EFFECT OF BIOFERTILIZERS ON EARLY ROOTING, GROWTH AND NUTRIENT STATUS OF BLACK PEPPER (*Piper nigrum* L.)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled "**Effect of biofertilizers on early rooting, growth and nutrient status of black pepper (*Piper nigrum* L.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Effect of biofertilizers on early rooting, growth and nutrient status of black pepper (*Piper nigrum* L.)"** is a record of research work done independently by **Mr. Ashithraj N.**, 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 him.

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Ashithraj N.

Humbly dedicated

to

GOD

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Introduction

INTRODUCTION

Black pepper, (*Piper nigrum* L.) "the King of spices" belonging to family *Piperaceae*, is a perennial climber and is a native of the moist evergreen forests of Western Ghats.

There are two morphologically different types of crop, viz. "bush pepper" obtained from regeneration of lateral cuttings (plagiotropic branches) and "vine pepper" resulting from planting of runner/terminal vines (orthotropic branches). Bush pepper is bushy in nature of its growth and yields within one year and throughout the year. The vine pepper is a climber and needs 3-4 years for bearing.

Among the various agronomic practices to improve and achieve stable production, availability of good planting material and better soil management through integrated nutrient management is of paramount importance. However, increased use of chemical fertilizers, pesticides and such high input technology is not sustainable in the long run because of the escalating prices of these inputs and the irreversible ill-effects they cause on the physical, chemical and biological properties of soil.

Use of biofertilisers is an alternative value-added sustainable technology for modern agriculture. Microbial fertilisers are biologically active products containing one or more specific microorganisms like bacteria, blue green algae (Cyanobacterium) or fungi. These organisms are capable of nitrogen fixation, phosphate solubilisation or phosphate mobilization.

Azotobacter, an aerobic gram-negative associative nitrogen-fixing bacteria is capable of fixing N, in the rhizosphere. It is also capable of elaborating small quantities of plant growth promoting substances like vitamin B and phytohormones like IAA (Nair and Najachandra, 1995).

Azospirillum sp. is a typical example of associative nitrogen fixing gram-negative bacterium. Its nitrogen fixing, NO_3 reductase and phytohormone producing activities stimulate better root growth and establishment. *Azospirillum sp.* is known to produce significant quantities of plant growth hormones such as gibberellins, cytokinins and auxins like IAA (Tien *et al.*, 1979) which naturally results in better plant growth.

Mycorrhiza literally means fungus roots. Arbuscular mycorrhiza is a obligate symbiont in nature requiring a suitable host plant for its growth. Plants with arbuscular mycorrhizal association are capable of mobilizing more of the available nutrients from soil by extending to several centimeters from root surface (Hayman, 1982). In tropical agriculture, where soils are generally poor in available phosphorus, the AM association will enable the host plant to absorb more P by greater exploration of soil, resulting in better access to soil pool of available phosphorus. The translocation of P by AM fungi is faster not only because of increased affinity of hypha for P ions but also due to decreased threshold concentration requirement for absorption from soil solution. AM association also aids in increased absorption of water, favouring better drought tolerance in transplanted seedlings and enhanced uptake of nutrient elements such as Zn, Mn, Cu, Fe, Mg and S.

In this context, an investigation was undertaken with the following objectives:

1) To study the comparative efficiency of different AM fungi inoculated on cuttings of runners and laterals.
2) Screening of effective *Azotobacter chroococcum* and *Azospirillum lipoferum* collected from different sources inoculated on cuttings of runners and laterals.
3) To study the effect of inoculation with AM fungi, Azotobacter and Azospirillum on growth and development of potted runner cuttings.
4) To study the interaction effect of combined inoculation of Azotobacter, Azospirillum and AMF on pepper.

Review of literature

REVIEW OF LITERATURE

2.1 Propagation in black pepper

Commercially, pepper is propagated through the cuttings taken from runners, creeping shoots on ground and orthotropic shoots i.e. erect growing shoots. Cuttings taken from fruiting branches produce bushy plants. Kurup (1956) suggested that single-node cuttings can be successfully used in the propagation of black pepper. Winters and Muzik (1963) opined that the rooting of lateral fruiting branch cuttings of *Piper nigrum* was markedly improved by treatment with rootone and planting in vermiculite. Shridhar and Shyam (1983) opined that two-node cuttings proved better for black pepper multiplication than one-node cuttings. Bavappa and Gurusinghe (1978) suggested that cuttings with two or three nodes from ground runners or primary vines root better. Single-node rooted and unrooted cuttings from bamboo splits recorded 91 and 73 per cent rooting respectively.

2.2 Microbial inoculants used in pepper

2.2.1 Arbuscular mycorrhizal fungi

AMF is capable of elaborating small quantities of growth hormones. *Glomus mosseae* produces auxins, gibberellin like substances and cytokinins in *in-vivo* culture (Barea and Azcon, 1982). Edriss, *et al.,* (1984) observed greater cytokinin levels in leaves of mycorrhizal sour orange as compared to non-mycorrhizal plants of similar dry weight and a higher P concentration in roots and leaves of *axenically* grown *citrus* lemon. Increased level of cytokinin like zeatin, zeatinriboside and dihydrozeatin levels in AMF seedlings were correlated with significant increase in total dry weight and improved P nutrition.

Anandaraj and Sarma (1994) reported that inoculation of *Glomus fasciculatum* on black pepper enhanced rooting per cent due to increased level of endogenous hormones in inoculated plants. A higher cytokinin production is reported in palmarosa when inoculated with AM fungi (Neelima and Janardhanan, 1996).

2.2.1.a Influence on root growth

Inoculation with *Glomus fasciculatum* in *Viburnum suspensum* and *Podocarpus macrophylla* increased root fresh weight by 111 per cent and 258 per cent respectively. Similarly, in *Pittosporum tobira,* inoculation with *Glomus mosseae* increased root fresh weight by 115 per cent (Crews *et al.,* 1978). In cashew, similar results were obtained on inoculation with *G. fasciculatum* (Krishna *et al.,* 1983).

Gendiah (1991) reported that inoculation of one-year-old Banati grape cuttings with *G. mosseae* resulted in increase in number of roots, root length and dry weight compared to two-year-old and one-year-old uninoculated control plants.

Black pepper is reported to be a host plant for *Glomus versiforme* (Berch and Fortin, 1983; Phillip and Iyer, 1994). In black pepper cv. Karimunda, inoculation of *Glomus fasciculatum* to single-node-runner cuttings, increased the root biomass, number and length (Anandaraj and Sarma, 1994).

Tisserant *et al.* (1992) reported that inoculation of *G. fasciculatum* on *Planatus acerifolia* transformed root system morphology from herringbone pattern to more dichotomous pattern after five weeks. Berta *et al.* (1995) reported that AMF inoculation on *Prunus cerasifera* increased root weight and specific root length. Giovametti *et al.* (1996) observed that inoculation of *in vitro* grown plum shoots with *G. mosseae* influenced root morphology and displayed more branched root system.

Inoculation with *G. mosseae* and *G. constrictus* caused greater vegetative development than *G. deserticola*. Douds *et al.* (1995) reported that inoculation of *G. intraradices* increased survival and rooting percentage of cuttings of foliage ornamental tree umbrella pine (*Sciadopitys verticillata* Sieb and Zucc).

2.2.1.b Water uptake

Hardie and Leyton (1981) showed that mycorrhizal clover roots had higher hydraulic conductivity than non-mycorrhizal roots on per unit root basis. Syversten and Graham (1985) reported that Carrizo citrange and sour orange seedlings grown in a low phosphorus sandy soil inoculated with *Glomus intraradices* had more than twice root hydraulic conductivity per unit root length than non-mycorrhizal seedlings under well watered conditions.

AMF inoculation delayed wilting by six to seven days in pepper cultivars subjected to moisture stress compared to uninoculated plants (Anon, 1991).

2.2.1.c Nutrient uptake

Mosse (1973) reported an increased uptake of relatively immobile elements especially P, Zn, Cu, S, Mn and Fe in AMF inoculated plants than uninoculated plants. Timmer and Leyden (1978) opined that AMF increased uptake of P, Zn and Cu in inoculated seedlings compared to P fertilized and uninoculated citrus plants. Gaddeda *et al.* (1984) found that P concentration in apple leaves increased from 0.04 to 0.19 per cent by inoculation with *Glomus fasciculatum* in Park dale soil with an exchangeable P content of 13 ppm.

AMF fungi inoculation increased the P content in rough lemon (Tang and Hao, 1984), strawberry (Hrselova *et al.*, 1987) and *Plantago major* spp.

Pleiosperma (Bass and Lambers, 1988). Higher uptake of N, K, Ca, Zn, Mg and 20 per cent increase in P content was observed in mycorrhizal papaya seedlings than non-mycorrhizal plants (Sukhada, 1988). Shivashankar *et al.* (1988) reported that mycorrhizal plant showed a significant increase in tissue N, P and leaf nitrate reductase activity, when compared with control.

In nutrient poor or moisture deficient soils, nutrient taken up by extra matrical hyphae can lead to improved plant growth and reproduction (Garbaye, 1994).AMF inoculated black pepper cuttings showed increased P and Zn contents against uninoculated plants (Thomas and Ghai, 1988 and Sivaprasad *et al.*, 1992).

Estrada *et al.* (1995) observed complete dependence of coffee (*Coffea arabica* L.v. *Columbia)* plants on AMF, *Glomus manihotis* in treatments with lowest rates of P.

2.2.1.d Vegetative Growth

Thomas and Ghai (1988) observed an increase in plant height, number of leaves and shoot dry weight of pepper, panniyur-1 on inoculation with different AMF fungi. Similar results were reported on inoculation with *Glomus fasciculatum* (Shivashanker and Iyer, 1988), *G. mosseae* (Bopaiah and Khader, 1989), *G. fasciculatum* and *G. etunicatum* (Sivaprasad *et al.*, 1992). Black pepper is reported to be a host plant for *G. versiformae (*Philip and Iyer, 1994). In a comparative study on effect of six known species and four indigenous isolates of AMF fungi on growth and nutrition of black pepper (*Piper nigrum* L.) cultivar Panniyur-1 in an acidic sandy loam soil of medium phosphorus status, the non indigenous AMF fungi, *Glomus epigaeum*, *G. fasciculatum* and *Gigaspora* margarita were superior to native isolates which increased growth considerably (Thomas and Ghai, 1988). Neelima and Janardhanan (1996) reported that among the four inoculated *Glomus* species., *G. aggregatum* and *G. fasciculatum* recorded higher dry weight of shoot and root in palmarosa. Anandaraj and Sarma (1994) observed that inoculation with AMF enhanced vegetative growth of four different varieties of pepper viz., Sreekara, Subhakara, Panniyur-1 and Kottanadan.

Enhancement of different vegetative parameters in citrus seedlings has been reported by many workers (Shanmugham *et al.*, 1981; Chandrababu and Shanmugham, 1983; Manjunath *et al.*, 1983; Maksoud *et al.*, 1988).

2.2.2 Azospirillum

2.2.2.a Hormone production

Among all diazotrophs, *Azospirillum* is known to produce significant quantities of plant growth hormones such as gibberellins, cytokinins and auxins like IAA (Tien *et al.*, 1979). Purushothuman *et al.* (1988) observed that *Azospirillum* secretes phytohormones in pure culture. They reported that initial growth response of *Azospirillum* inoculation might be more due to the secretion of growth promoting substances than biological nitrogen fixation. The major plant growth regulators produced by *Azospirillum* include IAA (Fallik and Okon, 1984), IBA (Fallik *et al.*, 1989) Indole – 3 – ethanol, Indole-3-methanol (Crozier *et al.*, 1988), unidentified indole compounds (Bashan and Levanony, 1990), several gibberellins (Bottine *et al.*, 1989) and cytokinins (Horemans *et al.*, 1986). Okon *et al.* (1995) suggested that the presence of *Azospirillum* in the rhizosphere affect the metabolism of endogenous phytohormones in the plant.

2.2.2.b Azospirillum in soil

Azospirillum is commonly seen in association with the roots of grasses, cereals, and many cotyledenous plants especially legumes or as free living soil organism. Dobereiner (1976) in a survey found that *Azospirillum* is a common inhabitant of tropics and their occurrence is dependent on soil pH. Soil pH of 5.6 – 7.2 encourages *Azospirillum* activity. But, optimum pH for maximum nitrogenase activity was reported at pH 6.7 – 7. But, in *Panicum maximum* roots, nitrogenase activity was detected even in acidic conditions of pH 5.2. The authors attributed this to the proliferation of *Azospirillum* with in the roots. Kumari *et al.* (1977) in a survey of Indian soils revealed that soil samples of varying texture and organic matter content had *Azospirillum,* but soil below pH 5.7 were *Azospirillum* free. Moreover, they also observed that sandy soils devoid of organic matter were not favourable for *Azospirillum* where as soils rich in organic matter were suitable for the bacterium. Bashan and Levanony (1988) observed that survivability of *Azospirillum* in Israeli soils was due to adherence of the organism to soil particles, especially in clay and organic matter of top soil. Levanony and Bashan (1991) observed that bacterium produced fibrillar material which immobilized it to a specific environment.

Bashan *et al.* (1995) studied the survival of *Azospirillum brasilense* in 23 soil types and detected presence of *Azospirillum* regardless of the soil types.

2.2.2.c Azospirillum in plants

Azospirillum grows on and inside the roots of several grain crops, forage grasses, and perennial crops such as coconut, pepper and rubber where it fixes nitrogen. It is considered as a good bacterium for the tropics as it can withstand high soil temperature. It consumes less energy and easily colonises on roots and surroundings. Subba Rao (1983) analysed roots of several orchard plants like banana, citrus, guava, jack fruit, mango, pomegranate, grape etc. and detected *Azospirillum spp.* in these samples.

Plantation crops like arecanut, cashew, cocoa, coconut, rubber and cardamom harboured *Azospirillum* (Govindan and Purushothuman, 1985). The root surface of these plants carried 0.21 to 46.6 x 10^6 cells/gram root of bacteria.

Root colonization usually occur on mucigel covered epidermis, root hairs and at points of lateral root emergence. There is evidence to suggest the existence of a recognition mechanism in *Azospirillum* similar to that of a pathogen and its host (Sumner, 1990).

Levanony and Bashan (1991) found that *A. brasilense* was capable of efficiently colonizing the elongation and root hair zones of tomato, pepper, cotton, soybean and wheat plants. Ghai and Thomas (1991) studied the occurrence of *Azospirillum* in coconut based farming systems such as high density multispecies cropping, multistoried cropping, mixed cropping with black pepper, coffee, tea etc. The root samples of total 26 plantation crops and intercrops included in study showed the presence of *Azospirillum*.

2.2.2.d Influence on root growth

Azospirillum colonization on roots of plants resulted in root elongation and growth. Increase in root elongation is reported in a number of crops like *Pennisetum sp.*, *Setaria italica* and *Triticum aestivum* by *Azospirillum* inoculation under both green house and field conditions (Kapulnik *et al.*, 1981). In C_3 (*Hordeum vulgare)* and C_4 *(Panicum miliaceum)* grasses different and mixed strains of *Azospirillum* increased the elongation of seminal roots (Okon and Kapulnik, 1986).

Patriquin, *et al.* (1983) reported that root hair number and differential root length increased by infection of maize roots with *Azospirillum*. Fallik and Okon (1984) observed that inoculation of maize seedlings with 10^7 colony forming unit (c.f.u) of *Azospirillum brasilense* per plant significantly increased root surface area, two weeks after sowing.

Azospirillum lipoferum inoculation improved rooting in one-year-old cuttings of pepper cv. Panniyur-1 (Govindan and Chandey, 1985). Fulchieir *et al.* (1993) reported that *A. lipoferum* enhanced root growth and root hair density in corn. Carletti *et al.* (1996), provided evidence that *Azospirillum* inoculation can increase the root area upto 52 per cent and root length upto 150 per cent compared to uninoculated control.

Kumar *et al.* (1998) observed significant increase in root biomass and increased fertilizer nitrogen uptake efficiency by sunflower (*Helianthus annus* L.) on *Azospirillum* inoculation.

2.2.2.e Shoot growth

Inoculations with *Azospirillum* have shown improvement in shoot growth and seedling height. Improvement in seedling height has been reported in *Syzygium aromaticum* (Rangarajan *et al.*, 1987) and casuarina (Subba Rao, 1993). *Azospirillum* treatment enhanced the growth and seedling establishment in oak as reported by Zadday and Perevolotsky (1993).

2.2.2.f Nutrient uptake

Azospirillum inoculation improves nutrient uptake by crops. Nitrate reductase activity of *Azospirillum* leading to increased absorption of NO_3 by crop plants is also responsible for better growth and yield (Kapulnik *et al.*, 1985). Two strains of *Azospirillum lipoferum*, studied for their effect on carrots showed that strongest effect was on increase of nitrogen content and then on mass and length of shoot

(Govedarica *et al.*, 1993). Application of *A. brasilense* as a root dip at transplanting stage to sweet orange substituted for at least one-fourth of the N-fertilizer requirement (Singh *et al.*, 1993). Jaskowska, (1995) observed in the rhizosphere of cereals the occurrence of *Azospirillum spp.* out of which 39 isolates were *A. brasilense* and 15 were *A. lipoferum.* Most isolates were active and effectively fixed nitrogen in laboratory conditions.

2.2.3 Azotobacter

Azotobacter can be used for nitrogen economy in the cultivation of many upland crops such as vegetables, cereals, sugarcane, plantation crops like coconut, arecanut, black pepper etc. and orchard crops.

The population of *Azotobacter* is relatively high in temperate soils rich in organic matter as compared to tropical soils especially of acidic nature. In Indian soils, the number of *Azotobacter* rarely exceed 10^4 to 10^5 g^{-1} and its occurrence depends on various soil factors like pH, moisture content, depth, availability of nutrients, organic matter content of soil etc. (Krishnamurthi, 1962). A positive correlation was found between *Azotobacter* population and organic matter, available phosphorus and pH. (Rangaswamy and Sadasivan, 1969). They also reported the predominance of *Azotobacter indicus* in acidic soil, while *Azotobacter chroococcum* in neutral alkaline soils.

Bopaiah (1987) in a study to find the occurrence of microorganisms associated with root regions of coconut reported the distribution of *Azotobacter* under both mono and mixed cropping systems of coconut. Hussain *et al.* (1987) observed significant difference in N and P content of grain and straw in maize due to presence of different strains of *Azotobacter* in unfertilized and fertilized soils. When rooted cuttings of pepper were dipped in peat based culture slurry of *Azotobacter* and grown for three months, plant height, shoot and root dry weight were highly increased (Bopaiah and Khader, 1989). Thomas *et al.* (1991) also reported the role of *Azotobacter* and *Beijerinckia* in coconut plantation soils. Martinez *et al.* (1994) reported that *A. chroococcum* populations were 1000-10,000 times higher in treated soils than in untreated soils.

Diverse groups of soil inhabiting microbes have associative and antagonistic effect on the population, survival and efficiency of *Azotobacter* in soil. Cellulolytic microorganisms are known to accelerate *Azotobacter* activity and its nitrogen fixing efficiency (Mishushtin and Shilinkova, 1971). *Cephalosporium spp.*, a common soil inhabitant, is known to inhibit the growth of *Azotobacter* (Iswaran and Rao, 1966). Synergistic effect on occurrence of vesicular arbuscular mycorrhizae due to proliferation of *Azotobacter* and other nitrogen fixing microorganisms has been reported in tomato (Secilia and Bagyaraj, 1987).

Martinez *et al.* (1994) observed an increase in yields of tomato and onion by *Azotobacter chroococcum* inoculation. Pareek *et al.* (1996) found that morphine yield increased in *Azotobacter* treated opium plants (*Papawer somniferum)* compared with uninoculated controls. *Azotobacter* inoculation produced significantly higher green forage and dry matter compared to no inoculation in forage sorghum (*Sorghum bicolor* L.) (Patel *et al.*, 1992 and Agarwal *et al.*, 1996). Simlarly *Azotobacter* inoculation showed effects on growth and yield of guinea grass (Sansamma, 1996). Similarly, Stajner *et al.* (1997) in a greenhouse study with sugar beet found that *Azotobacter* inoculation increased dry matter of the crop. Singh (1997) showed that yield of cashew increased due to *A. chroococcum* inoculation.

2.2.4 Dual inoculation

Dual inoculation with *Azotobacter* and P solubilizing bacteria in the rhizosphere of wheat increased *Azotobacter* population by two to four folds (Kundu and Gaur, 1980). A positive influence on plant height and shoot and root weight of rooted cuttings of black pepper was reported when combined inoculation of *A. brasilense* and *Glomus mosseae* was done on black pepper (Bopaiah and Khader, 1989). Dual inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria enhanced the yield of cotton significantly over single inoculation treatments (Prathibha *et al.*, 1995). Zaghoul *et al.* (1996) reported that wheat inoculation with *A. brasilense* + *Bacillus megatherium* var. *phosphaticum* or *Glomus mosseae* resulted in higher N and K uptake and different combinations gave differential results. Saleh and Nokhal (1998) in a field experiment to evaluate the effect of inoculation with a mixture of *Azotobacter, Azospirillum* and Bradyrhizobium and AMF on *Datura stramonium* observed that dual inoculation with symbiotic nitrogen fixers and AMF significantly increased dry matter, N content and alkaloid in plant.

Materials and methods

MATERIALS AND METHODS

The present experiment was conducted in the field of Central Nursery, attached to the Kerala Agricultural University Main Campus, Vellanikkara during 1999-2000. The details of materials used, methods adopted and observations recorded during the course of the study are presented below.

Vellanikkara is located at 10°31`N latitude and 76° 13` longitudes at an altitude of above 40.29 MSL The area enjoys a typical tropical climate with an average rainfall of about 2833 mm. The important weather parameters observed during the experimental period are presented in Appendix-4.

3.1 Experimental details

3.1.1 Crop and variety

Black pepper cv. Panniyur 1, a high yielding hybrid, was used for the experiment. The variety produces bold berries of medium quality and gives an average dry yield of 1.2 t ha^{-1}. It is not suited to heavily shaded areas.

3.1.2 Rooting medium

Potting mixture having 1:2:1 ratio of sand, soil and farmyard manure and filled in polythene bags (7″×5″) provided with 6 holes at the base, was used for planting the cuttings.

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3.2 Technical programme

3.2.1 Effect of microbial inoculations on establishment of pepper cuttings

3.2.1.a Source and preparation of cuttings

Mature, healthy plants of Panniyur-1 were selected. Two node cuttings from runner shoots (orthotropes) were used for producing vine pepper.

For bush pepper, one-year-old lateral branches (plagiotropes) with 4 nodes were used.

3.2.1.b Biofertilisers

The following species of AM fungi inoculum were collected from different sources and were used for the present investigation.

1. *Glomus mosseae*
2. *Glomus fasciculatum*
3. *Glomus etunicatum*
4. *Glomus monosporum*
5. AM fungi isolate from College of Agriculture, Vellayani

Source of *Azotobacter* and *Azospirillum* strains and inoculation

Azotobacter chroococcum and *Azospirillum lipoferum* collected from three different sources were used for the study. The sources were:

1. Regional Biofertilizer Development Centre, Bangalore, (RBDC).
2. TamilNadu Agricultural University, Coimbatore, (TNAU).
3. University of Agricultural Sciences, Bangalore, (UAS).

Five species of AM fungi and three strains each of *Azotobacter chroococcum* and *Azospirillum lipoferum* were inoculated on cuttings of runners and laterals and planted in polythene bags containing potting mixture.

Inoculation of potting media with AMF was done by uniformly, distributing a thin layer of inoculum @ 10 g/ polybag at two cm below the surface level so that the base of the bare cuttings were in contact with the inoculum.

Inoculation of pepper cuttings with *Azotobacter* and *Azospirillum* was done by dipping the cuttings in loose water slurry of the inoculum (500 g in 2.5 l water) for 20 minutes prior to planting.

Number of cuttings established after two months in the case of vine cuttings and six months for lateral cuttings was recorded.

3.2.1.c Experimental design and layout

Completely randomized design (CRD) was adopted for laying out the experiment. There were totally 24 treatments, out of which twelve were for runner cuttings and twelve for lateral cuttings. Each treatment was replicated four times with 100 plants in each replication.

After application of the treatments, polybags were arranged according to the layout plan and labelled.

Mist irrigation was given four times a day. Bordeaux mixture (1 per cent) was given as foliar spray at monthly intervals to check the incidence of fungal diseases.

3.2.1.d Observations recorded

Number of cuttings sprouted by the end of the experimental period was counted and expressed in percentage.

3.2.2 Effect of inoculation on growth of pepper vine cuttings

Rooted cuttings from experiment I were transferred to pots and growth observations were taken.

3.2.2.a Experimental design and layout

Completely randomized design was adopted for laying out the experiment. There were totally twelve treatments. Each treatment was replicated five times, with ten plants in each replication.

3.2.2.b Treatments

The treatments included, inoculation of both vine and bush cuttings with the following biofertilisers:

Azospirillum lipoferum, RBDC
Azospirillum lipoferum, TNAU
Azospirillum lipoferum, UAS
Azotobacter chroococcum, RBDC
Azotobacter chroococcum, TNAU
Azotobacter chroococcum, UAS
Glomus mosseae
Glomus fasciculatum

Glomus fasciculatum

Glomus etunicatum

Glomus monosporum

AM fungi isolate from College of Agriculture, Vellayani

3.2.2.c Observations recorded

A. Biometric observations

a) Root characters

Representative samples from each treatment and replications were uprooted 180 days after planting. These were carefully and thoroughly washed in running water to remove all adhering soil particles.

After removing the roots from the stem, the longest primary root was selected. Its length was measured from base to the tip of the root using measuring scale and expressed in centimeter.

b) Shoot characters

i) Vine length

Length of the longest shoot was taken from base of the shoot to the tip of emerging leaf using a measuring scale. It was expressed in centimeter.

ii) Number of leaves

Total number of leaves on the shoot was counted and recorded.

B. Nutrient analysis

The methods used for plant nutrient analysis is shown below:

Sl. No.	Nutrient	Method	Reference
a.	Nitrogen	Microkjeldhal digestion And distillation method	Jackson(1958)
b.	Phosphorus	Vanadomolybdophosphoric yellow colour method using Spectronic – 20	,,
c.	Potassium	Diacid extract using Flame photometer	,,

3.2.3 Effect of combined inoculation of Azotobacter, Azospirillum and AM fungi on pepper.

Rooted runner cuttings with best two AM fungi were reinoculated with promising strains of *Azotobacter* and *Azospirillum* and used for the study.

3.2.3.a Treatments

There were 27 treatments including single, double and triple inoculations and control with each treatment replicated twelve times.

For single inoculation, plants treated with the microbe in experiment-I were transferred to larger pots. For double and triple inoculations, plants having single inoculum were reinoculated with one or two of the microbes according to treatment requirement and transferred to larger pots. For all combination treatments involving AM fungi, plants treated with AM in experiment I were selected and reinoculated with *Azotobacter, Azospirillum* or both. Bacterial inoculum @ 5g per plant was applied in the pot at a depth of five cm so that, roots of the cuttings were in close contact with the inoculum.

3.2.3.b Treatment details

T_1	–	0	0	0
T_2	–	0	0	Al_1
T_3	–	0	0	Al_2
T_4	–	0	Ac_1	0
T_5	–	0	Ac_1	Al_1
T_6	–	0	Ac_1	Al_1
T_7	–	0	Ac_2	0
T_8	–	0	Ac_2	Al_1
T_9	–	0	Ac_2	Al_2
T_{10}	–	V_1	0	0
T_{11}	–	V_1	0	Al_1
T_{12}	–	V_1	0	Al_2
T_{13}	–	V_1	Ac_1	0
T_{14}	–	V_1	Ac_1	Al_1
T_{15}	–	V_1	Ac_1	Al_2
T_{16}	–	V_1	Ac_2	0
T_{17}	–	V_1	Ac_2	Al_1
T_{18}	–	V_1	Ac_2	Al_2
T_{19}	–	V_2	0	0
T_{20}	–	V_2	0	Al_1
T_{21}	–	V_2	0	Al_2
T_{22}	–	V_2	Ac_1	0
T_{23}	–	V_2	Ac_1	Al_1
T_{24}	–	V_2	Ac_1	Al_2
T_{25}	–	V_2	Ac_2	0
T_{26}	–	V_2	Ac_2	Al_1
T_{27}	–	V_2	Ac_2	Al_2

Al_1: *Azospirillum lipoferum* from University of Agricultural Sciences Banglore.

Al_2: *Azospirillum lipoferum* from TamilNadu Agricultural University Coimbatore.

Ac_1: *Azotobacter chroococcum* from University of Agricultural Sciences Bangalore.

Ac_2: *Azotobacter chroococcum* from TamilNadu Agricultural University Coimbatore.

V_1: *Glomus mosseae*

V_2: *Glomus fasciculatum*

Combination of the above inoculum were tried for the experiment

3.2.3.c Experimental design and lay out

3^3 factorial RBD

3.2.3.d Supporting structures

Rapid multiplication unit was erected with half split PVC pipes of length 125 cm and diameter 10cm and arranged criss-cross at 45° angle on a straight support. The vines were tied to the pipes at regular intervals. Sprinkler system was provided for irrigation.

3.2.3.e Observations recorded

Observations as shown in 3.2.2.d were taken. In addition to this, microbial spore count in the rhizosphere soil and root infection percentage by AMF were also taken. Details of the methods used are shown below.

A. Infection Percentage by AM fungi

Infection percentage was found by staining and detection procedure developed by Phillips and Hayman (1970). Root samples were cut into small bits and washed with water and cleared in 10 per cent KOH solution at 90°C for one hour. It was decanted and then neutralized with 2 per cent HCl. Root bits were stained with 0.05 per cent trypan blue in lacto-phenol (lactic acid, phenol, glycerol and water in the ratio 20:20:40:20) for five minutes. The stained root bits were observed under microscope for AMF colonisation. Then root infection percentage was calculated by the following formula:

$$\text{Per cent AMF infection} = \frac{\text{Number of root bits positive for colonisation}}{\text{Total number of roots bits observed}} \times 100$$

B. *Azotobacter* and *Azospirillum* colonisation

Population of *Azotobacter* and *Azospirillum* in the rhizosphere was estimated by using serial dilution technique. Soil samples from the rhizosphere of different treatments were taken and sieved in 2mm mesh. One gram of the soil was suspended in 99ml of sterilised water and serial dilutions of the suspension were prepared by further dilutions. One-ml aliquots of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were transferred to sterile test tubes containing *Azospirillum* Okon's medium (Appendix 1) and Jenson's N free medium (Appendix 2) for *Azotobacter*. The dilution and plating were done asceptically in a laminar flow chamber. The test tubes were incubated in a BOD incubator for three to four days at 31^{0}C. The positive tubes were counted and their number recorded. The population was calculated using MPN technique (Cochran, 1950).

Results

RESULTS

Results of the studies on the influence of various biofertilisers viz., *Azotobacter, Azospirillum* and AMF on establishment and growth of pepper cuttings are presented below.

4.1 Effect of AMF species on establishment and growth of pepper

4.1.1 Effect on lateral cuttings

Data on the effect of AMF on germination and growth of bush cuttings six months after planting is presented in Table 1 and Table 2.

4.1.1.1 On establishment

Among AMF treatments, germination percentage was highest in *G. fasciculatum* (34.4 per cent) followed by *G. mosseae* (31.5 per cent) and *G. monosporum* (31.2 per cent). Minimum germination was observed in the case of Vellayani isolate.

4.1.1.2 On growth

4.1.1.2.a Shoot length

Shoot length of bush cuttings was highest in treatment inoculated with *G. fasciculatum* (11.66 cm) followed by *G. monosporum* (8.94cm) and *G. mosseae* (7.98 cm).

Table 1. Germination percentage of lateral cuttings inoculated with AMF

Treatments	Germination (per cent)
Glomus mosseae	31.50
G. fasciculatum	34.40
G. etunicatum	29.60
G. monosporum	31.20
Vellayani isolate	20.00
Control	18.75

Table 2. Growth characters of rooted lateral cuttings inoculated with AMF

Treatments	Mean vine length (cm)	Mean number of leaves	Mean dry weight (g)	Mean root length (cm)
Glomus mosseae	7.98	3.06	7.20	4.80
G. fasciculatum	11.66	3.84	7.50	5.10
G. etunicatum	6.95	2.41	6.40	4.20
G. monosporum	8.94	3.33	6.60	4.40
Vellayani isolate	7.02	2.50	6.10	4.10
Control	6.48	2.35	5.80	3.00

Table 3. Nutrient content of lateral pepper cuttings under different AMF inoculations

Treatments	Mean nutrient content (per cent)		
	N	P	K
Glomus mosseae	2.04	0.131	1.933
G. fasciculatum	2.17	0.135	2.167
G. etunicatum	1.97	0.132	2.100
G. monosporum	2.05	0.128	2.067
Vellayani isolate	1.98	0.127	2.167
Control	1.96	0.117	1.933

4.1.1.2.b Number of leaves

In leaf number also *G. fasciculatum* proved to be the best among the different species (3.84). It was followed by G.*monosporum* (3.33) and G. *mosseae* (3.06).

4.1.1.2.c Dry weight

Dry weight was recorded highest in *G. fasciculatum* (7.5 g) inoculated plants followed by *G. mosseae* (7.2 g) inoculation.

4.1.1.2.d Root length

Highest root length was recorded for plants inoculated with *G. fasciculatum* (5.1 cm).

4.1.1.3 Nutrient content

Nutrient content of lateral cuttings is presented in Table 3. Compared to control, the difference in nutrient content of treatment plants was negligible.

4.1.2 Effect on runner cuttings

4.1.2.1 On establishment

The effect of AMF on germination of vine cuttings is presented in Table 4.

Among AMF treatments, germination per cent was highest with *G. mossae* (74.25 per cent) followed by *G. fasciculatum* (73.0 per cent). Other AMF fungi inoculated plants also performed better than uninoculated control. Inoculation with *G. monosporum* induced 71.5 per cent germination.

Table 4. Germination percentage of runner cuttings inoculated with AMF

Treatments	Germination (per cent)
Glomus mosseae	74.20
G. fasciculatum	73.00
G. etunicatum	67.50
G. monosporum	71.50
Vellayani isolate	70.00
Control	65.00

Table 5. Growth characters of rooted runner cuttings inoculated with AMF†

Treatments	Vine length (cm)	Number of leaves	Root length (cm)	Mean dry weight (g)
Glomus mosseae	36.30[a]	8.40[ab]	31.50	19.50
G. fasciculatum	28.30[b]	7.05[cd]	37.10	20.40
G. etunicatum	17.01[d]	6.60[d]	29.88	17.60
G. monosporum	18.95[d]	5.20[e]	24.56	15.80
Vellayani isolate	17.04[d]	5.02[e]	22.94	14.42
Control	16.52[d]	4.80[e]	24.44	12.37
	S	S	NS	

† Associated with Table 12 & 19
Figures with same alphabets do not differ significantly at 5 % level

Table 6. Nutrient content of runner pepper cuttings under different AMF inoculations

Treatments	Mean nutrient content (per cent)		
	N	P	K
Glomus mosseae	2.06	0.125	2.067
G. fasciculatum	2.03	0.127	2.060
G. etunicatum	2.03	0.133	2.243
G. monosporum	2.05	0.131	2.127
Vellayani isolate	2.19	0.119	2.273
Control	1.99	0.125	2.043

4.1.2.2 On growth

The effect of AMF inoculation on growth of rooted runner cuttings is presented in Table 5.

4.1.2.2.a Shoot length

Application of AMF produced significant difference on shoot length of vine cuttings, six months after planting.

Plants inoculated with *G. mosseae* (36.3 cm) was significantly superior to uninoculated control and was followed by *G. fasciculatum* (28.3 cm).

4.1.2.2.b Number of leaves

Number of leaves also differed significantly in AMF inoculated plants. The treatment inoculated with *G. mosseae* (8.4) was significantly superior over control followed by *G. fasciculatum* (7.05) inoculated treatment.

4.1.2.2.c Dry weight

Dry weight recorded was highest for the plants inoculated with *G. fasciculatum* (20.4 g) followed by *G. mosseae* (19.5 g).

4.1.2.2.d Root length

Root length as influenced by inoculation with AMF were found to be non-significant.

4.1.2.3 Nutrient content

Nutrient content of AMF inoculated pepper vines, six months after planting is presented in Table 6. The treatments were found to be non-significant.

4.1.2.4 Effect of AMF on growth six months after inoculation

Based on the performance in the initial experiment, G.*fasciculatum* (V_2) and *G.mosseae* (V_1) were selected for further studies. Plants inoculated with these fungi were transferred to big pots and the growth characters were studied for a further period of three and half months. The observations on growth are presented in Table 7. *G.fasciculatum* showed overall better performance when compared to *G.mosseae* and control. *G. fasciculatum* recorded higher vine length (94.98cm), vine dry weight (28.70g), number of leaves (31.58) and leaf dry weight (24.43 g). It also showed maximum root length (55.37), root weight (12.13 g) and root- shoot ratio (0.228).

Nitrogen content was found to be higher for *G. fasciculatum* with 1.99 per cent. Phosphorus content was recorded maximum for *G.mosseae* which recorded 0.23 per cent. Potassium content was found to be non-significant.

4.2 Effect of *Azotobacter* strains on establishment and growth of pepper

Different strains of *Azotobacter chroococcum* were inoculated on pepper cuttings and their effect on establishment as well as growth was studied. Inoculation was done on lateral and runner cuttings separately.

4.2.1 On lateral cuttings

Results of the study on establishment and growth of lateral cuttings is presented below.

Table 7. Growth characters nine months after inoculation with AMF species†

Treatments	Shoot character				Root character			Nutrient content (per cent)		
	Vine length (cm)	Vine dry weight (g)	Number of leaves	Leaf dry weight	Root length (cm)	Root weight (g)	Root shoot ratio	N	P	K
Control	88.66^{j}	28.70^{j}	24.41^{i}	20.87^{n}	45.47^{h}	9.33^{g}	0.204^{i}	0.960^{j}	0.192^{i}	2.090
V_1	94.08^{ij}	28.00^{ij}	26.08^{i}	24.37^{m}	54.00^{fg}	11.33^{de}	0.217^{fgh}	1.987^{kl}	0.234^{f}	2.023
V_2	94.98^{hi}	28.70^{hij}	31.58^{fgh}	24.43^{n}	55.37^{ef}	12.13^{cd}	0.228^{abc}	1.997^{jkl}	0.237^{ef}	2.130
	S	S	S	S	S	S	S	S	S	NS

† Associated with Table 14, 21, 23 & 25

Figures with same alphabets do not differ significantly at 5 % level

4.2.1.1 On establishment

The effect of *A. chroococcum* on germination of lateral cuttings six months after planting is presented in Table 8.

Among *A. chroococcum* inoculated treatments, germination percentage was highest in treatment inoculated with TNAU strain ,Ac-2 and recorded 26.3 per cent

4.2.1.2 On growth

Data on the effect of *A. chroococcum* on growth of bush cuttings six months after planting is presented in Table 9.

4.2.1.2.a Shoot length

Shoot length of bush cuttings was highest in treatments inoculated with Ac-2 (10.77 cm).

4.2.1.2.b Number of leaves

Number of leaves recorded was highest in treatment inoculated with Ac-2 (3.87).

4.2.1.2.c Dry weight

Dry weight recorded was highest for Ac-1 (6.9 g) treated plants.

4.2.1.2.d Root length

Root length was recorded highest for plants inoculated with Ac-1 (3.5 cm).

4.2.1.3 Nutrient content

Nutrient contents in lateral cuttings six months after inoculation is presented in Table 10.Influence on nutrient content was found to be non-significant.

4.2.2 Effect on vine cuttings

4.2.2.1 On establishment

The same bacterial strains were inoculated on vine cuttings and the germination percentage was noted after three months of inoculation. The data is presented in Table 11.

Among *A. chroococcum* treated vine cuttings, germination per cent was highest in plants inoculated with Ac_2 (79.75 per cent) followed by Ac_1 (79.25 per cent)

4.2.2.2 On growth

The effect of *A. chroococcum* inoculation on growth of vine cuttings is presented in Table 12.

4.2.2.2.a Shoot length

Inoculation of *A. chroococcum* produced significant difference in shoot length and number of leaves produced in pepper, six months after planting. Treatment inoculated with Ac_1 produced highest vine length (25.18cm) and leaf number (8.6). It was followed by Ac_2, with a vine length of 22.31cm and leaf number of 8.3. The treatment effect on root length was non-significant.

Table 8. Germination percentage of lateral cuttings inoculated with *Azotobacter chroococcum* strains

Treatments	Germination (per cent)
A. chroococcum RBDC	20.00
A. chroococcum TNAU	26.30
A. chroococcum UAS	24.32
Control	18.75

Table 9. Growth characters of rooted lateral cuttings inoculated with *Azotobacter chroococcum* strains

Treatments	Mean vine length (cm)	Mean number of leaves	Mean dry weight (g)	Mean root length (cm)
A. chroococcum RBDC	7.46	2.45	6.40	3.20
A. chroococcum TNAU	10.77	3.87	6.50	3.10
A. chroococcum UAS	7.61	2.65	6.90	3.50
Control	6.48	2.35	5.80	3.00

Table 10. Nutrient content of lateral pepper cuttings under different *Azotobacter chroococcum* inoculations

Treatments	Nutrient content (per cent)		
	N	P	K
A. chroococcum RBDC	2.01	0.123	2.067
A. chroococcum TNAU	2.05	0.119	2.000
A. chroococcum UAS	2.02	0.118	2.200
Control	1.96	0.117	1.933

Table 11. Germination percentage of runner cuttings inoculated with *Azotobacter chroococcum* strains

Treatments	Germination (per cent)
A. chroococcum RBDC	76.25
A. chroococcum TNAU	79.75
A. chroococcum UAS	79.25
Control	65.00

Table 12. Growth characters of rooted runner cuttings inoculated with *Azotobacter chroococcum* strains†

Treatments	Vine length (cm)	Number of leaves	Root length (cm)	Mean dry weight (g)
A. chroococcum RBDC	22.14[cd]	8.10[abc]	26.52	16.32
A. chroococcum TNAU	22.31[cd]	8.30[ab]	26.87	17.50
A. chroococcum UAS	25.18[bc]	8.60[a]	30.78	17.90
Control	16.52[d]	4.80[e]	24.44	12.37
	S	S	NS	

† Associated with Table 5 & 19
Figures with same alphabets do not differ significantly at 5 % level

Table 13. Nutrient content of runner pepper cuttings under different *Azotobacter chroococcum* inoculations

Treatments	Nutrient content (per cent)		
	N	P	K
A. chroococcum RBDC	1.97	0.124	2.050
A. chroococcum TNAU	1.99	0.127	2.153
A. chroococcum UAS	2.01	0.125	2.117
Control	1.99	0.125	2.043
	NS	NS	NS

4.2.2.3 Nutrient content

Nutrient content of the treated plants is presented in Table 13. The effect of inoculum was found to be non-significant.

4.2.2.4 Effect of *Azotobacter* strains on growth six months after inoculation

Based on the performance in the preliminary experiment, two strains of *A. chroococcum,* Ac_1 and Ac_2 were selected. The plants inoculated with these strains in the first experiment were repotted and permitted to grow for a further period of three and half months .The shoot growth, root growth and nutrient content were recorded and are presented in Table 14.

A.chroococcum UAS (Ac_1) showed higher vine length (98.15cm) and root-shoot ratio (0.171) when compared to TNAU strain (Ac_2). But, parameters like vine dry weight (29.98g), number of leaves (29.08) and leaf dry weight (25.57g) were highest for Ac_2. It also showed higher root length (45.67cm) and root weight (9.48g) and was found to be non-significant. But, root-shoot ratio recorded was showing poor performance (0.169) when compared to control (0.204).

Nitrogen and phosphorus content was found to be significantly superior for *Azotobacter* inoculated plants over control. Nitrogen content recorded was higher for Ac_2 with 2.03 per cent. Phosphorus content observed was higher for Ac_1 (0.19 per cent) Potassium content was found to be non-significant.

Nitrogen and phosphorus content was found to be significantly superior for *Azospirillum* inoculated plants over control.

Table 14. Growth characters nine months after inoculation with *Azotobacter chroococcum* strains†

Treatments	Shoot character				Root character			Nutrient content (per cent)		
	Vine length (cm)	Vine dry weight (g)	Number of leaves	Leaf dry weight	Root length (cm)	Root weight (g)	Root shoot ratio	N	P	K
Control	88.66^{j}	28.70^{j}	24.41^{i}	20.87^{n}	45.47^{h}	9.33^{g}	0.204^{i}	0.960^{j}	0.192^{i}	2.090
Ac_1	98.15^{fghij}	29.93^{ghi}	27.16^{hi}	25.17^{lm}	45.10^{h}	9.40^{g}	0.171^{l}	2.027^{ijk}	0.193^{hi}	2.030
Ac_2	96.28^{ghi}	29.98^{ghi}	29.08^{ghi}	25.57^{klm}	45.67^{h}	9.48^{g}	0.169^{l}	2.037^{ijk}	0.194^{ghi}	2.043
	S	S	S	S	NS	S	S	S	S	NS

† Associated with Table 7, 21, 23 & 25

Figures with same alphabets do not differ significantly at 5 % level

4.3 Effect of *Azospirillum* strains on establishment and growth of pepper

Another nitrogen fixing bacteria, *Azospirillum lipoferum* was also tried on cuttings of pepper. Both lateral and runner cuttings were used and were inoculated with three strains of the bacterium. Growth and establishment characters were studied as in the case of *Azotobacter*.

4.3.1 Effect on lateral cuttings

The observations on establishment and growth of lateral cuttings six months after planting as effected by *A. lipoferum* inoculation is presented below.

4.3.1.1 On establishment

Data on the effect of strains of A. *lipoferum* on germination of lateral cuttings is presented in Table 15. Among *A. lipoferum* treatments, the highest percentage germination was recorded withAl_2 (25 per cent) followed by Al_1 (24.6 per cent)

4.3.1.2 On growth

The data on the effect of *A. lipoferum* strains on growth of lateral cuttings six months after planting is presented in Table 16.

4.3.1.2.a Shoot length

Shoot length of bush cuttings was highest in treatment inoculated with Al_2 (11.32 cm) followed by RBDC strain, Al_3 (9.51 cm).

Table 15. Germination percentage of lateral cuttings inoculated with *Azospirillum lipoferum* strains

Treatments	Germination (per cent)
A. lipoferum RBDC	23.40
A. lipoferum TNAU	25.00
A. lipoferum UAS	24.62
Control	18.75

Table 16. Growth characters of rooted lateral cuttings inoculated with *Azospirillum lipoferum* strains

Treatments	Mean vine length (cm)	Mean number of leaves	Mean dry weight (g)	Mean root length (cm)
A. lipoferum RBDC	9.51	3.40	6.20	3.40
A. lipoferum TNAU	11.32	3.91	6.40	4.10
A. lipoferum UAS	8.18	3.18	6.30	3.50
Control	6.48	2.35	5.80	3.00

Table 17. Nutrient content of lateral pepper cuttings under different *Azospirillum lipoferum* inoculations

Treatments	Mean nutrient content (per cent)		
	N	P	K
A. lipoferum RBDC	2.05	0.128	2.133
A. lipoferum TNAU	2.15	0.125	1.900
A. lipoferum UAS	2.05	0.124	2.133
Control	1.96	0.117	1.933

4.3.1.2.b Number of leaves

Number of leaves produced by lateral cuttings was highest in Al_2 (3.91) followed by Al_3 (3.4).

4.3.1.2.c Dry weight

Dry weight recorded was highest for treatment inoculated with Al_2 (6.4 g) followed by Al_1 (6.3 g).

4.3.1.2.d Root length

Root length of bush vines was highest for treatment inoculated with Al_2 (4.1 cm).

4.3.1.3 Nutrient content

Nutrient content of *Azospirillum* inoculated lateral plants is presented in Table 17. The change in content of nutrients as influenced by *Azospirillum* inoculation was found to be non-significant.

4.3.2 Effect on pepper runner cuttings

4.3.2.1 On establishment

Data on the effect of *Azospirillum lipoferum* strains on germination of vine cuttings three months after planting is presented in Table 18.

Among *Azospirillum lipoferum* inoculated treatments, germination per cent was maximum in UAS strain, Al_1 (86 per cent) followed by TNAU strain, Al_2 (77.5 per cent).

4.3.2.2 On growth

The Table 19 presents data on the effect of *Azospirillum lipoferum* strains on growth of vine cuttings six months after planting.

4.3.2.2.a Shoot length

Inoculation with *A. lipoferum* produced significant difference in shoot length of vine cuttings six months after planting. Shoot length of plants inoculated with Al_1 (41.8 cm) was significantly superior to uninoculated control and on par with Al_2 (39.6 cm).

4.3.2.2.b Number of leaves

The number of leaves also differed significantly in *A. lipoferum* inoculated plants when compared with control and was found to be significantly superior. Plants inoculated with Al_1 recorded higher leaf number of 9.1and was on par with treatment Al_2 (9.06).

4.3.2.2.c Dry weight

Dry weight recorded was higher for the treatment inoculated with Al_1(24.1 g) followed by Al_2 (22.56 g).

4.3.2.2.d Root length

Increase in root length as influenced by inoculation with *Azospirillum* was found to be non-significant.

Table 18. Germination percentage of runner cuttings inoculated with *Azospirillum lipoferum* strains

Treatments	Germination (per cent)
A. lipoferum RBDC	68.00
A. lipoferum TNAU	77.50
A. lipoferum UAS	86.00
Control	65.00

Table 19. Growth characters of rooted runner cuttings inoculated with *Azospirillum lipoferum* strains†

Treatments	Vine length (cm)	Number of leaves	Root length (cm)	Mean dry weight (g)
A. lipoferum RBDC	27.52[bc]	7.30[bcd]	25.90	20.40
A. lipoferum TNAU	39.60[a]	9.06[a]	27.08	22.56
A. lipoferum UAS	41.80[a]	9.10[a]	36.52	24.16
Control	16.52[d]	4.80[e]	24.44	12.37
	S	S	NS	

† Associated with Table 5 & 12

Figures with same alphabets do not differ significantly at 5 % level

Table 20. Nutrient content of rooted pepper cuttings under different *Azospirillum lipoferum* inoculations

Treatments	Nutrient content (per cent)		
	N	P	K
A. lipoferum RBDC	2.14	0.125	2.073
A. lipoferum TNAU	2.15	0.135	2.140
A. lipoferum UAS	2.04	0.127	2.190
Control	1.99	0.125	2.043
	NS	NS	NS

4.3.2.3 Nutrient content

The Table 20 provides data on nutrient content of the plants. Nutrient content as influenced by *Azospirillum* inoculation was not significant.

4.3.2.4 Effect of *Azospirillum* strains on growth, six months after inoculation

Based on the performance in the preliminary experiment, two strains of *A. lipoferum*, Al_1 and Al_2 were selected. Plants inoculated with these strains in the first experiment were repotted and permitted to grow for a further period of three and half months. Shoot growth, root growth and nutrient content are presented in Table 21.

Significant difference in growth was noticed even after repotting. Al_2 inoculated vines performed better when compared to Al_1, but the difference was non-significant. Control plants showed better root-shoot ratio of 0.204 compared to *Azospirillum* inoculated vines.

Nitrogen and phosphorus content were found to be significantly superior for *Azospirillum* inoculated plants over control. Al_2 recorded higher nitrogen and phosphorus content of 2.04 per cent and 0.19 per cent. Potassium content was found to be non-significant.

4.4 Effect of multiple microbial inoculation on growth of pepper

4.4.1 Double inoculation

After studying the effect of single inoculations combinations of biofertilisers were tried for their effect on growth of pepper. In combinations involving AMF, inoculation with AMF was done initially on the vine cuttings at the time of planting. Bacterial inoculation was done six months after the first inoculation.

Table 21. Growth characters nine months after inoculation with *Azospirillum lipoferum* strains†

Treatments	Shoot character				Root character			Nutrient content (per cent)		
	Vine length (cm)	Vine dry weight (g)	Number of leaves	Leaf dry weight	Root length (cm)	Root weight (g)	Root shoot ratio	N	P	K
Control	88.66^{j}	28.70^{j}	24.41^{i}	20.87^{n}	45.47^{h}	9.33^{g}	0.204^{j}	0.960^{j}	0.192^{i}	2.090
Al_1	97.57^{fghij}	30.10^{ghi}	32.33^{efgh}	25.47^{klm}	46.03^{h}	9.43^{g}	0.169^{l}	2.037^{ijk}	0.195^{ghi}	2.130
Al_2	100.60^{efgh}	30.12^{ghi}	32.42^{efgh}	25.70^{klm}	45.77^{h}	9.50^{fg}	0.170^{l}	2.047^{ij}	0.196^{ghi}	2.112
	S	S	S	S	S	S	S	S	S	NS

† Associated with Table 7, 14, 23 & 25

Figures with same alphabets do not differ significantly at 5 % level

In combinations of *Azotobacter* and *Azospirillum*, inoculation with one of the bacterium was done initially and the other was introduced six months after. The treatment combinations are shown below.

Treatments

V_1 Ac_1	V_2 Ac_1	Ac_1 Al_1
V_1 Ac_2	V_2 Ac_2	Ac_1 Al_2
V_1 Al_1	V_2 Al_1	Ac_2 Al_1
V_1 Al_2	V_2 Al_2	Ac_2 Al_2

The study was continued for three and half months and the results are presented in Table 22 and 23.

In general, the double combinations were superior to single inoculations in all growth measurements.

Among double combination treatments, combination of AMF and nitrogen-fixing bacteria was superior to the combination of two nitrogen fixing bacteria. On comparing AM fungi, G.*mosseae* (V_1) and bacterial combination, treatment combination of V_1 Al_2 was superior to its all other combinations. On comparing AMF *Glomus fasciculatum* (V_2) combined with nitrogen fixing bacteria, *A. lipoferum* Al_2 proved to be superior to all its other combinations.

4.4.1.a Shoot length

Length of pepper vines was recorded at fortnightly intervals and monthly increment in length was found out and is presented in Table 22.

Table 22. Monthly increment in vine length for double combination treatments

Treatments	Vine length increment (cm)				
	30 DAP	60 DAP	90 DAP	105 DAP	Total
V_1Ac_1	15.426	14.283	19.326	13.712	62.847
V_1Ac_2	14.107	15.574	15.564	14.788	60.033
V_1Al_1	15.008	13.897	18.899	14.452	62.257
V_1Al_2	17.711	11.998	19.425	15.997	65.132
V_2Ac_1	16.013	10.492	17.650	15.481	59.636
V_2Ac_2	15.461	14.357	19.859	14.357	64.054
V_2Al_1	14.357	11.044	17.669	15.462	58.532
V_2Al_2	17.670	12.148	19.884	15.459	65.161
Ac_1Al_1	12.229	14.432	16.676	13.340	56.698
Ac_1Al_2	14.452	11.117	18.809	11.517	55.895
Ac_2Al_1	13.341	14.232	16.686	13.341	57.610
Ac_2Al_2	14.402	12.788	15.167	15.455	57.810
Control	11.990	12.820	12.980	7.692	45.530
	NS	NS	NS	NS	NS

Thirty days after planting, treatment combination of V_1Al_2 recorded highest increment of 17.71cm.

Sixty days after planting, treatment with V_1Ac_2 recorded highest increment of 15.57cm.

Ninety days after planting, V_2Al_2 recorded highest increment (19.88 cm).

After 105 days, V_1Al_2 combination was found to give the highest increment in vine length of 15.99cm.

The highest total vine length increment was also recorded by V_2Al_2 treatment combination (65.16cm).

Comparing all double treatment combinations, combination of V_2Al_2 recorded highest shoot length of 117.30cm followed by treatment with V_1Al_2 (116.50cm) and V_2Ac_2 (114.20cm).

4.4.1.b Vine dry weight

Treatment combination of V_2Al_2 recorded highest vine dry weight of 36.27 g followed by V_1Al_2 (36.10 g).

4.4.1.c Number of leaves

Maximum number of leaves was recorded for treatment combination of V_2Al_2 (38.75) and was on par with V_1Al_2 (38.38).

4.4.1.d Leaf dry weight

Combination of V_2Al_2 recorded highest leaf dry weight of 30.20 g followed by V_1Al_2(29.90 g).

4.4.1.e Root length

Treatment with V_2Al_2 recorded maximum root length of 61.90cm followed by V_1Ac_2 (61.50cm) and were on par with each other.

4.4.1.f Root weight

Combination treatment of V_2Al_2 recorded highest root weight of 13.27 g followed by V_2Ac_2 (13.20g) and V_2Al_1 (12.97 g).

4.4.1.g Root shoot ratio

Treatment combination of V_1Ac_1 recorded highest root-shoot ratio of 0.221 followed by V_2Ac_1 (0.219).

4.4.1.h Nutrient content

In vine pepper plants nitrogen and phosphorus content were found to be significantly influenced by biofertilizer application while potassium content was found to be non-significant.

Nutrient content of vine pepper plants as influenced by inoculation with biofertilisers is given in Table 23.

Table 23. Growth characters of double combination treatments[†]

Treatments	Shoot character				Root character			Nutrient content (per cent)		
	Vine length (cm)	Vine dry weight (g)	Number of leaves	Leaf dry weight	Root length (cm)	Root weight (g)	Root shoot ratio	N	P	K
V_1Ac_1	111.9^{cdef}	30.57^{ghi}	34.25^{cdef}	25.97^{kl}	60.00^{de}	12.47^{c}	0.221^{efg}	2.317^{f}	0.235^{f}	2.140
V_1Ac_2	108.8^{defg}	30.53^{ghi}	32.66^{defg}	28.30^{hi}	61.50^{cd}	12.57^{c}	0.213^{h}	2.190^{gh}	0.236^{f}	2.126
V_1Al_1	113.6^{cde}	35.57^{cdef}	37.16^{bcdef}	28.53^{ghi}	59.80^{de}	12.77^{c}	0.199^{ij}	2.357^{ef}	0.242^{cde}	2.167
V_1Al_2	116.5^{bcd}	36.10^{bcdef}	38.38^{abcd}	29.90^{f}	60.20^{de}	12.90^{c}	0.195^{j}	2.350^{ef}	0.243^{bcd}	2.236
V_2Ac_1	114.1^{cde}	32.47^{fghi}	35.66^{cdef}	26.33^{kl}	59.40^{def}	12.87^{c}	0.219^{fgh}	2.150^{h}	0.244^{bcd}	2.090
V_2Ac_2	114.2^{cde}	34.47^{defg}	37.58^{abcde}	26.70^{jk}	57.93^{def}	13.20^{c}	0.215^{gh}	2.353^{e}	0.243^{bcd}	2.224
V_2Al_1	109.8^{defg}	35.50^{cdef}	38.52^{abcd}	29.70^{fg}	60.43^{de}	12.97^{c}	0.199^{ij}	2.350^{ef}	0.241^{de}	2.219
V_2Al_2	117.3^{bcd}	36.27^{bcdef}	38.75^{abcd}	30.20^{f}	61.90^{cd}	13.27^{c}	0.200^{ij}	2.380^{e}	0.234^{f}	2.154
Ac_1Al_1	107.0^{defg}	30.47^{ghi}	32.50^{efg}	25.87^{kl}	48.37^{h}	10.47^{efg}	0.186^{k}	2.170^{h}	0.196^{ghi}	2.007
Ac_1Al_2	106.6^{defg}	33.33^{fgh}	35.75^{cdef}	29.30^{fgh}	49.13^{gh}	10.57^{ef}	0.169^{l}	2.057^{l}	0.200^{g}	2.234
Ac_2Al_1	108.1^{defg}	32.67^{fghi}	35.66^{cdef}	27.63^{ij}	49.57^{gh}	10.46^{efg}	0.173^{l}	2.190^{gh}	0.196^{ghi}	2.130
Ac_2Al_2	110.3^{defg}	34.17^{efg}	37.24^{bcdef}	29.60^{fg}	48.57^{h}	10.67^{e}	0.168^{l}	2.327^{ef}	0.197^{ghi}	2.115
Control	88.66^{j}	23.80^{j}	24.41^{i}	20.87^{n}	45.47^{h}	9.33^{h}	0.204^{i}	1.960^{i}	0.192^{i}	2.090
	S	S	S	S	S	S	S	S	S	NS

| Associated with Table 7, 14, 21 & 25

Figures with same alphabets do not differ significantly at 5 % level

Nitrogen content was highest in the combination of V_2Al_2 with 2.380 per cent. Phosphorus content was recorded maximum for treatment V_2Ac_1 with 0.244 per cent. Potassium content was found to be non-significant.

4.4.2 Triple Combination

The following combinations of bio-fertilizers were tried for their effect on growth of pepper vines. Inoculation with AMF was done initially on the vine cuttings at the time of planting to which bacterial cultures were introduced six months after.

Treatments

$V_1Ac_1Al_1$	$V_2Ac_1A_{l1}$
$V_1Ac_1Al_2$	$V_2Ac_1Al_2$
$V_1Ac_2Al_1$	$V_2Ac_2Al_1$
$V_1Ac_2Al_2$	$V_2Ac_2Al_2$

Table 24 provides data on increase in vine length of pepper at monthly intervals. Data on other growth parameters and nutrient contents are presented in Table 25. The data indicates superiority of treatment combinations over control.

4.4.2.a Shoot length

For triple combination treatments, the increase in vine length of pepper vine at monthly intervals were taken and the data are presented in Table 24.

At Thirty days after planting, treatment combination of $V_2Ac_2Al_2$ recorded maximum increment of 21.67 cm.

Table 24. Monthly increment in vine length for triple combination treatments

Treatments	Vine length increment (cm)				
	30 DAP	60 DAP	90 DAP	105 DAP	Total
$V_1Ac_1Al_1$	17.555	20.856	14.253	16.457	69.121
$V_1Ac_1Al_2$	15.215	20.846	15.360	19.749	17.170
$V_1Ac_2Al_1$	17.505	19.749	15.320	19.839	72.413
$V_1Ac_2Al_2$	17.306	17.205	20.767	17.600	74.878
$V_2Ac_1Al_1$	17.298	18.451	16.145	19.613	71.498
$V_2Ac_1Al_2$	18.451	19.599	17.298	19.609	74.958
$V_2Ac_2Al_1$	18.454	17.317	17.276	20.758	73.804
$V_2Ac_2Al_2$	21.673	16.827	16.887	20.469	75.855
Control	11.990	12.820	12.980	7.692	45.530
	NS	NS	NS	NS	NS

At Sixty days after planting treatment with $V_1Ac_1Al_1$ recorded highest increment of 20.85 cm.

At ninety days after planting the highest vine length increment was recorded by the treatment combination of $V_1Ac_2Al_2$ (20.76 cm).

At 105 days after planting combination treatment of $V_2Ac_2Al_1$ recorded highest vine length increment of 20.75 cm.

The highest total vine length increment was recorded by the combination of $V_2Ac_2Al_2$ (75.85cm) followed by the combination $V_2Ac_1Al_2$ (74.95 cm). The total vine length was also highest for the combination $V_2Ac_2Al_2$ (132.9cm).

On comparing shoot length of triple combination treatments, combinations with *G.fasciculatum* (V_2) was superior to combinations with *G.mosseae* (V_1) and triple combination treatment of $V_2Ac_2Al_2$ was significantly superior to rest of the treatments.

4.4.2.b Vine dry weight

The treatment combination of $V_2Ac_2Al_1$ recorded highest vine dry weight of 40.37 g.

4.4.2.c Number of leaves

The combination treatment of $V_2Ac_1Al_2$ recorded maximum number of leaves of 43.33.

Table 25. Growth characters of triple combination treatments[†]

Treatments	Shoot character				Root character			Nutrient content (per cent)		
	Vine length (cm)	Vine dry weight (g)	Number of leaves	Leaf dry weight	Root length (cm)	Root weight (g)	Root shoot ratio	N	P	K
$V_1Ac_1Al_1$	117.5^{bcd}	38.67^{abcde}	39.08^{abcd}	31.80^{e}	71.43^{ab}	15.93^{b}	0.226^{bcd}	2.453^{d}	0.246^{bcd}	2.014
$V_1Ac_1Al_2$	121.7^{abcd}	39.70^{abc}	40.92^{abc}	35.80^{cd}	69.10^{ab}	15.23^{b}	0.202^{i}	2.237^{g}	0.248^{bcd}	2.248
$V_1Ac_2Al_1$	119.6^{abcd}	38.77^{abcde}	38.91^{abcd}	31.37^{e}	71.63^{ab}	15.73^{b}	0.224^{cde}	2.380^{e}	0.246^{bcd}	2.184
$V_1Ac_2Al_2$	126.2^{abc}	39.00^{abcd}	40.50^{abc}	36.53^{bc}	72.33^{ab}	16.17^{b}	0.214^{h}	2.533^{bc}	0.244^{bcd}	2.190
$V_2Ac_1Al_1$	126.1^{abc}	39.67^{abc}	41.75^{ab}	36.87^{abc}	69.93^{ab}	17.50^{a}	0.228^{abc}	2.583^{ab}	0.245^{bd}	2.077
$V_2Ac_1Al_2$	130.2^{ab}	40.27^{ab}	43.33^{a}	37.47^{ab}	66.80^{bc}	17.97^{a}	0.231^{ab}	2.630^{a}	0.249^{b}	2.184
$V_2Ac_2Al_1$	129.6^{ab}	40.37^{ab}	40.66^{abc}	34.80^{d}	72.07^{ab}	17.54^{a}	0.234^{a}	2.493^{cd}	0.248^{bc}	2.480
$V_2Ac_2Al_2$	132.9^{a}	42.20^{a}	42.66^{ab}	38.00^{a}	73.30^{a}	17.73^{a}	0.222^{def}	2.610^{a}	0.256^{a}	2.236
Control	88.66^{j}	23.80^{j}	24.41^{i}	20.87^{n}	45.47^{h}	9.33^{h}	0.204^{l}	1.960^{j}	0.192^{i}	2.090
	S	S	S	S	S	S	S	S	S	NS

| Associated with Table 7, 14, 21 & 23

Figures with same alphabets do not differ significantly at 5 % level

4.4.2.d Leaf dry weight

Leaf dry weight observed was maximum for treatment $V_2Ac_2Al_2$ and recorded 38.00g.

4.4.2.e Root length

Treatment combination of $V_2Ac_2Al_2$ recorded highest root length of 73.30cm.

4.4.2.f Root weight

Combination treatment of $V_2Ac_1Al_2$ recorded highest root weight of 17.97 g.

4.4.2.g Root shoot ratio

Root-shoot ratio recorded was highest for treatment combination of $V_2Ac_2Al_1$, which was 0.234.

4.4.2.h Plant analysis

In vine pepper plants, nitrogen and phosphorus content were found to be significantly influenced by biofertilizer application while potassium content was found to be non-significant.

Nutrient content of vine pepper plants as influenced by inoculation with biofertilisers is given in Table 25.

Nitrogen content was highest in the combination of $V_2Ac_1Al_2$ with 2.63 per cent and was significantly superior to all other treatments. Phosphorus content was recorded maximum for treatment $V_2Ac_2Al_2$ with 0.25 per cent. Potassium content was found to be non-significant.

4.4.3 Microbial characteristic of rhizosphere

4.4.3.a AMF spore count

Table 26 presents data on the microbial properties of the rhizosphere.

Spore counts of AM fungi taken from the root rhizosphere of pepper vine showed that all of them including control gave spore counts of various degrees.

In single inoculation, rhizosphere of pepper vines with treatment *G. fasciculatum* (V_2) gave maximum spore count of 35.0 cfu/g soil.

In double combination, treatment with V_2Al_2 showed maximum spore count of 51.30cfu/g soil in its rhizosphere.

In triple combination, treatment with $V_2Ac_2Al_1$ recorded maximum spore count (72.3 cfu/g soil).

4.4.3.b Root infection percentage by AM fungi

It was observed that pepper plants inoculated with AM fungi showed better root infection percentage compared to uninoculated vines.

In single inoculation, treatment with *G. fasciculatum* (V_2) gave better root infection percentage of 43.33 per cent

In double combination, treatment with V_2Al_2 recorded highest root infection percentage of 56.67 per cent

Among triple combinations, treatment with $V_2Ac_2Al_2$ gave the maximum root infection percentage of 66.67per cent

Uninoculated plants showed very low root infection percentage.

4.4.3.c *Azotobacter* in pepper rhizosphere soil

Data on spore count of *Azotobacter chroococcum* in the rhizosphere soil of vine pepper plants as influenced by various treatments is presented in Table 26.

In single inoculation treatments, Ac_2 recorded highest of 3.5693cfu/g soil.

Among double combinations, treatment with V_2Ac_2 recorded highest of 3.9138 cfu/g soil.

Azotobacter was maximum in triple combination of $V_2Ac_1Al_1$ (5.2304 cfu/g soil) followed by $V_2Ac_2Al_1$ and $V_1Ac_1Al_2$(4.9637 cfu/g soil).

4.4.3.d *Azospirillum* spore count in pepper rhizosphere soil

The data on *Azospirillum lipoferum* in the rhizosphere soil of vine plants as influenced by various treatments is presented in Table 26.

Among single inoculations, Al_2 recorded highest of 2.6031 cfu/g soil.

Among double combinations, treatment with V_2Al_2 recorded highest of 4.3802 cfu/g soil.

Azospirillum recorded was highest in the triple combination of $V_2Ac_1Al_2$ (5.672 cfu/g soil) followed by triple combination of $V_2Ac_2Al_2$ (5.3802 cfu/g soil).

Table 26. Microbial properties of the rhizophere

Treatments	AM Fungi		*Azospirillum*	*Azotobacter*
	Root infection percentage (mean)	Mean spore count (cfu/g soil)	*Microbial count (cfu/g soil)	*Microbial count (cfu/g soil)
T_1 – Control	11.67	16.60	1.3222	1.3222
T_2 –Al_1	11.67	18.30	2.2576	1.6627
T_3 –Al_2	11.67	20.00	2.6031	1.6627
T_4 –Ac_1	11.67	21.60	1.6627	2.6031
T_5 – Ac_1Al_1	13.33	21.60	4.0792	2.2576
T_6 – Ac_1Al_1	11.67	24.00	4.2304	2.2555
T_7 – Ac_2	11.67	18.30	4.0792	3.5693
T_8 – Ac_2Al_1	13.33	23.30	3.1464	3.2555
T_9 – Ac_2Al_2	11.67	23.30	1.3222	3.2070
T_1 – V_1	41.67	25.00	2.6031	1.3222
T_{11} – V_1Al_1	48.33	40.00	3.8325	1.6627
T_{12} – V_1Al_2	51.6	41.6	3.9777	1.3222
T_{13} – V_1Ac_1	45.00	38.33	4.2304	1.7853
T_{14} – $V_1Ac_1Al_1$	30.00	30.00	3.8325	1.7853
T_{15} – $V_1Ac_1Al_2$	60.00	58.70	4.4149	4.9637
T_{16} – V_1Ac_2	30.0	38.60	4.0792	3.6533
T_{17} – $V_1Ac_2Al_1$	41.66	56.60	4.8325	4.7853
T_{18} – $V_1Ac_2Al_2$	-48.33	52.30	5.0413	4.2304
T_{19} – V_2	43.33	35.0	2.2576	1.7853
T_2 – V_2Al_1	53.33	50.00	3.8325	1.3222
T_{21} – V_2Al_2	56.67	51.30	4.3802	2.2555
T_{22} – V_2Ac_1	50.00	43.30	1.3222	1.3222
T_{23} – $V_2Ac_1Al_1$	63.33	40.00	4.6721	5.2304
T_{24} – $V_2Ac_1Al_2$	65.0	68.30	5.6720	3.6021
T_{25} – V_2Ac_2	41.66	41.60	2.2576	3.9138
T_{26} – $V_2Ac_2Al_1$	65.00	72.30	4.5185	4.9637
T_{27} – $V_2Ac_2Al_2$	66.67	65.00	5.3802	4.0797

*Logarithmically transformed value

Discussion

DISCUSSION

Black pepper, the 'King of spices' is an important export oriented spice crop of our country. But shortage of planting material, poor initial growth and low survival of rooted cuttings are the bottlenecks for successful cultivation of pepper in India.

Studies have shown that beneficial organisms like *Azotobacter* and *Azospirillum* improves rooting and initial growth of many horticultural crops (Rekar *et al.*, 1930; Ami *et al.*, 1995). *Azotobacter* is capable of fixing 15-20 kg $Nha^{-1}year^{-1}$ and can be exploited for nitrogen economy for the cultivation of many crops (Nair and Peethambaran.,2000). *Azospirillum* is reported to fix 15-25 kg $Nha^{-1}year^{-1}$ in association with plants. It also produces significant quantities of gibberellic acid, cytokinins and auxins naturally (Tien *et al.*, 1979). Recent studies have revealed the beneficial role of AM fungi in improving the plant growth through improved nutrition and growth hormone production. Pepper being a transplanted crop, inoculation of AM fungi in nursery stage can help to get vigorous transplants. Keeping these views in consideration, two experiments were conducted to study the effect of biofertilisers viz.; AM fungi, *Azotobacter* and *Azospirillum* on rooting, early growth and vegetative growth of pepper cuttings. The results obtained are discussed in this chapter.

5.1 Influence of AMF on pepper

5.1.1 On germination

Mycorrhizal treatments recorded higher rooting per cent both in vine and lateral cuttings over control plants.In runner cuttings, *G. mosseae* recorded 14.2 per cent more germination followed by *G. fasciculatum* which accounted for 12.3 per cent more germination over control (Fig.1) .For lateral cuttings, AMF treatments with G.*fasciculatum*, G. *mosseae* and G. *monosporum* improved rooting percentage by 83 per cent, 68 per cent and 66 per cent respectively over control (Fig.2). It is reported that mycorrhizal fungi are capable of elaborating small quantities of growth hormones including auxins which may help in inducing root production (Barea and Azcon., 1982). Anandaraj and Sarma (1994) also observed enhanced rooting in *G. fasciculatum* inoculated pepper cuttings. They attributed this effect to increased level of endogenous hormones.

5.1.2 *On vegetative growth*

Pepper cuttings inoculated with AMF, also enhanced the vegetative growth parameters such as vine length and number of leaves (Fig.3). This is in agreement with the findings of Hartmann *et al.* (1993) as reported in various plants. Shoot length and number of leaves were 71 per cent and 46 per cent respectively more in *G. fasciculatum* over control plants while inoculation of *G. mosseae* resulted in 119.7 per cent and 75 per cent increase in shoot length and leaf number respectively. *G. fasciculatum* and *G.mosseae*, which improved the vegetative characters in pepper, were selected for further studies of AMF cultures.

Plants inoculated with the selected species were transferred to larger pots and allowed to grow for an extended period of three and half months. During this period, growth performance recorded was better for *G. fasciculatum* inoculated plants though initially *G. mosseae* appeared to be the best inoculum. Between the two species, number of leaves and root-shoot ratio were significantly higher for *G. fasciculatum* inoculation .

However, inoculation with AMF had no significant effect on root length and nutrient content of plants. This is contradictory to the reports of Tang and Hao

Fig. 1. Germination percentage of runner cuttings inoculated with AMF

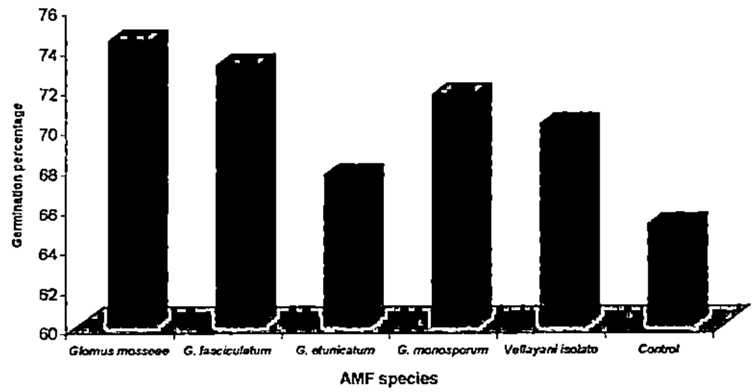


Fig. 2. Germination percentage of lateral cuttings inoculated with AMF

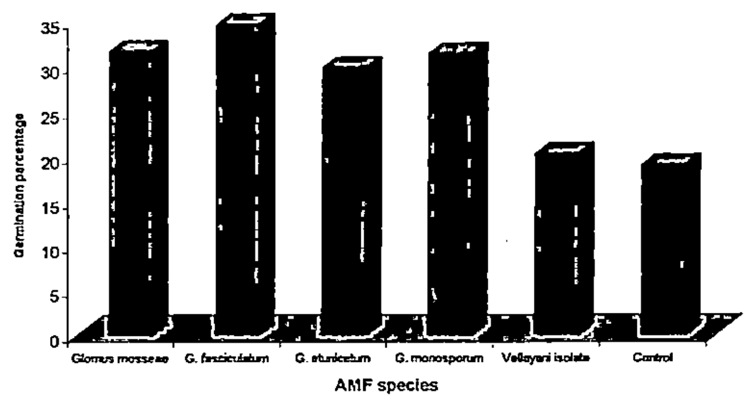


Fig. 3. Vine length and leaf number for runner as affected by AMF inoculations

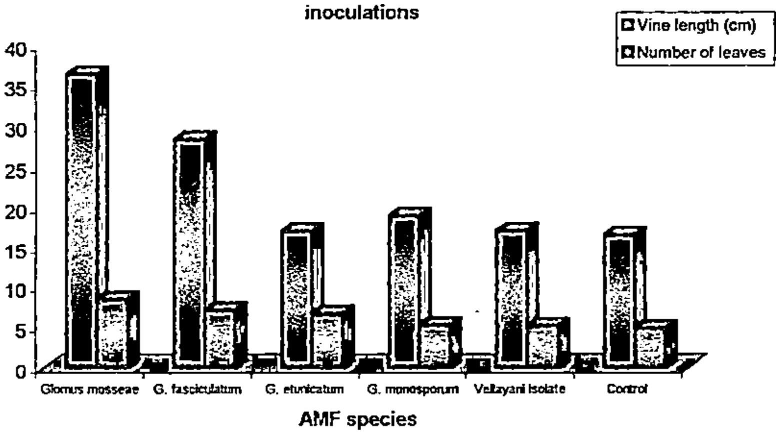


Plate 1. Effect of biofertilizers on plant characters

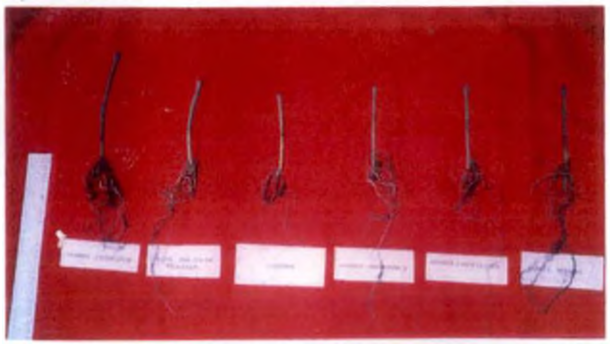
a) Shoot characters

AMF

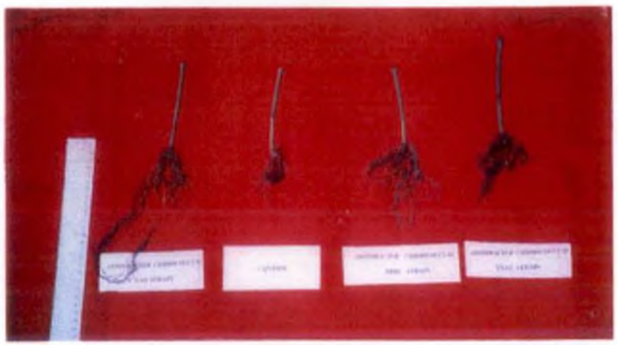
Azotobacter chroococcum

Azospirillum lipoferum

b) Root characters



AMF



Azotobacter chroococcum

Azospirillum lipoferum

(1984). The observed non-significant influence may be due to poor development within the short period of observation.

The overall influence of AMF on establishment and growth of pepper can be summarised as follows:

The effect of AMF treatment on improving sprouting was very conspicuous in lateral cuttings though it was not so marked with runner cuttings. The species *G. mosseae* and *G. fasciculatum* appeared to be more promising. In early vegetative growth, *G. mosseae* was found to have more influence. Later G. *fasciculatum* took over the position. Vegetative characters like vine length, leaf number, root length, dry weight etc. showed a significant increase as a result of inoculation. Nitrogen and phosphorus content were also found to increase.

5.2 Influence of *Azotobacter* on pepper

Pepper cuttings inoculated with *Azotobacter chroococcum* improved the overall vegetative growth parameters when compared to uninoculated control.

5.2.1 On germination

Treatments inoculated with *Azotobacter chroococcum* strains from UAS and TNAU recorded highest rooting per cent over control plants. *Azotobacter chroococcum* inoculants from TNAU (Ac_2) and UAS (Ac_1) improved the sprouting percentage in vine cuttings by 26.6 per cent and 17.3 per cent respectively over control plants (Fig.4). In lateral cuttings (Fig.5), Ac_2 and Ac_1 enhanced the germination per cent by 40.2 per cent and 29.7 per cent respectively over control plants. This influence of *Azotobacter* may be attributed to the production of small quantities of growth promoting substances as reported by Nair and Najachandra (1995).

Fig. 4. Germination percentage of runner cuttings inoculated with *Azotobacter chroococcum* strains

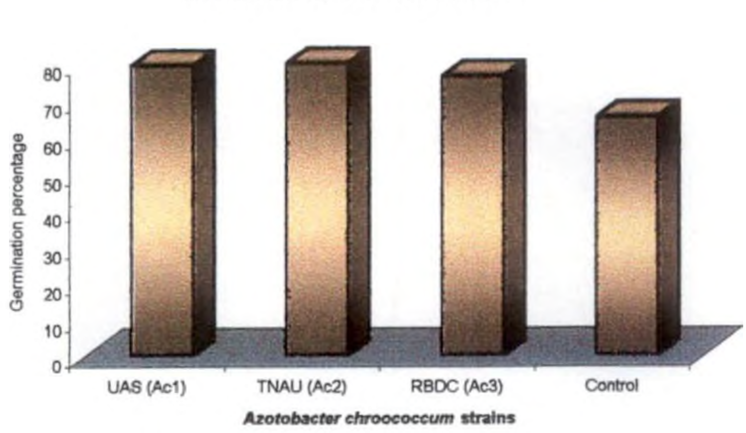


Fig. 5. Germination percentage of lateral cuttings inoculated with *Azotobacter chroococcum* strains

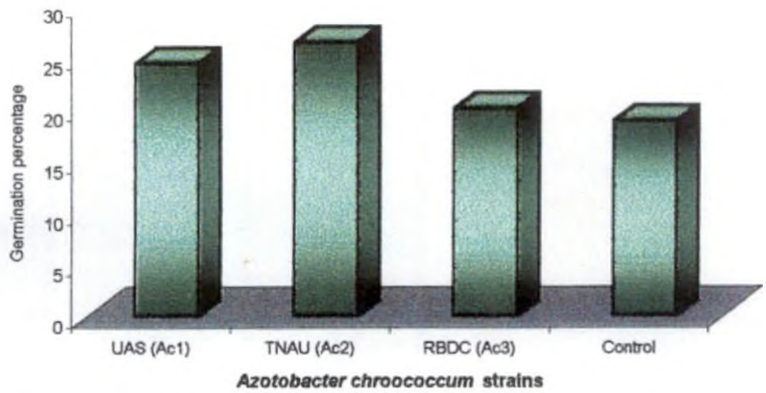
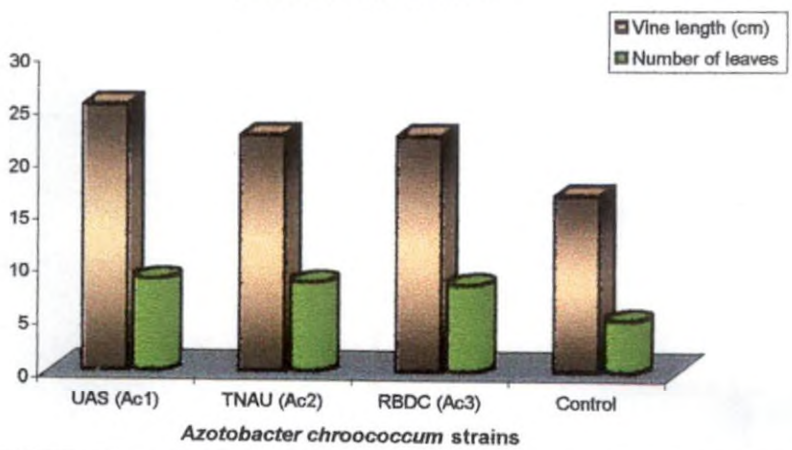


Fig.6. Vine length and leaf number as affected by different strains of *Azotobacter chroococcum*



5.2.2 On growth

After six months of planting, shoot length and number of leaves recorded were 35 per cent and 72 per cent more with TNAU strain (Ac_2) and 52 per cent and 79 per cent more with UAS strain (Ac_1) (Fig.6). Similar results of enhanced growth by *Azotobacter* inoculation were reported in pepper by Bopaiah and Khader (1989), in forage sorghum by Agarwal *et al.*(1996) . The enhancement of growth in treated plants when compared to control may be due to production of phytohormones since no significant effect on nitrogen content was observed due to inoculation. *Azotobacter* besides nitrogen fixation is also reported to produce plant growth promoting substances and phytohormones but the extent of production varies according to different isolates (Nair and Najachandra, 1995).

Based on the relative performance *Azotobacter*, UAS (Ac_1) and *Azotobacter* TNAU (Ac_2) were selected for further studies of the *Azotobacter* strains.

Plants inoculated with these strains of *A. chroococcum* were repotted in larger pots and growth observations were taken for a further period of three and half months. For all growth parameters the strains were found to be on par with each other, which indicated that source difference was not at all important in later stages of inoculation. Over control plants, the treated plants were significantly superior in terms of vine length, dry weight and leaf number.

By the end of the experiment, nitrogen content appeared to be significantly higher in treated plants. This is in agreement with the findings of Nair and Peethambaran (2000) in different crops.

5.3 Influence of *Azospirillum* on pepper

Vegetative growth characters of vine cuttings such as rooting per cent, vine length and number of leaves were improved by *Azospirillum* inoculation.

5.3.1 On germination

In vine cuttings treatments inoculated with *Azospirillum lipoferum* strains from TNAU and UAS recorded better rooting per cent over control plants. In runner cuttings, *Azospirillum* inoculum from UAS (Al_1) and TNAU (Al_2) improved the sprouting percentage by 32.3 per cent and 19.2 per cent respectively over control (Fig.7). In lateral cuttings, $Al_1$and Al_2 recorded higher growth by 31 per cent and 33.3 per cent over control (Fig.8). Govindan and Purushothuman (1985) observed that *Azospirillum* secretes phytohormones and these phytohormones might have improved the sprouting percentage.

5.3.2 On vegetative growth

Azospirillum colonisation improved vegetative growth parameters of pepper plants when compared to control. Similar results have been reported in black pepper by Govindan and Chandy (1985). In vine pepper cuttings, shoot length and number of leaves were 153 per cent and 89.5 per cent respectively more in UAS strain (Al_1) over control while for TNAU strain (Al_2) it recorded 140 per cent and 88.7 per cent respectively more over control (Fig.9). *Azospirillum* strains from UAS and TNAU were selected for further inoculation studies. Plants treated with the selected strains of inoculum were transferred to larger pots and growth measurements were continued for three and half months. As in the case of *Azotobacter*, *Azospirillum* strains were also on par with each other. When compared to control, the treated plants were found to be superior in shoot and root growth and nitrogen content. The

Fig. 7. Germination percentage of runner cuttings inoculated with *Azospirillum lipoferum* strains

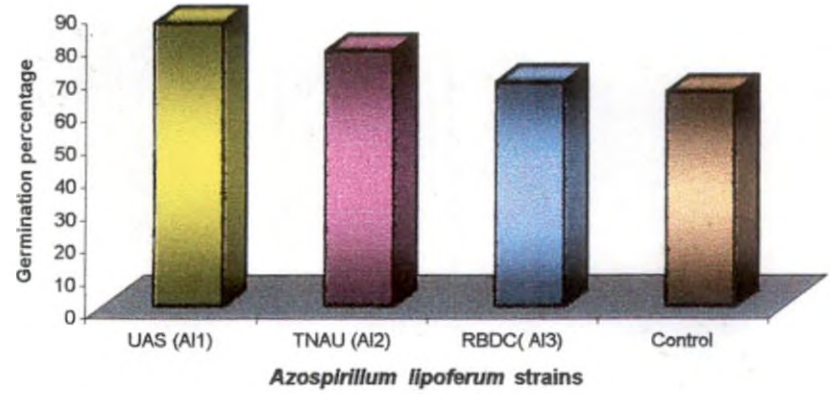


Fig. 8. Germination percentage of lateral cuttings inoculated with *Azospirillum lipoferum* strains

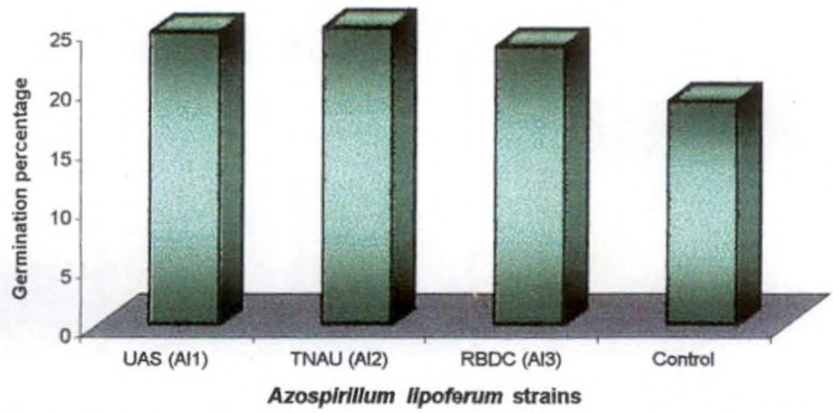
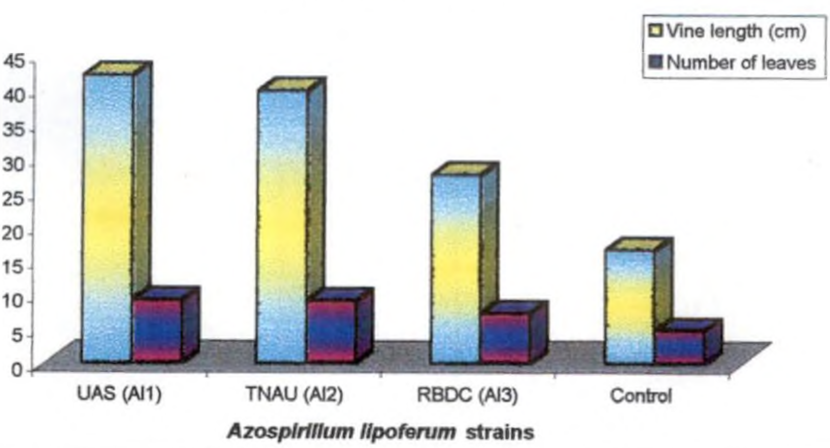


Fig. 9. Vine length and leaf number as affected by different strains of *Azospirillum lipoferum*



better growth performance may be due to the secretion of growth promoting substances in addition to biological nitrogen fixation (Purushothuman et al., 1988).

From the study on the influence of nitrogen fixing bacteria on pepper, the following conclusions may be drawn. Both the microbes increased rooting percentage, vine length, leaf number and nutrient content.

Azospirillum lipoferum was more efficient than Azotobacter chroococcum in improving establishment and overall vegetative growth. This may be because of the fact that our environmental conditions are more matching to the requirements of *A.lipoferum*. Again, it is a better root coloniser than the free-living Azotobacter (Paul, 1995).

5.4 Multiple inoculation

5.4.1 Dual inoculation

Rooted cuttings inoculated initially with AMF were reinoculated using free-living nitrogen fixers and allowed to grow for three and a half months. Double inoculation involving only *Azotobacter* and *Azospirillum* were also tried. In all cases, double inoculation was found to be superior to inoculation with a single microbe. Double inoculation of V_2Al_2 increased vine length, leaf number and root length by 32.3 per cent, 49 per cent and 42 per cent respectively. Inoculation with AMF and nitrogen fixing bacteria was found to be more promising than two nitrogen fixing bacteria. The best combination found was AMF with *Azospirillum lipoferum*. Zaghoul et al. (1996) have reported the importance of dual inoculation with *G. mosseae* and *A. brasilense* in increasing the uptake of nutrients in wheat. Bopaiah and Khader (1989) observed a positive influence on plant height and shoot and root weight of rooted cuttings of black pepper. Saleh and Nokhal (1988) have also reported the beneficial effects of dual inoculation. The presence of AMF might have influenced root development, which in turn facilitated the bacterial colonization.

5.4.2 Triple inoculation

Inoculation with three microbes proved to be better than single and double inoculations. Triple inoculation improved the overall performance in pepper. Triple combination of $V_2Ac_2Al_2$ gave the highest vine length, vine dry weight and root length which corresponds to an increase of 49.8 per cent, 77.3 per cent, 61.2 per cent respectively over control. The observed significance on growth parameters may be due to the beneficial effect of AMF on root proliferation coupled with growth promoting characters of nitrogen fixing bacteria. Improvement in growth became more pronounced when two bacteria were involved because of additive effect.

5.4.3 Combination effect

All treatments inoculated with double combination of inoculum were compared with respect to each inoculum and the treatments showing highest increment in vine length with respect to both inoculum involved are presented in Figure 10

For double combinations highest increment with respect to two inoculum was for V_1Al_2 thirty days after planting which was 17.71cm. V_2Ac_2 recorded the highest increment sixty days after planting (15.56cm). Ninety days after planting, the highest increment was for V_2Al_2 with 19.88cm. Hundred and five days after planting, V_2Al_1 recorded maximum height increment of 15.46 cm. The total plant height difference for double combination with respect to two inoculum was maximum for treatment combination of V_2Al_2 (65.16cm).

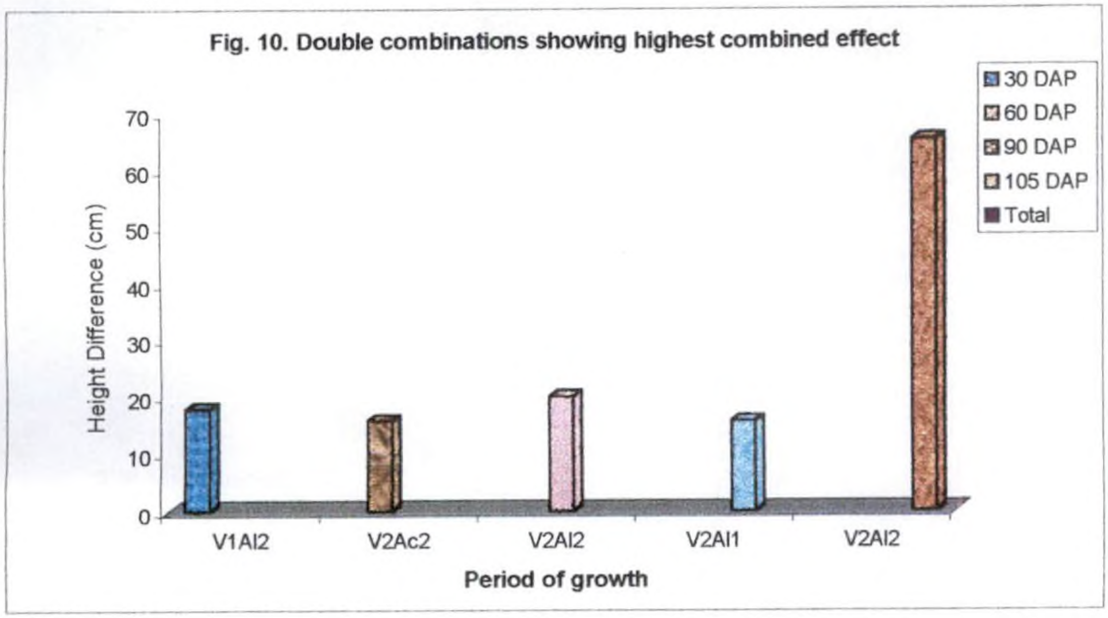


Fig. 10. Double combinations showing highest combined effect
30 DAP
60 DAP
90 DAP
105 DAP
Total
Height Difference (cm)
70
60
50
40
30
20
10
0
V1Al2
V2Ac2
V2Al2
V2Al1
V2Al2
Period of growth

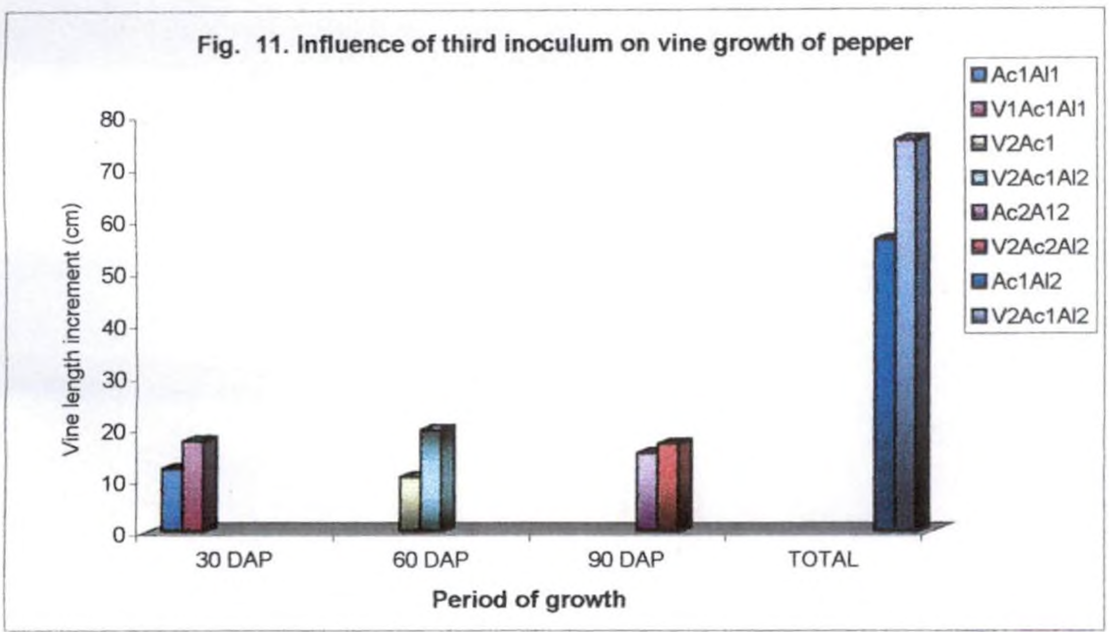


Fig. 11. Influence of third inoculum on vine growth of pepper
Ac1Al1
V1Ac1Al1
V2Ac1
V2Ac1Al2
Ac2A12
V2Ac2Al2
Ac1Al2
V2Ac1Al2
Vine length increment (cm)
80
70
60
50
40
30
20
10
0
30 DAP
60 DAP
90 DAP
TOTAL
Period of growth

Improvement in growth of double inoculum treatments with minimum growth as influenced by triple inoculation during different stages of growth is presented in Fig. 11.

For double inoculation, the minimum increment in vine length observed thirty days after planting was for the combination Ac_1Al_1 (12.22 cm) and gave a better increment of 43.5 per cent when inoculated with the third AMF inoculum. Sixty days after planting treatment with V_2Ac_1 had a vine length increment of 10.49cm, which gave a better increment of 86 per cent when combined with the third *Azospirillum* inoculum. Ninety days after planting minimum increment was observed with treatment Ac_2Al_2 (15.165cm) and gave a better increment of 42.9 per cent when inoculated with the third AMF inoculum. The minimum increment in total plant height was observed with treatment Ac_1Al_2 (55.89cm) and gave a better increment of 34.1 per cent when third inoculum AMF was also incorporated. This may be due to the synergistic effect of AMF on bacterial population.

The combined effect of inoculum for triple combination treatments is given in Fig.12. During thirty days after planting maximum increment with respect to three inoculum was for triple combination of $V_2Ac_2Al_2$ (21.67cm). At sixty days after planting highest increment in plant height recorded with respect to three inoculum was maximum for triple combination of $V_1Ac_1Al_2$ (20.84cm). At ninety days after planting highest increment in plant height recorded with respect to three inoculum was maximum for triple combination of $V_1Ac_2Al_2$ (20.76cm). During hundred and five days after planting increment in plant height difference recorded was maximum with respect to three inoculum was for triple combination of $V_2Ac_2Al_1$(20.75cm).Total plant height difference recorded with respect to two inoculum was maximum for triple combination of $V_2Ac_2Al_2$ (75.85 cm).The overall influence of multiple inoculation can be summarised as follows.

Plate 2. Combination effect of biofertilizers on pepper roots

$V_2Ac_2Al_1$

$V_2Ac_2Al_2$

V_2Al_2

Control

All combinations in spite of the components performed better than single inoculum. Combinations involving an AMF and nitrogen-fixing bacteria were more efficient than two bacteria. Triple combinations were more promising than double combinations.

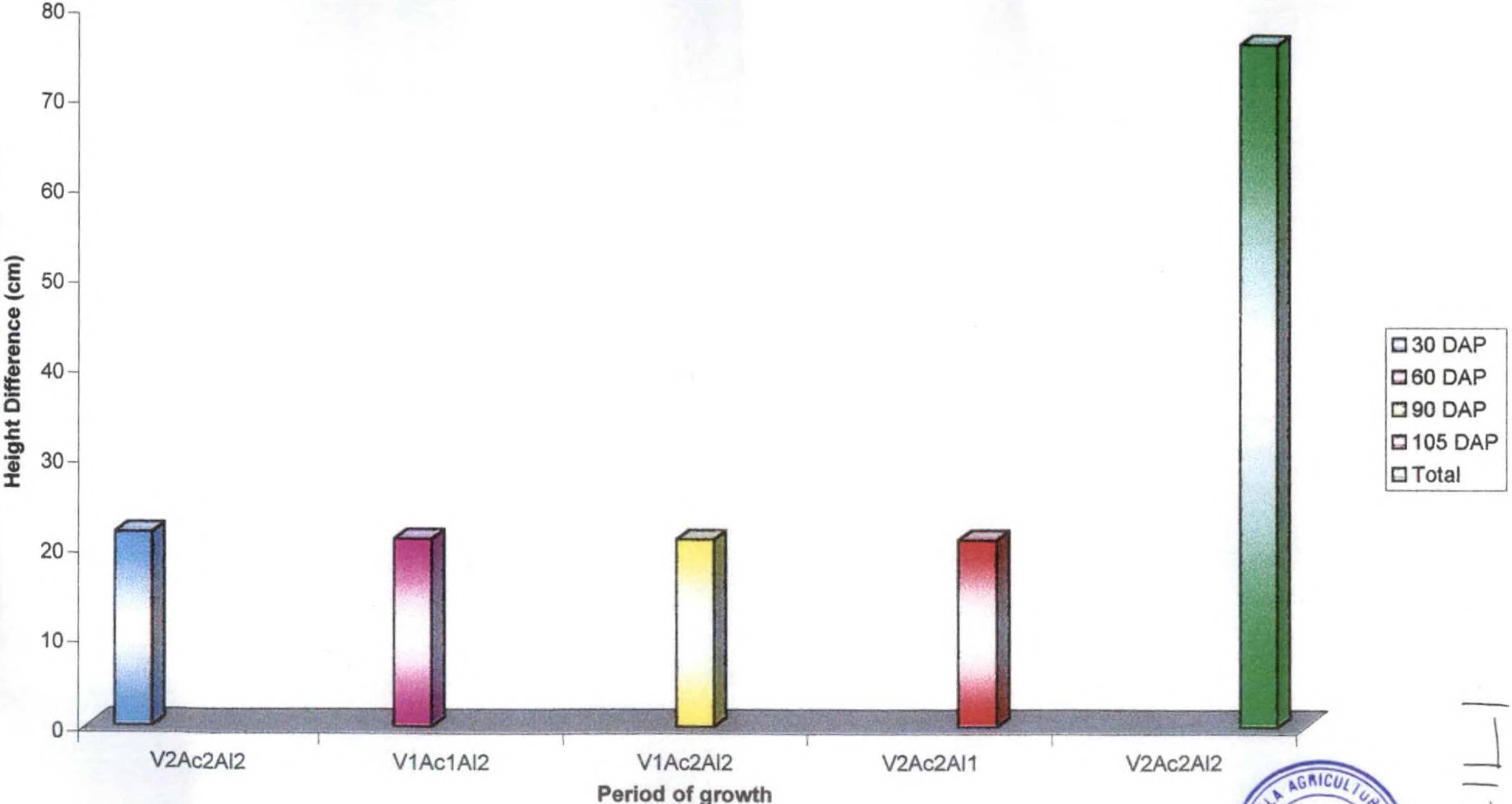
5.4.4 Microbial population

For AMF, the spore count in the rhizosphere as well as root infection percentage was maximum for vine inoculated with triple combination of $V_2Ac_2Al_1$ Similar results with synergistic effect on occurrence of AMF due to proliferation of *Azotobacter* and other nitrogen fixing microorganisms has been reported in tomato (Secelia and Bagyaraj, 1987)

In general the spore count observed was low for *Azotobacter* and comparatively better for *Azospirillum*. The occurrence of *Azotobacter* is largely determined by many soil factors such as pH, moisture content, nutrient availability, organic matter content etc (Krishnamurthy,1962). *Azotobacter chroococcum* population is low in acidic soils and it proliferates in neutral to alkaline soils (Rangaswamy and Sadasivan, 1969). The unfavourable soil conditions might have contributed to the low spore count of *Azotobacter* recorded in the experiment. The highest *Azotobacter* spore count recorded was for triple combination of $V_2Ac_1Al_1$. Similar results are reported in *Azotobacter* by AMF combination (Azcon *et al.*, 1978).

Similarly, for *Azospirillum* bacteria, the occurrence is also controlled by soil pH. It can grow in acidic conditions also. Under acidic condition the bacteria proliferates within the roots (Dobereiner *et al.*, 1976). That might be the reason for low spore count of *Azospirillum*. The *Azospirillum* spore count recorded was highest for the triple combination of $V_2Ac_1Al_2$. Similar results were also reported for *Azospirillum* in combination with AMF (Prathibha *et al.*, 1995).

Fig. 12. Triple combinations showing highest combined effect



Summary

SUMMARY

An experiment was conducted in the Department of Agronomy, College of Horticulture, Vellanikkara during 1999-2000 to study the effect of various biofertilisers on establishment and growth of pepper cuttings. Both lateral and runner cuttings were used in the study. The biofertilisers tried included five species of the arbuscular mycorrhizal fungus, *Glomus sp.* and three strains each of the bacteria *Azotobacter chroococcum* and *Azospirillum lipoferum*. After a preliminary screening tried on establishment and early growth of cuttings, two species/strains each of the AMF and the bacteria were selected. Single, double and triple combinations of these microbes were then tried on further growth of established runner cuttings. Salient features of the investigations are summarised below.

Black pepper responds well to inoculation with biofertilisers. With regard to establishment, the influence of *Azotobacter* and *Azospirillum* were comparable. Effect of AMF inoculation was not very conspicuous in runner cuttings, but with laterals, it seemed very promising. Among different species of *Glomus*, *G. mosseae* and *G. fasciculatum* recorded higher percentage germination followed by *G.monosporum*.

Both in *A. chroococcum* and *A. lipoferum*, strains from TNAU, Coimbatore and UAS, Bangalore were more efficient when compared to that from RBDC, Bangalore.

Vine length, number of leaves and dry weight were significantly higher in plants inoculated with the selected species/ strains of AMF and nitrogen fixing bacteria. Nitrogen and phosphorus content also increased significantly. However, K content was not at all affected by the microbial colonisation. AMF inoculation had positive, significant difference on root length whereas bacterial treatment did not show any significant effect. Between the two species of AMF, *G. fasciculatum* was more effective in improving vegetative growth. Similarly, comparing the N fixers, *A.lipoferum* was more promising with little difference between the strains at the final stage of observation.

Multiple inoculation studies showed that double and triple inoculations were always superior to single inoculum and a nitrogen fixing bacteria were more useful than two nitrogen fixing bacteria. However, addition of second nitrogen fixers to a combination of AMF and bacteria further improved its performance.

The best combined effect was shown by the combination of *G.fasciculatum* with *Azospirillum lipoferum*.

All these results relate to the work done for a period of less than one year, in pots. A recommendation on species and strains needs further investigations with repeated inoculations under field condition.

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*Originals not seen

Appendices

Appendix-1

Okon's media for *Azospirillum*
K_2HPO_4 – 6gm
KH_2PO_4 – 4gm
Distilled water – 500ml
$MgSO_4$. $7H_2O$ – 0.2g
NaCl – 0.1g
$CaCl_2$ – 0.02g
NH_4Cl – 1g
Malic Acid – 5g
NaOH – 3g
Yeast – 0.05g
$Na_2M_0O_4$ – 0.002g
$MnSO_4$. H_2O – 0.0001g
H_3BO_3 – 0.0014g
$Cu(NO_3)_2$ – 0.0004g
$ZnSO_4$ – 0.0021g
$FeCl_3$ – 0.002g
Agar – 6g
Distilled water – 500ml

Appendix-2

Jenson's media for *Azotobacter*
Mannitol – 20gm
K_2HPO_4 – 0.2gm
$MgSO_4$ – 0.2gm
NaCl – 0.2gm
K_2SO_4 – 0.1gm
$CaCO_3$ – 5gm
Agar – 18 gm
pH – 7

Appendix-3

Physio-chemical properties of potting mixture

Characters	Values	Methodology
Organic carbon (%)	2.9	Walkley & Black Method (Soil Survey staff, 1992)
PH	6.7	(1: 2.5 Soil water suspension) (Jackson, 1973)
Available Nitrogen (ppm)	200	Available Permanganate Method (Subbaiah & Asija, 1956)
Available Phosphorus (ppm)	21	Bray I extractant Ascorbic Acid Reductant Method (Soil Survey staff, 1992)
Available Potassium (ppm)	712	Neutral Normal Ammonium Acetate Extractant, Flame Photometry (Jackson, 1973)

Appendix-4

Weekly rainfall (mm), evaporation (mm), surface air temperature (°C) relative humidity (%) and sunshine hours (h day^{-1}) at COH, Vellanikara from April 1999 to April 2000 (Latitude 10°31'N, Longitude 76°13' and Altitude 40.29 MSL)

Week No.	Rain fall (mm)	Evapo-ration (mm)	Surface air temperature(°C)			Relative humidity (%)		Sunshine Hours (h da^{-1})
			Max	Min	Mean	Morning	Evening	
1st	5.2	25.1	32.0	25.9	29.0	90	59	4.2
2nd	35.0	30.7	33.6	25.8	29.7	89	59	6.3
3rd	37.0	21.6	31.0	25.2	28.1	90	66	6.4
4th	51.6	22.6	30.4	25.1	27.8	88	74	5.5
5th	221.2	20.1	29.0	23.8	26.4	95	85	2.6
6th	143.2	21.5	29.8	3.5	16.7	96	75	5.0
7th	134.7	22.7	29.1	2.8	16.0	94	81	4.8
8th	170.9	17.3	28.1	22.7	25.4	95	81	1.8
9th	114.8	20.6	29.6	23.2	26.4	95	76	5.1
10th	21.6	26.4	30.9	23.0	27.0	92	67	8.9
11th	114.7	0.5	29.6	23.1	26.4	95	80	3.7
12th	124.6	18.0	29.0	22.9	26.0	96	76	3.1
13th	326.5	12.6	26.9	22.8	24.9	97	92	3.2
14th	182.8	13.0	27.7	22.7	25.2	95	83	1.1
15th	194.1	17.1	28.7	23.3	26.0	95	84	2.7
16th	121.5	20.8	29.5	23.7	26.6	95	74	5.2
17th	8.9	24.7	30.6	24.1	27.4	93	69	7.5
18th	3.2	25.1	30.0	23.6	26.8	93	69	6.9
19th	7.1	20.6	30.0	23.6	26.8	93	71	5.3
20th	18.3	18.7	30.0	23.2	26.6	92	67	4.9
21st	10.1	27.6	31.0	23.0	27.0	90	65	8.1
22nd	0.0	31.0	32.6	23.4	28.0	90	56	8.5
23rd	0.0	28.2	32.9	23.8	28.4	93	60	6.4
24th	80.5	19.6	30.5	23.1	26.8	95	71	4.8
25th	185.7	23.8	31.5	23.6	27.6	95	75	6.8
26th	161.6	16.8	29.5	23.3	26.4	95	80	2.9
27th	38.8	19.6	31.3	23.5	27.4	93	74	5.5
28th	41.9	20.3	29.6	22.7	26.2	96	7	6.2
29th	2.8	22.4	31.4	22.1	26.8	87	62	7.8
30th	0.0	26.6	31.1	22.1	26.6	74	46	10.1
31st	4.0	29.4	31.1	23.5	27.3	79	62	6.3
32nd	0.0	36.4	31.9	23.7	27.8	76	55	8.7
33rd	0.0	34.3	31.8	21.6	26.7	79	49	9.4
34th	0.0	31.5	31.8	22.6	27.2	78	50	8.1
35th	0.0	44.8	31.4	22.6	27.0	72	47	8.7
36th	0.0	49.0	31.4	23.4	27.4	68	43	8.8
37th	0.0	6.9	32.2	23.8	28.0	71	45	9.6
38th	0.0	6.5	31.9	24.3	28.1	73.6	50.7	7.6
39th	0.0	6.2	33.5	22.4	28.0	78	37	9.5
40th	0.0	5.9	33.8	22.1	28.0	82	39	9.9
41st	0.0	7.1	33.7	19.9	26.8	74	41	10.1
42nd	0.0	4.5	33.2	22.9	28.1	92	57	7.2
43rd	0.0	6.0	34.2	23.0	28.6	80	44	9.3
44th	0.0	4.7	33.2	22.6	27.9	90	59	8.7
45th	0.0	5.5	33.9	23.8	28.9	81	47	8.0
46th	0.0	5.4	35.1	23.3	29.2	93	49.3	9.5
47th	0.0	5.3	34.8	23.9	29.4	88.6	50	9.6
48th	0.0	6.0	35.9	23.8	29.9	89	43.7	9.9
49th	0.0	5.8	36.3	24.7	30.5	84	55	9.2
50th	0.0	3.2	33.3	23.6	28.5	91.1	61	5.5
51st	0.0	4.4	34.2	24.9	29.6	89	58	7.6
52nd	0.0	4.6	34.1	24.5	29.3	88	58	8.4
53rd	0.0	4.8	34.2	25.1	29.7	88	54	6.9

EFFECT OF BIOFERTILIZERS ON EARLY ROOTING, GROWTH AND NUTRIENT STATUS OF BLACK PEPPER (*Piper nigrum* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

With an objective of studying the effect of various biofertilizers on establishment and growth of pepper cuttings, an experiment was conducted in the College of Horticulture, Vellanikkara during 1999-2000. Both lateral and runner cuttings were inoculated with five species of Glomus and three strains each of *A.lipoferum* and *A.chroococcum*. Observations were taken on germination and different growth parameters. Based on the early performance two species of *Glomus* and two strains each of *A.chroococcum* and *A.lipoferum* were selected. Single, double and triple combinations of these selected microbes were studied further.

Multiple inoculation was found to be superior over single inoculations. Combinations involving AMF and nitrogen fixing bacteria were showing better results compared to two nitrogen fixing bacteria. Comparing the two species of AMF, *G.fasciculatum* was more effective in improving overall growth characters. Among nitrogen fixers, *A.lipoferum* was more promising.

Combination effect of *G.fasciculatum* and *A.lipoferum* was appreciably good. However, addition of *A.chroococcum* with *A.lipoferum* and AMF further improved the growth performance of crop.