diversity was lower for fungal antagonistic bacteria inside roots than in the rhizosphere of potato (Berg *et al*., 2005). Balan (2009) isolated 37 bacterial and 14 fungal endophytes from anthurium plants.

2.7.2 Isolation of endophytes

Isolation procedures for endophytes are mainly of two types, they are vacuum extraction methods and homogenization methods. In vacuum extraction method the sap is extracted using the vacuum extraction apparatus and spread plate method is used for plating. Whereas homogenization or trituration methods invariably involves surface sterilization followed by homogenization under aseptic condition. The triturate is then serially diluted and plated (Gardner *et al.,* 1982; Gagne *et al.,* 1987). Bell *et al.* (1995) isolated xylem inhabiting bacteria from grape vine by vacuum extraction and homogenization method. They compared the efficiency of the two methods. The size of the population varied with the method of extraction: 2.65×10^2 to 3.46×10^3/ml xylem sap with vacuum extraction, 3.83×10^3 to 1.31×10^4/g xylem tissue with homogenization. Thus trituration or homogenization technique is considered as ideal for isolation of endophytes because it will yield higher number of endophytic bacteria (Hallmann *et al.,* 1997; Uppala, 2007; Balan, 2009; Kurian, 2011).

Sodium hypochlorite is commonly used for disinfecting plant surfaces during endophyte isolation. Residual sodium hypochlorite may affect the growth or induce mutagenesis or death of microorganisms, hence the plants bits are washed in three

changes of sterile buffer or treated with it is necessary to rinse the tissues with sodium thiosulfate to remove all the residual sodium hypochlorite (Miche and Balandreau, 2001).

2.8 MICROORGANISMS FOR GROWTH PROMOTION

Role of microorganisms in plant growth promotion is well documented. The rhizosphere and endosphere microbes play a major role in soil and plant health, induced systemic resistance and biological control of plant diseases.

2.8.1 Plant growth promoting rhizosphere microorganisms

Bacteria and fungi live around roots and feed on root exudates and dead root cells. These interact in different ways during their growth and establishment in the vicinity of plant root system like antagonism, competition and induced resistance. Antagonism is mainly brought about by antibiosis *i.e.* the inhibition of microbial growth by diffusible antibiotics and volatile organic compounds, toxins, and biosurfactants *etc.* Parasitism is another mechanism involved in antagonism may involve production of extracellular cell wall-degrading enzymes such as chitinases and β-1,3- glucanase (Compant *et al.,* 2005; Haas and Defago, 2005). Excretion of chitinases and glucanases by species of *Trichoderma* and *Streptomyces* has been shown to play an important role in mycoparasitism of phytopathogenic fungi (Whipps, 2001). In biocontrol aspects competition occurs when antagonist directly competes with pathogens for resources such as nutrients, oxygen, space *etc*.

Several species of *Pseudomonas* has the capacity for extracellular conversion of glucose to gluconic acid and 2-ketogluconic acid to sequester glucose effectively and gives a competitive advantage over microorganisms that lack the ability to utilise these compounds (Gottschalk, 1986). Competition for iron is one of the limiting factor for existence of plant pathogens in rhizosphere. Under iron limiting conditions, certain bacterial species produce a range of iron chelating compounds (siderophores)

which have very high affinity for ferric iron limiting the available iron to pathogens, thereby their growth is restricted (O' Sullivan and O' Gara, 1992; Loper and Henkels, 1999). Competition for nutrients, especially for carbon leads to fungistasis *i.e.* inhibition of fungal spore germination in soil (Alabouvette *et al.,* 2006). Induced resistance is yet another mechanism by which rhizosphere bacteria can reduce the activity of pathogenic microorganisms by activating the plant to better defend itself, a phenomenon termed "induced systemic resistance", "ISR" (Shoda, 2000; Van Loon, 2007).

Species of *Pseudomonas* and *Bacillus* can produce growth regulators that induce the plants to have greater numbers of fine roots there by increasing the absorptive surface for uptake of water and nutrients. The phytohormones they produce include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement (Salisbury, 1994). This hormone is very commonly produced by PGPRs. Plant growth promoting rhizobacteria (PGPR) and plant-growth-promoting fungi (PGPF) can exert positive effects on plants by various mechanisms, some of them being direct positive effect on seed germination, root development, mineral nutrition and/or water utilization (Dobbelaere *et al*., 2003).

Canbolt *et al.* (2006) studied plant growth of barley (*Hordeum vulgare*). As influenced by three bacterial species isolated from the rhizosphere of barley and wheat. Seed inoculation of barley with *Bacillus* RC01, *Bacillus* RC02, *Bacillus* RC03 and *Bacillus* M-13 increased root weight by 16.7, 12.5, 8.9 and 12.5 per cent as compared to the control (without bacteria inoculation and mineral fertilizers) and shoot weight by 34.7, 34.7, 28.6 and 32 per cent respectively. Bacterial inoculation increased the total biomass to the tune of 20.3–25.7 per cent over the control as compared with 18.9 and 35.1 per cent increase in total biomass by P and NP application.

Yedidia *et al*. (1999) reported that *Trichoderma*-treated plants were more developed than non-treated plants it induced systemic resistance in cucumber plants. The studies of Lo and Lin (2002) revealed that some species of *Trichoderma* can enhance plant growth. Strains of *Trichoderma* spp significantly increased 26 to 61per cent of seedling height, 85-209 per cent of root exploration, 27 to 38per cent of leaf area and 38 to 62per cent of root dry weight of bitter gourd. Similarly *Trichoderma* strains also increased seedling growth of loofah and cucumber.

Plant growth-promoting bacteria (PGPB) are associated with many, plant species and are commonly present in many environments. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces and the closely adhering soil interface, the rhizosphere (Kloepper and Schroth, 1978; Kloepper *et al*., 1999). PGPR that increase the availability of nutrients in the rhizosphere there is ample evidence that the mode of action of many PGPR is by increasing the availability of nutrients for the plant in the rhizosphere (Glick, 1995). The method by which these increases take place involve solubilization of unavailable forms of nutrients and/or siderophore production which helps facilitate the transport of certain nutrients (ferric iron).Root surfaces there are various suitable nutrient rich niches attracting a great diversity of microorganisms, including phytopathogens. Competition for these nutrients and niches is a fundamental mechanism by which PGPB protects plants from phytopathogens (Duffy, 2001).

PGPR may benefit the host by causing plant growth promotion or biological disease control. The same strain of PGPR may bring about cause both growth promotion and biological control (Kloepper, 1993; Glick and Bashan, 1997). Many of the soil borne plant pathogens are negatively affected by PGPR, including *Rhizoctonia solani.* In most of these cases, biological control results from production of metabolites by bacteria such as antibiotics, hydrogen cyanide, iron-chelating siderophores, and cell wall-degrading enzymes, which directly inhibit the pathogen.

2.8.2 Plant Growth Promoting Endophytes (PGPE)

Plant growth effects attributed by endophytes include growth and developmental promotion (Kloepper *et al*., 1991) growth inhibition (Schippers *et al*., 1990) growth stimulation indirectly through the biocontrol of phytopathogens in the root zone (DéFago *et al*., 1990), growth stimulation through the direct production of phytohormones (Holland, 1997), indirect growth stimulation through the induction of phytohormone synthesis by the plant (Lazarovits and Nowak, 1997), growth promotion through the enhanced availability of minerals, altered susceptibility to frost damage (Gagné *et al*., 1989), and increased plant susceptibility to other pathogens (Schippers *et al*., 1990). Endophytes directly provided their hosts with secondary metabolites, thereby contributing to their chemical defense, or they might have transferred the corresponding genes to the host genome or vice versa (Wink, 2008).

Kloepper *et al.* (1980) isolated two strains of fluorescent *Pesudomonas* spp. from potato periderms and celery roots. They reported that these two isolates significantly increased growth of potato plants up to 500per cent. Sturz *et al*. (1998) isolated from clover and potato. Of the plant growth promoting bacteria, 63 per cent increased shoot height, 66 per cent increased shoot wet weight, and 55 per cent increased root wet weight.

Rangeshwaran *et al*. (2008) isolated five endophytic bacteria from healthy chick pea (*Cicer arietinum* L.) plants. Higher growth promotion was noticed in chickpea plants treated with *B. megaterium*, *E. agglomerans* and *Bacillus sp.* and the plants treated with endophyte survived well in the presence of the wilt pathogen, *R. solani.* Uppala (2007) and Balan (2009) reported that, increased biometric parameters in endophytes treated plants. Kurian (2011) reported that endophytes treated cocoa plants showed increased seedling height, number of leaves, girth at collar, fresh and dry weight of shoot.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Characterization of rhizosphere and endophytic microflora from organically grown amaranth for management of leaf spot diseases' was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during 2012-2014. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 ISOLATION AND CHARACTERIZATION OF THE PATHOGEN

The infected leaves with symptoms of leaf spot were collected from the field experiment, washed under tap water and cut into small pieces (2-5 mm) containing both the diseased and healthy tissue. These bits were surface sterilized using one per cent sodium hypochlorite solution for 60 seconds and washed in three changes of sterile water. The surface sterilized leaf bits were placed on Petri plates containing Potato Dextrose Agar (PDA) and incubated at room temperature. The fungal growth was noticed 24 h after inoculation. The isolate was purified by hyphal tip method and subcultured and maintained pure cultures for further studies.

The pathogenicity of fungal isolate obtained was proved by artificial inoculation on amaranth seedlings raised in earthern pots. Mycelial discs (5 mm) of the isolate from seven day old culture grown on PDA were placed on the lower surface of the leaves of three week old seedlings after giving pin pricks. Moistened cotton was placed over the mycelial disc and the seedlings were covered with polythene covers with air holes. Observations on the symptoms developed were recorded. The organism was re-isolated from the infected leaves. The cultural and morphological characters of the isolate was studied and compared with that of the original one. The isolate was purified by hyphal tip method and maintained on PDA slants for further study.

3.1.1 Symptomatology

Symptoms produced by the pathogen on amaranth were studied both under natural and artificial condition. For studying the symptomatology under natural condition the plants in the field experiment were observed daily and the initial symptoms were recorded. Infected leaves were tagged and observed continuously and observed that till the leaf spots developed in to typical blight lesions or shot holes. Under artificial condition the amaranth seedlings were inoculated with pathogen and observed for initiation and development of symptoms.

3.2 QUANTITATIVE ESTIMATION OF MICROFLORA IN ORGANIC SOURCES OF NUTRIENTS

Organic sources of nutrients used for the field experiment were farm yard manure, neem cake, vermicompost and poultry manure. To estimate the total microflora in these organic sources of nutrients these subjected to standard serial dilution plate technique. One gram samples were taken from each organic nutrient source and serially diluted to up to 10^{-6}. For isolation of fungi and actinomycetes 10^{-4} dilution was used and for bacteria 10^{-6} was used. The media used were Martin's Rose Bengal Streptomycin Agar, Nutrient Agar and Kenknight's Agar for fungi, bacteria and actinomycetes respectively. Microbial suspension (1 ml) of respective dilution was added to sterile Petri dish and 15 ml molten cooled medium was added. The plates were then incubated at room temperature. Three replications were kept for each sample. After incubation the fungal, bacterial and actinomycetes colonies were counted at 24 h, 48 h and on 7^{th} day respectively.

3.3 EFFECT OF ORGANIC SOURCES OF NUTRIENTS ON GROWTH, YIELD AND LEAF BLIGHT OF AMARANTH

A field experiment was conducted in the Department of Olericulture, College of Horticulture, Vellanikkara during August to November of 2013 to evaluate the

effect of different organic sources of nutrients on the incidence and severity of leaf spot diseases of amaranth.

The experiment details are furnished below

Design : RBD

Treatments : 5

Replication : 4

Plot size : 3 × 3 m

Spacing : 35 × 35 cm

Variety : Arun

Season : August – November 2013

Treatments

T_1- NPK as per POP recommendation (50:50:50 N: P_2O_5 : K_2O kg ha^{-1})

T_2 – Farm yard manure equivalent to 50 kg ha^{-1} of N

T_3 - Neem cake equivalent to 50 kg ha^{-1} of N

T_4 – Vermicompost equivalent to 50 kg ha^{-1} of N

T_5 - Poultry manure equivalent to 50 kg ha^{-1} of N

3.3.1 Preparation of nursery

Nursery was raised during the third week of July, the beds were prepared by ploughing and mixing with farm yard manure, two beds of 2 × 1 m size were prepared and amaranth seeds were sown in 20 rows in each bed and spreaded a thin layer of fine sand on top.

3.3.2 Preparation of main field

The field was prepared and the whole field was divided into four blocks. Each block was then divided into 5 plots size 3 × 3 m each. Bunds of 50 cm width were made on the four sides of each plot to separate it from the neighbouring ones. The treatments were applied to each plot at random in each block. Before the treatment application soil samples were collected from each plot for estimation of soil microflora. Then ridges of width 40 cm were taken at 35 cm apart. Three weeks old seedlings were transplanted to main field at a spacing of 35 × 35 cm.

3.3.3 Effect of organic sources of nutrients on growth and yield of amaranth

Ten plants were selected at random from each plot and biometric observations *viz.* shoot length, number of branches, number of leaves, girth at collar, yield was recorded at 30, 60 and 75 DAT. The length of shoot was measured from the collar region to the growing tip of the plant. The number of branches and total number of leaves were counted and girth at collar region was recorded at periodic intervals. Fresh root weight and root length were recorded at 75 DAT. For recording the root length, length of the longest root was measured using a ruler. The fresh root weight was recorded in an electronic balance after removing the soil.

3.3.4 Effect of organic sources of nutrients on leaf spot diseases of amaranth

The plants in the field experiment were observed daily for occurrence of any foliar diseases and the only disease observed was leaf blight caused by *Rhizoctonia solani*. The disease incidence and severity of amaranth plants were recorded at 30, 60 and 75 days after transplanting (DAT). Number of plants infected in each plot was recorded to calculate per cent disease incidence. The per cent disease incidence (PDI) was calculated using the formula,

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

The per cent disease severity (PDS) was calculated using 0 -7 scale (Celine *et al*., 2002). For calculating disease severity ten amaranth plants were selected at random from each plot and ten leaves were selected from each plant (4 leaves from top, 3 leaves from middle and 3 leaves from bottom).

Table 1. Score chart for assessment of disease severity

Score	**Per cent leaf area infected**
0	No incidence
1	Up to 25 per cent
3	> 25 to 50 per cent
5	> 50 to 75 per cent
7	> 75 per cent

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

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

3.4 ISOLATION AND ENUMERATION OF RHIZOSPHERE MICROFLORA

Rhizosphere soil samples of amaranth were collected from each plot before transplanting, 30, 60 and 75 DAT. The samples (10 g) were used for isolation and enumeration of microflora *viz.* fungi, bacteria, fluorescent pseudomonads and actinomycetes using serial dilution plate technique as mentioned in 3.2. The dilutions and media used for fungi, bacteria and actinomycete were same as that in 3.2. For fluorescent pseudomonads King'B Agar medium and 10^{-6} dilution was used.

Representative colonies based on colony morphology were selected, purified and maintained for further studies.

Thus a total of 30 fungal, 38 bacterial, 7 fluorescent pseudomonads and 4 actinomycetes isolates were sub cultured, purified and maintained.

3.5 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROBES FROM AMARANTH

Endophytic microbes were isolated from the stem and roots of randomly selected amaranth plants. Plants for isolation of endophytes were selected randomly from each plot. The whole plant was uprooted and brought to the laboratory. The plants were washed thoroughly under running tap water. The stem at 10 cm height from the base and roots were cut into small bits using a sterilized knife. The bits (1g) were surface sterilized using one per cent sodium hypochlorite solution for three minutes and washed in three changes of sterile water under aseptic condition. Finally, the bits washed in 0.02 M in sterile potassium phosphate buffer of pH 7.0. An aliquot of 1 ml taken from the final buffer wash was poured in to sterile Petri plates and cooled molten PDA was poured over it and this served as sterility check. The samples were discarded if any growth was observed on sterility check within 24 h. The samples were triturated with a sterile mortar and pestle in 9 ml of sterile buffer (Mc Inroy and Klopper, 1995). From this serial dilutions up to 10^{-2} of the triturate were made in sterile buffer. The dilution used for isolation was 10^{-2} for all the microbes. From the diluted (10^{-2}) triturate 1 ml was transferred to Petri plates and then respective medium was poured. The medium used were Martin's Rose Bengal Streptomycin Agar, Nutrient Agar, King's B and Kenknight's Agar for fungi, bacteria, fluorescent pseudomonads and actinomycetes respectively. Three replications were maintained for each sample. Then the plates were incubated at room temperature. Observations were recorded daily as in 3.4. A total of 18 fungal,

23 bacterial and 3 fluorescent pseudomonad colonies were selected based on colony morphology and maintained pure culture for future studies.

3.6 *IN VITRO* ANTAGONISTIC EFFECT OF MICROORGANISMS AGAINST THE PATHOGEN

The antagonistic effect of rhizosphere and endosphytic microbes towards the pathogen was assessed under *in vitro*. Initially the isolates were subjected to preliminary screening to test whether they are antagonistic or not. The isolates which showed antagonism were further evaluated by dual culture method.

3.6.1 Preliminary screening of microorganisms against *R. solani*

A total of 48 fungal, 61 bacterial, 10 fluorescent pseudomonads and 4 actinomycete isolates were subjected to preliminary screening. To test the antagonistic effect of fungal isolates, mycelial disc (8 mm) of the pathogen taken from seven day old culture was placed in the centre of Petri plate and mycelial discs of two different test isolates were placed on both sides of pathogen one cm away from the edge of the plate. In the case of bacterial isolates, an eight mm disc of the pathogen was placed in the centre of a PDA plate. Then four bacterial isolates were inoculated as line of streak one each on four sides of the pathogen, one cm away from the edge of the plate. PDA plate with pathogen alone was maintained as control. The plates were incubated at room temperature and observations were recorded till growth of pathogen in control was completed. Those isolates which exhibited antagonistic activity against the pathogen were selected for further evaluation.

3.6.2 *In vitro* evaluation of antagonistic fungal isolates

A total of 25 antagonistic fungal isolates including 17 from rhizosphere and eight from endosphere were subjected to further evaluation using dual culture method (Skidmore and Dickinson, 1976) to select the isolate having the highest antagonism against *R. solani*. Mycelial disc (8 mm) of the pathogen taken from a seven old

culture was placed on mediated plates two cm away from the edge of Petri plates. Then mycelial disc of antagonistic fungal isolate was placed on the opposite side of the plate, four cm away from the pathogen. Mediated Petri plate with pathogen alone served as control. The plates were kept for incubation at room temperature and observations were recorded daily till the growth of pathogen in the control was full. The per cent inhibition was calculated using the formula suggested by Vincent (1927).

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

PI = Per cent inhibition

C = Growth of the pathogen in control (cm)

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

Out of the 25 fungal isolates, the endophytic isolate which exhibited cent per cent inhibition of the pathogen was selected for further studies.

3.6.3 *In vitro* evaluation of antagonistic bacterial isolates

A total of 36 bacterial isolates were tested individually using dual culture assay (Utkhede and Rahe, 1983) in which 24 were from rhizosphere and 12 from endosphere. Mycelial disc (8 mm) of the pathogen taken from seven day old culture was placed at the centre of PDA plate and the bacterial isolates were inoculated as a line of streak on both sides of the pathogen, one cm away from the edge of the Petri plate. Plates with pathogen alone served as control. The plates were incubated at room temperature, and growth of pathogen was recorded daily till there was full growth in control. The per cent inhibition was calculated as in case of 3.6.2

Out of the 36 bacterial isolates, three isolates which showed the highest level of inhibition of the pathogen were selected for future use.

3.7 CHARACTERIZATION AND IDENTIFICATION OF SELECTED ANTAGONISTS

The isolates selected based on *in vitro* evaluation were subjected to cultural, morphological and biochemical characterization.

3.7.1 Characterization of selected fungal isolate

The cultural and morphological character of the selected fungal antagonist EF-8 was studied. The fungal isolate was inoculated on PDA and incubated at room temperature till it attained full growth. The cultural characters like colour, growth, and texture of the colony and morphological characters like width of hyphae, size of the conidiophores and conidial characters were recorded.

3.7.2 Characterization of selected bacterial antagonists

The cultural, morphological and biochemical characters of selected bacterial antagonists *viz.,* EB-2, RB-14 and RB-21 were studied. These were subjected to Gram's reaction and 18 biochemical tests as described below.

3.7.2.1 Gram's reaction

For Gram's staining, 48 h old culture of selected bacterial isolates was taken and a smear is made on clean glass slide then heat-fixed the culture by passing it over a flame. The fixed smear was flooded with crystal violet solution and allowed to remain for one minute. Gently rinsed off the crystal violet solution under running tap water. The culture was flooded with iodine solution and kept for one minute and then the iodine solution was rinsed off with tap water. Again the culture was flooded with 95 per cent ethanol (decolourizer) for 15 seconds and washed off the decolourizer under running tap water. Finally, the culture was flooded with safranin stain for 30 seconds, washed off the safranin stain under running tap water and dried the slide on blotting paper and then the culture examined under microscope.

3.7.2.2 Indole production test

Inoculated the peptone water broth containing tubes (5 ml) with bacterial cultures and kept for 48 h at room temperature. After 48 h one ml Kovac's reagent was added to the tubes and shaken for few minutes. Then tubes were observed for pink or cherry red ring formation above the culture medium.

3.7.2.3 Methyl red test

Inoculated the glucose phosphate broth with 48 h old bacterial culture and kept for incubation for 48 h at room temperature and after that added 1 ml of methyl red reagent to it. Observed for the colour change.

3.7.2.4 Voges-Proskauer test

Inoculated the 5 ml glucose phosphate broth containing tubes with 48 h old bacterial culture and incubated for 48 h at room temperature. After incubation 1 ml 40 per cent potassium hydroxide and 3 ml 5 per cent α-naphthol solution is added and observed for development of pink colour in 2-5 minutes.

3.7.2.5 Starch hydrolysis

One day old bacterial culture was streaked on nutrient agar containing 0.4 per cent starch mediated plates and kept for incubation for 48 h at room temperature. Flood the plates with Gram's iodine solution and kept for 30 seconds. Examined the plates for the presence of clear zone around the line of growth of bacterium.

3.7.2.6 Catalase test

One loop full of bacterial culture was collected from 24 h old bacterial culture and placed it on to a clean glass slide. One drop of 3 per cent H_2O_2 was placed on the smear using a dropper and observed for effervescence.

3.7.2.7 Oxidase test

The 24 h old bacterial culture was spot inoculated on oxidase disc and change in colour of the disc from white to dark blue was observed.

3.7.2.8 Characterization using HiAssorted^{TM} Biochemical Test Kit

HiAssorted^{TM} Biochemical Test Kit for Gram negative rods were also used for characterization and compared with the interpretation chart given in the manual. It is a standardized colorimetric identification system and test kit contains twelve tests which include seven conventional biochemical and five carbohydrate utilization tests. The tests include: citrate utilization, lysine utilization, ornithine utilization, urease test, phenylalanine deamination, nitrate reduction, H_2S production and mode of utilization of sugars *viz.,* glucose, adonitol, lactose, arabinose and sorbitol. Bacterial suspension (50 µl) was added to each well and colour change was observed after 24 h.

3.8 EVALUATION OF ORGANIC SOURCES OF NUTRIENTS AND SELECTED ANTAGONISTS AGAINST LEAF BLIGHT OF AMARANTH

A pot culture experiment was conducted to study the efficacy of organic sources of nutrients and four selected antagonists on the management of leaf blight of amaranth. The details of experiment are furnished below.

Design : CRD

Treatments : 12

Replications : 3

Plants/replication : 6

Variety : Arun

Season : June- August of 2014

Treatments

T_1 - NPK as per POP recommendation (50:50:50 N: P_2O_5 : K_2O kg ha^{-1})

T_2 - FYM equivalent to 50 kg ha^{-1} of N

T_3 - Neem cake equivalent to 50 kg ha^{-1} of N

T_4 - Vermi compost equivalent to 50 kg ha^{-1} of N

T_5 - Poultry manure equivalent to 50 kg ha^{-1} of N

T_6 - RB-21 + T_1

T_7 - EB-2 + T_1

T_8 - RB-14 + T_1

T_9 - EF-8 + T_1

T_{10} - *Trichoderma viride (*KAU) + T_1

T_{11} - *Pseudomonas fluorescens* (KAU) + T_1

T_{12} - Absolute control

***** T_6 to T_9 – Selected antagonists from *in vitro* evaluation

Amaranth seedlings were raised in nursery as mentioned in 3.3.1 and three week old seedlings were transplanted to poly bags (40 × 40 cm) with potting mixture and two seedlings were planted in each bag.

In the case of T_6 to T_{12} and T_1 potting mixture was prepared by mixing soil and sand in the ratio 1:1 and fertilizers were applied as per POP recommendation of KAU. Where as in T_2 to T_5 potting mixture was prepared as above and respective organic sources of nutrients equivalent to 50 kg ha^{-1} of N was added. Further the

fertilizers and organic sources of nutrients were applied at 15 and 30 DAT. In T_6 to T_{11} inoculum of respective microorganism was applied by seedling dip at the time of planting, soil drenching (30ml/plant) and foliar spray at 15 and 30 DAT.

3.8.1 Preparation of bacterial inocula

The three selected bacterial isolates, EB-2, RB-21 and RB-14 and *Pseudomonas fluorescens* (KAU) were mass multiplied in Kings'B liquid medium for which the medium was inoculated with 48 h old bacterial culture and incubated in room temperature for 48 h with constant shaking on a rotary shaker. After 48 h the broth culture was diluted three times with sterile water so as to get 10^8 cfu ml^{-1}.

3.8.2 Preparation of fungal inocula

The inocula of the selected fungal isolate EF-8 and *Trichoderma viride* (KAU) were prepared by inoculation on potato dextrose liquid medium and incubated at room temperature for ten days. When sporulated, the fully grown sporulated mycelial mat along with the medium was taken and mixed thoroughly using a blender, it was then diluted with sterile water so as to get 10^6 cfu ml^{-1}.

3.8.3 Effect of organic sources of nutrients and selected antagonist on growth, yield and leaf blight of amaranth

During the pot culture experiment, per cent disease incidence, severity and biometric observations were recorded at 15, 30 and 45 DAT as in the case of field experiment (3.3.3 and 3.3.4) root length, root weight, girth at collar were recorded at 45 DAT.

3.9 METEOROLOGICAL OBSERVATIONS

The meteorological observations during the period of experiment were recorded.

3.10 STATISTICAL ANALYSES

The data collected from various experiments were subjected to analysis of variance using statistical package, MSTAT (Freed, 1986). The data on population of rhizosphere microflora were analyzed by analysis of covariance (ANOCOVA) taking pre-treatment population count as covariate. The means were compared using DMRT (Duncan's Multiple Range Test). Correlation analysis was performed on data on population of rhizosphere microflora and yield of amaranth.

Results

4. RESULTS

The present study "Characterization of endophytic and rhizosphere microorganisms on organically grown amaranth for management of leaf spot diseases" was conducted in the Dept of Plant Pathology, College of Horticulture. The study consisted of both field and pot culture experiments. In the field experiment, amaranth plants were grown with different types of organic sources of nutrients to replace 50 Kg $ha^{-1}$of the N requirement. Antagonistic endophytic and rhizosphere microbes were isolated during the field experiment and the potential antagonists against *Rhizoctonia solani* were selected and tested in the pot culture experiment.

4.1 ISOLATION AND CHARACTERIZATION OF THE PATHOGEN

The fungus causing leaf blight of amaranth was isolated from infected leaves as this was the only disease observed during the field experiment. The isolate was purified by hyphal tip method. For further studies the pathogen was grown on PDA and cultural and morphological characters were studied. At initial stage hyphae were hyaline later turned to light brown. Hyphae were characteristically branched and arise at right angles from below the septa and had distinct constriction at the point of origin. The mycelial growth was cream coloured but in later stages it turned to light brown (Plate 1). Sclerotia were produced on the surface of the culture after 7 to 10 days, and were irregularly shaped, light brown to dark brown coloured.

The pathogenicity of the isolate was confirmed by inoculation on healthy amaranth seedlings on which it produced the symptoms of leaf blight. The pathogen was reisolated from the inoculated leaves and grown on PDA. The cultural and morphological characters of the pathogen was same as that of the original one. Thus the Koch's postulates were proved and based on cultural and morphological characters along with pathogenicity on amaranth the isolate was identified as *Rhizoctonia solani* Kuhn.

Plate 1. Isolation of leaf blight pathogen

a. Leaf blight of amaranth

b. Culture of *Rhizoctonia solani*

The symptoms of leaf blight were studied under natural as well as artificial conditions. In natural condition the symptoms of disease initiated as water soaked lesions which developed into small irregular whitish cream spots. Under high humidity the spots enlarged with greyish centre and irregular brown margins. Later severe infection led to shot hole symptoms and defoliation. Under artificial condition the symptoms were same as that of natural condition except that translucent irregular green patches were formed after two days of inoculation which further developed into whitish cream spots.

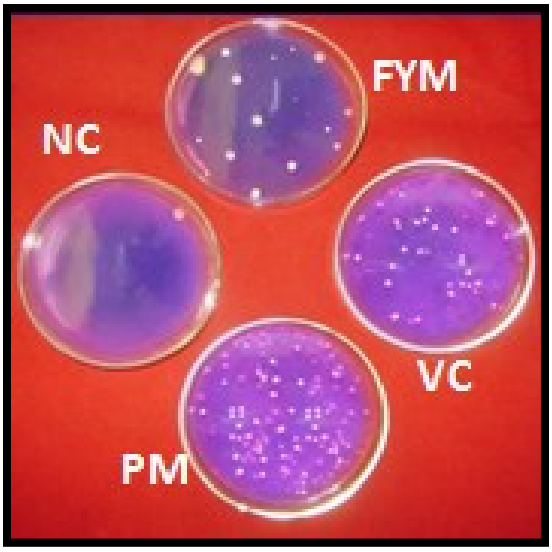
4.2 QUANTITATIVE ESTIMATION OF MICROFLORA IN ORGANIC SOURCES OF NUTRIENTS

The fungi, bacteria and actinomycetes present in different organic sources of nutrients were estimated. Results showed that poultry manure contains the highest number of fungi, bacteria and actinomycetes followed by vermicompost, farm yard manure and neem cake. The population of fungi, bacteria and actinomycetes varied among organic sources of nutrients (Plate 2). The fungal population ranged from 2.33×10^4 cfu g^{-1} in neem cake to 139.67×10^4 cfu g^{-1} in poultry manure, bacteria 4.67×10^6 cfu g^{-1} in neem cake to 16.67×10^6 cfu g^{-1} in poultry manure. The population of actinomycetes showed variation ranging from 1.33×10^4 cfu g^{-1} in neem cake to 3.67×10^4 cfu g^{-1} in poultry manure (Table 2).

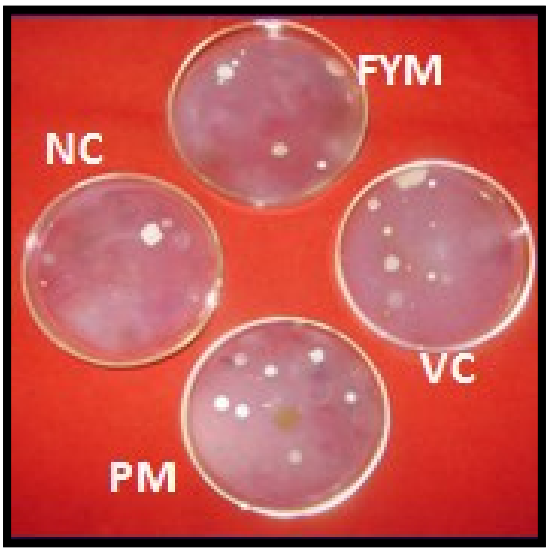
4.3 EFFECT OF ORGANIC SOURCES OF NUTRIENTS ON GROWTH, YIELD AND LEAF BLIGHT OF AMARANTH

The field experiment to study the influence of different organic sources of nutrients like farm yard manure, neem cake, vermicompost and poultry manure on leaf spot of amaranth was conducted in the field of Department of Olericulture, College of Horticulture, Vellanikkara (Plate 3). Observations on per cent disease incidence and severity and biometric observations were recorded during the experiment after each harvest.

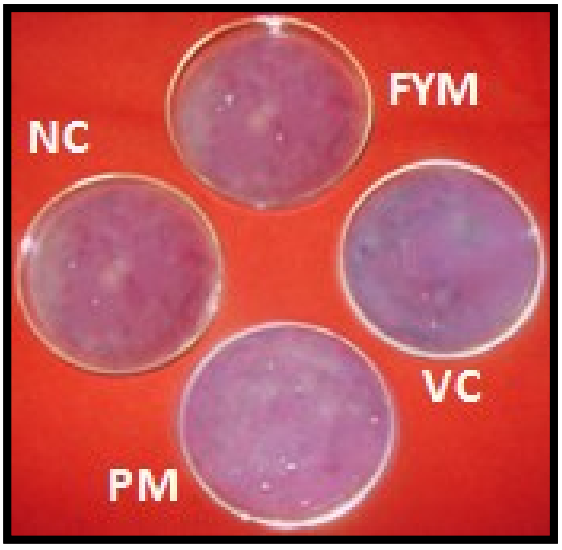
Plate 2.Isolation and enumeration of microflora from organic sources of nutrients



a. Fungi



b. Bacteria



FYM- Farm yard manure

NC- Neem cake

VC- Vermicompost

PM- Poultry manure

c. Actinomycetes

Table 2. Population of microflora in organic sources of nutrients

Sl. No.	Organic source of nutrients	Fungi* $\times 10^4$ cfu g^{-1}	Bacteria $\times 10^6$ cfu g^{-1}	Actinomycetes $\times 10^4$ cfu g^{-1}
1	Farm yard manure	18.00	6.67	1.67
2	Neem cake	2.33	4.67	1.33
3	Vermicompost	41.00	10.33	2.33
4	Poultry manure	139.67	16.67	3.67

*Mean of three replications

Plate 3. Experimental field

4.3.1 Effect of organic sources of nutrients on growth and yield of amaranth

Biometric observations such as shoot length, number of branches, number of leaves, and girth at collar were recorded at 30, 60 and 75 DAT. Root length and root weight were recorded at 75 DAT.

4.3.1.1 Shoot length

Data presented in Table 3 revealed that the shoot length of amaranth plants grown under different organic treatment varied significantly. It was observed that at plants in poultry manure (T_5) showed maximum shoot length during the period of experiment (41.08 cm, 53.57 cm and 62.76 cm respectively), which was superior to all other treatments. It was followed by NPK (T_1) with shoot length 34.75 cm, 47.57 cm and 58.49 cm at 30, 60 and 75 DAT respectively. At 75 DAT T_5 was on par with T_1. At 30 and 60 DAT the lowest shoot length was recorded in neem cake (T_3) (27.90 cm and 40.92 cm) whereas at 75 DAT lowest shoot length was observed in farm yard manure (T_2) (53.65 cm).

4.3.1.2 Number of branches

The number of branches of amaranth at different intervals is furnished in Table 4. The result shows that there was significant difference in the number of branches among the treatments at 30 and 60 and 75 DAT. At different stages of observation poultry manure (T_5) (7.72, 8.02 and 8.23) recorded the highest number of branches, which was superior to all other treatments. It was followed by T_1, T_2, T_4 at 30 DAT, and these were on par. At 60 DAT, all the treatments except T_5 were on par. Neem cake (T_3) recorded the lowest number of branches at 30 and 75 DAT.

Table 3. Effect of different organic sources of nutrients on shoot length of amaranth

Sl. No.	Treatment	Shoot length (cm)*		
		30 DAT	60 DAT	75 DAT
1	T_1- NPK	34.75^{b}	47.57^{b}	58.49^{ab}
2	T_2- Farm yard manure	31.37^{bc}	43.47^{cd}	53.65^{c}
3	T_3- Neem cake	27.90^{c}	40.92^{d}	54.21^{bc}
4	T_4- Vermicompost	32.78^{bc}	44.82^{bc}	57.27^{bc}
5	T_5- Poultry manure	41.08^{a}	53.57^{a}	62.76^{a}

* Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

Table 4. Effect of different organic sources of nutrients on number of branches of amaranth

Sl. No.	Treatment	Number of branches*		
		30 DAT	60 DAT	75 DAT
1	T_1- NPK	6.07^{b}	6.65^{b}	7.17^{b}
2	T_2- Farm yard manure	5.45^{bc}	6.02^{b}	6.75^{bc}
3	T_3- Neem cake	4.97^{c}	6.02^{b}	6.25^{c}
4	T_4- Vermicompost	5.22^{bc}	6.32^{b}	6.50^{bc}
5	T_5- Poultry manure	7.72^{a}	8.02^{a}	8.23^{a}

* Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

4.3.1.3 Number of leaves

The data on number of leaves of amaranth grown in different treatments are given in Table 5. It was found that number of leaves among the treatments were significantly at different intervals of observation. The number of leaves in different treatments ranged from 14.12 to 36.30 at 30 DAT, 32.60 to 65.87 at 60 DAT and 50.92 to 110.93 at 75 DAT.

At 30, 60 and 75 DAT poultry manure (T_5) was superior among the treatments with maximum number of leaves (36.30, 65.87 and 110.93 leaves per plant) which was followed by T_1 (26.25, 46.29 and 73.86 leaves per plant) except for at 75 DAT the second highest number of leaves was observed in farm yard manure (T_2) (75.80 leaves per plant). At 75 DAT T_1 was on par with T_2 and T_4. The least number of leaves were observed in neem cake (T_3) (14.12, 32.60 and 50.92 leaves per plant) at all stages of observation.

4.3.1.4 Girth at collar

The girth at collar of amaranth plants in the experiment at different intervals of observation differed significantly among the treatments (Table 6). It was observed that at 30 DAT (2.7 cm), 60 DAT (4.82 cm) and 75 DAT (5.38 cm) plants in poultry manure (T_5) showed maximum girth at collar which was followed by NPK (T_1) (2.60, 3.9 and 5.22 cm respectively) and these were on par at 30 and 75 DAT. At 30 DAT, the minimum girth at collar of 2.14 cm was recorded in vermicompost (T_4). At 60 and 75 DAT, the lowest girth at collar was recorded in neem cake (T_3) (3.00 cm and 3.83 cm respectively). At 75 DAT poultry manure (T_5) (5.38 cm) was on par with all other treatments except vermicompost (T_4) (4.31 cm) and neem cake (T_3) (3.83 cm).

Table 5. Effect of different organic sources of nutrients on number of leaves of amaranth

Sl. No.	Treatment	Number of leaves per plant*		
		30 DAT	60 DAT	75 DAT
1	T_1- NPK	26.25^{b}	46.29^{b}	73.86^{b}
2	T_2- Farm yard manure	24.07^{bc}	43.34^{bc}	75.80^{b}
3	T_3- Neem cake	14.12^{d}	32.60^{d}	50.92^{c}
4	T_4- Vermicompost	17.52^{cd}	39.25^{c}	64.87^{b}
5	T_5- Poultry manure	36.30^{a}	65.87^{a}	110.93^{a}

* Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

Table 6. Effect of different organic sources of nutrients on girth at collar of amaranth

Sl. No.	Treatment	Girth at collar (cm)*		
		30 DAT	60 DAT	75 DAT
1	T_1- NPK	2.60^{a}	3.90^{b}	5.22^{a}
2	T_2- Farm yard manure	2.23^{b}	3.70^{bc}	4.95^{ab}
3	T_3- Neem cake	2.16^{b}	3.00^{d}	3.83^{c}
4	T_4- Vermicompost	2.14^{b}	3.45^{c}	4.31^{bc}
5	T_5- Poultry manure	2.70^{a}	4.82^{a}	5.38^{a}

* Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

4.3.1.5 Root length

Root length of amaranth plants in different treatments are given in Table 7. Root length was recorded at 75 DAT. The data revealed that the treatments differ significantly with each other in root length. The effect of different treatments showed that plants grown in poultry manure (T_5) had maximum root length (18.62 cm) and it was superior over other treatments and the lowest root length was recorded in neem cake (T_3) (12.09 cm).

4.3.1.6 Root weight

The data on root weight of amaranth was recorded at 75 DAT and presented in Table 7. It was observed that plants grown with poultry manure as organic source of nutrient had the maximum root weight (22.07 g) and it was followed by NPK (T_1) (19.25 g) which were on par with T_5. The roots of plants from neem cake (T_3) had the lowest root weight (10.47 g).

4.3.1.7 Yield

Yield of amaranth plants are given in Table 8. The data revealed that the yield was significantly different among the treatments. It was found that at 30, 60 and 75 DAT maximum yield was recorded in poultry manure (T_5) (2.21, 4.64 and 10.81 kg) which was superior to all other treatments. At 30, 60 and 75 DAT T_5 was followed by T_1 (1.68, 3.31 and 7.77 kg).The lowest yield was recorded by T_3 at 30, 60 and 75 DAT (0.44, 1.4 and 3.91 kg).

Table 7. Effect of different organic sources of nutrients on root length and root weight of amaranth

Sl. No.	Treatment	Root length (cm)* at 75 DAT	Root weight (g) at 75 DAT
1	T_1- NPK	16.84^{b}	19.25^{a}
2	T_2- Farm yard manure	15.47^{c}	15.32^{b}
3	T_3- Neem cake	12.09^{e}	10.47^{c}
4	T_4- Vermicompost	13.97^{d}	12.95^{bc}
5	T_5- Poultry manure	18.61^{a}	22.07^{a}

*Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

Table 8. Effect of different organic sources of nutrients on yield of amaranth

Sl. No.	Treatment	Yield (kg)*		
		30 DAT	60 DAT	75 DAT
1	T_1- NPK	1.68^{b}	3.31^{b}	7.77^{b}
2	T_2- Farm yard manure	0.99^{c}	3.12^{b}	6.86^{b}
3	T_3- Neem cake	0.44^{d}	1.4^{d}	3.91^{c}
4	T_4- Vermicompost	0.75^{cd}	2.07^{c}	5.10^{c}
5	T_5- Poultry manure	2.21^{a}	4.64^{a}	10.81^{a}

*Mean of four replications, Values followed by same superscript are not significantly different by DMRT (P=0.05)
DAT- Days after transplanting

4.3.2 Effect of organic sources of nutrients on leaf blight of amaranth

The per cent disease incidence (PDI) and severity were recorded and 100 per cent disease incidence was noticed during different intervals of observation. The per cent disease severity (PDS) of leaf blight of amaranth grown under different organic source of nutrients was calculated. There was no significant difference in the PDS among treatments (Table 9). In all treatments PDS gradually increased throughout the crop period. At 30 DAT, the per cent disease severity ranged from 42.93 in poultry manure (T_5) to 52.20 per cent in neem cake (T_3). However, poultry manure treated plants exhibited 10.08 per cent reduction over control. At 60 DAT, PDS increased in all the treatments and it is ranged from 54.18 in NPK (T_1) to 63.30 per cent in neem cake (T_3). At 75 DAT, it ranged from 59.13 in NPK (T_1) to 64.98 per cent in neem cake (T_3). The maximum disease severity was observed on plants in T_3 (52.2%, 63.3% and 64.97%) at 30, 60 and 75 DAT. The minimum PDS was noticed in T_5 (42.93%) at 30 DAT, in T_1 (54.17%) at 60 DAT and in T_4 (61.00%) at 75 DAT.

Table 9. Effect of organic sources of nutrients on leaf blight of amaranth

Sl. No.	Treatment	Per cent disease severity*					
		30 DAT	Per cent reduction over control	60 DAT	Per cent reduction over control	75 DAT	Per cent reduction over control
1	T_1- NPK	47.73[a]	--	54.18[a]	--	59.13[a]	--
2	T_2- Farm yard manure	48.45[a]	-1.51	59.57[a]	-9.70	64.11[a]	-8.47
3	T_3- Neem cake	52.20[a]	-9.37	63.30[a]	-16.42	64.98[a]	-9.95
4	T_4- Vermicompost	52.00[a]	-8.97	57.35[a]	-5.71	61.00[a]	-3.179
5	T_5- Poultry manure	42.93[a]	10.08	58.75[a]	-8.25	64.33[a]	-8.84

* Mean of four replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
*DAT- Days after transplanting

4.4 ISOLATION AND ENUMERATION OF RHIZOSPHERE MICROFLORA

The population of rhizosphere microflora was estimated at periodical intervals *i.e.* pre-treatment, 30, 60 and 75 days after transplanting (DAT). It was noticed that after application of organic sources of nutrients the population of microflora increased during the experiment. In general, bacteria were predominant followed by fungi and actinomycetes. The population after transplanting was subjected to analysis of covariance, taking population before treatment as covariate.

4.4.1 Isolation and enumeration of rhizosphere fungi

Population of fungi in rhizosphere was estimated and presented in Table 10. The pre-treatment fungal population was not significantly different; however it varied from 16.67 to 20.17 × 10^4 cfu g^{-1} (Fig. 1). At 30 DAT the fungal population varied significantly among the treatments, which ranged from 17.38 to 27.75 × 10^4 cfu g^{-1}. It was found that T_4 (27.75 × 10^4 cfu g^{-1}) had the highest fungal population which was followed by T_2 (26.50 × 10^4 cfu g^{-1}) and T_5 (24.62 × 10^4 cfu g^{-1}) and these were on par. Lowest fungal population was noticed in T_1 (17.38 × 10^4 cfu g^{-1}). At 60 DAT also the fungal population varied significantly with the highest in T_5 (42.96 × 10^4 cfu g^{-1}) which was superior to all other treatments. It was followed by T_3 (27.46 × 10^4 cfu g^{-1}) and T_2 (27.02 × 10^4 cfu g^{-1}) which were on par. The least fungal population was in T_1 (16.29 × 10^4 cfu g^{-1}) which was on par with T_4 (21.02 × 10^4 cfu g^{-1}). It was noticed that fungal population decreased during 75 DAT in all treatments except in T_1, in which slight increase in population was observed. At 75 DAT, the maximum fungal population was observed in T_5 (30.05 × 10^4 cfu g^{-1}) (Plate 4. a). It was followed by T_2 (21.74 × 10^4 cfu g^{-1}) and T_4 (17.95 × 10^4 cfu g^{-1}) which were on par. The least population was noticed in T_3 (17.64 × 10^4 cfu g^{-1}) which was on par with T_1 (17.64 × 10^4 cfu g^{-1}).

Table 10. Population of rhizosphere fungi

Sl. No.	Treatment	Fungi x 10^4 cfu g^{-1}*			
		Pre-treatment	**30 DAT****	**60 DAT**	**75 DAT**
1	T_1- NPK	16.84^a	17.38^b	16.29^c	17.72^c
2	T_2- Farm yard manure	18.67^a	26.50^a	27.02^b	21.74^b
3	T_3- Neem cake	16.67^a	19.127^b	27.46^b	17.64^c
4	T_4- Vermicompost	20.17^a	27.75^a	21.02^c	17.95^b
5	T_5- Poultry manure	19.84^a	24.62^a	42.96^a	30.05^a

* Values followed by same superscript are not significantly different
** Treatment mean adjusted to pre-treatment population before for ANOCOVA
cfu – colony forming units
DAT- Days after transplanting

4.4.2 Isolation and enumeration of rhizosphere bacteria and fluorescent pseudomonads

Population of bacteria was also assessed during, before treatment application, 30, 60 and 75 DAT. In general bacterial population increased due to the application of treatments. By observing the data it was evident that the bacterial population varied throughout the period of experiment. Before treatment application it was not significantly different and ranged from 6.42 to 8.84 × 10^6 cfu g^{-1} for bacteria and 4.86 to 7.21 × 10^6 cfu g^{-1} for fluorescent pseudomonads. The population varied significantly among the treatments throughout the period of experiment (Fig. 2 and Fig. 3). At 30 DAT, the bacterial population ranged from 10.68- 21.98 × 10^6 cfu g^{-1} and the highest count was in T_5 (21.98 × 10^6 cfu g^{-1}) followed by T_2 (21.65 × 10^6 cfu g^{-1}) and these were on par. The least population was observed in T_1 (10.68 × 10^6 cfu g^{-1}). The population of fluorescent pseudomonads was also the highest in T_5 (39.58 × 10^6 cfu g^{-1}) which was followed by T_3 (32.98 × 10^6 cfu g^{-1}) and these were on par. The lowest population was noticed in T_1 (11.73 × 10^6 cfu g^{-1}). At 60 DAT bacterial population ranged from 12.12 to 81.05 × 10^6 cfu g^{-1} and fluorescent pseudomonads ranged from 11.39 - 64.81 × 10^6 cfu g^{-1}. The population of bacteria and fluorescent pseudomonads were the highest in T_5 (81.05 × 10^6 cfu g^{-1} and 64.81 × 10^6 cfu g^{-1} respectively). The lowest bacterial count was recorded in T_1 (12.12 × 10^6 cfu g^{-1}) and fluorescent pseudomonads in T_3 (10.58 × 10^6 cfu g^{-1}). At 75 DAT, the highest population of bacteria was in T_5 (21.84 x 10^6 cfu g^{-1}) and it was on par with T_2 (20.16 × 10^6 cfu g^{-1}). The least population was found in T_1 (12.21 × 10^6 cfu g^{-1}). At the same time population of fluorescent pseudomonads were also higher in T_5 (53.48 × 10^6 cfu g^{-1}) followed by T_2 (24.95 × 10^6 cfu g^{-1}) and T_4 (15.55 × 10^6 cfu g^{-1}) and least was in T_1 (9.18 × 10^6 cfu g^{-1}) (Plate 4. b and Plate 4. c) (Table 11).

Fig. 1. Population of rhizosphere fungi

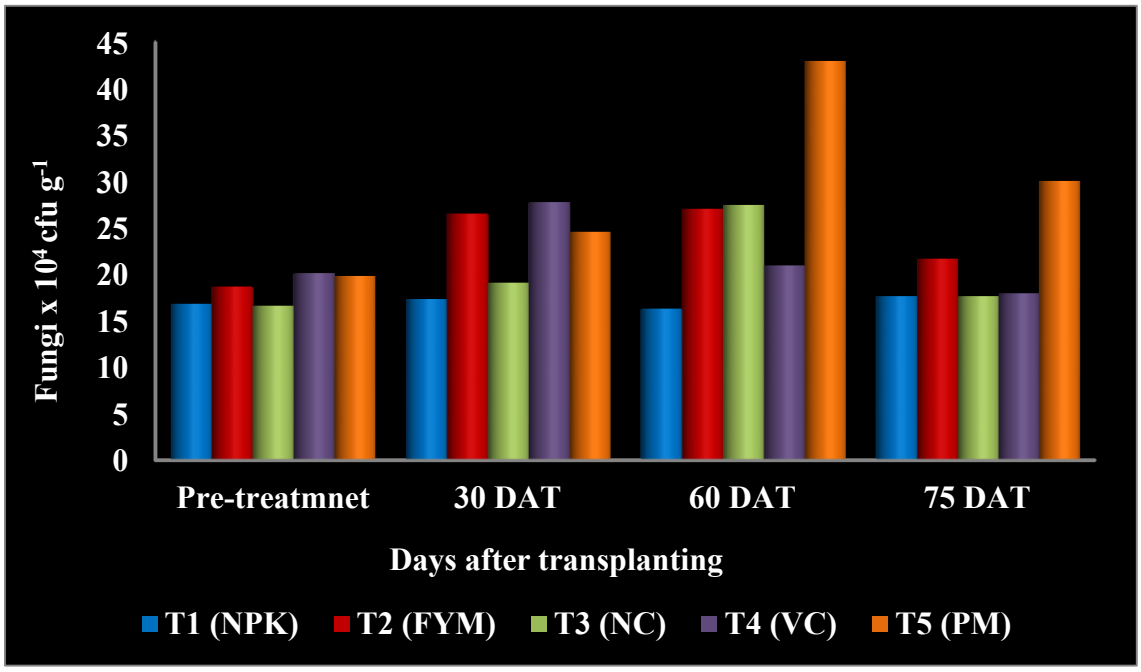
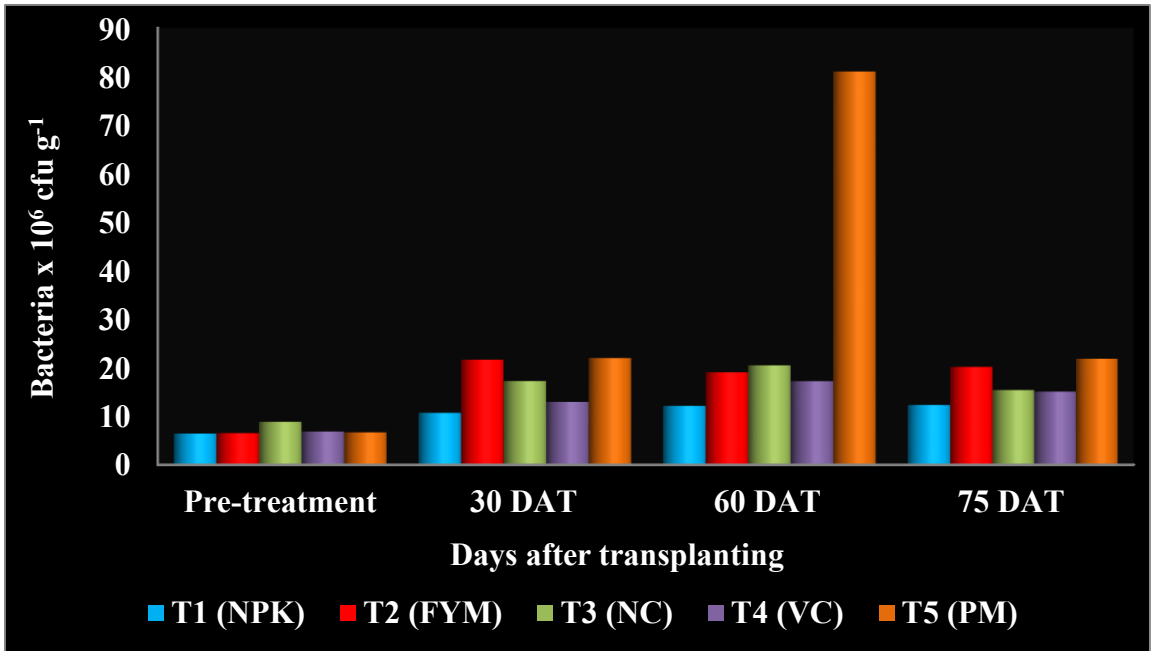


Fig. 2. Population of rhizosphere bacteria



FYM- Farm yard manure, NC- Neem cake, VC- Vermicompost, PM- Poultry manure

Table 11. Population of rhizosphere bacteria and fluorescent pseudomonads

Sl. No.	Treatment	Bacteria and fluorescent pseudomonads x 10^6 cfu g^{-1}*							
		Pre-treatment		30 DAT**		60 DAT		75 DAT	
		Bacteria	Fluorescent pseudomonads	Bacteria	Fluorescent pseudomonads	Bacteria	Fluorescent pseudomonads	Bacteria	Fluorescent pseudomonads
1	T_1- NPK	6.42^{a}	5.75^{a}	10.68^{c}	11.73^{d}	12.12^{c}	11.39^{c}	12.31^{c}	9.18^{c}
2	T_2- Farm yard manure	6.50^{a}	4.86^{a}	21.65^{a}	21.92^{bc}	19.05^{b}	20.20^{b}	20.16^{ab}	24.95^{b}
3	T_3- Neem cake	8.84^{a}	7.21^{a}	17.25^{b}	32.98^{ab}	20.48^{b}	10.58^{c}	15.39^{bc}	13.39^{c}
4	T_4- Vermicompost	6.83^{a}	5.93^{a}	12.94^{c}	18.17^{cd}	17.22^{bc}	12.02^{c}	15.06^{c}	15.55^{bc}
5	T_5- Poultry manure	6.67^{a}	7.12^{a}	21.98^{a}	39.58^{a}	81.05^{a}	64.81^{a}	21.84^{a}	53.48^{a}

*Values followed by same superscript are not significantly different

** Treatment mean adjusted to pre-treatment population for ANOCOVA

DAT - Days after transplanting

4.4.3 Isolation and enumeration of rhizosphere actinomycetes

The population of actinomycetes was not significantly different before treatment application. However, before treatment application population ranged from 2.67 to 3.67 × 10^4 cfu g^{-1}. At 30 DAT, population varied significantly and it was the highest in T_4 (9.71 × 10^4 cfu g^{-1}) followed by T_3 (8.66 × 10^4 cfu g^{-1}) and these were on par. The least actinomycetes count was recorded by T_2 (6.52 × 10^4 cfu g^{-1}). At 60 DAT also the population was significantly different among the treatments and it ranged from 5.95 to 13.17 × 10^4 cfu g^{-1}. The highest population was observed in T_4 (13.17 × 10^4 cfu g^{-1}) which was followed by T_2 (9.60 × 10^4 cfu g^{-1}), T_5 (9.47 × 10^4 cfu g^{-1}) and T_1 (8.73 × 10^4 cfu g^{-1}). The minimum population was recorded in T_3 (5.25 × 10^4 cfu g^{-1}) (Plate 4. d). It was noticed that at 75 DAT there was decreased population of actinomycetes in all treatments except neem cake (T_3), however, there was no significant difference (Fig. 4) (Table 12).

4.5 CORRELATION BETWEEN RHIZOSPHERE MICROFLORA AND YIELD OF AMARANTH

A correlation analysis was conducted to assess the relation between the population of rhizosphere microflora and yield of amaranth during the period of experiment (Table 13). It was observed that, there is significant positive correlation between population of rhizosphere fungi, bacteria and fluorescent pseudomonads at 60 and 75 DAT with yield. However, significant negative correlation was observed between population of actinomycetes at 30 DAT with yield at 60 and 75 DAT.

Fig. 3. Population of rhizosphere fluorescent pseudomonads

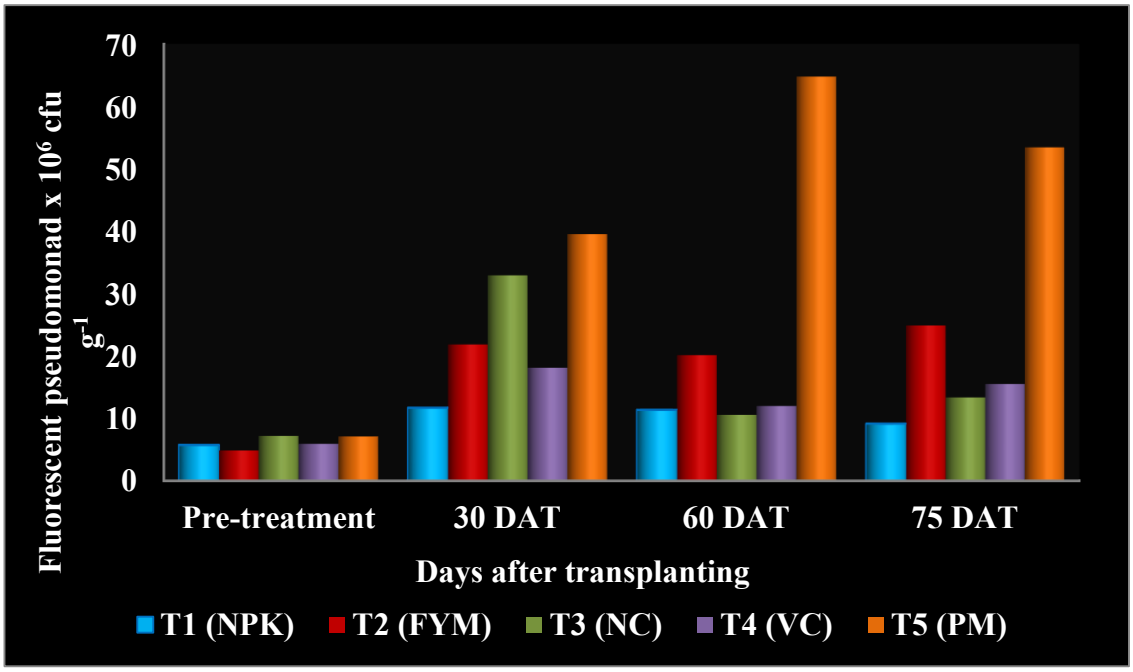


Fig. 4. Population of rhizosphere actinomycetes

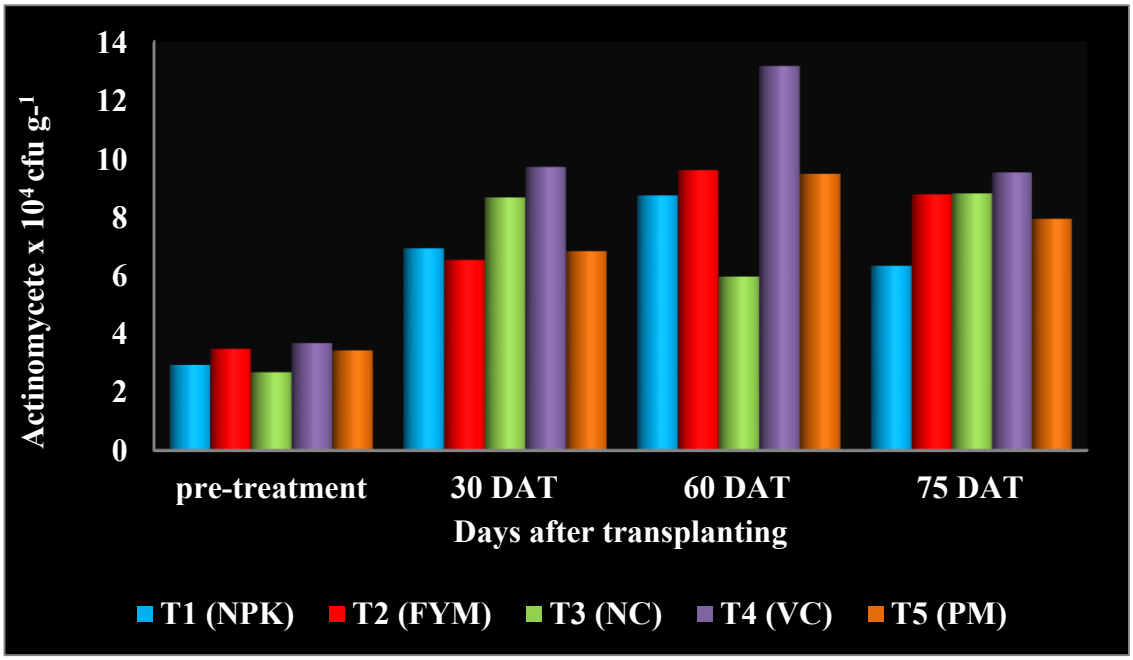


Table 12. Population of rhizosphere actinomycetes

Sl. No.	Treatment	Actinomycetes × 10^4 cfu g^{-1}*			
		Pre-treatment	30 DAT**	60 DAT	75 DAT
1	T_1- NPK	2.92^a	6.92^b	8.73^{ab}	6.32^a
2	T_2- Farm yard manure	3.47^a	6.52^b	9.60^{ab}	8.77^a
3	T_3- Neem cake	2.67^a	8.66^a	5.95^b	8.80^a
4	T_4- Vermicompost	3.67^a	9.71^a	13.17^a	9.52^a
5	T_5- Poultry manure	3.42^a	6.82^b	9.47^{ab}	7.93^a

* Values followed by same superscript are not significantly different
** Treatment mean adjusted to pre-treatment population for ANOCOVA
cfu – colony forming
DAT- Days after transplanting

Table 13. Correlation between rhizosphere microflora and yield of amaranth

Microorganism		Yield		
		30 DAT	**60 DAT**	**75 DAT**
Fungi	30 DAT	-	-	-
	60 DAT	-	0.487	0.496
	75 DAT	-	-	0.785
Bacteria	30 DAT	-	-	-
	60 DAT	-	0.651	0.746
	75 DAT	-	-	-
Fluorescent pseudomonads	30 DAT	-	-	-
	60 DAT	-	0.720	0.781
	75 DAT	-	-	0.720
Actinomycetes	30 DAT	-	-0.581	-0.445
	60 DAT	-	-	-
	75 DAT	-	-	-

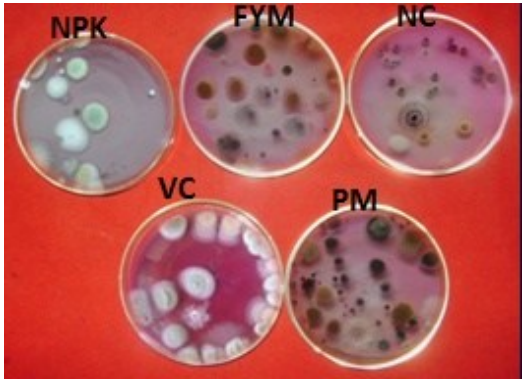
DAT- Days after transplanting

4.6 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROFLORA

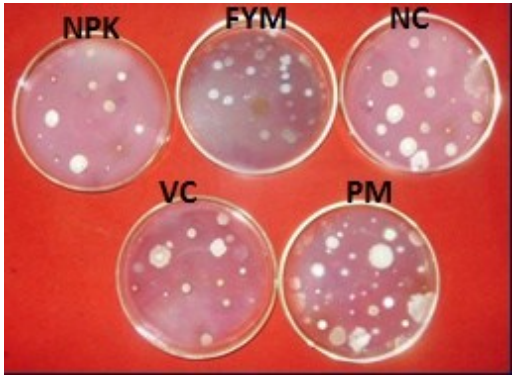
The endophytic microorganisms were isolated from stem and roots of amaranth plants (Plate 4. e and 4. f). The samples were collected from each treatment and endophytes were isolated by trituration method. The endophytes were few in number when compared to rhizosphere microorganisms.

The results are presented in Table 14. From the data it is evident that bacteria are predominant in the endosphere of amaranth plants. The endophytic fungal and bacterial population showed significant difference among treatments and in general, both fungi and bacteria are more in roots. The population of fungi in the stem ranged from 1.33 to 4.33 x 10^2 cfu g^{-1} and it was the highest in poultry manure (T_5) (4.33 × 10^2 cfu g^{-1}). In the case of roots maximum endophytic fungi were present in plants grown in farm yard manure (T_2) (12.33 × 10^2 cfu g^{-1}) followed by vermicompost (T_4) (10.66 × 10^2 cfu g^{-1}) and poultry manure (T_5) (10.00 × 10^2 cfu g^{-1}) and these were on par. Endophytic bacterial population in stem, the highest population was in T_2 (14.00 × 10^2 cfu g^{-1}) which was immediately followed by T_5 (13.67 × 10^2 cfu g^{-1}) which were on par. In roots the highest endophytic bacterial population was found in poultry manure (T_5) (28.33 × 10^2 cfu g^{-1}) which was followed by T_2, T_3, T_4 and T_1 The endophytic fluorescent pseudomonad population was the highest in poultry manure (T_5) both in stem (3.67 × 10^2 cfu g^{-1}) and roots (4.33 × 10^2 cfu g^{-1}). The lowest fluorescent pseudomonad population was noticed in T_1 (1.33 and 2.33 × 10^2 cfu g^{-1} respectively). Actinomycetes could not be isolated from any of the samples.

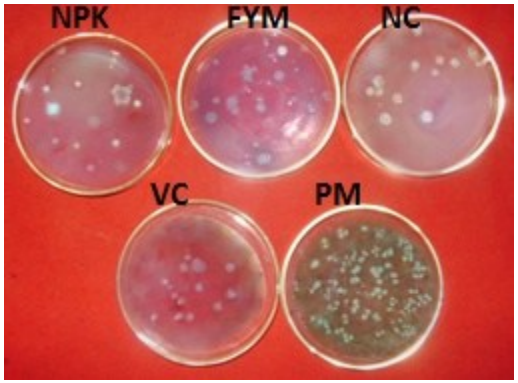
Plate 4. Isolation and enumeration of rhizosphere and endophytic microflora



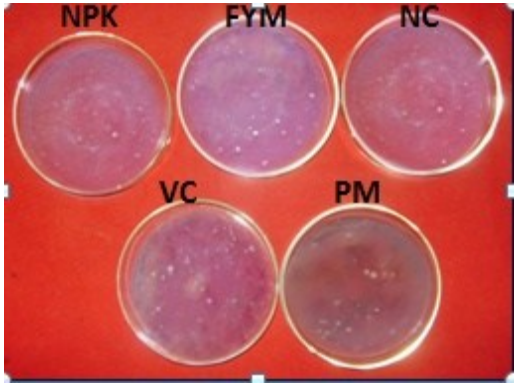
a. Rhizosphere fungi



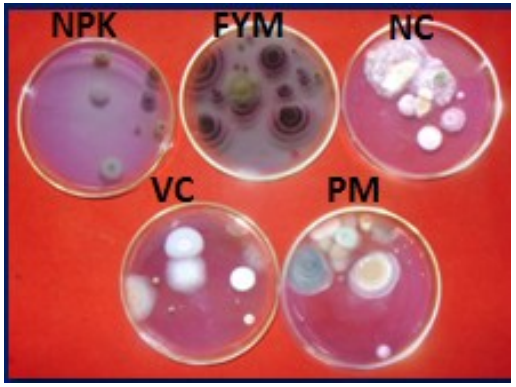
b. Rhizosphere bacteria



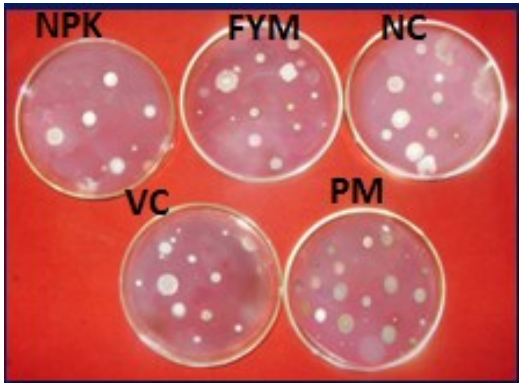
c. Rhizosphere fluorescent pseudomonad



d. Rhizosphere actinomycetes



e. Endophytic fungi



f. Endophytic bacteria

Table 14. Population of endophytic microbes from stem and roots of amaranth

Sl. No.	Treatment	Fungi ($\times 10^2$ cfu g^{-1})		Bacteria ($\times 10^2$ cfu g^{-1})		Fluorescent pseudomonad ($\times 10^2$ cfu g^{-1})	
		stem	root	stem	root	stem	root
1	T_1- NPK	1.33^{b} (1.14)	7.67^{c} (2.77)	9.00^{b} (2.99)	5.67^{d} (2.37)	1.33^{c} (1.12)	2.33^{b} (1.53)
2	T_2- Farm yard manure	2.33^{b} (1.52)	12.33^{a} (3.51)	14.00^{a} (3.74)	19.33^{b} (4.40)	2.67^{b} (1.63)	3.00^{b} (1.73)
3	T_3- Neem cake	2.33^{b} (1.52)	9.33^{b} (3.05)	10.67^{b} (3.26)	18.00^{bc} (4.24)	2.00^{bc} (1.41)	2.67^{b} (1.63)
4	T_4- Vermicompost	1.67^{b} (1.28)	10.66^{ab} (3.26)	9.33^{b} (3.04)	13.67^{c} (3.68)	2.33^{b} (1.53)	2.67^{b} (1.12)
5	T_5- Poultry manure	4.33^{a} (2.07)	10.00^{abc} (3.15)	13.67^{a} (3.69)	28.33^{a} (5.32)	3.67^{a} (1.92)	4.33^{a} (2.08)

* Values in the parenthesis are square root transformed, values followed by same superscript are not significantly different
cfu – colony forming units
DAT- Days after transplanting

4.7 *IN VITRO* EVALUATION OF MICROORGANISMS AGAINST THE PATHOGEN

4.7.1 Preliminary screening of microorganisms against *R. solani*

A total of 123 microbial isolates including 48 fungal, 61 bacterial, 10 fluorescent pseudomonads and 4 actinomycetes were subjected to preliminary screening in *in vitro* for inhibition of *R. solani*. In preliminary screening 25 fungal and 36 bacterial isolates showed antagonism against *R. solani* and these was further evaluated individually using dual culture method (Plate 5. a).

4.7.2 *In vitro* evaluation of antagonistic fungi

Among the fungal isolates from rhizosphere 17 showed antagonism against *R. solani*. These include five each from T_2 (RF-1, RF-2, RF-14, RF-21 and RF-24) and T_5 (RF-3, RF-6, RF-11, RF-12 and RF-18). Four were from T_3 (RF-5, RF-8, RF-15 and RF-27), two from T_4 (RF-7 and RF-10) and one from T_1 (RF-23). The data depicts that per cent inhibition of the pathogen *R. solani* by selected antagonist varies among antagonists. Among the 17 antagonistic fungi RF-21 (80.37%) had maximum per cent inhibition against *R. solani.* However four fungal isolates (RF-3, RF-18, RF-24 and RF-10) exhibited per cent inhibition ranging from 60-80 per cent. Eleven isolates showed per cent inhibition from 40-60 per cent and the rest showed per cent inhibition less than 40 per cent (RF-6).(Table 15)

There were eight endophytic fungal isolates which exhibited antagonistic activity against *R. solani* in preliminary screening. Among the eight endophytic fungal isolates two each were from T_5 (EF-8 and EF-12), T_2 (EF-2, and EF-10) and T_4 (EF-5 and EF-11). From T_1 (EF-7) and T_3 (EF-3) one each were selected. The data presented in the Table 16 revealed that EF-8 had the highest inhibition of cent per cent of the pathogen and it was superior among the tested isolates (Plate 5.b).

Table 15. *In vitro* evaluation of rhizosphere fungi against *R. solani*

Sl. No.	Fungi	Per cent inhibition*
1	RF-1	55.85^{cd}
2	RF-2	48.08^{ef}
3	RF-3	64.83^{b}
4	RF-5	40.66^{gh}
5	RF-6	38.08^{h}
6	RF-7	49.93^{ef}
7	RF-8	53.25^{de}
8	RF-10	61.11^{bc}
9	RF-11	41.32^{gh}
10	RF-12	44.66^{fg}
11	RF-14	51.80^{de}
12	RF-15	55.75^{cd}
13	RF-18	63.00^{b}
14	RF-21	80.37^{a}
15	RF-23	48.21^{ef}
16	RF-24	61.82^{bc}
17	RF-27	59.70^{bc}

* Values followed by same superscript are not significantly different
RF – Rhizosphere Fungus

Table 16. *In vitro* evaluation of endophytic fungi against *R. solani*

Sl. No.	Fungi	Per cent inhibition*
1	EF-2	66.6^{b}
2	EF-3	47.93^{ef}
3	EF-5	42.54^{f}
4	EF-7	60.36^{bc}
5	EF-8	100.00^{a}
6	EF-10	56.62^{cd}
7	EF-11	46.20^{ef}
8	EF-12	51.80^{de}

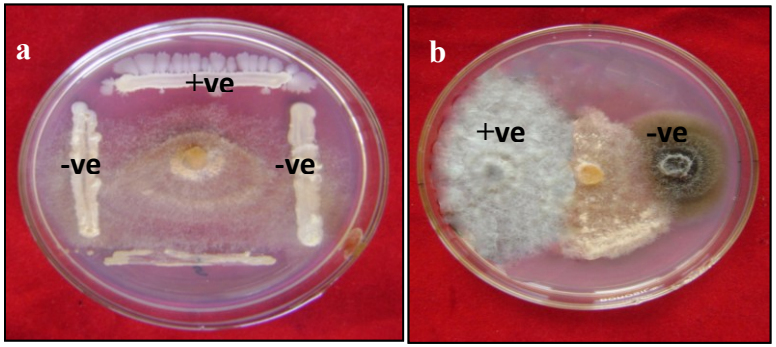
* Values followed by same superscript are not significantly different
EF- Endophytic Fungus

4.7.3 *In vitro* evaluation of antagonistic bacteria

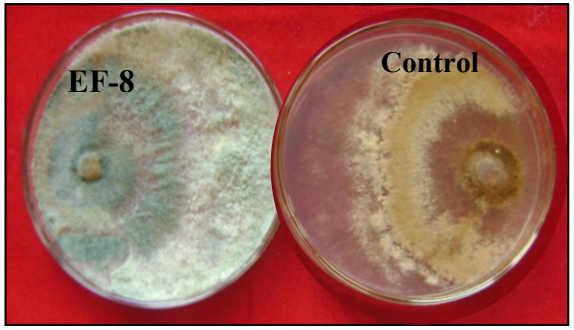
Out of 36 bacterial isolates showed antagonistic activity against the pathogen under preliminary screening 24 were from rhizosphere. There were eight bacterial isolates from T_2 (RB-9, RB-14, RB-20, RB-22, RB-27, RB-28, RB-33 and RB-39), five isolates from T_5 (RB-11, RB-12, RB-17, RB-21 and RB-35), four each from T_3 (RB-3, RB-10, RB-16 and RB-18), from T_4 (RB-5, RB-7, RB-15 and RB-34) and three from T_1 (RB-8, RB-13 and RB-19). *In vitro* evaluation of selected isolates revealed (Table 17) that RB-21 (65.33%) had the highest per cent inhibition against *R. solani* (Plate 5. c) and it was immediately followed by RB-14 (63.33%), RB-27 (62.33%), RB-15 (62.00%), RB-12 (62.00%), RB-8 (61.06%) and RB-20 (60.33%). There were eight bacterial isolates those expressed per cent inhibition ranging from 60-65 per cent and fourteen isolates shown 40-60 per cent inhibition. The least per cent inhibition against *R. solani* was expressed by RB-22 (32.33%).

A total of 12 endophytic bacterial isolates were selected from preliminary screening and subjected to *in vitro* evaluation of antagonistic ability against *R. solani*. These included five isolates from T_2 (EB-2, EB-15, EB-17, EB-18 and EB-20), two each from T_1 (EB-8 and EB-19), T_3 (EB-13 and EB-14), T_5 (EB-1 and EB-3) and one from T_4 (EB-7). The results revealed that EB-2 (62.66%) having maximum per cent inhibition against *R. solani* and it was immediately followed by EB-14 and EB-3 (59.00% each). Among the selected endophytes six has shown 50-60 per cent inhibition and three endophytic bacterial isolates exhibited 40-50 inhibition and the rest showed less than 40 per cent inhibition against *R. solani.* The least per cent inhibition was exhibited by EB-13 (28.66%) (Table 18).

Plate 5. ***In vitro*** **evaluation of rhizosphere and endophytic microflora against** ***R. solani***



a. Preliminary screening of fungal (a) and bacterial (b) isolate against ***R. solani***



b. ***In vitro*** **evaluation of fungal isolate against** ***R. solani***

c. ***In vitro*** **evaluation of selected bacterial isolates against** ***R. solani***

Table 17. *In vitro* evaluation of rhizosphere bacteria against *R. solani*

Sl. No.	Bacteria	Per cent inhibition*
1	RB-3	44.33^{h}
2	RB-5	59.06^{bcdef}
3	RB-7	55.66^{efg}
4	RB-8	61.06^{abcd}
5	RB-9	36.33^{i}
6	RB-10	58.66^{bcdef}
7	RB-11	59.33^{bcdef}
8	RB-12	62.00^{abc}
9	RB-13	57.66^{cdefg}
10	RB-14	63.33^{ab}
11	RB-15	62.00^{abc}
12	RB-16	56.00^{defg}
13	RB-17	58.33^{bcdef}
14	RB-18	47.00^{h}
15	RB-19	52.66^{g}
16	RB-20	60.33^{abcde}
17	RB-21	65.33^{a}
18	RB-22	32.33^{i}
19	RB-27	62.33^{abc}
20	RB-28	58.33^{bcdef}
21	RB-33	60.00^{bcdef}
22	RB-34	56.00^{defg}
23	RB-35	56.66^{defg}
24	RB-39	55.00^{fg}

* Values followed by same superscript are not significantly different
RF- Rhizosphere Bacteria

Table 18. *In vitro* evaluation of endophytic bacteria against *R. solani*

Sl. No.	Bacteria	Per cent inhibition
1	EB-1	54.00[b]
2	EB-2	62.66[a]
3	EB-3	59.00[ab]
4	EB-7	37.66[d]
5	EB-8	56.33[b]
6	EB-13	28.66[e]
7	EB-14	59.00[ab]
8	EB-15	37.00[d]
9	EB-17	46.66[c]
10	EB-18	41.66[cd]
11	EB-19	56.33[b]
12	EB-20	45.66[c]

* Values followed by same superscript are not significantly different
EB- Endophytic Bacteria

4.8 CHARACTERIZATION OF SELECTED ANTAGONISTS

Based on the efficacy in inhibiting *R. solani* under *in vitro* condition three bacterial and one fungal isolates are selected for further studies. The selected antagonists are EB-2, RB-14, RB-21 and EF-8.

4.8.1 Characterization of selected fungal antagonist

Cultural and morphological characters of the selected fungal antagonist (EF-8) were studied by growing on PDA. Colonies were fast growing, smooth surfaced, and primarily watery translucent, sparse mycelial mat. The colour of the colony changed from whitish green to dark green. The mycelium was hyaline, septate, profusely branched and smooth walled. Conidiophores were long, flexuous highly branched and the width of the conidiophore measured 5.61 to 5.87 µm. Subglobose to ovoid and light green coloured conidia are produced on phialides which are short ampulliform, bulged at the middle and narrower at the base. The size of phialides were 5.89-6.22 × 3.32-3.48 µm. The phialides were arised at right angles to the bearer. Based on conidia and conidiophores characters, the fungus was identified as *Trichoderma* sp. (Plate 6. b and 6. c).

4.8.2 Characterization of selected bacterial antagonists

The cultural, morphological and biochemical characters of selected three bacterial isolates *viz.,* RB-14, RB-21 and EB-2 were studied in detail. The cultural characters were studied in detail by streaking on nutrient agar. The colonies of EB-2 were cream coloured, circular, smooth, slimy and convex with undulating margin whereas RB-14 had yellowish green colour, raised with smooth edged colonies. The colonies of RB-21 were greenish yellow coloured, raised with undulating margin. All the three isolates were rod shaped on microscopic observation.

The isolates were further subjected to biochemical characterization. All together 18 tests were conducted for determining the biochemical reaction of each

isolate. The results are presented in Table 19. All tree isolates EB-2, RB-21 and RB-14 showed positive results for catalase, citrate utilization, oxidase, ornithine utilization, urease but they were unable to utilize adonitol, lactose and sorbitol. However, they can utilize glucose and arabinose. The isolates exhibited negative reaction to methyl red, Voges-Proskauer, adonitol, lactose and sorbitol tests. The isolate EB- 2 was positive to indole, lysine utilization and negative to phenylalanine deamination, nitrate reduction, H_2S production while RB-14 and RB-21 were found negative to indole test but they were able to hydrolyze starch. RB-14 expressed weak reaction to lysine utilization and H_2S production. EB-2 and RB14 were negative to phenylalanine deamination, nitrate reduction. EB-2 and RB-21 were negative to H_2S production. RB-21 was positive to phenylalanine deamination and nitrate reduction. Based on cultural, morphological and biochemical properties RB-21 and RB-14 were tentatively identified as fluorescent *Pseudomonas* sp. and EB-2 is a non-fluorescent *Pseudomonas* sp.

Table 19. Cultural, morphological and biochemical characters of selected bacterial isolates

Sl. No.	Cultural/ morphological/ biochemical test	Bacterial isolates		
		EB-2	RB-14	RB-21
1	Grams staining	-	-	-
2	Configuration	rod	rod	rod
3	Colony shape	round	irregular	round
4	Margin	irregular	undulating	entire
5	Elevation	umbonate	raised	raised
6	Colour	cream	yellowish green	greenish yellow
7	Pigment production	-	+	+
8	Indole	+	-	-
9	Catalase	+	+	+
10	Methyl red	-	-	-
11	Voges-Proskauer	-	-	-
12	Oxidase	+	+	+
13	Starch hydrolysis	-	+	+
14	Citrate utilization	+	+	+
15	Lysine utilization	+	V	+
16	Ornithine utilization	+	+	+
17	Urease	+	+	+
18	Phenylalanine deamination	-	-	+
19	Nitrate reduction	-	-	+
20	H_2S production	-	+	-
21	Glucose	+	+	+
22	Adonitol	-	-	-
23	Lactose	-	-	-
24	Arabinose	+	+	+
25	Sorbitol	-	-	-

- : Negative reaction
+: Positive reaction
V: weak reaction

4.9 EVALUATION OF ORGANIC SOURCES OF NUTRIENTS AND SELECTED ANTAGONISTS AGAINST GROWTH, YIELD AND LEAF BLIGHT OF AMARANTH

A pot culture experiment was conducted to study the efficacy of organic sources of nutrients and selected potential antagonists from the field experiment for management of leaf blight of amaranth (Plate 6. a).

4.9.1 Effect of organic sources of nutrients and selected antagonists on growth and yield of amaranth

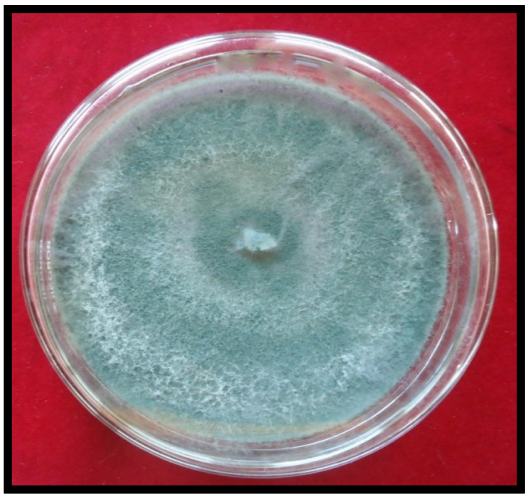
Biometric observations like shoot length, number of leaves, number of branches were recorded at 15, 30 and 45 DAT. Yield was recorded at 30 and 45 DAT and girth at collar, root length and root weight were taken at 45 DAT.

4.9.1.1 Shoot length

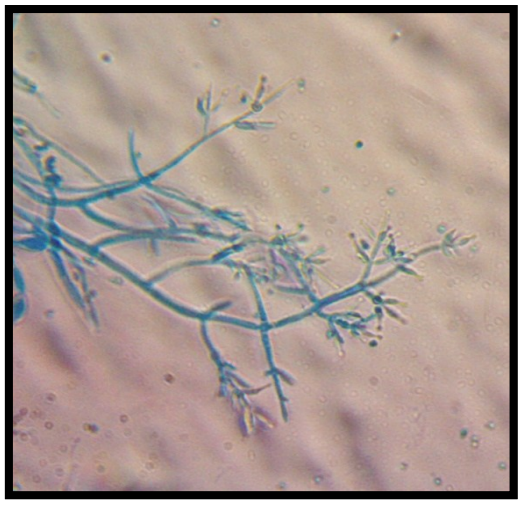
The impact of different treatments on shoot length of amaranth is presented in Table 20**.** It was observed that shoot length of plants was significantly different among treatments. At 15 and 30 DAT, the highest shoot length was in T_{10}. At 45 DAT also it was on par with T_9. AT 15 DAT the shoot length ranged from 9.25 cm to 19.12cm among the treatments. The maximum shoot length was observed in T_{10} (19.12 cm) it was followed by T_9 (18.02 cm) and T_5 (17.99 cm) and these were on par. The lowest shoot length was recorded in T_{12} (9.25 cm). At 30 DAT, T_{10} (50.55 cm) showed the highest shoot length and it was immediately followed by T_9 (50.50 cm), T_1 (44.55 cm), T_5 (43.26 cm) and these were on par. Shoot length at 30 DAT among treatments ranged from 50.55cm to 23.66 cm. The minimum shoot length was observed in T_{12} (23.66 cm). At 45 DAT T_9 (62.00 cm) recorded the highest shoot length followed by T_{10} (61.92cm), T_5 (59.51 cm), T_8 (58.87 cm) and these were on par. The lowest shoot length was recorded in T_{12} (30.78 cm).

Plate 6. Evaluation of organic sources of nutrients and selected antagonists against leaf blight of amaranth

a. Pot culture experiment



b. Pure culture of EF-8



c. Conidia and conidiophores of EF-8 (*Trichoderma* sp.)

Table 20. Effect of organic sources of nutrients and selected antagonists on shoot length of amaranth

Sl. No.	Treatment	Shoot length (cm)*		
		15 DAT	**30 DAT**	**45 DAT**
1	T_1- NPK	15.03^{bc}	44.55^{ab}	49.43^{cd}
2	T_2- Farm yard manure	15.59^{b}	39.49^{bcd}	51.31^{c}
3	T_3- Neem cake	13.58^{c}	30.80^{ef}	37.49^{fg}
4	T_4- Vermicompost	15.58^{b}	34.44^{bc}	46.05^{cde}
5	T_5- Poultry manure	17.99^{a}	43.26^{ab}	59.51^{a}
6	T_6- RB-21+T_1	15.15^{bc}	32.05^{de}	44.16^{def}
7	T_7- EB-2 +T_1	13.71^{c}	33.33^{cde}	40.74^{ef}
8	T_8- RB-14 +T_1	16.19^{b}	46.44^{b}	58.87^{ab}
9	T_9- EF-8 +T_1	18.02^{a}	50.50^{a}	62.00^{a}
10	T_{10}- *Trichoderma viride* (KAU) + T_1	19.12^{a}	50.55^{a}	61.92^{a}
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	15.93^{b}	39.61^{b}	52.15^{bc}
12	T_{12}- Absolute control	9.59^{d}	23.66^{d}	30.78^{g}

*Mean of three replication, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria
EB- Endophytic Bacteria
EF- Endophytic Fungus
DAT- Days after transplanting

4.9.1.2 Number of leaves

The influence of various treatments on number of leaves of amaranth is furnished in Table 21. It varied among treatments at 15 DAT, 30 DAT and 45 DAT. AT 15 DAT highest number of leaves were observed in T_{10} (10.24) followed by T_9 (9.76) and these were on par. AT 15 DAT the number of leaves ranged from 10.24 to 6.49. The lowest number of leaves was recorded in T_{12} (6.49). The same trend was found at 30 DAT, also where T_{10} (46.64) had the maximum number of leaves followed by T_{11} (44.11), T_9 (42.99) and these were on par. The number of leaves among treatments ranged from 46.67 to 17.62. The minimum number of leaves was recorded in T_{12} (17.62). At 45 DAT also T_{10} (58.82) had the maximum number of leaves followed by T_{11} (54.51) which were on par. The treatments with lowest number of leaves were T_{12} and T_3 (31.66 and 30.73).

4.9.1.3 Number of branches

The data presented in Table 22 revealed that at 15 DAT highest number of branches was in T_5 (1.44) which was followed by T_9 (1.41), T_1 (1.40), T_{10} (1.30) and T_{11} (1.00). At the same time branching was not started in treatments like T_{12}, T_6, T_3, T_7 and T_8. At 30 DAT, the highest number of branches was observed in T_9 (3.73) immediately followed by T_{10} (3.69) and these were on par. The lowest number of branches was observed in T_{12} (1.49). At 45 DAT, the maximum number of branches was observed on T_{10} (5.24). It was followed by T_9 (5.18), T_{11} (4.56), T_1 (4.51) and these were on par. The lowest number of branches was observed in T_{12} (2.94) at 45 DAT.

Table 21. Effect of organic sources of nutrients and selected antagonists on number of leaves of amaranth

Sl. No.	Treatment	Number of leaves*		
		15 DAT	30 DAT	45 DAT
1	T_1- NPK	8.43^{de}	26.67^{f}	46.43^{de}
2	T_2- Farm yard manure	8.74^{cd}	33.95d	44.00^{ef}
3	T_3- Neem cake	7.22^{fg}	22.27^{g}	30.73^{g}
4	T_4- Vermicompost	8.64^{cde}	33.32^{d}	44.37^{ef}
5	T_5- Poultry manure	9.27^{bc}	39.58^{c}	52.25^{bc}
6	T_6- RB-21+T_1	8.55^{cde}	30.55^{de}	40.47^{f}
7	T_7- EB-2 +T_1	7.92^{ef}	28.79^{ef}	40.07^{f}
8	T_8- RB-14 +T_1	9.27^{bc}	40.90^{bc}	49.56^{cd}
9	T_9- EF-8 +T_1	9.76^{ab}	42.99^{abc}	54.23^{b}
10	T_{10}- *Trichoderma viride* (KAU) + T_1	10.24^{a}	46.64^{a}	58.82^{a}
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	9.06^{bcd}	44.11^{ab}	54.51^{ab}
12	T_{12}- Absolute control	6.49^{g}	17.62^{h}	31.66^{g}

*Mean of three replication, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria
EB- Endophytic Bacteria
EF- Endophytic Fungus
DAT- Days after transplanting

Table 22. Effect of organic sources of nutrients and selected antagonists on number of branches of amaranth

Sl. No.	Treatment	Number of branches*		
		15 DAT	30 DAT	45 DAT
1	T_1- NPK	1.40	2.67^{cd}	4.51^{ab}
2	T_2- Farm yard manure	0.66	2.80^{cd}	4.05^{bc}
3	T_3- Neem cake	0.00	2.00^{ef}	2.99^{d}
4	T_4- Vermicompost	0.81	2.94^{cd}	4.26^{bc}
5	T_5- Poultry manure	1.44	3.09^{bc}	4.16^{bc}
6	T_6- RB-21+T_1	0.00	2.53^{cde}	3.76^{bcd}
7	T_7- EB-2 +T_1	0.00	2.34^{de}	3.57^{cd}
8	T_8- RB-14 +T_1	0.00	2.64^{cd}	3.85^{bc}
9	T_9- EF-8 +T_1	1.41	3.73^{a}	5.18^{a}
10	T_{10}- *Trichoderma viride* (KAU) + T_1	1.30	3.69^{ab}	5.24^{a}
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	1.00	3.05^{c}	4.56^{ab}
12	T_{12}- Absolute control	0.00	1.49^{f}	2.94^{d}

*Mean of three replication, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria
EB- Endophytic Bacteria
EF- Endophytic Fungus
DAT- Days after transplanting

4.9.1.4 Girth at collar

The data presented in Table 23. shows that girth at collar significantly varied among treatments. It was ranged from 3.99 cm to 2.12 cm. The girth at collar was the highest in T_{10} (3.99 cm) and it was followed by T_5 (3.94 cm), T_9 (3.93 cm), T_4 (3.80 cm) and T_8 (3.80 cm) and these were on par. The lowest girth at collar was observed in T_{12} (2.12 cm).

4.9.1.5 Root length

The effect of different treatments on root length is furnished in Table 23. The root length of different treatments ranged from 19.39 cm to 12.40 cm. it was the highest in T_{10} (19.39 cm) and followed by T_1 (19.20 cm), T_9 (18.95 cm), T_4 (18.16 cm), T_8 (17.88 cm), T_2 (17.69 cm) these were on par. The lowest root length was observed in T_{12} (12.40 cm).

4.9.1.6 Root weight

Root weight of amaranth plants varied among different treatments (Table 23) which ranged from 2.03gm to 8.42 gm. The highest root weight was observed on T_5 (8.45 g) followed by T_{10} (8.27 g), T_4 (7.49 g), T_9 (7.38 g), T_{11} (7.180 g) and these were on par. The lowest root weight was recorded in T_{12} (2.03 g).

4.9.1.7 Yield

The yield of amaranth recorded (Table 24) and was significantly different among treatments. It was the highest in T_{10} during the experiment. At 30 DAT T_{10} was on par with T_9 (0.20 kg) and T_8 (0.18 kg). The T_{12} weighed the least yield 0.33 kg. At 45 DAT the yield ranged from 0.43 kg to 0.18 kg. The highest yielder T_{10} was followed by T_1 (0.16 kg), T_5 (0.15 kg), T9 (0.14 kg), T_8 (0.13 kg), T_4 (0.12 kg) and these were on par. The lowest yield was observed in T_{12} (0.43 kg).

Table 23. Effect of organic sources of nutrients and selected antagonists on girth at collar, root length and root weight of amaranth

Sl. No.	Treatment	Girth at collar (cm)	Root length (cm)	Root weight (g)
1	T_1- NPK	3.42b^{cd}	19.22^{a}	7.69^{ab}
2	T_2- Farm yard manure	3.10^{d}	17.69^{abc}	5.13^{de}
3	T_3- Neem cake	2.14^{e}	15.10^{d}	4.00^{e}
4	T_4- Vermicompost	3.80^{ab}	18.16^{a}	7.49^{ab}
5	T_5- Poultry manure	3.94^{ab}	15.50^{d}	8.42^{a}
6	T_6- RB-21+T_1	2.90^{d}	15.44^{d}	4.00^{e}
7	T_7- EB-2 +T_1	3.25^{cd}	15.99^{cd}	6.57^{bc}
8	T_8- RB-14 +T_1	3.80^{abc}	17.88^{ab}	5.97^{cd}
9	T_9- EF-8 +T_1	3.93^{ab}	18.95^{a}	7.38^{ab}
10	T_{10}- *Trichoderma viride* (KAU) + T_1	3.99^{a}	19.39^{a}	8.27^{a}
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	3.08^{d}	16.19^{bcd}	7.18^{abc}
12	T_{12}- Absolute control	2.12^{e}	12.40^{e}	2.03^{f}

* Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria
EB- Endophytic Bacteria
EF- Endophytic Fungus
DAT- Days after transplanting

Table 24. Effect of organic sources of nutrients and selected antagonists on yield of amaranth

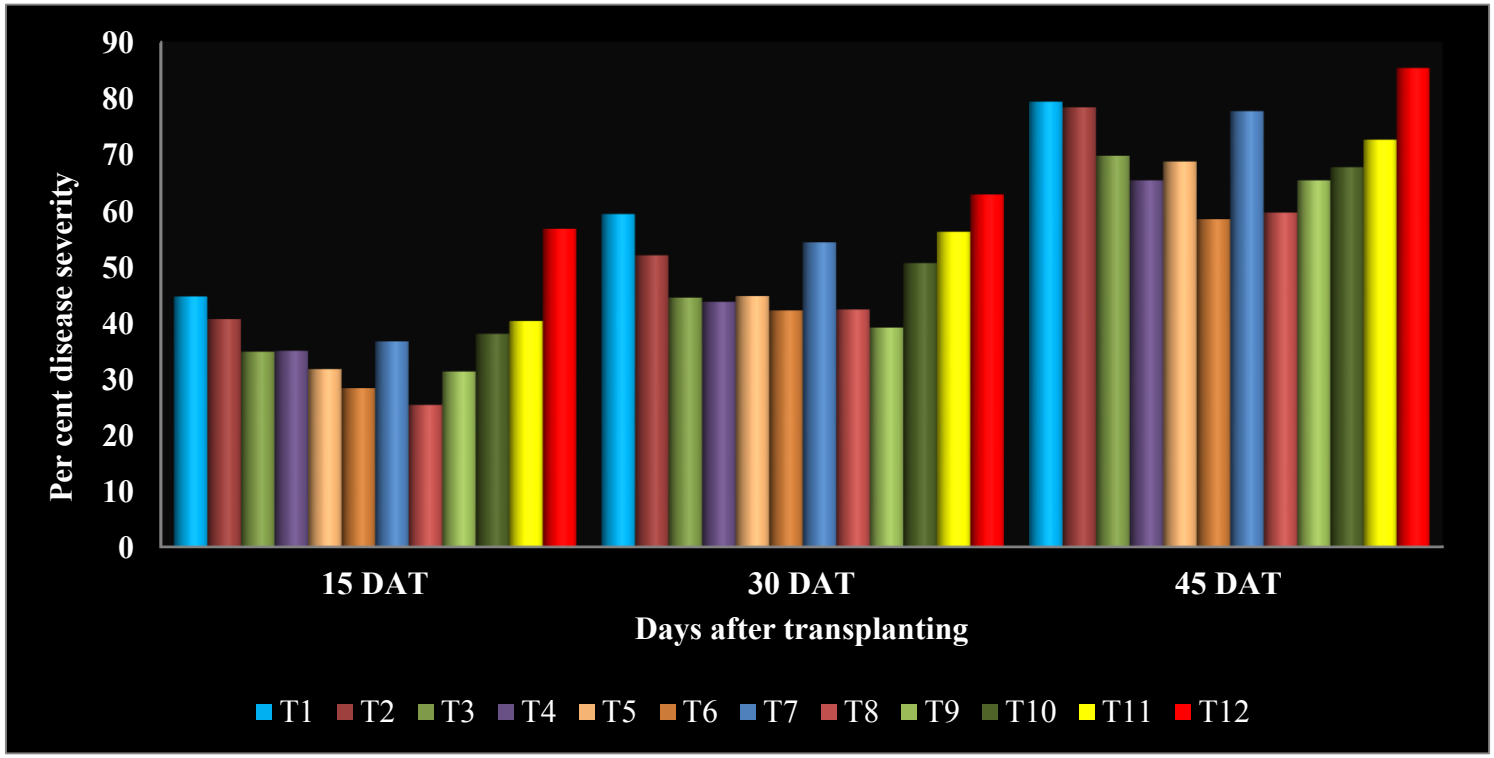
Sl. No.	Treatment	Yield (Kg)*	
		30 DAT	45 DAT
1	T_1- NPK	0.15^{bc}	0.16^{ab}
2	T_2- Farm yard manure	0.09^{d}	0.11^{cd}
3	T_3- Neem cake	0.07^{de}	0.09^{de}
4	T_4- Vermicompost	0.10^{cd}	0.12^{abcd}
5	T_5- Poultry manure	0.15^{bc}	0.15^{abc}
6	T_6- RB-21+T_1	0.11^{cd}	0.10^{cd}
7	T_7- EB-2 +T_1	0.11^{cd}	0.12^{bcd}
8	T_8- RB-14 +T_1	0.18^{ab}	0.13^{abcd}
9	T_9- EF-8 +T_1	0.20^{ab}	0.14^{abcd}
10	T_{10}- *Trichoderma viride* (KAU) + T_1	0.21^{a}	0.18^{a}
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	0.09^{d}	0.11^{cd}
12	T_{12}- Absolute control	0.03^{e}	0.04^{e}

* Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria
EB- Endophytic Bacteria
EF- Endophytic Fungus

4.9.2 Effect of organic sources of nutrients and selected antagonists against leaf blight of amaranth

The effect of different nutrient sources and selected antagonists on per cent disease incidence and severity of leaf blight of amaranth were recorded. The observations were taken during 15, 30 and 45 DAT. The disease incidence was 100 per cent during different stages of observation. There was significant difference among treatments in per cent disease severity (Fig. 5). The details are presented in Table 25. Throughout the crop period disease severity increased among treatments. At 15 DAT, T_8 (25.41%) was superior among the treatments with lowest disease severity and 43.13 per cent reduction over control. It was followed by T_6 (36.39%), T_9 (29.78%), T_5 (28.81%). The highest disease severity was observed in T_{12} (56.78%). At 30 DAT, T_9 (39.20%) was superior over other treatments with 33.96 per cent reduction over control followed by T_8 (42.42%) and T_6 (42.27%).the highest disease severity was observed in T_{12} (62.83%). At 45 DAT, T_6 (58.50%) recorded the least disease severity with 26.35 per cent reduction over control followed by T_8 (59.66%), T_9 (65.40%) and highest disease severity was recorded in T_{12} (85.40%).

Fig. 5. Effect of organic sources of nutrients and selected antagonists against leaf blight of amaranth



T_1 – NPK, T_2 – FYM, T_3 - Neem cake, T_4 – Vermicompost, T_5 - Poultry manure

T_6 - RB-21+ T_1, T_7 - EB-2+ T_1, T_8 - RB-14 + T_1, T_9 - EF-8 + T_1, T_{10} - *Trichoderma viride* (KAU) + T_1

T_{11} - *Pseudomonas fluorescens* (KAU) + T_1, T_{12} - Absolute control

Table 25. Effect of organic sources of nutrients and selected antagonists against leaf blight of amaranth

Sl. No.	Treatment	Per cent disease severity					
		15 DAT	PROC	30 DAT	PROC	45 DAT	PROC
1	T_1- NPK	44.75^{g}	-	59.42^{ef}	-	79.41^{ef}	-
2	T_2- Farm yard manure	40.73^{f}	8.97	52.08^{c}	12.35	78.40^{def}	1.27
3	T_3- Neem cake	34.93^{d}	21.90	44.83^{b}	24.51	69.79^{cd}	12.12
4	T_4- Vermicompost	35.08^{d}	21.56	44.54^{b}	24.99	65.41^{abc}	17.64
5	T_5- Poultry manure	31.83^{c}	28.81	43.79^{b}	26.25	68.76^{c}	13.42
6	T_6- RB-21+T_1	28.43^{b}	36.39	42.27^{ab}	28.81	58.50^{a}	26.35
7	T_7- EB-2 +T_1	36.75^{de}	17.84	54.37^{de}	8.48	77.72^{def}	2.31
8	T_8- RB-14 +T_1	25.41^{a}	43.13	42.42^{ab}	28.56	59.66^{ab}	24.86
9	T_9- EF-8 +T_1	31.40^{c}	29.78	39.20^{a}	33.96	65.40^{abc}	17.65
10	T_{10}- *Trichoderma viride* (KAU) + T_1	38.10^{ef}	14.83	50.69^{c}	14.67	67.76^{bc}	14.68
11	T_{11}- *Pseudomonas fluorescens* (KAU) + T_1	40.39^{f}	9.72	56.26^{de}	5.30	72.63^{cde}	8.54
12	T_{12}- Absolute control	56.78^{h}	-26.83	62.88^{f}	-5.813	85.40^{f}	-7.55

* Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0.05)
RB- Rhizosphere Bacteria, EB- Endophytic Bacteria, EF- Endophytic Fungus, DAT- Days after transplanting
PROC- Per cent Reduction Over Control

4.10 METEOROLOGICAL DATA DURING EXPERIMENT

During the field and pot culture experiments meteorological parameters which directly influence disease were recorded. In general rain fall and mean relative humidity were higher during pot culture experiment. Monthly average rain fall ranged from 82 to 369.8 mm during the field experiment. Whereas during the pot culture experiment it ranged from 469.8 to 768.00 mm. The highest amount of rain fall was received in July 2014 during pot culture experiment. The mean relative humidity was also higher during pot culture experiment, which ranged from 85 to 87 per cent compared to 73 to 85 per cent during the field experiment (Table 26).

Table 26. Meteorological data during field and pot culture experiments

Parameter	2013				2014		
	August	September	October	November	June	July	August
Mean relative humidity (%)	84	85	83	73	85	87	87
Rain fall (mm)	305.9	344.1	369.8	82.0	469.8	768.0	599.8

Discussion

5. DISCUSSION

Organic farming is an eco-friendly system of cultivation which can maintain the soil health in terms of soil biological fertility and productivity besides producing quality produce which can fetch high price in the market (Krishnakumar *et al.,* 2005). This is particularly true in the case of a leafy vegetable like amaranth. Incorporation of organic sources of nutrients not only improves the physical properties of the soil but they also prevent the increase of soil borne pathogens by supporting the saprophytic and beneficial microflora in the soil. Pathogen suppression in soil is of microbial origin mainly brought about by biological buffering affected by the beneficial microflora. Accordingly the density and diversity of soil microflora is an indicator of soil health. But the application of fungicides and fertilizers causes depletion of microbial population. This can be prevented by substitution of fertilizers with organic sources of nutrients. Moreover, most of the popular biocontrol agents were isolated from rhizosphere of crop plants.

Amaranth is the most popular leafy vegetable in Kerala. It is rich in vitamin C, A and iron in addition, it also provides protein, calcium, folic acid and phosphorus. Plant parts like leaves, stem and seeds are edible and are being used as food all over the world. Depending on the species of amaranth colour of leaves and shoots varies from green to deep red or brown. Foliar diseases are major constraints in the amaranth cultivation. Being a leafy vegetable, use of fungicides is not advisable for amaranth. Hence there is a need for effective biological control against the diseases.

Studies conducted elsewhere have revealed the importance of organic farming in improving the growth and yield of amaranth and also success of biological control against soil borne diseases. Hence it is ideal to have a detailed investigation on the effect of various organic sources of nutrients as well as the effect of beneficial microflora in reducing the disease.

In the present investigation effect of various organic sources of nutrients on the foliar diseases, growth and yield of amaranth was studied. Role of organic sources of nutrients on rhizosphere microflora, in turn affecting crop growth and disease reduction was also assessed. Antagonistic potential of the different rhizosphere and endophytic microbes isolated from amaranth was tested under *in vitro* and *in vivo* conditions.

5.1 ISOLATION AND IDENTIFICATION OF THE PATHOGEN

During the study amaranth plants grown in the field experiment were observed for occurrence of any foliar disease. But only disease noticed was leaf blight. The pathogen was isolated from infected leaves and the characters studied. Initially, the hyphae were hyaline later turned to light brown. Hyphae are characteristically branched and arise at right angles from below the septa and had distinct constriction at the point of branching. Sclerotia were produced on the surface of the mycelium after 7 to 10 days, and were irregularly shaped, light brown to dark brown coloured. The symptoms were similar under natural and artificial conditions. Based on cultural and morphological characters of the pathogen along with pathogenicity test on amaranth plants it is identified as *Rhizoctonia solani* (Kuhn). Results confirmed the findings by Nayar *et al*., (1996), Gokulapalan *et al*, (2000), Smitha (2000), Priyadarsini (2003) and Uppala (2007).

5.2 QUANTITATIVE ESTIMATION OF MICROFLORA IN ORGANIC SOURCES OF NUTRIENTS

Results of the present investigation showed that poultry manure contains the maximum number of fungi, bacteria and actinomycetes followed by vermicompost, farm yard manure and neem cake. This may be due to the fact that these contain high amount of nutrients (Ayeni *et al*., 2012) as indicated by the high rate of growth of plants grown with these organic sources nutrients. The microbial population was the lowest in neem cake in which the growth of amaranth plants was also the lowest.

5.3 EFFECT OF ORGANIC SOURCES OF NUTRIENTS ON GROWTH, YIELD AND LEAF BLIGHT OF AMARANTH

A field experiment was conducted to study the influence of different organic sources of nutrients like farm yard manure, neem cake, vermicompost and poultry manure on leaf blight of amaranth.

5.3.1 Effect of organic sources of nutrients on growth and yield of amaranth

Biometric parameters like shoot length, leaves, number of branches, girth at collar, root length, root weight, shoot weight all these were the highest in plants grown poultry manure (T_5) as organic nutrient source. It was superior to all other treatments in the case of number of branches, number of leaves and shoot weight. Better performance of amaranth by application of poultry manure has been reported by earlier workers (Geethakumari *et al.,* 2005; Okokoh and Bisong, 2011). Higher content of nutrients and higher population of soil microflora definitely leads to better performance of any crop. However in the case of biometric characters and yield of amaranth, poultry manure was immediately followed by NPK. It shows that supply of nutrients is also very important in the plant growth. But presence of beneficial microflora alone has brought about the enhanced performance in poultry manure as compared to NPK. Other organic sources of nutrients like farmyard manure, vermicompost and neem cake gave lesser yield compared to NPK. This result confirms the importance of major nutrients. (Maerere *et al.,* 2001).

5.3.2 Effect of organic sources of nutrients on leaf blight of amaranth

The experiment was conducted during August to November 2013. A total of 768.63 mm rain fall was received and there was 40 rainy days during the period of experiment. The average relative humidity was above 80 per cent till the final harvest (Table 26). These climatic factors were conducive for the disease and there was 100 per cent disease incidence at different intervals of observation. The per cent disease severity gradually increased during the period of experiment. However, there was no

significant difference among the treatments. The disease severity ranged from 42.9 per cent to 52.20 per cent at 30 DAT. It was noticed that the lowest disease severity was in poultry manure in which there was only 10.08 per cent reduction over control. Since poultry manure supported the highest population of microflora among the different organic sources of nutrients used, the competition from these microbes might have reduced the activity of the pathogen. The growth and yield of amaranth was also the highest in poultry manure which may be due to the release of nutrients contained in it which supported increase in microflora and in turn reduced the disease severity (Cooperband, 2002). Maximum disease severity was observed in neem cake which contained the lowest population of microflora.

Organic sources of nutrients were applied so as to replace nitrogen (N) in the recommended dose for amaranth. Disease was greater at low level of balanced nutrition than at normal level (Bloom and Couch, 1960). However, higher level of potassium decreases disease severity (Sarhan and Jalal, 1988.) In the present study disease severity was the lowest in poultry manure (T_5) which was followed by farm yard manure. The nutrient content in poultry manure is 1.2:1.4:0.8 and in farm yard manure it is 1:0.5:1 per cent of N:P_2O_5:K_2O respectively. The content of N and K_2O are almost equal in these two treatments. Hence these provided balanced nutrition even though applied according to the N content. Where as in the case of neem cake (T_3) which contains 5:1:1.5 per cent N:P_2O_5:K_2O respectively the potassium supplied was only 30 per cent of the required quantity. Accordingly the highest disease severity was recorded in neem cake. In the case of vermicompost also the nutrient content was 1.2:0.1:0.5 which provided only 42 per cent of the required quantity of potassium.

5.4 ISOLATION AND ENUMERATION OF RHIZOSPHERE MICROFLORA

The density and diversity of soil microbial population is an indicator of soil health. Soil microorganisms play a very important role in soil fertility and plant

health. Even though application fertilizers improve crop production, recently concerns have been raised about the environmental problems and the long term sustainability of the practice (Nakhro and Dkhar, 2010). On the other hand use of organic materials increased nutrient status, microbial activity and productive potential of soil (Kang *et al.,* 2005). Owing to rapid growth habit microbial community is a more reactive component of ecosystem to essential stress than plants and animals (Panikov, 1999). Hence changes in microbial communities are indicators of ecological disturbances. Root exudates produced by plants also influence rhizosphere microflora and these are plant specific (Grayston *et al.,* 1998).

In the present study it was noticed that after application of organic sources of nutrients the population of microflora increased during the period of experiment. The pre-treatment fungal population was not significantly different but there was an increasing trend after application of treatments up to 60 days after transplanting. After that there is a decline in the fungal population in all treatments except NPK (T_1). During crop growth, nutrient uptake by plants increases leading to insufficient supply of nutrients for fungi. Hence the fungal population decreased when the crop growth is at its peak (Swer *et al.,* 2011). In the present study it was observed that population of rhizosphere fungi was the highest in poultry manure (T_5) at 60 and 75 DAT. At 30 DAT it was the highest in T_4 (vermicompost) but on par with farmyard manure (T_2) and poultry manure (T_5). Hence it may be assumed that poultry manure is the best for supporting the rhizosphere fungi. However the fungal population was the least in plots treated with fertilizer alone (T_1). Hence organic sources of nutrients are preferred over fertilizer as it activates the microbial biomass (Emede *et al.,* 2012). During the experiment the fungal population differed significantly among the treatments. This may be due to the type and amount of organic sources of nutrients supplemented and the degree of decomposition (Swer *et al.,* 2011). The lower fungal population before treatment application is attributed to lack of vegetation and lack organic sources of nutrients. At 75 DAT, there was an increase in fungal population

in T_1, which indicate that, the effect of inorganic sources of nutrients on fungi persisted only up to 75 days and afterwards, the fungi could resume to the previous level of population density.

The bacterial population continuously increased up to 60 days after treatment application except in NPK (T_1) in which at 75 DAT slight increase of bacterial population was noticed. Before treatment application the population of bacteria and fluorescent pseudomonads were not significantly different. Among all the treatments poultry manure (T_5) had the highest population of bacteria and fluorescent pseudomonads which were followed by farm yard manure (T_2). In the present investigation, earlier it was found that, there is very high population of bacteria in poultry manure (Table 1). The increase of bacterial population may be due to availability of greater organic carbon after application of organic sources of nutrients in the soil (Fraser *et al.,* 1994). Krishnakumar *et al.* (2005) reported that fertilizer treated soils contain lower bacterial count than organic sources of nutrients treated ones. Propagule densities of fluorescent pseudomonads were significantly higher in soils amended with poultry manure than with fertilizers (Bulluck and Ristaino, 2001). Hence addition of poultry manure improves soil health to a great extent. However, presence of higher number of enteric pathogenic food borne bacteria is also reported when poultry manure or farm yard manure is added to the soil (Machado *et al.,* 2006). Hence there is a risk of contamination of produce by contact with soil, rain splashes and during cultural operations when these are used as source of nutrients.

The population of actinomycetes in rhizosphere was not significantly different. After treatment application actinomycetes count in soil increased up to 60 days. Throughout the crop period higher actinomycetes population was found in vermicompost (T_4). Even though the population of actinomycetes was slightly more in poultry manure and farm yard manure, it was not reflected in the rhizosphere. At 30 DAT the lowest population was in farmyard manure (T_2) but it was on par with NPK (T_1) and poultry manure (T_5). So also, there was no significant difference in the

population after 75 DAT. The results are in accordance with the earlier reports by Nakhro and Dkhar, (2010) and Sudhakaran *et al.* (2013) where actinomycetes were more in organic sources of nutrients treated than in fertilizer treated soil. The fungal and bacterial population in vermicompost was less compared to other treatments but the actinomycetes population was more in rhizosphere of vermicompost. This may be due to less competition of fungal and bacterial population in rhizosphere of vermicompost treated plants led to increase in population of actinomycetes.

5.5 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROFLORA

One of the objectives of the study was isolation of endophytes from amaranth plants. Endophytes were isolated from stem and roots. Bacterial communities were observed to be predominant in plant samples and the highest count was recorded in roots. It is known that endophytes mainly originate from rhizosphere and hence their population is more in roots. Similar results were reported by many workers (Uppala, 2007 and Amaresan *et al.,* 2014). The highest population of endophytes was present in plants grown with poultry manure as organic nutrient source. The bacterial population from stem and roots were maximum in farmyard manure (T_2). However, it was on par with poultry manure (T_5). Endophytes were more in plants grown with organic sources of nutrients. Amending organic sources of nutrients increase microbial count in soil. Several reports say that most of the endophytic genera are same as those in rhizosphere (Ryan *et al.,* 2008). Population of endophytes in plants from poultry manure was more and it may be more due to the fact that, after application of poultry manure population of rhizosphere microbes enhanced, which in turn led to increase in endophytic population. Many workers reported that high organic carbon in poultry manure served as food for microbes and this enhanced the population of rhizosphere microflora and this lead to increased endophytic population (Mshelia and Degri, 2014).

5.6 *IN VITRO* EVALUATION OF MICROORGANISMS AGAINST THE PATHOGEN

Even though results of *in vitro* studies may not be directly translated to field conditions. They are important for making intelligent guesses about likely candidates as biological control agents (Mejia *et al.,* 2008). Microbes from endosphere and rhizosphere collectively help the plants to resist pathogen attack. But about many of them are showing direct antagonism to pathogens. Based on cultural and morphological characters 48 fungal, 61 bacterial, 10 fluorescent pseudomonads and four actinomycetes were isolated from rhizosphere and endosphere of amaranth plants grown in different treatments. In general higher number of fungi and bacteria were present in soil treated with poultry manure and farmyard manure. There was more endophyte population also in plants from these treatments. Out of 30 rhizosphere fungal isolates, 11 and 10 were from poultry manure and farm yard manure respectively. In the case of bacteria also 13 rhizosphere bacterial isolates were from farm yard manure treated plots followed by 10, nine, eight each from poultry manure, vermicompost and neem cake respectively. These results shows that poultry manure and farm yard manure support more variability of fungi and bacteria also. Hence in the present study all the 123 isolates were subjected to preliminary screening to identify those organisms which have antagonism against *R. solani*. It was found that all together 61 isolates were antagonistic towards the pathogen in varying degrees. These were subjected to further evaluation using dual culture method to identify the best suitable candidates for biocontrol of leaf blight of amaranth.

Out of 48 fungal isolates rhizosphere contributed 62.50 per cent and endosphere 37.50 per cent. However, 17 fungal isolates from rhizosphere showed antagonism against *R. solani* under *in vitro* condition. Out of these, five each were from farm yard manure and poultry manure. The results suggest that farm yard manure and poultry manure contains not only more number of fungi but also there are more antagonists in them. Among the 17 fungal antagonists from rhizosphere five

isolates showed an antagonistic activity higher than 60 per cent. In this, two each were from poultry manure, farmyard manure and one from vermicompost. Moreover, of the rhizosphere fungal isolates RF-21 (80.37%) had maximum per cent inhibition against *R. solani in vitro*. It was isolated from farm yard manure. Other isolates like RF-3 and RF-18 are from poultry manure, RF-24 from farm yard manure and RF-10 from vermicompost also exhibited per cent inhibition above 60 per cent. From the rhizosphere of potato fungal isolates with more than 60 per cent inhibitory effect on mycelial growth of *R. solani* has been earlier reported by Lahlali *et al.* (2007).

Altogether 18 endophytic fungal isolates were collected, out of which only eight showed antagonism and in these two each were from poultry manure (T_5) (EF-8 and EF-12), farm yard manure (T_2) (EF-2 and EF-10) and the rest is from NPK (T_1) (EF-7) and neem cake (T_3) (EF-3). Of the antagonistic endophytic fungi EF-8, isolated from plants grown in poultry manure treated plots recorded the highest per cent inhibition of 100 per cent within three days of inoculation. Later in the study EF-8 was identified as *Trichoderma* sp. Several workers reported the antagonistic activity of *Trichoderma* sp. against *R. solani* under *in vitro* condition. It can produce extra cellular enzymes (Haran *et al.,* 1996) or antifungal antibiotics or compete with pathogens for food, space *etc.* (Ghisalberti and Rowland, 1993). Moreover, it promotes plant growth (Inbar *et al.,* 1994), and induces resistance in plants (De Meyer *et al.,* 1998; Grondona *et al.,* 1997).

Altogether 71 bacterial isolates were collected from rhizosphere and endosphere. Among these, 45 were from rhizosphere out of which only 24 *i.e.* 53.30 per cent showed antagonism towards *R. solani in vitro*. However, only 12 *i.e.* 46.15 per cent of endophytic bacterial isolates out of 26 seemed to be antagonistic to *R. solani.* From rhizosphere eight bacterial isolates (RB-8, RB-12, RB-14, RB-15, RB-20, RB-21, RB-27 and RB-33) showed per cent inhibition above 60 per cent. Out of 24 rhizosphere antagonistic bacterial isolates 33 per cent was from farm yard manure and 20.80 per cent from poultry manure. Earlier results revealed that poultry

manure treated rhizosphere soil contains more number of bacteria but the antagonistic bacteria are more in farm yard manure. Under dual culture technique RB-21 showed the highest antagonism against *R. solani* which was isolated from poultry manure. Next to RB-21, RB-14 from farm yard manure showed the highest antagonism under *in vitro* condition. Smitha (2000) and Priyadarshini (2003) also reported that bacterial isolates from rhizosphere exhibited antagonism against *R. solani*. In the field experiment however, less disease severity and more growth rate of amaranth plants was observed in poultry manure (T_5). This suggests that there is general growth promotion and disease suppression by the saprophytic microflora even though they are not antagonistic. More over RB-21 which showed the highest antagonism against *R. solani* under dual culture was also isolated from poultry manure.

Out of 36 bacterial isolates which showed antagonistic property against *R. solani* 33 per cent was from endosphere. Moreover, five of them were from farm yard manure (T_2). *In vitro* evaluation of selected endophytic bacterial isolates against *R. solani* showed highest per cent inhibition in EB-2 (62.66%) and it was isolated from farm yard manure (T_2). Antagonistic endophytic bacteria against *R. solani* has been reported earlier by Rosales *et al.* (1993) in rice, Chen *et al.* (1995) in cotton, Sturtz *et al*. (1998) in clover and potato, Rangeshwaran *et al.* (2002) in chick pea, sunflower and chilli, Lahlali *et al.* (2007) in potato and Uppala (2007) in amaranth. For further studies RB-21, RB-14 and EB-2 were selected. Later in the studies these were identified as fluorescent and non-fluorescent *Pseudomonas* sp. There are many reports of *Pseudomonas* sp. as good biocontrol agents. Several workers reported various strains of *Pseudomonas fluorescens* which produce varying amounts of antifungal compounds. They also show mycoparasitism (Cook and Baker, 1983), production of siderophores (Elad and Baker, 1985), production of cell wall degrading enzymes chitinase and *β*-1,3-glucanase (Lim *et al.,* 1991; Velazhahan *et al.,* 1999; Nagarajkumar *et al.,* 2004) endochitinase and chitobiosidase (Nielson and Sorensen, 1999).

5.7 CHARACTERIZATION OF SELECTED ANTAGONISTS

Based on the efficacy in inhibiting *R. solani* under *in vitro* condition one fungal isolate and three bacterial isolates were selected for further studies. The selected antagonists are EB-2, RB-14, RB-21 and EF-8. Attempts were made to identify the selected microorganisms based on cultural and morphological characters.

5.7.1 Characterization of selected fungal antagonist

Based on cultural and morphological characters, the selected fungal antagonist (EF-8) was identified as *Trichoderma* sp. There are earlier reports of *Trichoderma* sp. existing as endophyte in amaranth (Uppala, 2007).

5.7.2 Characterization of selected bacterial antagonists

Based on Gram's reaction and results of 18 biochemical tests RB-21, RB-14 and EB-2 were tentatively identified as fluorescent pseudomonads and non-fluorescent pseudomonad respectively (Stanley *et al.,* 1989). Existence of the fluorescent pseudomonads and non-fluorescent pseudomonad in rhizosphere and endosphere of plant is well documented (O' Sullivan and O' Gara, 1992; Loper and Henkels, 1999; Dobbelaere *et al*., 2003).

5.8 EVALUATION OF ORGANIC SOURCES OF NUTRIENTS AND SELECTED ANTAGONISTS AGAINST GROWTH, YIELD AND LEAF BLIGHT OF AMARANTH

The main objective of this study was management of leaf blight of amaranth by biological control. In order to evaluate the selected antagonists from *in vitro* studies in comparison with the organic sources of nutrients, a pot culture experiment was conducted.

5.8.1 Effect of organic sources of nutrients and selected antagonists on growth and yield of amaranth

As in the case of field experiment, in pot culture experiment also there was significant difference among the treatments in various biometric characters studied. *Trichoderma viride* (T_{10}) recorded the highest value in all the parameters except shoot length at 45 DAT, number of branches at 15 and 30 DAT and root weight at 45 DAT. This is in accordance with growth promoting effect of *Trichoderma* sp reported by several workers (Baker, 1991; Kleifeld and Chet, 1992; Altomare *et al*., 1999; Lo and Lin, 2002). Several mechanisms are involved in growth promotion by microorganisms they are, enhancing the supply of mineral nutrients, prevention of the activity of deleterious microorganisms and direct growth promotion by production of phyto hormones (Welbaum *et al.,* 2004).

5.8.2 Effect of organic sources of nutrients and selected antagonists against leaf blight of amaranth

The pot culture experiment was conducted during June to August 2014 when the climatic conditions were highly conducive for the disease. During the experiment a total rain fall of 1441.6 mm was received and the relative humidity was above 85 per cent (Table 26). Hence there was 100 per cent disease incidence at different intervals of observation and PDS ranged from 56.78 at 15 DAT to 85.40 at 45 DAT. In this experiment the first five treatments were the same as that in the field experiment. Here also it was found that, there is less disease severity in plants grown with poultry manure as organic source of nutrient with 28.81, 26.25 and 13.42 per cent reduction over control at 15, 30 and 45 DAT respectively. During field experiment the highest PDS observed was 64.98. But here the maximum PDS is 85.40. The higher per cent disease severity in pot culture experiment might be due to the higher rain fall and relative humidity experienced during the period. Even though there was significant difference in the population of microflora, there was no

significant difference among the treatments in field experiment on per cent disease severity. But in the pot culture experiment the selected antagonistic microorganisms had a significant effect on the disease. These findings suggest the need of selective isolation of antagonists for successful biological control. In the pot culture experiment the plants treated with EB-2, RB-21, RB-14 and EF-8 exhibited less disease severity. So also those treated with *Trichoderma viride.* At 30 DAT EF-8 was the best treatment with 43.13 per cent reduction in PDS over control which was earlier in the study identified as *Trichoderma* sp. Ability of *Trichoderma* sp. to suppress various soil borne pathogens is well documented and the different mechanisms are production of antifungal antibiotics (Ghisalberti and Rowland, 1993), promotion of plant growth (Inbar *et al.,* 1994), extra cellular enzymes (Haran *et al.,* 1996), and induced resistance in plants (Grondona *et al.,* 1997; De Meyer *et al.,* 1998). In addition to that, Shalini and Kotasthane (2007) also reported that major mechanism of *Trichoderma* sp. to suppress *R. solani* is by competition. Even though various species of *Trichoderma* are very efficient in reducing soil borne plant diseases the efficiency is lesser against foliar diseases owing to lack of medium for survival. But the isolate EF-8 being an endophyte it can give better control in the case of foliar pathogens too.

Disease reduction by rhizosphere and endophytic bacteria is also reported by many workers. They also promote plant growth and offer systemic protection against plant diseases (Zhou and Paulitz, 1994). The isolates EB-2 (non-fluorescent *Pseudomonas* sp.), RB-14 (fluorescent *Pseudomonas* sp.), RB-21 (fluorescent *Pseudomonas* sp.) and EF-8 (*Trichoderma* sp.) gave 62 to 100 per cent inhibition of *R. solani* in *in vitro* evaluation. These isolates gave significant reduction of the disease in the pot culture experiment also. This shows the efficacy of rhizosphere and endophytic antagonists to control the plant pathogen. The isolate EF-8 (*Trichoderma* sp.) obtained from endosphere of amaranth grown with poultry manure as organic source of nutrient was highly effective (100%) against the pathogen in *in vitro* and it

recorded the least disease severity *in vivo* during 30 and 45 DAT. Similarly the isolate RB-14 (fluorescent *Pseudomonas* sp.) obtained from farm yard manure treated plots also showed high antagonistic activity (63.33%) in *in vitro* and it recorded the least disease severity *in vivo* at 15 DAT. Hence these two isolates may be further evaluated and used for the biological management of the disease.

In conclusion, less disease severity and higher growth rate was observed in plants grown with poultry manure as source of nutrient in field experiment. It can be interpreted that there is general growth promotion and disease suppression by the saprophytic microflora even though they are not antagonistic. Although there was significant difference in the population of microflora, there was no significant difference among the treatments in field experiment on per cent disease severity. But in the pot culture experiment the selected antagonistic microorganisms recorded a significant reduction in disease severity. These findings suggest the need of selective isolation of antagonists for successful biological control. There was higher population and variability of rhizosphere microflora when organic sources of nutrients are used in place of fertilizers. Out of 30 rhizosphere fungal isolates, 11 were from poultry manure and 10 from farm yard manure. In the case of bacteria 13 rhizosphere bacterial isolates were from farm yard manure treated plots followed by ten, nine and eight each from poultry manure, vermicompost and neem cake respectively. The result shows that poultry manure and farm yard manure support more variability of fungi and bacteria. In addition to that, out of 24 rhizosphere antagonistic bacterial isolates 33.00 per cent was from farm yard manure and 20.80 per cent from poultry manure. The isolate EF-8 (*Trichoderma* sp.) recorded 100 per cent inhibition of the pathogen in dual culture, it gave the highest reduction in disease severity in pot culture experiment and also it was the second best in growth promotion. Hence this isolate may be further evaluated and used for the biological management of the disease.

Summary

6. SUMMARY

Amaranth is the most important and popular leafy vegetable of Kerala. Rapid growth, quick rejuvenation after each harvest, higher yield per unit area and easiness in cultivation make it the cheapest vegetable which is rightly described as the 'poor man's spinach'. It is a rich source of vitamins and minerals. Among the diseases of amaranth, leaf blight caused by *Rhizoctonia solani* is a major threat for growers. Even though chemical control is promising especially under intensive cropping programmes, use of fungicides causes environmental and health hazards. It is especially true in a leafy vegetable like amaranth. Therefore sustainable biological methods are needed for disease management. Hence, the main objective of this study was to find out the effect of different organic sources of nutrients on leaf spot diseases of amaranth and to evaluate the antagonistic potential of rhizosphere and endophytic microflora towards *Rhizoctonia solani* (Kuhn) causing leaf blight. The study consisted both field and pot culture experiments. Here are the findings of the investigation summarized below:

1. The fungus causing leaf blight disease was isolated and its pathogenicity proved. The cultural and morphological characters of the pathogen along with the pathogenicity on amaranth confirmed it as *Rhizoctonia solani* (Kuhn).
2. Quatitative estimation of microflora in organic sources of nutrients revealed that poultry manure contains the highest number of fungi, bacteria and actinomycetes followed by vermi compost, farm yard manure and neem cake.
3. The study on effect of different organic sources of nutrients like farm yard manure, neem cake, vermi compost and poultry manure on leaf spot of amaranth showed that there was no significant difference in the per cent disease severity (PDS) among treatments. In all treatments PDS gradually increased throughout the crop period.

4. Biometric parameters like shoot length, number of leaves, number of branches, girth at collar, root length, root weight and shoot weight were the highest in plants grown with poultry manure (T_5) as organic nutrient source.
5. The isolation and enumeration of rhizosphere microflora revealed that after application of organic sources of nutrients the population of microflora increased in the rhizosphere. But the population was the lowest in fertilizer (T_1) treated plots.
6. In general, bacteria were predominant followed by fungi and actinomycetes. The pre-treatment fungal, bacteria, fluorescent pseudomonads and actinomycetes population was not significantly different. But the population varied significantly among the treatments throughout the period of experiment.
7. It was noticed that fungal population decreased after 60 DAT in all treatments except in T_1, in which slight increase in population was observed.
8. The endophytic microorganisms were isolated from stem and roots of amaranth plants grown in different organic sources of nutrients. The data revealed that endophytes were few in number when compared to rhizosphere microorganisms and bacteria are predominant in the endosphere of amaranth plants.
9. The endophytic fungal and bacterial population showed significant difference among treatments and in general, both fungi and bacteria are more in roots. Actinomycetes could not be isolated from any of the samples.
10. Rhizosphere and endophytic microbes were isolated during the field experiment. Altogether 123 microbial isolates including 48 fungal, 61 bacterial, 10 fluorescent pseudomonads and 4 actinomycetes were collected from rhizosphere and endosphere.
11. Preliminary screening under *in vitro* condition showed that 25 fungal and 26 bacterial and 10 fluorescent pseudomonad isolates are antagonistic to *R. solani*.
12. Among the fungal isolates, 17 from rhizosphere and eight from endosphere showed antagonism against *R. solani*. The maximum per cent inhibition of *R.*

solani was expressed by EF-8 (100.00%) and it was followed by RF-21 (80.37%). However six fungal isolates RF-3, RF-18, RF-24, RF-10, EF-2 and EF-7 exhibited per cent inhibition above 60 per cent.

13. A total of 24 rhizosphere and 12 endophytic bacterial isolates exhibited antagonism against *R. solani* in which RB-21(65.33%) had the highest per cent inhibition against *R. solani* and it was immediately followed by RB-14 (63.33%). There were nine bacterial isolates which expressed per cent inhibition between 60 and 65 per cent. From endosphere EB-2 (62.66%) showed maximum per cent inhibition against *R. solani*.
14. Based on the efficacy in inhibiting *R. solani* under *in vitro* condition three bacterial and one fungal isolate *viz.,* EB-2, RB-14, RB-21 and EF-8 respectively were selected for further studies.
15. Based on conidia and conidiophore characters, the fungal isolate (EF-8) was identified as *Trichoderma* sp.
16. Based on cultural, morphological and biochemical properties RB-21 and RB-14 were tentatively identified as fluorescent *Pseudomonas* sp. and EB-2 as non-fluorescent *Pseudomonas* sp.
17. Pot culture experiment on efficacy of organic sources of nutrients and selected antagonists on leaf blight of amaranth revealed that there was 100 per cent disease incidence in all the treatments. However, there was significant difference in the case of per cent disease severity.
18. During the period of pot culture experiment the plants treated with the selected antagonist *viz.,* RB-21, RB-14 and EF-8 and *Trichoderma viride* exhibited less disease severity compared to other treatments. However, the plants treated with EF-8 (T_9) showed the lowest per cent disease severity followed by plants in RB-14 (T_8).
19. It was observed that during the period of experiment T_{10} had the highest value for most of the biometric parameters followed by T_9 (EF-8).

20. The isolates EF-8 (*Trichoderma* sp.) and RB-14 (fluorescent *Pseudomonas* sp.) were highly effective against *R. solani* under *in vitro* and *in vivo* condition. More over these gave very good growth promoting effect. Hence these two isolates may be further evaluated and used for the biological management of the disease.

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Appendices

APPENDIX I

1. **Potato Dextrose Agar medium (PDA)**

Potato	:	200.0 g
Dextrose	:	20.0 g
Agar	:	20.0 g
Distilled water	:	1000 ml

2. Nutrient Agar medium (NA)

Peptone	:	5.0g
Beef extract	:	1.0g
Sodium Chloride	:	5.0g
Agar	:	20.0g
Distilled water	:	1000 ml
pH	:	6.5 to 7.

3. Martin's Rose Bengal Agar medium

Dextrose	:	10.0 g
Peptone	:	5.0 g
KH_2PO_4	:	1.0 g
$MgSO_4$	:	0.5 g
Agar	:	20.0 g
Rose Bengal	:	0.03 g
Streptomycin	:	30.0 mg (added aseptically)
Distilled water	:	1000 ml

4. Kenknight's Agar medium (pH 7.0)

Dextrose	:	1.0 g
KH_2PO_4	:	0.1 g
$NaNO_3$	:	0.1 g
KCl	:	0.1g
$MgSO_4$	:	0.1g
Agar	:	20.0 g
Distilled water	:	1000 ml

5. King's B medium

Peptone	:	20.0 g
Glycerol	:	10.0 ml
K_2HPO_4	:	10.0 g
$MgSO_4.7H_2O$	:	1.5 g
Agar	:	20.0 g
Distilled water	:	1000 ml
pH	:	7.2 – 7.4

APPENDIX II

I. BUFFER USED FOR ISOLATION OF ENDOPHYTIC MICROFLORA

0.02 M Potassium Phosphate buffer: 7 pH

* Stock solutions

A- 3.5 g of dibasic K_2HPO_4 in1000 ml

B- 2.7 g of monobasic KH_2PO_4 in 1000 ml

For getting 0.02 M Potassium Phosphate buffer of pH 7, Mix 61 ml of solution A with 39 ml of solution B and 100 ml water.

II. MEDIA USED FOR BIOCHEMICAL TESTS

1. **Peptone water broth**

NaCl	: 1.4 g
$NaHCO_3$	: 0.02 g
KCl	: 0.04 g
$CaCl_2$	: 0.04 g
KH_2PO_4	: 0.24 g
$Na_2HPO_4. 2H_2O$	: 0.88 g
Peptone	: 0.8 g
Distilled water	: 1000 ml

2. Starch agar medium

Beef extract	: 3.0 g
Peptone	: 5.0 g
NaCl	: 5.0 g
Agar	: 20.0 g
10% Starch solution	: 200 ml
Distilled water	: 1000 ml

Abstract

CHARACTERIZATION OF RHIZOSPHERE AND ENDOPHYTIC MICROFLORA FROM ORGANICALLY GROWN AMARANTH FOR MANAGEMENT OF LEAF SPOT DISEASES

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ABSTRACT OF A THESIS

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ABSTRACT

Amaranth is the most important and popular leafy vegetable of Kerala. Rapid growth, quick rejuvenation after each harvest, higher yield per unit area and easiness in cultivation make it the cheapest vegetable which is rightly described as the 'poor man's spinach'. However, leaf spots and blights caused by fungal pathogens are serious threats to amaranth growers. The present investigation was carried out to explore the influence of various organic sources of nutrients on leaf spot diseases and to characterize potential antagonists from organically grown amaranth for management of these diseases. During the study, the pathogen causing leaf blight disease was isolated as this was the only foliar disease observed on amaranth plants grown for the field experiment. The cultural and morphological characters of the pathogen along with pathogenicity on amaranth confirmed it as *Rhizoctonia solani* (Kuhn).

Population of fungi, bacteria and actinomycetes present in different organic sources of nutrients *viz.,* farm yard manure, neem cake, vermicompost and poultry manure were estimated. Poultry manure contained the highest number of microflora per unit weight. A field experiment was conducted to study the effect of different organic sources of nutrients on leaf blight, growth and yield of amaranth. The results revealed that, there was no significant difference among organic sources of nutrients on disease severity. However, rate of growth indicated by various biometric parameters was the highest in plants grown with poultry manure (T_5) as organic nutrient source. The population of rhizosphere microflora was estimated at periodical intervals *i.e.* pre-treatment, 30, 60 and 75 days after transplanting. The results revealed that, after application of organic sources of nutrients the microflora increased in the rhizosphere up to 60 days after transplanting. Bacteria were predominant in rhizosphere followed by fungi and actinomycetes. The pre-treatment fungal, bacterial, fluorescent pseudomonad and actinomycete population was not significantly different. But the population varied significantly among the treatments

throughout the period of experiment. The endophytic microorganisms from stem and roots showed that endophytes were lesser in number compared to rhizosphere microorganisms and bacteria are predominant in the endosphere of amaranth plants. In general, both fungi and bacteria are more in roots. Actinomycetes could not be isolated from any of the samples.

Based on colony characters, 123 different isolates were collected from rhizosphere and endosphere of amaranth plants in which 25 fungal and 36 bacterial isolates showed antagonistic activity against *R. solani*. These were further evaluated under dual culture. From rhizosphere the bacterial isolate RB-21(65.33%) had the highest per cent inhibition against *R. solani* immediately followed by RB-14 (63.33%). From endosphere EB-2 (62.66%) showed maximum per cent inhibition and the fungal isolate, EF-8 recorded cent per cent inhibition of the pathogen. These were selected for evaluation in pot culture experiment.

A pot culture experiment was conducted to study the efficacy of organic sources of nutrients and selected antagonists on management of leaf blight of amaranth. Even though there was 100 per cent disease incidence in all the treatments, significant difference was observed in the case of per cent disease severity. The plants treated with RB-14 (T_8) at 15 DAT, EF-8 (T_9) at 30 DAT and RB-14 (T_6) at 45 DAT exhibited less disease severity during the period of experiment. It was observed that T_{10} (*Trichoderma viride* of KAU) had the highest value for most of the biometric parameters and T_9 (EF-8) was the second best in promoting plant growth. Based on cultural, morphological and biochemical properties, the selected bacterial isolates, RB-21 and RB-14 were tentatively identified as fluorescent *Pseudomonas* sp. and EB-2 as non-fluorescent *Pseudomonas* sp. Based on conidia and conidiophore characters, the fungal isolate (EF-8) was identified as *Trichoderma* sp.