EFFECT OF *AZOSPIRILLUM* INOCULATION ON ESTABLISHMENT AND GROWTH OF BUSH PEPPER

BY

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DECLARATION

I hereby declare that this thesis entitled "Effect of Azospirillum inoculation on establishment and growth of bush pepper" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me any degree, diploma, associateship, fellowship, or other similar title, of any other University of Society.

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CERTIFICATE

Certified that this thesis, entitled, "Effect of Azospirillum inoculation on establishment and growth of bush pepper " is a record of research work done independently by Smt. YAMINI VARMA. C.K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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INTRODUCTION

Introduction

Pepper is one of the most important spices produced in India. It accounts for nearly 60 percent of the foreign exchange earned by our country from export of spices. The world demand of pepper by 2000 A.D will be around 250,000 metric tonnes. Inorder to meet this high demand, the annual production of pepper has to be increased substantially by expanding the present area under cultivation.

Bush pepper cultivation is a novel way of growing black pepper in homestead. It has also got an advantage in that it can meet a major part of the domestic demand for pepper. An important drawback in the cultivation of bush pepper is the difficulty in getting sufficient quantity of planting material, as the farmers are often reluctant to part with fruiting branches from a good yielding pepper vine. Further these branches are also shy in rooting. The rooting percentage can however be increased by using plant hormones like Indole butyric acid and Ceradix. But these products can be effectively substituted with certain bacteria capable of producing significant quantity of phytohormones like Indole acetic acid, Gibberellins and cytokinins. The present study was mainly intended to explore the possibility of using Azospirillum for this

purpose. The effect of subsequent inoculation with this diazotroph on establishment and early growth of bush pepper also formed a part of this investigation.

The approved technical programme were as follows:

1. Isolation of native strains of Azospirillum from pepper rhizosphere by enrichment culture technique using nitrogen free semi solid malate agar medium.
2. Estimation of production of phytohormones like Indole acetic acid and gibberellins by Azospirillum under in vitro conditions.
3. Use of Azospirillum for root induction in Panniyur-1 and Karimunda varieties of bush pepper.
4. Effect of Azospirillum inoculation on establishment and growth of Panniyur-1 and Karimunda varieties of bush pepper.

REVIEW OF LITERATURE

Review of literature

Pepper, "The king of spices" is a native of Indo-Malayan tropical forest region. It grows well from low lands to high lands up to 1200 m above mean sea level. According to spices Board Report (1994) the area under pepper cultivation in Kerala is around 1,69,670 hectares with an annual production of 41,560 metric tonnes. The corresponding figures for India and for the world are 1,74,870 hectares and 42,690 metric tonnes and 3,48,700 hectares and 2,25,873 metric tonnes respectively.

Pepper is a climber with strong stems and nodal adventitious climbing roots. It is normally propagated from runner shoots produced at the base of mother plants. However, the laterals or plageotrophs can also be used for vegetative propagation. Cooper (1955) reported a rapid method for propagating black pepper. In this procedure, leaf cuttings with petiole and attached bud were dipped in IBA of 2mg/ml concentration. This resulted in 75 percent rooting in 21 days in a standard cacao propagator with coir dust as the medium. Nambiar and Kurian(1963) found that pepper cuttings selected from mother vines in the month of March gave 90-95 percent rooting in bamboo baskets with 80-90 percent stand on transplanting after two months. Choudhary and Phadnis (1971) noted that higher rooting was induced in pepper by

using IBA of 55 ppm concentration. Bavappa and Gunasinghe (1978) tested the percentage of success in rooting of different types of pepper cuttings in local varieties without any specific treatment. They found that lateral branches produced only 13.1 percent rooting.

Wahid (1981) observed that planting of single node cuttings with one leaf in a rooting medium of 7 parts of soil and 3 parts of farm yard manure gave 84.5 percent rooting 4 weeks after planting. Senanayake and Kirthisinghe (1983) observed that two cuttings of orthotropic shoots in pepper under 35, 50,75 or 95 percent shaded condition with irrigation produced highest number of roots, largest leaf area and greatest dry weight at 95 days of plant growth. Zaubin (1981) got best rooting results in Indonesia by treating the cuttings having adventitious roots with Rhizopan AA (0.1 percent Indole Butyric acid). Pillay et al (1982) reported that two nod cuttings of runner shoots of Panniyur-1 produced early roots after treatment with IBA 1000 ppm for 45 seconds. This not only increased the length and weight of roots, but also boosted the shoot growth and number of leaves. Hegde (1984) also suggested that small pepper cuttings of Panniyur-1 with one node could be used for multiplication of pepper by pepper with 25 ppm IBA.

Litzenlerger and Lip (1961) reported that Eupatorium odoratum a common weed in Cambodia could be used

effectively as an organic manure to improve the yield of black pepper. Pillay and Nair (1977) observed that digging around the vines to a diameter of 6 feet every year increased the yield of black pepper. Kato et al (1980) compared the effect of mulching black pepper with rice husk,hay and saw dust at the rate of 34 tonnes per hectare with no mulching .They got better yield after mulching with rice husk. Nybe et al (1989) reported that for increasing yield in Panniyur-1 the addition of 10 kg green leaves and 500g of lime in the month of July per vine was a good practice. Pillay (1992) also reported that the application of 10 kg of organic manure in the form of green leaves, compost or well rotten cattle manure to each pepper vine annually increased the yield of pepper. According to NRCS research highlights (1993-94) the application of oil cakes equivalent to a nutrient ratio of NPK at the rate of 1:0.5:2g per 10 kg soil increased the spiking intensity, berry volume and yield in two varieties of pepper namely Panniyur-1 and Karimunda. The use of groundnut cake at the rate of 10g per 10kg pot at bimonthly intervals also increased the yield by about 184 percent over control treatment. Sivaraman et al (1994) found that irrigating pepper vines from November to December till the end of March and withholding irrigation till monsoon break increased pepper yield by 50 percent. They further observed that the

application of farm yard manure, well decomposed compost or green leaves at the time of onset of monsoon significantly increased the yield of pepper.

Bush Pepper

Menon (1949) reported that in some localities of Travancore and North Kanara, farmers used planting material from the fruiting branches and such plants began to bear in the second year and gave onwards. In comparison to this, the vegetative runners began to yield only from sixth year onwards. Pillay and Chandy (1980) observed that if fruiting branches of pepper were used for propagation, the resulting plants had a bushy nature. Later such plants came to be known as bush pepper.

Irulappan et al (1981) reported that the percentage of rooting in Panniyur-1 branches could be increased from 31.3 to 84.3 percent by using 5000 ppm IBA. Geetha et al (1989) showed that the lateral shoots of pepper collected in the month of May were best for rooting with 85 success after treatment with Ceradix B(2). They further noted that the mean time taken for rooting of laterals ranged from 40-45 days in various treatment trials. According to NRCS (1990) for the propagation of bush pepper, young healthy laterals taken must be initially dipped in 0.2

percent copper oxychloride solution for 20-30 minutes and pruned to 2-4 nodes length with one topmost leaf prior to planting in poly bags containing moist coir dust. After 35-50 days these cuttings were planted in poly bags with soil, sand and farm yard manure in 1:1:1 proportion. Varieties such as Panniyur-1, Karimunda, Kuthiravally, Aimpiriyan, and Kottanadan were most suitable for bush pepper production and the average yield from these varieties ranged from 50 to 1000 g from second year of planting.

Sivaraman et al (1994) noticed that lateral cuttings of pepper planted in poly bags produced 5-6 healthy roots over a period of 35-45 days after planting and that the average yield of green pepper varied from 300-400g per year during the second year of bearing.

Geetha (1990) evaluated the differences in nutrient uptake by bush pepper and vine pepper. She reported that the differences were in the magnitude of response to applied nutrients especially N, K, Ca, Mg, and S. The vine pepper required more quantities of these nutrients as compared to bush pepper consequent to higher biomass production.

Azospirillum - an associative diazotroph

The nitrogen fixing bacterium, Spirillum lipoferum was first described by Beijerinck in 1925. Later Becking

(1963) isolated a strain of Spirillum from African soils resembling Beijerinck's S. lipoferum and Dobereiner et al (1976) isolated Azospirillum from the surface sterilized roots of field grasses. It was Dobereiner and Day (1976) who defined associative symbiosis as an intracellular relationship between a dinitrogen fixing bacterium and plant roots. Lakshmikumari et al (1976) reported the association of Azospirillum with the roots of several crop plants in India such as maize, sorghum, rice, sugar cane and forage grasses. Venkateswarlu and Rao (1985) isolated Azospirillum from roots of diverse desertic plant species having C_3, C_4 and CAM photo synthetic path ways. These cultures had identical morphological and physiological characters similar to A. lipoferum in their ability to utilise glucose and 2 keto glutaric acid as sole carbon source for growth.

Sixteen strains of Azospirillum sp. were obtained by Padshetty et al (1986) from the rhizosphere soil and root cuttings of wheat. Similarly Purushothaman (1988) got two promising isolates of A. lipoferum C-13 and IPI with relatively high rates of nitrogenase activity from root tissues of rice. Khan and Hossain (1990) observed that both roots and stems of rice and grasses harboured Azospirillum. Lukin et al (1992) reported greater density of Azospirillum in the rhizosphere of barely which decreased with increase in distance from root surface.

Govindan and Chandy (1985) first isolated the diazotroph Azospirillum from black pepper rhizosphere. Later Bopaiah and Khader (1989) also isolated Azospirillum from surface sterilized roots of black pepper.

Dobereiner et al (1976) and Okon et al (1976) observed that certain strains of Azospirillum required low levels of yeast extract for growth on mineral media and that such strains exhibited good growth with glucose as a carbon source whereas strains that did not require yeast extract failed to grow on glucose as a sole source of carbon. Kries and Tarrand (1978) first suggested the existence of more than one group among S. lipoferum. Tarrand et al (1978) made a detailed investigation of different S. lipoferum strains from various sources for substrate utilisation, flagellation and DNA base composition. Based on these studies they described a new genus Azospirillum with two specific groups I and II. These bacteria had the following general characteristics such as vibroid form with diameter of about 1 micrometer and single polar flagellum in liquid medium, numerous lateral flagella on solid medium and capable of using different salts of organic acids like malate, succinate, pyruvate and lactate as carbon sources. Two species of Azospirillum were also described. The group I consisted of species Azospirillum brasilense sp.nov. which did not require biotin for growth on nitrogen free medium.

However the bacterium was incapable of utilising glucose as the sole source of carbon. The second group consisted of the species Azospirillum lipoferum capable of using glucose as a sole source of carbon. However, their growth on semisolid nitrogen free agar medium was dependent on the addition of low concentration of biotin. Later a 3rd species A. amazonense was proposed by Magalhaes et al (1983). This species was recognised upon isolation by the formation of deep diffuse pellicle in nitrogen free semisolid malate medium, which did not migrate to the surface of the medium. Dobereiner (1983) summarised the main characteristics of all the 3 species of Azospirillum. A. brasilense and A. lipoferum grew well only in medium with pH above 6.8 while A. amazonense grew poorly in such a medium. Out of the three species, A. lipoferum was positive for biotin requirements. A. amazonense was capable of utilising sucrose as a carbon source. The colony type on potato agar was white for A. amazonense and pink for A. lipoferum and A. brasilense.

Kavimandan et al (1978) isolated Spirillum lipoferum from wheat stem which grew well on nitrogen free malate medium. Tien et al (1979) studied various effects of Azospirillum brasilense on growth of pearl millet. Nalini (1980) found overwhelming evidence for intracellular colonization of Azospirillum in wheat and sorghum. Similarly, Patriquin et al (1988) also observed the presence

of Azospirillum in cortical stellar cells, epidermis and xylem vessels of maize. Umali et al (1980) studied the association of A. brasilense grass root hairs and concluded that root recognition was associated with root exudates. Baldani and Dobereiner (1980) suggested that only specific species were capable of endosymbiotic association with particular host plants. Azospirillum isolates from field grown corn roots were always A. lipoferum while those from wheat and rice were A. brasilense. Further they observed that when maize, wheat and rice were grown in plots using non sterile uninoculated soil which contained both the Azospirillum species, infection of inner roots showed considerable specificity. Thus in maize, 58 percent isolations made were that of A. lipoferum.

Lakshmikumari et al (1976) confirmed nitrogen fixation by Azospirillum not only by conventional microkjeldahl assay but also by the more definite methods of acetylene reduction assay and isotopic enrichment. Subba Rao (1980) reported that the nitrogen fixing ability of Indian isolates of Azospirillum sp. were higher than that of Brazilian ones. Further he cultured this bacteria on ammonium chloride containing liquid medium and used the same for field application after mixing with a sterilized carrier material of soil and FYM in 1:1 proportion. Subha Rao et al (1980) later isolated two strains of A. brasilense from

roots of rice and the weed Cynodon dactylon which did not possess denitrifying character and fixed maximum amount of nitrogen. Fages (1990) optimized the process for manufacturing commercial inoculum of Azospirillum lipoferum. He observed that bacterial survival could be enhanced by the addition of skim milk and controlled air-dehydration of alginate beads which were later powdered for inoculation with more than 10 billion cells per gram of carrier material.

Gafny et al (1986) reported that the adherence of Azospirillum brasilense to corn roots increased during the first 90 minutes and attained a maximum level within 45 hours. Zaady and Okon (1990) found that maize root exudates contained D-fructose, which caused greater adsorption of A. brasilense to the roots. Lukin et al (1992) also observed greatest density of Azospirillum in the immediate vicinity of root surface. Dobereiner et al (1976) reported that Azospirillum sp. generally occurred only in soils with pH around 7.0. However sporadic occurrence could be observed even in soils with pH 4.8. Similar results were reported by Beerkeem and Bohlool (1980). They found that eventhough optimal pH for root association in tropical grasses was 7.0 for Azospirillum sp. root association could be observed even in soils with pH 3.2. Khan and Hossain (1991) isolated Azospirillum from Chittagong soils of Bangladesh which had

optimal growth at pH between 6.8-7.5. Day and Dobereiner (1976) reported that optimum pH for nitrogen dependent growth of Azospirillum was between 6.8-7.8 and that nitrogen fixation reduced to a considerable extent at pH below 5.5 and above 8.0. Later Day and Mishra (1983) reported that the optimum pH for growth and acetylene reduction activities of Azospirillum was between 7 and 7.5. However, Purushothaman and Oblisami (1985) have observed the presence of Azospirillum in alkaline and saline soils with pH 8 to 8.8, as well.

Gopalaswamy and Vidhyasekaran (1987) standardised a combined seed, seedling and field application method of Azospirillum in rice. In this procedure, 60 Kg seeds were initially soaked for 24 hours in 60 litres of water containing 6 Kg peat based inoculum of Azospirillum. For seedling treatment, the seedling roots were dipped in 400 litres of water containing 6kg of inoculum for 20 minutes prior to transplanting. Subsequently another 6Kg of inoculum mixed with 15Kg sand was broadcast in the main field. They observed that the split application of A. brasilense through seed, seedling and soil application resulted in better grain and straw yield in rice. Later Gopalaswamy et al (1989) tested another procedure for the use of A. lipoferum on direct sown rice. In this procedure , a peat based inoculum of A. lipoferum at the rate of 2kg/ha was mixed with 100kg

of rice seeds and dried in shade for 20 minutes before sowing in the main field. For soil application, 4kg/ha of peat based inoculum, after mixing with 15kg powdered FYM was used. This also resulted in higher grain and straw yields in rice besides increasing root biomass plant height and productive tillers.

Day et al (1975) reported that Azospirillum fixed 1.7 kg of nitrogen per hectare per day in elephant grass. (Pennisetum purpureum). Tjepkema and Burris (1976) tested seven Wisconsin prairie grasses for nitrogenase activity by Azospirillum and found an activity equivalent to 2.9 and 3.6 kg nitrogen per year in association with Sporobolus heterolopis and Panicum virgatum. Nery et al (1978) observed significant difference in nitrogenase activity among wheat cultivars ranging from 111 to 3523 of C_2H_4 per gram dry root per hectare. Based on this, they estimated a potential for daily nitrogen fixation of 800g nitrogen per hectare by Azospirillum. Khan and Hossain (1990) reported that Azospirillum isolates fixed about 2.9-7.3 mg nitrogen per 50ml of malate containing broth culture.

Charyulu and Rao (1979) studied the influence of various soil factors on nitrogen fixation by Azospirillum species. The application of rice straw to alluvial laterite in acid - sulphate Pokkali soils under submerged conditions was found to enhance the population of nitrogen fixing

Azospirillum and they concluded that nitrogen fixation by Azospirillum was governed by fluctuations in soil conditions such as redox potential, pH and organic matter content, Subba Rao et al (1980) used two strains of A. brasilence from rice which did not possess denitrifying character but fixed maximum amount of nitrogen of 28-36 mg nitrogen per gram of calcium malate for a saving of 40 kg nitrogen per hectare on inorganic nitrogen application. Saha et al (1985) studied the effect of inoculation of Azospirillum lipoferum on nitrogen fixation in rhizosphere soil, their association with root, yield and nitrogen uptake by Mustard (Brassica juncea). The inoculation significantly increased nitrogen content in rhizosphere soil at early stage of plant growth. This was accompanied by increased association of bacteria in rhizosphere soil, root surface washing and surface sterilized macerated roots. Significant increases in yield and nitrogen uptake were also observed due to Azospirillum inoculation.

Production of phytohormones by diazotrophs

Brown and Burlingham (1968) found that Azotobacter chroococcum produced indole - 3-acetic acid, gibberellins and cytokinins in association with plants . Lee et al (1970) observed that IAA production by Azotobacter vinelandii was

reduced in the presence of combined nitrogen in both shaking and stationary culture conditions. Barea and Brown (1974) reported that Azotobacter paspali produced growth hormones which increased growth and yield of crop plants like tomato, lettuce, Paspalum notatum, Lolium perenne and Centrosema pubescens grown in pots containing compost. Abbass and okon (1993) studied the plant growth promotion effect of Azotobacter paspali in plant rhizosphere. There was formation of more root hairs that were longer and thicker than control plants. Root surface area was also higher due to bacterial inoculation. Reynders and Vlassak (1979) obtained A. brasilense strains capable of IAA production from tryptophan. They were initially grown in Dobereiner's semisolid malate medium and after 6 days 1 µM tryptophan was added aseptically. Auxin production was tested after 24 hours of inoculation at 28^{o}C using wheat coleoptile straight growth test. The extent of auxin production was statistically significant over tryptophan untreated Azospirillum culture. Further, thin layer chromatography of indole containing substances in tryptophan treated culture of A. brasilense strain S_{11} showed the presence of newly produced indole acetic acid along with unconverted tryptophan. Tien et al (1979) observed that IAA and indole lactic acid were produced by A. brasilense from tryptophan and that IAA production increased from 1-100 µg/ml according

to increase in tryptophan concentration. The production of phytohormone increased till the bacteria reached stationary phase of growth. Hartmann et al (1983) also got isolates of Azospirillum which produced IAA in culture broth upto 16 µg/ml. Govindan and Purushothaman (1984) reported that the addition of 0.05 percent tryptophan under aerated conditions of incubation helped the production of more IAA and GA by Azospirillum from pearl millet. These were equivalent to 13.586 µg/ml of IAA and 0.56 µg/ml of GA3 respectively in shake culture technique Kolb and Martin (1985) have also reported the production of IAA by Azospirillum species. Crozier et al (1988) quantitatively analysed the extent of IAA production by 20 strains of A. brasilense and A. lipoferum and reported a production level of 26.1 µg/ml culture broth after Salkowski assay. Mascarua et al (1988) isolated Azospirillum from rhizosphere soil and roots of Cactaceae species growing under arid conditions. They identified the most active strains in terms of indole acetic acid production (36.5-77 µg/ml) as A. brasilense. In comparison to this A. lipoferum isolated from Opuntica roots produced only 6.5 to 17.5 mg/ml of IAA. Fallik and Okon (1989) made identification and qualification of IAA and IBA production by A. brasilense in maize. Bottini et al (1989) identified the gibberellins produced by A. lipoferum as A1, A3 and iso A3. They found that based on estimations

by bioassay, the A. lipoferum culture produced 20-40 pg GA_3 equivalent. The amount of iso GA_3 was greater than that of the GA1 and GA3. They suggested that the identification of bioactive GA's from pure culture of Azospirillum provided a more complex basis for assessing the promotive effects of Azospirillum inoculation on growth and yield of higher plants. Gorden and Paleg (1957) standardised the method for quantitative determination of IAA by colourimetric methods. In this procedure, to 1ml of organic solvent containing IAA, 2ml of Salper's reagent was added dropwise with continuous agitation of the mixture. The absorbency was measured at 535nm after 35 minutes, for diethyl ether and 530nm after 60 minutes for methanol as solvent reagents.

Root induction by Azospirillum

Dewan and Subha Rao (1979) studied the effect of Azospirillum inoculation on rice seedling. The root biomass production was better in sterilized soil than in unsterilized soil. In general seed inoculation enhanced weight, length and volume of roots at all stages of plant growth in unsterilized and sterilized soil. Tien et al (1979) reported that IAA production by A. brasilense caused densely covered root hair formation in pearl millet. Inbal and Feldman (1982) found that Azospirillum affected root

growth and function by producing plant growth regulators in wheat plant. Venkateswarlu and Rao (1983) showed that inoculation of pearl millet with A. brasilense increased growth and dry matter production, root growth and number of lateral roots and root hairs.

Govindan and Chandy (1985) studied the utilisation of the diazotroph Azospirillùm for inducing rooting in pepper cuttings. Inoculation of Azospirillum increased the number of roots per cutting (7.4/cutting), total length of root (34.5 cm) and root dry weight (0.05 g) as compared to zero values for the control treatment. Besides, 80 percent of the inoculated cuttings also germinated when compared to only 40 percent in the untreated control. Eventhough IBA induced greater number of roots (16 per cutting), bacterial inoculation was found to favour the production of more healthy and strong roots, a trait desirable for better establishment of rooted pepper cuttings.

Kapulnik et al (1985) observed that inoculation of wheat seedlings with 10^5-10^6 colony forming units of Azospirillum caused largest root induction and root surface area. They further reported that the increased total shoot and root weight, plant height, number of fertile tillers and grain yield in wheat , than control treatments. Bashan (1986) studied the effect of inoculation of Azospirillum brasilense. Cd on wheat and found that inoculation increased

plant dry weight, number of tillers per plant, spikelet fertility, harvest index and grain yield. Jeyaraman and Ramaiah (1986) found that application of 75kg Nitrogen per hectare along with root dipping of Azospirillum at a rate of 1 kg of inoculant per ha recorded significantly higher grain yield, and application of 50 kg N along with Azospirillum produced comparable grain yield in rice as compared to application of recommended level of 100 kg nitrogen per hectare alone. Jeyaraman and Purushothaman (1988) found that Azospirillum lipoferum inoculation at 1kg per ha with 75 kg nitrogen per ha significantly increased the grain yield in wet season rice. Mahapatra and Sharma (1988) found that dipping rice seedlings in 2 percent solution of Azospirillum increased rice yield 2q per ha over control. Also 60kg N per ha along with Azospirillum gave 15q per ha more yield than control. Purushothaman (1988) reported that inoculation with two isolates of Azospirillum lipoferum C-13 and IPI increased grain and straw yield significantly in rice. Okon and Kapulnik (1986) noted that at a concentration of 10^5-10^7 cfu of Azospirillum per plant, the inoculation increased the number of root hairs, its thickness, length and branching.

Hadas and Okon (1987) studied the effect of A. brasilense inoculation on root morphology and respiration in tomato seedlings. There was significant increase in root

length, top and root dry weight and total leaf area due to Azospirillum inoculation, compared to uninoculated controls. An inoculum concentration of $1x10^8$ to $5x10^8$ colony forming units per ml also stimulated the appearance of root hairs. Fallik et al (1988) also got increased root hair development and root branching in maize due to Azospirillum inoculation. Fallik and Okon (1989) reported that Azospirillum at 10^7 cfu $plant^{-1}$ significantly increased the root surface area from the second week in maize plants. Lee et al (1989) reported that inoculation with A. amazonense Y1 accelerated growth of underground parts of maize plant more than upper ground parts. Zaady et al (1993) studied that the extent of growth promotion in maize by inoculation with aggregated and single cell suspensions of A. brasilense and reported that fructose grown bacterial cells adsorbed in higher numbers with maize roots than malate grown cells.

Morgenstern and Okon (1988) studied the effect of A. brasilense and auxin on root morphology in seedling of Sorgham bicolor x S.sudanense. Scanning electron photographs of inoculated roots revealed increase in root diameter and in the density and length of root hairs. Inoculation caused asymmetric growth of root tips and shortened the distance between first root hair and root tip. Okon et al (1988) concluded that positive effect of Azospirillum on plant growth was mainly from a general effect on root growth and

function. Purushothaman (1988) also made a similar observation based on increase in root surface area and enhanced root proliferation due to Azospirillum inoculation. Bopaiah and Khader (1989) studied the effect of combined inoculation of Azotobacter, Azospirillum and VA mycorrhizae together resulted in higher plant height, shoot and root weight in black pepper.

MATERIALS AND METHODS

Materials and Methods

The present study on the effect of Azospirillum inoculation on establishment and growth of bush pepper was conducted in College of Agriculture, Kerala Agricultural University, Vellayani, Trivandrum during 1992-94 with the following technical programme.

1. Isolation and screening of native strains of Azospirillum from pepper rhizosphere for phytohormone production.
2. Use of selected isolates of Azospirillum for root induction, establishment and growth of bush pepper.

1. Isolation of Azospirillum from pepper rhizosphere

Native strains of Azospirillum were isolated from 25 cultivars of Pepper maintained at the germplasm collection centre of Pepper Research station of Kerala Agricultural University at Panniyur. They were as follows:

1. Arakkulammunda
2. Arivally
3. Balankotta
4. Cheriyakaniyakkadam
5. Chumala
6. Kulluvally
7. Kaniyakkadan
8. Kanjiramundy
9. Karimunda
10. Karivally

11. Kottanadan
12. Kottaram
13. Kumbhakkodi
14. Kuthiravally
15. Narayakkodi
16. Neelagiri
17. Neelamundi
18. Panniyur-1
19. Panniyur-2
20. Panniyur-3
21. Panniyur-4
22. Perumkodi
23. Poonjarmunda
24. Thulakkodi
25. Veluthanamban

The different rhizosphere samples with one or more healthy root lets were collected in triplicate in the month of April 1992.

Azospirillum was isolated by enrichment culture technique using nitrogen free semisolid malate agar medium of following composition.

Nitrogen free semisolid malate agar medium (Baldani and Dobereiner, 1980)

A. Macronutrient solution

Malic acid	- 5.0g
$K_2 HPO_4$	- 0.5g
$MgSO_4$	- 0.2g
$NaCl_2$	- 0.1g
$CaCl_2$	- 0.02g
Trace element solution	- 2.0ml

Alcoholic solution of bromothymol blue (5%)	- 2.0ml
Fe EDTA (1.64% W/N aqueous)	- 4.0ml
Vitamin solution	- 4.0ml
KOH	- 4.0g
Agar	- 1.75g
Distilled water	- 1000 ml
pH	- 6.8

B. Trace element solution.

$Na_2\ MoO_4$	- 200mg
$MnSO_4.H_2O$	- 280 mg
H_3PO_3	- 280 mg
$ZnSO_4.7H_2O$	- 24 mg
$CuSO_4$	- 8 mg
Distilled water	- 200 ml

C. Vitamin Solution

Biotin	- 10 mg
Pyridoxine	- 20 mg
Distilled water	- 100 mg

D. Preparation of Fe EDTA

26.1 g of EDTA was dissolved in 1N KOH solution end 24.9 g of $FeSO_4$ was added to this. The volume was

made up to 1 litre. This solution was kept overnight to produce a stable complex for further use.

A vertical semisolid gel was prepared by dispensing 5ml of the above medium in 10 ml test tubes and sterilizing the same in an autoclave at 121^{o}C for 15 minutes. Healthy rootlets from various rhizosphere samples were initially separated and washed gently with sterile tap water to remove all adhering soil particles. These were then cut into small bits of 0.5 cm length and aseptically transferred to previously prepared nitrogen free semisolid malate agar medium in test tubes. Each root bit was carefully placed just below the surface of the medium by using a sterile inoculation needle. These tubes were incubated at 36 $\pm$ 1^{o}C for 5 days in an incubator. The presence of micro aerophilic nitrogen fixing bacterium was indicated by the development of a pale dense subsurface pellicle together with a change in colour of the medium from yellowish green to blue. Such cultures were further enriched by repeated transfer to fresh nitrogen free semisolid malate agar medium until a clear distinct subsurface pellicle was formed in each tube. These were then streaked on potato infusion agar medium of following composition.

Potato infusion agar medium (Baladani and Dobereiner(1980)

Potato	- 200.0g
Malic acid	- 2.5 g
KOH	- 2.0 g
Sucrose	- 2.5 g
Vitamin solution	- 1.0ml
Alcoholic solution of bromothymol blue (5%)	- 2.0ml
Agar	- 15.0g
Distilled water	- 1000 ml
pH	- 7.0

The potato infusion broth was prepared by boiling 200g of peeled and sliced potatoes for 30 minutes in 200 ml water and filtering the broth through a piece of clean cotton. The remaining nutrient components were added to this and after making up the volume to 1000 ml and adding the required quantity of agar, the medium was sterilized in 250 ml conical flasks in an autoclave at 121^{o}C for 15 minutes.

The different isolates of bacterium which showed a distinct subsurface pellicle formation in nitrogen free semisolid malate agar medium were streaked on this medium. The plates were incubated at 36 $\pm$ 1^{o}C for 48 hours in an incubator and observed for the development of typical dry wrinkled colonies of Azospirillum species . These cultures were them checked once for purity by Gram staining.

Procedure for Gram staining

A thin smear of different isolates were initially prepared, heat fixed and stained with 0.2% ammonium oxalate crystal violet for 1 minute. The slides were gently washed in tap water and treated with Gram iodine solution for 30 seconds. They were decolourised with 95 percent ethyl alcohol for 30 seconds with gentle agitation. The slides were then washed in tap water and counter stained with 2.5 percent safranin for 30 seconds. These were then washed again in tap water, blot dried and examined under a microscope.

Composition of different stains and reagents

1. Ammonium oxalate crystal violet

Solution A

Crystal violet	-	0.2g
Ethyl alcohol (95%)	-	20.0ml

Solution B

Ammonium oxalate	-	0.8g
Distilled water	-	80.0ml

Solution A and B were mixed together to prepare the ammonium oxalate crystal violet stain.

2. Gram iodine solution

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

3. Counter stain

Safranin (25% solution in 95% ethanol)	- 10.0ml
Distilled water	- 90.0ml

The pure cultures of Azospirillum were maintained on malate agar slants supplemented with 0.3 percent ammonium chloride. These were stored in a refrigerator at $4^{o}c$ for further use.

2. Production of phytohormones by Azospirillum

2.1.a. Preliminary screening for production of indole acetic acid

Sixteen cultures of Azospirillum isolated from following cultivars of pepper along with an isolate from Tamil Nadu Agricultural University, Coimbatore (TN) were screened for the production of indole acetic acid (IAA) under in vitro conditions.

1. Arakkulammunda
2. Arivally
3. Balankotta
4. Chumala
5. Kaniyakkadan
6. Karimunda
7. Karivally
8. Kottanadan
9. Kumbhakkodi
10. Kuthiravally
11. Neelamundi
12. Panniyur-1
13. Panniyur-2
14. Panniyur-3
15. Perumkodi
16. Thulakkodi

Each isolate of Azospirillum was inoculated into 10ml of sterilized malate broth supplemented with 0.3 percent ammonium chloride and filter sterilized L.Tryptophan (SISCO Research Laboratories Private Ltd, Bombay) to a final concentration of 100 µg/ml. These were incubated in dark at 36 ± 1°C in an incubator for 7 days. At the end of incubation period, the broth was centrifuged at 6000 rpm to sediment the bacterial cells. The resulting supernatent was used for testing the production of IAA by the method of Zhandrer and Mahadevan (1968). In this procedure, to 5ml of cell free supernatent, 1.6 ml of methanol and 13.4 ml of Salper's reagent were added and incubated in dark for 1 hour at room temperature.

Composition of Salper's reagent

Ferric chloride (0.5m)	-	1.0 ml
Perchloric acid (35%)	-	50.0ml

The presence of IAA in the cell free culture broth was indicated by the development of a pink colour of the reaction mixture at the end of incubation period.

2.1.b. Quantitative estimation of IAA

Azospirillum cultures which tested positive for the production of IAA during the preliminary screening were further selected along with the standard culture (TN) for the quantitative estimation of this phytohormone under in vitro conditions. The procedure adopted for this was same as that of preliminary screening except that the cultures were initially grown in 250 ml conical flasks containing 100ml of sterilized malate broth supplemented with 0.3 percent ammonium chloride and filter sterilized L-Tryptophan to a final concentration of 100 µg/ml. The quantity of IAA produced was estimated by measuring the colour intensity at 535nm in a Klett-Summerson colourimeter after incubation at different time intervals ranging from one to five weeks in dark at 36 $\pm$ 1^{o}C in a B.O.D incubator and comparing the values with that of a standard curve.

2.1.c. Preparation of standard curve

A standard curve was prepared by using an aqueous solution of IAA (E.Merck (India) Pvt Ltd, Bombay) of different concentrations such as 10, 20, 30, 40 and 50 µg/ml. To 5 ml of each of this dilution 1.6ml of methanol and 13.4 ml of Salper's reagent were added to get a final volume of 20ml. These tubes were incubated in dark for 1 hour at room temperature. The colour intensity was measured at 535nm using Klett-Summerson colourimeter. The standard curve was prepared by plotting the colourimeter readings on Y axis and concentration of IAA (µg/ml) on X axis. The quantity of IAA produced by different isolates of Azospirillum was determined with the help of this standard curve. The final values were expressed as micro gram of IAA per millilitre of culture broth. The local isolate which produced maximum IAA together with the TN culture were selected for further studies.

2.2 Preliminary screening for production of gibberellins

Sixteen cultures of Azospirillum isolates along with the TN culture were tested for the production of gibberellins under in vitro conditions. They were initially grown in sterile malate broth containing 0.3% ammonium

chloride in test tubes and incubated at 36 ± 1°C in an incubator for 7 days. At the end of incubation period, the broth was centrifuged at 6000 rpm to sediment the bacterial cells and the resulting supernatent was used for spot testing the production of gibberellins by the method of Bird and Pugh (1958).

A drop of each of the cell free culture extract was initially spotted in the centre of a clean Whatman number 42 filter paper with the help of 1 ml pipette. After air drying, the filter paper was dipped in a saturated aqueous solution of potassium permanganate and washed immediately in tap water. The development of a brown colour in the spotted area of filter paper indicated the presence of gibberellins in the culture filtrate. A filter paper spotted with 1 ppm solution of a standard sample of gibberellins (Qualigens Fine Chemicals, Glaxo India Ltd, Bombay) was used as a check for comparing the results.

3. Biotin requirements and pH sensitivity of selected cultures of *Azospirillum*

3.1 Biotin requirement

The ability of the local isolate of *Azospirillum* which produced maximum IAA and TN culture to grow in

nitrogen free semisolid malate medium with or without 0.04 percent biotin and sucrose medium with 0.04 percent biotin were tested. The composition of sucrose medium was same as that of malate agar except that in place of malic acid , 10g of sucrose was used. The two cultures of Azospirillum were stab cultured in these media and incubated at 36 ± 1°C for 5 days in a B.O.D incubator. The results were recorded as the presence or absence of bacterial growth in each of the above medium.

3.2. pH sensitivity

The native isolate of Azospirillum selected earlier along with the TN culture were tested for their pH sensitivity under in vitro conditions. They were grown in test tubes using malate broth containing 0.3 percent ammonium chloride, and with the pH initially adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0. The tubes were inoculated with 0.1 ml of 72 hour broth culture of respective Azospirillum culture initially grown at pH 7 and incubated at 36 ± 1°C in an incubator. Three replications were maintained for each treatment . The extent of growth was measured at different time intervals of 24,48,72,96 and 120 hours by using a UV-visible Spectrophotometer at 600nm.

4. Mass production of *Azospirillum*

Mass production of *Azospirillum* was done in a modified malate broth of following composition.

malic acid	- 5.0g
K_2HPO_4	- 0.5g
$MgSO_4.7H_2O$	- 0.2g
NaCl	- 0.1g
$CaCl_2$	- 0.02g
Trace element solution	- 2.0ml
FeEDTA	- 4.0ml
Vitamin solution	- 4.0ml
KOH	- 4.0g
NH_4Cl	- 3.0g
Distilled water	- 1000ml
pH	- 6.8

The medium after sterilization in an autoclave at 121°C for 15 minutes was supplemented with filter sterilized L Tryptophan solution to a final concentration of 100 µg/ml. This was then inoculated with the required *Azospirillum* cultures and incubated for 72 hours at 36 $\pm$ 1°C in an incubator. The resulting cultures were used either directly as broth culture or as a carrier based inoculation after mixing with finely powdered (105 micron mesh size) and sterilized wood charcoal.

5. Use of *Azospirillum* for root induction of bush pepper

The native isolate of *Azospirillum* which produced maximum IAA under *in vitro* conditions (isolate 34) along with the standard culture (TN) were used for root induction study in bush pepper. Two year old lateral shoots of approximately 3 node length having one top leaf from two varieties of pepper namely Panniyur-1 and Karimunda were used as the planting material. The potting mixture consisted of farm yard manure, sand and soil in ratio of 1:1:1. Two Kg of this potting mixture was filled in polypropylene bags of 8x15 cm size. Three cuttings were maintained in each bag with following treatment combinations.

1. *Azospirillum* treatments
   a) Dipping pepper cuttings in 72 hour broth culture of isolate 34 and TN culture for 15 minutes prior to planting.
   b) Dipping pepper cuttings in a water slurry of carrier based inoculum of *Azospirillum* isolate 34 and TN culture for 15 minutes prior to planting.

2. Hormone treatments
   a) Dipping pepper cuttings in 1000 ppm aqueous solution of IBA for 45 seconds prior to planting.

b) Dipping moistened end of pepper cuttings in Ceradix prior to planting.

3. Control treatment

a) Control treatment without Azospirillum and hormone treatment in unsterilized potting mixture.

b) Control treatment without Azospirillum and hormone treatment in sterilized potting mixture.

The experiment was laid out in CRD with 10 replications each for each treatment. The cuttings were grown in shade for 3 months with regular irrigation. Following observations were taken at the end of growth period after carefully depotting each cutting from polypropylene bags.

1) Percentage of rooted cuttings

$$= \frac{\text{Number of rooted cuttings/replication}}{\text{Total number of cuttings/replication}} \times 100$$

The final value was expressed as the mean of 10 replications.

2) Number of roots

These were counted individually for each cutting

and the final value was expressed as the mean of 10 replications for each treatment.

3) Fresh and dry weight of roots

The fresh and dry weight of roots were taken after carefully separating out the roots from the cuttings. They were washed gently in tap water to remove all adhering soil particles and blot dried before taking the fresh weight in a Metler Single Pan Balance. The samples were then dried at $60^{o}c$ in a drying oven to a constant weight for determining the dry weight.

4) Percentage of germinated cuttings

The initiation of new leaf formation by way of fresh sprout development was taken as the criterion for germination of rooted cuttings. This was calculated as follows:

$$\frac{\text{Number of germinated cuttings/replication}}{\text{Total number of cuttings/replication}} \times 100$$

5) Fresh and dry weight of shoot

The fresh weight of shoot was taken in a Metler Single Pan Balance after cuttings the shoot portion into small bits of 5 centimetre in length. The samples were then

dried at 60°C in a drying oven to a constant weight for determining the dry weight.

6) Shoot to root ratio

$$\text{Shoot to root ratio} = \frac{\text{Fresh weight of shoot}}{\text{Fresh weight of root}}$$

6. Effect of *Azospirillum* inoculation on establishment and growth of bush pepper

The initial root induction of lateral shoot from pepper varieties such as Panniyur-1 and Karimunda were done by the procedure described earlier. However, only the best bacterial and hormone treatments from the previous experiment were only selected for root induction purpose. These were as follows:

1. Dipping pepper cuttings in a water slurry of carrier based inoculum of *Azospirillum* isolate 34 for 15 minutes prior to planting.
2. Dipping pepper cuttings in 1000 ppm aqueous solution of IBA for 45 seconds prior to planting.
3. Control treatment without any *Azospirillum* or hormone application.

Twenty replications were maintained for both

varieties of pepper. Plants were grown in shade for 3 months with regular irrigation for initial root induction. The rooted cuttings alone were selected for further studies on establishment and growth of bush pepper. These cuttings were transplanted to fresh potting mixture of farm yard manure, sand and soil in 1:1:1 proportion in larger polypropylene bags of 20x22 cm size. The potting mixture was used either as such or after supplementing with a carrier based inoculum of Azospirillum isolate 34 at the rate of 25 and 100g per kg of potting mixture or chemical fertilizer in the form of vegetable mixture (10:10 (5):10 by T. Stanes and Co Ltd, coimbatore) at the rate of 0.5 g/kg of potting mixture.

The experiment was laid out in CRD with 5 replications for each treatment. One rooted cutting was maintained in each polypropylene bag. The different observations were taken at 180 days of plant growth.

1) Percentage establishment of bush pepper

$$= \frac{\text{Number of established cuttings}}{\text{Total number of cuttings in each treatment}} \times 100$$

The development of fresh leaves was taken as the criterion for establishment of each cutting.

2) Number of roots

These were counted individually for each cutting and the final value was expressed as the mean of 5 replications for each treatment.

3) Fresh and dry weight of roots

The fresh and dry weight of roots were taken after carefully separating out the roots from each cutting. They were washed gently in tap water to remove all adhering soil particles and blot dried before taking the fresh weight in a Metler Single Pan Balance. The samples were then dried at 60 ^{o}c in a drying oven to a constant weight for determining the dry weight.

4) Number of leaves

This was counted as the mean of 5 replications for each treatment.

5) Fresh and dry weight of shoot

The fresh weight of shoots were taken in a Metler Single Pan Balance, after cutting the shoot portion into small bits of 5 cm in length. The samples were then dried at 60^{o}C in a drying over to a constant weight for determining the dry weight.

6) Shoot to root ratio

$$\text{Shoot to root ratio} = \frac{\text{Fresh weight of shoot}}{\text{Fresh weight of root}}$$

7) Length of branches

The length was determined after measuring the length of each branch in centimetres.

8) Fresh and dry weight of branch

The fresh and dry weights of branches were determined by the same procedure as that of the fresh and dry weight of shoot.

9) Total fresh and dry weight

The total fresh and dry weights of pepper cuttings were determined by adding the fresh and dry weights of shoot, branches and roots for each treatment.

Due to the random nature of berry formation in Pepper cuttings, observations on yield were not taken during this experiment.

7) Statistical Analysis

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

RESULTS

Results

1. Isolation of *Azospirillum* from pepper rhizosphere

Azospirillum was isolated from sixteen different cultivars of pepper (Table 1). All these isolates were gram negative and were given a code number which was same as the serial numbers of the replicated tube (1 to 75) used for initial isolation.

2. Production of phytohormones by *Azospirillum*

2.1. Preliminary screening for production of indole acetic acid and gibberellins

Sixteen native isolates of *Azospirillum* and one exotic isolate from Tamil Nadu Agricultural University (TN) were screened for production of Indole acetic acid (IAA) and gibberellins under *in vitro* conditions. Out of this, six native isolates-isolate numbers 34, 39, 41, 55, 60, 64 and the TN culture produced IAA (Table 2). These cultures were therefore selected for quantitative estimation of IAA under *in vitro* conditions using malate broth supplemented with L-Tryptophan. However, none of these isolates produced gibberellins in the above medium.

2.2. Quantitative estimation of IAA

Azospirillum isolate 34 from the pepper cultivar

Table 1. Isolation of *Azospirillum* from different cultivars of Pepper

Sl.No.	Pepper cultivars	Isolation
1.	Arakkulammunda	+
2.	Arivally	+
3.	Balankotta	+
4.	Cheriyakaniyakkadam	-
5.	Chumala	+
6.	Kalluvally	-
7.	Kaniyakkadam	+
8.	Kanjiramundy	-
9.	Karimunda	+
10.	Karivally	+
11.	Kottanadam	+
12.	Kottaram	-
13.	Kumbhakkodi	+
14.	Kuthiravally	+
15.	Narayakkodi	-
16.	Neelagiris	-
17.	Neelamundi	+
18.	Panniyur - 1	+
19.	Panniyur - 2	+
20.	Panniyur - 3	+
21.	Panniyur - 4	-
22.	Perumkodi	+
23.	Poonjarmunda	-
24.	Thulakkodi	+
25.	Veluthanambam	-

Table 2. Preliminary screening of Azospirillum for the production of IAA and Gibberellins

Sl. No.	Isolate No.	Pepper cultivars	Production of IAA	Production of GA
1.	55	Arakkulammunda	+	-
2.	43	Arivally	-	-
3.	39	Balankotta	+	-
4.	60	Chumala	+	-
5.	21	Kaniyakkadam	-	-
6.	34	Karimunda	+	-
7.	3	Karivally	-	-
8.	24	Kottanadam	-	-
9.	51	Kumbhakkodi	-	-
10.	69	Kuthiravally	-	-
11.	18	Neelamundi	-	-
12.	64	Panniyur - 1	+	-
13.	53	Panniyur - 2	-	-
14.	41	Panniyur - 3	+	-
15.	26	Perumkodi	-	-
16.	29	Thulakkodi	-	-
17.	TN	TNAU culture	+	-

Karimunda produced maximum amount of IAA equivalent to 69 µg/ml of culture broth after incubation for 14 days in dark at 36 ± 1°C in an incubator (Table 3, Figures 1 and 2). The production of this phytohormone by isolate 60 and TN culture were also higher. These were 48 and 46 µg/ml respectively. But this ability was comparatively low in isolate 39 which produced only 17 µg of IAA under identical growth conditions. The period of incubation also had an effect on IAA production. The amount of IAA produced reached a peak level during second week of incubation and it declined subsequently. Thus in case of isolate 34 the quantity of IAA produced varied from 45, 69, 38, 31 and 26 µg/ml of culture broth during the first, second, third, fourth and fifth week of incubation. (Table 3, Figure 2). A similar trend was also observed in remaining native isolates and in TN culture.

The Azospirillum isolate 34 and TN culture were only used for the preliminary study on root induction in bush pepper.

3. Biotin requirement and pH sensitivity of selected cultures of Azospirillum

3.1 Biotin requirement

Azospirillum isolate 34 did not require biotin for growth. However, this isolate did not grow in sucrose medium

Fig.1 – Standard curve for quantitative estimation of indole acetic acid

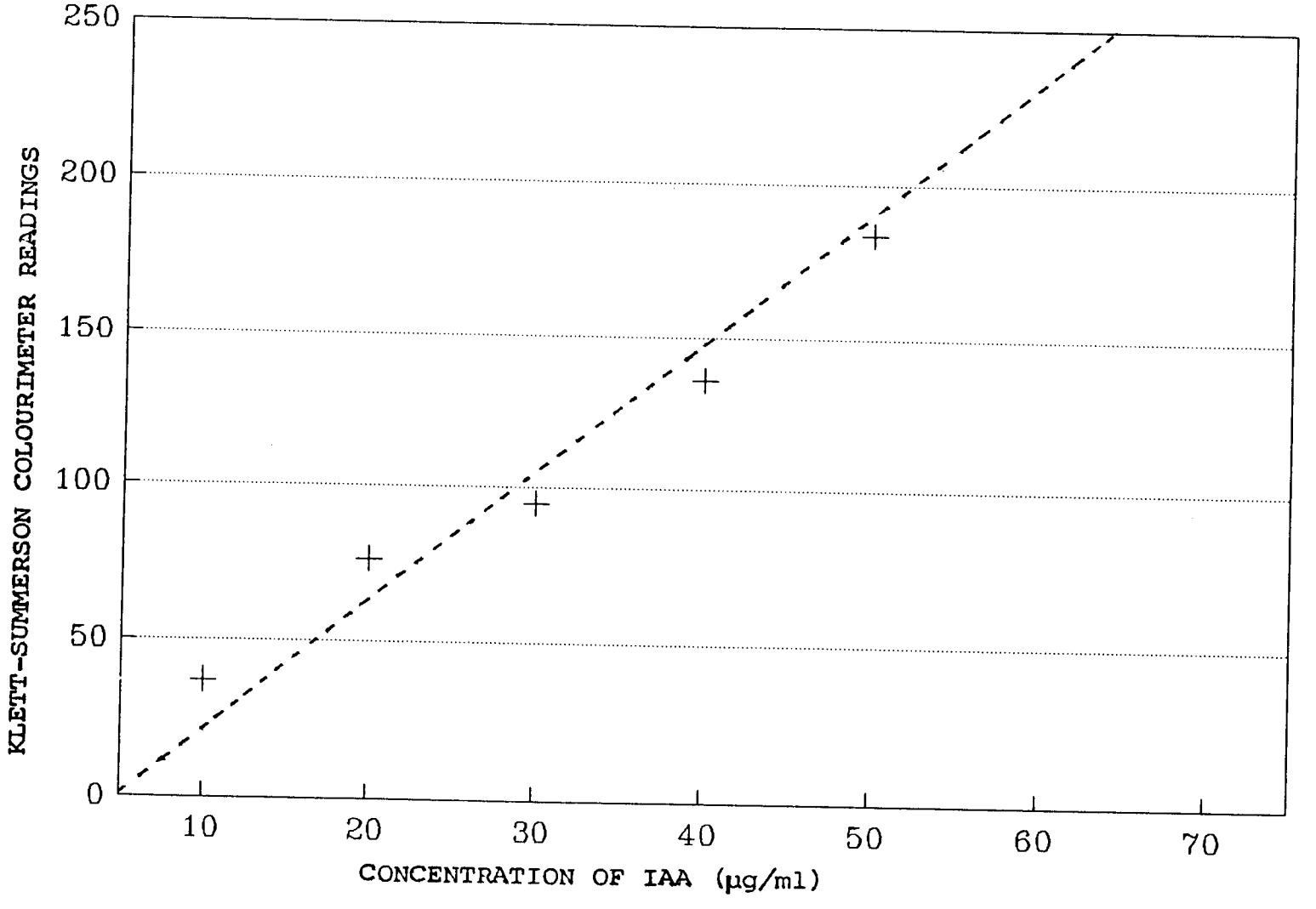

Fig.2 – Quantitative estimation of IAA produced by different isolates of Azospirillum.

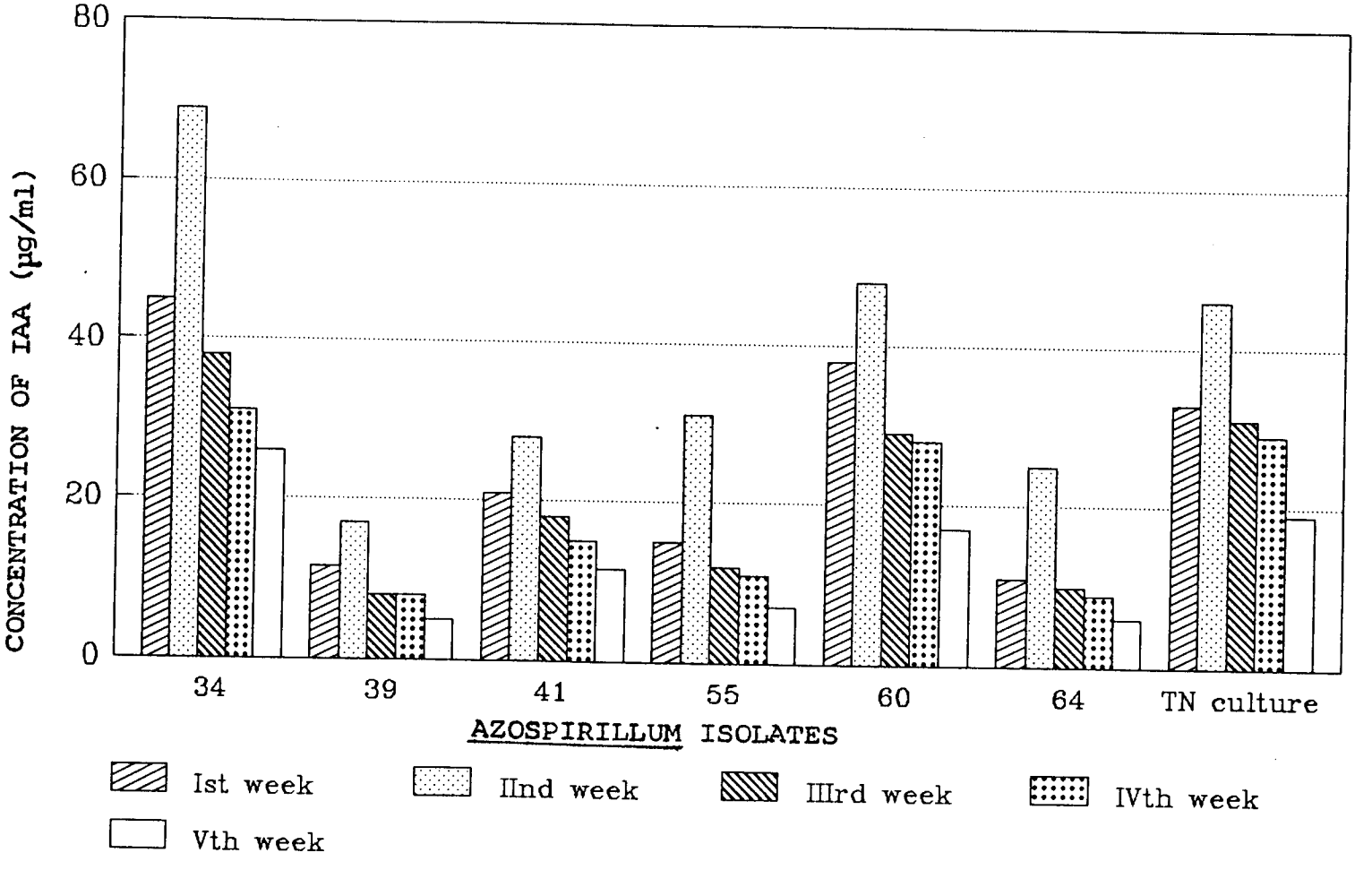

Table 3. Production of IAA (µg/ml) by selected isolates of *Azospirillum*

Azospirillum isolates	*Concentration of IAA at different time intervals				
	1st week	2nd week	3rd week	4th week	5th week
34	45.0	69.0	38.0	31.0	26.0
39	11.5	17.0	8.0	8.0	5.0
41	21.0	28.0	18.0	15.0	11.5
55	15.0	31.0	12.0	11.0	7.0
60	38.0	48.0	29.0	28.0	17.0
64	11.0	25.0	10.0	9.0	6.0
TN culture	33.0	46.0	31.0	29.0	19.0

*Mean of 3 replications.

Table 4. Biotin requirement of selected cultures of Azospirillum in malate and sucrose medium

Growth medium	Growth characteristics	
	Isolate 34	TN culture
1. Semisolid nitrogen free malate agar medium with biotin	+	+
2. Semisolid nitrogen free malate agar medium without biotin	+	-
3. Semisolid nitrogen free sucrose agar medium with biotin	-	+

(Table 4). On the other hand the TN culture grew only in biotin supplemented medium and it was capable of utilising both malate and sucrose as carbon substrates. Based on the requirement of biotin for growth, Azospirillum isolate 34 was tentatively identified as an isolate of Azospirillum brasilense and TN culture as Azospirillum lipoferum.

3.2 pH Sensitivity

The pH sensitivity of Azospirillum isolate 34 and TN culture in malate broth showed certain variations with the native isolate exhibiting better growth at pH 6.0-7.0, and the exotic isolate TN at pH 7.0-8.0. The isolate 34 had maximum growth at pH 6.0 with an optical density value of 0.689 after incubation for 120 hours (Table 5, Figure 3). The growth of TN culture was more at pH 8.0, with an optical density value of 0.284 after 96 hours of culture growth. (Table 6, Figure 4). In general, growth of both the isolates of Azospirillum were less at pH below 5.0.

4. Mass Production and maintenance of Azospirillum

Pure cultures of different isolates of Azospirillum were maintained in malate agar medium supplemented with 0.3 percent ammonium chloride. Mass production of selected cultures were also done in this medium after supplementing with L-Tryptophan. The resulting

Table 5. *pH sensitivity of Azospirillum isolate 34 under in vitro conditions

pH	Time intervals (hours)				
	24	48	72	96	120
4.0	0.009	0.055	0.015	0.042	0.041
5.0	0.011	0.082	0.014	0.042	0.054
6.0	0.309	0.488	0.398	0.594	0.689
7.0	0.319	0.425	0.352	0.340	0.349
8.0	0.322	0.398	0.274	0.276	0.305

*OD values - Mean of 3 replications.

Fig.3 – Effect of pH on growth of <u>Azospirillum</u> isolate 34

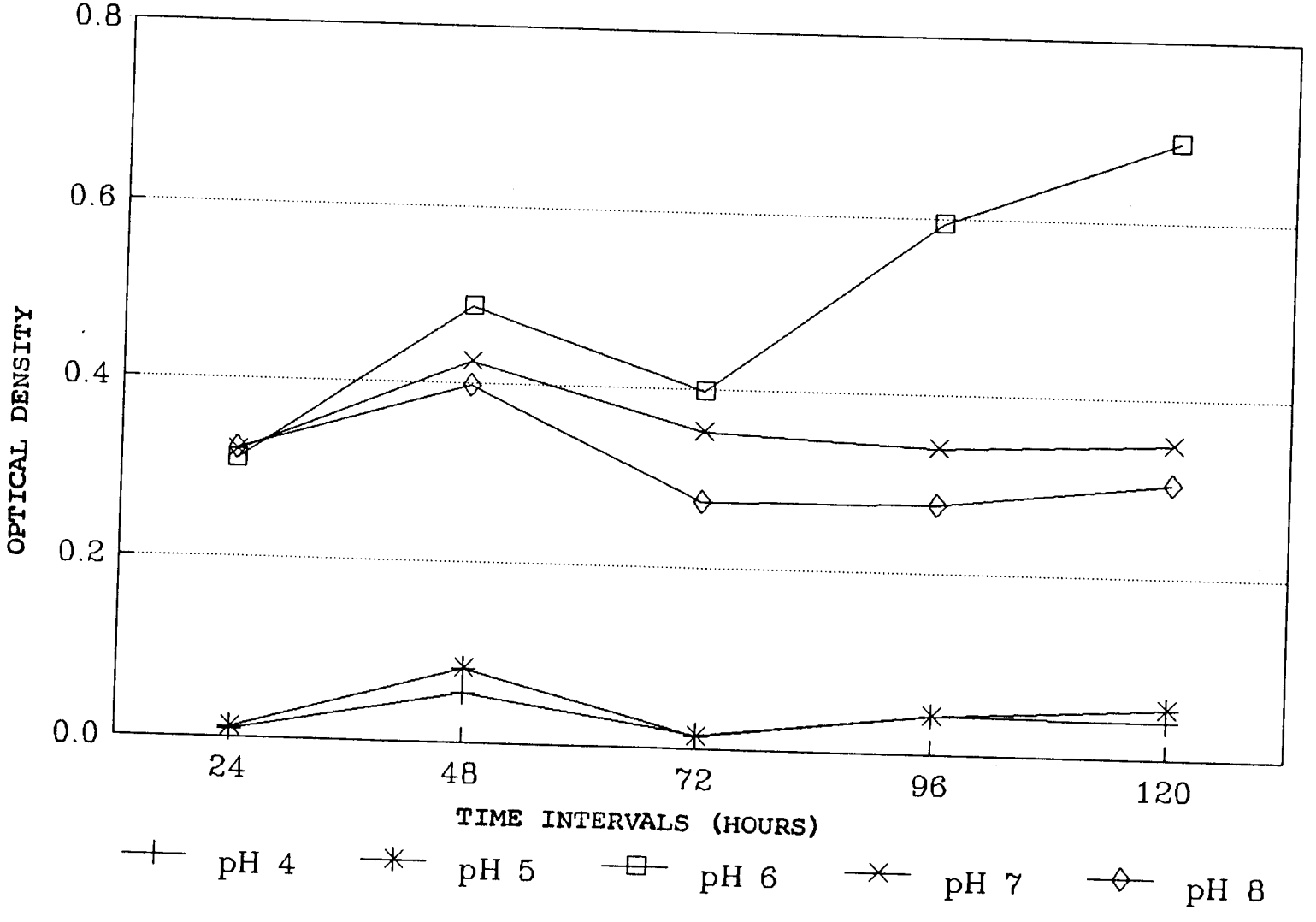

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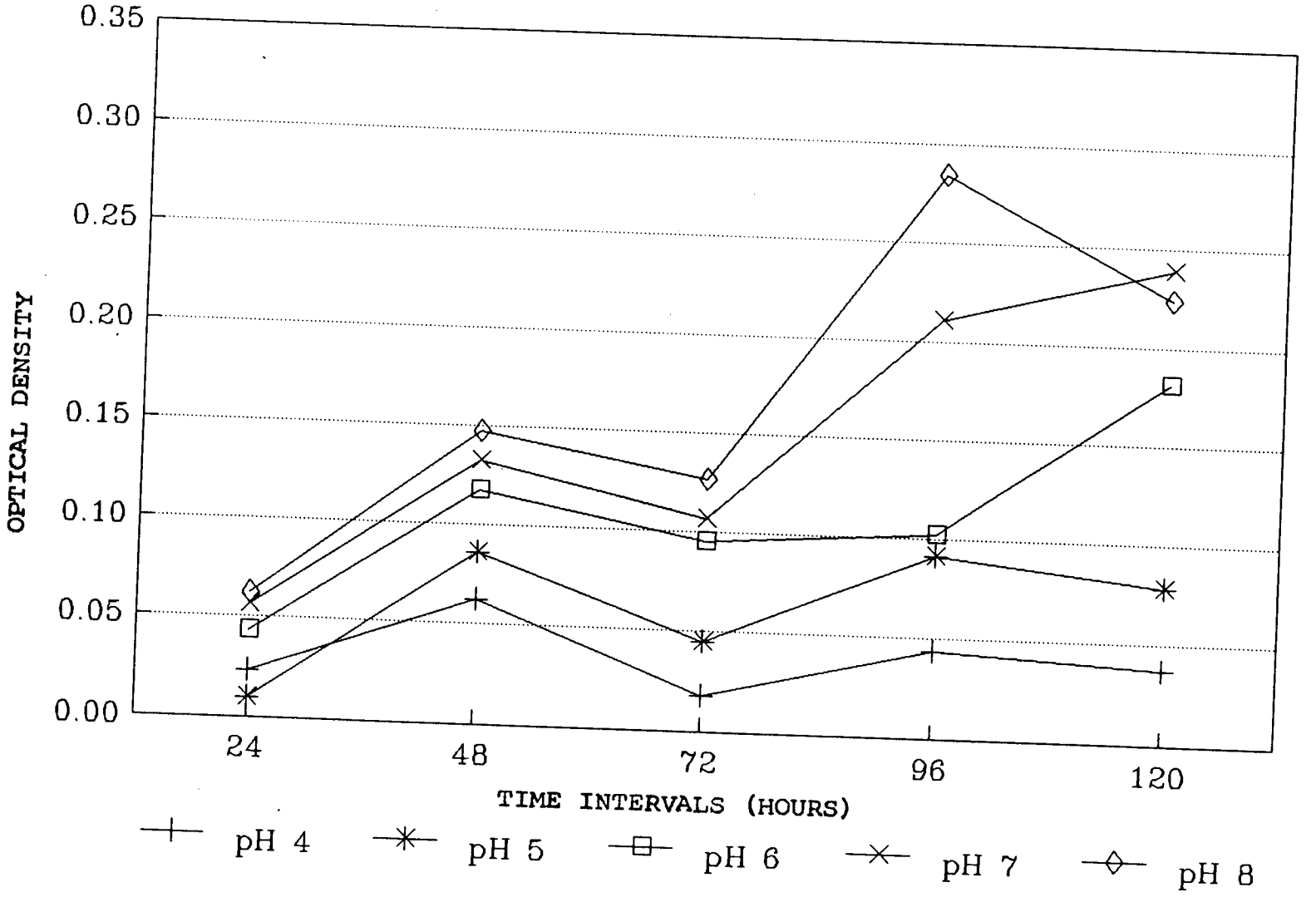
Table 6. *pH sensitivity of TN culture under *in vitro* conditions

pH	Time intervals (hours)				
	24	48	72	96	120
4.0	0.023	0.062	0.018	0.044	0.037
5.0	0.010	0.086	0.045	0.091	0.078
6.0	0.043	0.117	0.095	0.102	0.181
7.0	0.056	0.132	0.106	0.211	0.239
8.0	0.062	0.147	0.126	0.284	0.224

*OD values - Mean of 3 replications.

Fig.4 – Effect of pH on growth of TN culture



broth cultures were used either as such in the form of broth inoculum or after mixing with sterile wood charcoal powder as carrier based inoculum.

5. Use of *Azospirillum* for root induction in bush pepper

The extent of root induction in Panniyur-1 and Karimunda varieties of bush pepper due to inoculation of *Azospirillum* isolate 34 and TN culture were compared with that of IBA and Ceradix treatments. Pepper cuttings raised in unsterilized and sterilized potting mixture without any bacterial or hormone treatment served as control.

The effect of *Azospirillum* and IBA treatments on root induction in bush pepper were significant. But this effect was more in Panniyur-1 variety. Here 26.8 percent rooted cuttings were obtained after treatment with 1000 ppm IBA (Table 7, Figure 5). The percentage of rooted cuttings produced, 23.3 percent by *Azospirillum* isolate 34 carrier based inoculum treatment was also higher and statistically on par with IBA application. The mean number of roots produced per cutting and root dry weight were also more after bacterial inoculation. These were 3.5 and 0.68 g respectively compared to 3.3 and 0.59 g (Table 7, Figure 5) in IBA treatment.

Root formation in Karimunda also followed a pattern similar to that of Panniyur-1. However, the

Table 7. *Effect of Azospirillum inoculation on root induction in bush pepper variety - Panniyur I

Treatments	Percentage of rooted cuttings	Number of roots per cutting	Dry weight of roots (g)
1. Azospirillum treatment			
Broth culture			
34	3.5	0.8	0.11
TN	7.0	0.5	0.21
Carrier based inoculum			
34	23.3	3.5	0.68
TN	3.5	0.3	0.21
2. Hormone treatment			
IBA	26.8	3.3	0.59
Ceradix	7.0	0.9	0.16
3. Control treatment			
Unsterilized potting mixture	5.4	0.3	0.05
Sterilized potting mixture	0.0	0.0	0.00
CD (0.01)	14.9	2.0	0.40

*Mean of 10 replications.

Fig.5 – Effect of Azospirillum inoculation on root induction in bush pepper

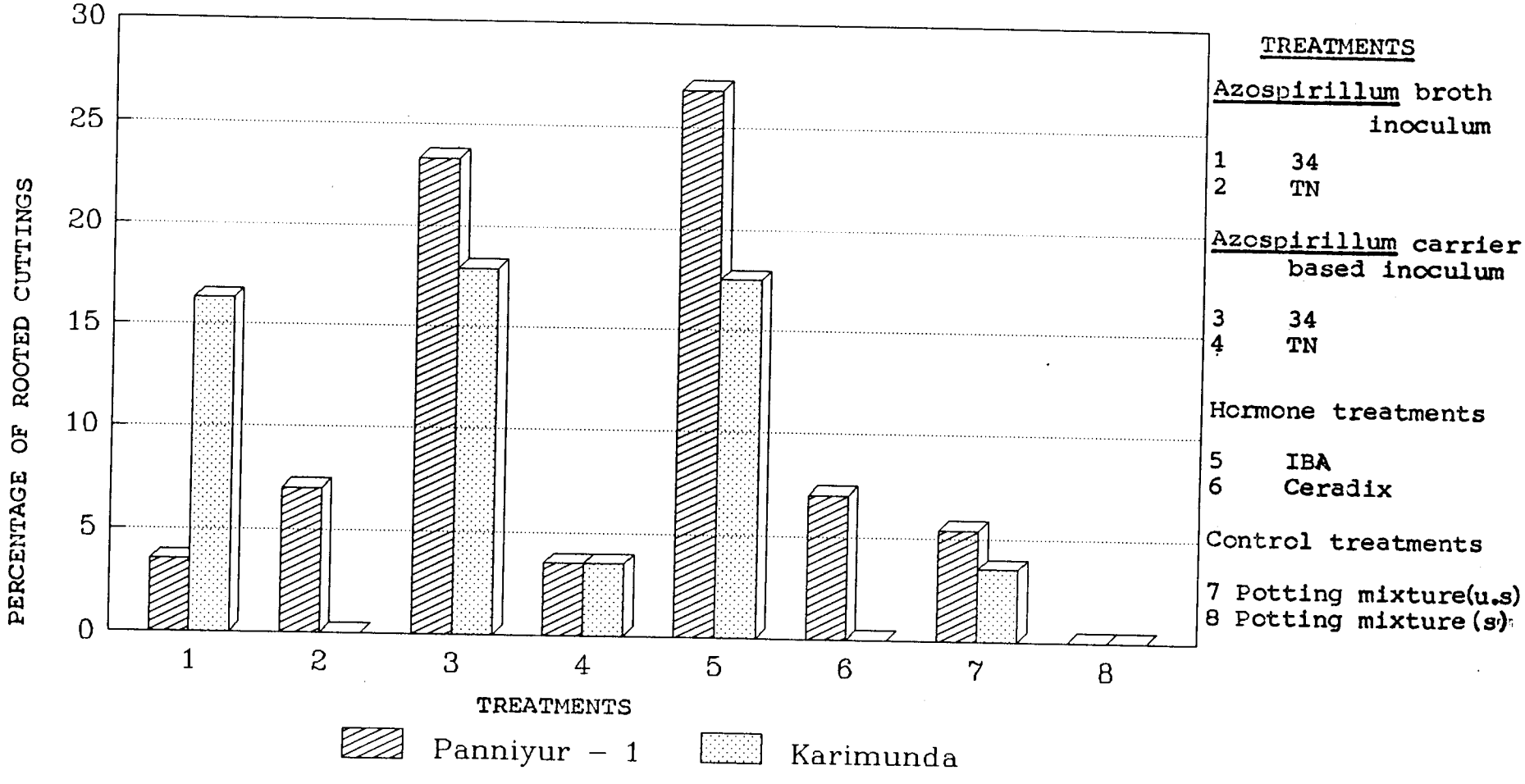


Plate I

Root induction in Panniyur-1 by Azospirillum isolate 34 broth inoculum.

CO - Unsterilized potting mixture

CD - Ceradix treatment

34 AZO BR - Azospirillum isolate 34 broth inoculation.

Plate 2

Root induction in Panniyur-1 by Azospirillum isolate 34 carrier based inoculation.

CO - Unsterilized potting mixture

CD - Ceradix treatment

34 AZ CA - Azospirillum isolate 34 carrier based inoculum.

Plate 1

Plate 2

Plate 3

Root induction in Panniyur-1 by Azospirillum TN broth inoculum.

CO - Unsterilized potting mixture

CD - Ceradix treatment

TN AZO BR - Azospirillum TN broth inoculum

Plate 4

Root induction in Panniyur -1 by Azospirillum TN carrier based inoculum.

CO - Unsterilized Potting mixture

CD - Ceradix treatment

TN AZO CA - Azospirillum TN carrier based inoculum.

Plate 3

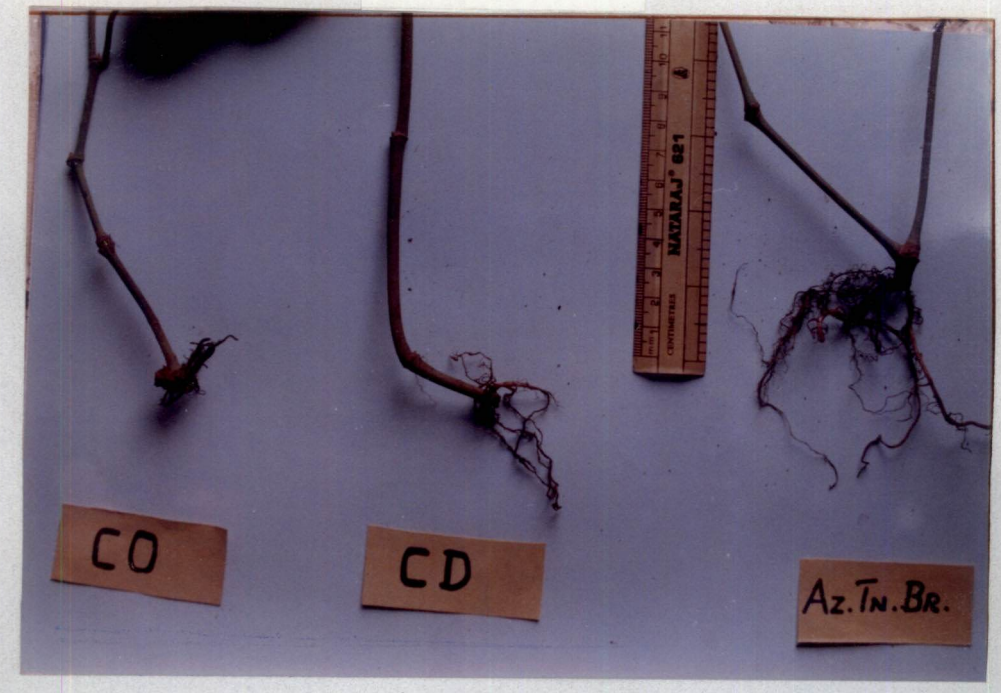


Plate 4

Plate 5

Root induction in Panniyur-1 by IBA treatment

ACO - Sterilized potting mixture

CO - Unsterilized potting mixture

IBA - 1000 ppm IBA treatment

Plate 6

Root induction in Karimunda by IBA treatment

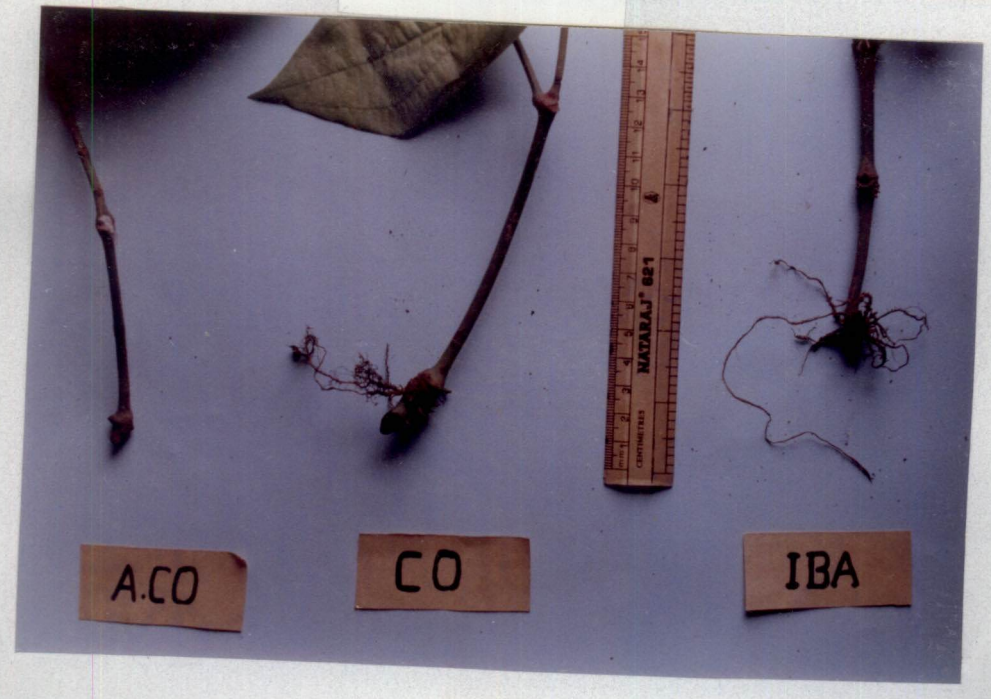
ACO - Sterilized potting mixture

CO - Unsterilized potting mixture

IBA - 1000 ppm IBA treatment.

Plate 5

Plate 6



treatment effect of Azospirillum isolate 34 carrier based inoculum was uniformly superior for all the three rooting parameters studied. The percentage of rooted cuttings obtained 17.9 percent, mean number of roots per cutting, 2.1 and root dry weight, 0.53 g, were all maximum after bacterial inoculation (Table 8, Figure 5). The effect of IBA application was also on par with Azospirillum treatment except for dry weight of roots. The remaining treatments had no significant effect on root induction in both Panniyur-1 and Karimunda varieties of bush pepper.

The formation of new leaf primordia by way of fresh sprout formation was taken as the criterion for germination of pepper cuttings. But there were no significant difference between treatments. The percentage of germinated cuttings obtained was maximum due to Azospirillum isolate 34 treatment. This was 12.4 percent in both Panniyur-1 and Karimunda varieties (Figure 6). In Panniyur-1 the increase in fresh weight of shoot and root were statistically significant in Azospirillum isolate 34 carrier based inoculum and IBA treatments, when compared to control. These were 3.39 and 0.71 g respectively for bacterial treatment and 3.63 and 0.62 g for IBA treatment (Table 9). The fresh weight of shoot and shoot to root ratio were however higher in the hormone treatment.

Table 8. *Effect of Azospirillum inoculation on root induction in bush pepper variety - Karimunda

Treatments	Percentage of rooted cuttings	Number of roots per cutting	Dry weight of roots (g)
1. Azospirillum treatment			
Broth culture			
34	16.3	1.2	0.37
TN	0.0	0.0	0.00
Carrier based inoculum			
34	17.9	2.1	0.53
TN	3.5	0.5	0.10
2. Hormone treatment			
IBA	17.5	1.9	0.41
Ceradix	0.0	0.0	0.00
3. Control treatment			
Unsterilized potting mixture	3.5	0.3	0.09
Sterilized potting mixture	0.0	0.0	0.00
CD (0.05)	13.6	1.5	0.36

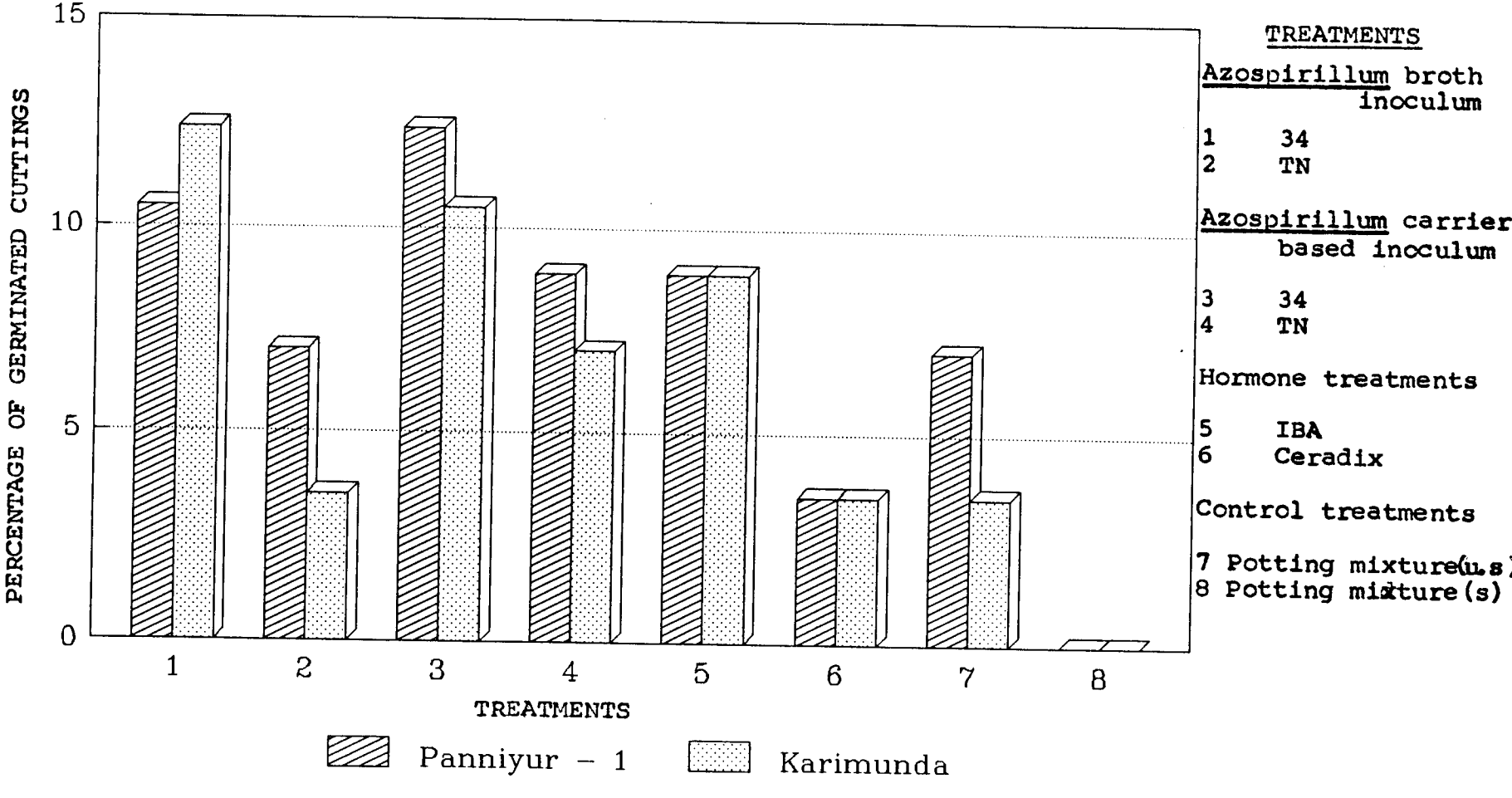
*Mean of 10 replications.

Table 9. *Effect of Azospirillum inoculation on germination and growth of bush pepper variety - Panniyur I

Treatments	Percentage of germinated cuttings	Fresh weight of shoot (g)	Fresh weight of root (g)	Shoot to root ratio
1. Azospirillum treatment				
Broth culture				
34	10.5	2.38	0.12	0.2
TN	7.0	2.32	0.22	0.6
Carrier based inoculum				
34	12.4	3.39	0.71	1.7
TN	8.9	1.29	0.12	0.2
2. Hormone treatment				
IBA	8.9	3.63	0.62	3.7
Ceradix	3.5	1.47	0.18	0.5
3. Control treatment				
Unsterilized potting mixture	7.0	1.39	0.05	0.7
Sterilized potting mixture	0.0	0.68	0.00	0.0
CD (0.01)	14.2	1.59	0.42	1.7

*Mean of 10 replications.

Fig.6 – Effect of Azospirillum inoculation on germination in bush pepper



In Karimunda, the fresh weight of shoot was maximum in Azospirillum isolate 34 broth culture treatment. Maximum germination was also obtained in this treatment (Table 10). But the fresh weight of root was higher in Azospirillum isolate 34 carrier based inoculum treatment. The fresh weight of 0.57 g in this treatment and 0.54 g in IBA treatment were significant when compared to control treatment. In Karimunda also, the shoot to root ratio of 1.56 was maximum in IBA treatment (Figure 7).

6. **Effect of** Azospirillum inoculation on establishment and growth of bush pepper

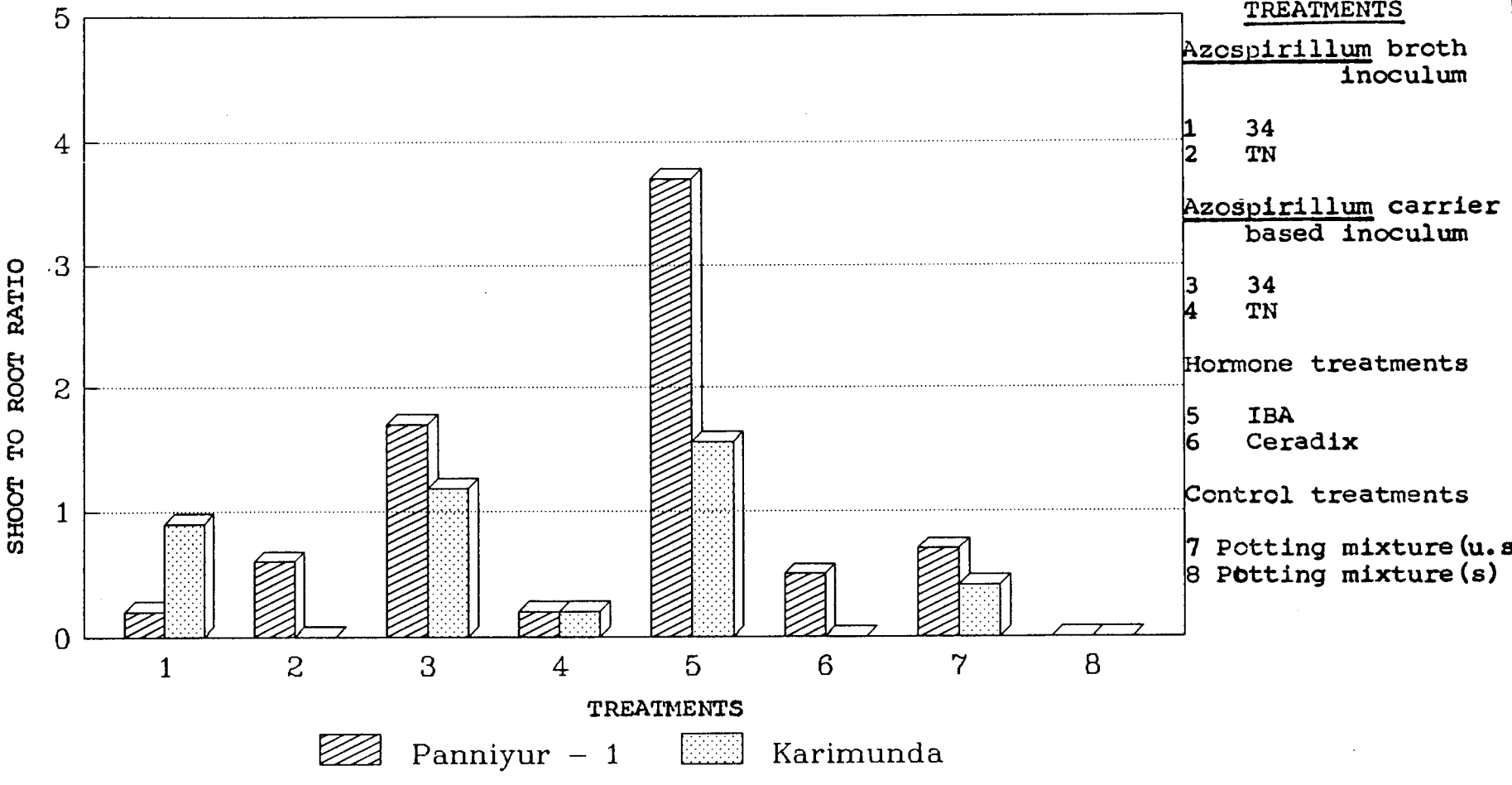
The study on establishment and growth of bush pepper was conducted by using rooted cuttings of both Panniyur-1 and Karimunda varieties. These were selected from 3 groups of pepper cuttings maintained separately for 3 months after pretreatment with Azospirillum isolate 34 carrier based inoculum, (Group 1 Plants) or 1000 ppm IBA (Group 2 plants) for root induction. Pepper cuttings grown in unsterilized potting mixture without any bacterial or hormone treatment (Group 3 plants) served as control. Twenty rooted cuttings were randomly selected from each of the above 3 groups of plants for transplanting to fresh potting mixture supplemented with Azospirillum isolate 34 carrier based inoculum at the rate of 25 and 100g per kg of potting mixture and with and without chemical fertilizer in

Table 10. *Effect of Azospirillum inoculation on germination and growth of bush pepper variety - Karimunda

Treatments	Percentage of germinated cuttings	Fresh weight of shoot (g)	Fresh weight of root (g)	Shoot to root ratio
1. Azospirillum treatment				
Broth culture				
34	12.4	2.74	0.39	0.90
TN	3.5	1.34	0.00	0.00
Carrier based inoculum				
34	10.5	2.06	0.57	1.18
TN	7.0	2.42	0.11	0.20
2. Hormone treatment				
IBA	8.9	2.15	0.54	1.56
Ceradix	3.5	1.36	0.00	0.00
3. Control treatment				
Unsterilized potting mixture	3.5	1.08	0.09	0.41
Sterilized potting mixture	0.0	1.36	0.00	0.0
CD (0.01)	12.9	1.58	0.39	0.99

*Mean of 10 replications.

Fig.7 – Effect of Azospirillum inoculation on shoot to root ratio in bush pepper



the form of vegetable mixture at the rate of 0.5g per kg of potting mixture. The different observations were taken at 180 days of plant growth.

6.1. Effect of Azospirillum and fertilizer application on root growth in bush pepper

In Panniyur-1, the mean number of roots produced per cutting, 17.6, fresh and dry weight of roots, 21.58g and 7.4g respectively, were maximum in the pepper cuttings pretreated with Azospirillum for initial root induction and grown subsequently in potting mixture supplemented with 100g Azospirillum inoculum alone (100g Azo 34-F). A similar effect was observed for IBA treated cuttings when raised in potting mixture supplemented with 25 g Azospirillum culture. In this treatment, the average number of roots produced per cutting, fresh and dry weight of roots were 13.8, 16.74g and 5.35g respectively (Table 11, Figure 8). The above treatments were also statistically significant when compared to corresponding control. The use of chemical fertilizer in the form of vegetable mixture did not have a significant effect on rooting parameters. However, the number of roots produced per cutting, fresh and dry weight of roots in the 100g Azo 34+F of group 1 and group 2 plants, fresh and dry weight of roots in the 25g Azo 34+F and the number of roots in the 100g Azo 34+F of group 2 plants were

Table 11. *Effect of Azospirillum and fertilizer application on root growth in bush pepper variety - Panniyur I

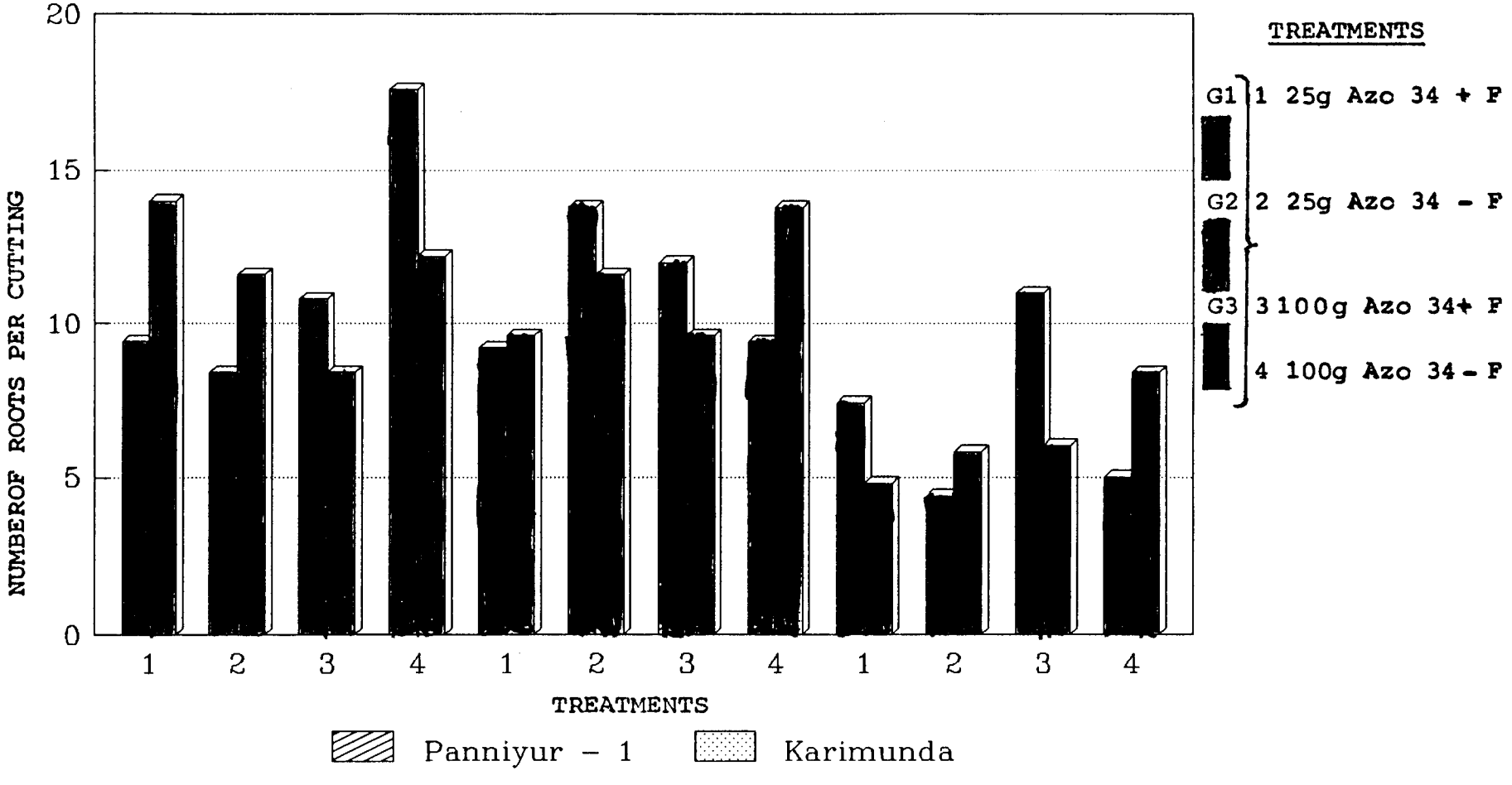
Treatments in potting mixture	Number of roots	Fresh weight of roots (g)	Dry weight of roots (g)
G.1 - RI with Azospirillum			
25g Azo 34 + F	9.4	11.05	3.74
25g Azo 34 - F	8.4	9.93	3.47
100g Azo 34 + F	10.8	12.71	4.62
100g Azo 34 - F	17.6	21.58	7.40
G.2 - RI with IBA			
25g Azo 34 + F	9.2	14.90	5.05
25g Azo 34 - F	13.8	16.74	5.35
100g Azo 34 + F	12.0	16.09	5.52
100g Azo 34 - F	9.4	11.82	4.04
G.3 - Control treatment			
25g Azo 34 + F	7.4	8.98	3.07
25g Azo 34 - F	4.4	4.80	1.62
100g Azo 34 + F	11.0	11.43	3.98
100g Azo 34 - F	5.0	6.57	2.25
CD (0.05)	7.7	8.92	3.09

*Mean of 5 replications.

RI - Root induction.

Gl, G2, G3 - Group 1, Group 2 and Group 3 plants.

Fig.8 – Effect of Azospirillum and fertilizer application on root growth in bush pepper



statistically on par with the best treatment of 100g Azo34-F of group I pepper cuttings.

In Karimunda, the number of roots produced per cutting, 14.0 fresh and dry weight of roots 15.72g, 5.45g respectively were statistically significant in the 25g Azo 34+F of group I plants (Table 12, Figure 8) In most of remaining cases, the treatment effect on various rooting parameters were statistically on par with above treatment. Lastly between the two varieties of bush pepper, Panniyur-1 responded better than Karimunda, in terms of number of roots produced per cutting and fresh and dry weight of roots at 180 days of plant growth.

6.2. Effect of Azospirillum and fertilizer application on germination and shoot growth in bush pepper

The production of new leaves was taken as the criterion for germination of pepper cuttings. In both Panniyur-1 and Karimunda varieties, the percentage of germination varied from 80-100 percent. In treatments such as 25g Azo 34+F and 100g Azo 34-F of Panniyur-1 and 25g Azo 34-F of Karimunda of group 2 and 25g Azo 34-F of Karimunda of group 3 plants, 100 percent germination was obtained (Tables 13 and 14, Figure 10). The use of chemical fertilizer in the form of vegetable mixture did not have uniform effect on germination.

Table 12. *Effect of Azospirillum and fertilizer application on root growth in bush pepper variety - Karimunda

Treatments in potting mixture	Number of roots	Fresh weight of roots (g)	Dry weight of roots (g)
G.1 - RI with Azospirillum			
25g Azo 34 + F	14.0*	15.72*	5.45*
25g Azo 34 - F	11.6	15.10	5.23
100g Azo 34 + F	8.4	9.46	3.29
100g Azo 34 - F	12.2	13.33	4.63
G.2 - RI with IBA			
25g Azo 34 + F	9.6	11.77	4.10
25g Azo 34 - F	11.6	12.20	4.25
100g Azo 34 + F	9.6	11.46	3.97
100g Azo 34 - F	13.8	15.57	5.36
G.3 - Control treatment			
25g Azo 34 + F	4.8	4.63	1.61
25g Azo 34 - F	5.8	6.77	2.31
100g Azo 34 + F	6.0	5.90	2.05
100g Azo 34 - F	8.4	10.94	3.76
CD (0.05)	8.3	10.61	3.68

*Mean of 5 replications.

RI - Root induction.

Gl, G2, G3 - Group 1, Group 2 and Group 3 plants.

Table 13. *Effect of Azospirillum and fertilizer application on germination and shoot growth in bush pepper variety - Panniyur I

Treatments in potting mixture	percentage of germination	Number of leaves	Fresh weight of shoot (g)	Dry weight of shoot (g)
G.1 - RI with Azospirillum				
25g Azo 34 + F	100	11.2	23.10	10.51
25g Azo 34 - F	80	10.4	25.07	11.35
100g Azo 34 + F	80	14.2	18.54	8.61
100g Azo 34 - F	100	28.0	34.07	15.49
G.2 - RI with IBA				
25g Azo 34 + F	80	15.2	23.16	10.33
25g Azo 34 - F	100	24.6	29.10	13.48
100g Azo 34 + F	100	25.0	34.44	15.06
100g Azo 34 - F	80	16.0	20.40	9.25
G.3 - Control treatment				
25g Azo 34 + F	80	7.6	15.25	6.82
25g Azo 34 - F	80	5.6	13.91	6.16
100g Azo 34 + F	80	12.6	18.71	8.13
100g Azo 34 - F	80	6.4	14.74	6.71
CD (0.05)		14.4	15.03	6.80

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

Table 14. *Effect of Azospirillum and fertilizer application on germination and shoot growth in bush pepper variety - Karimunda

Treatments in potting mixture	percentage of germination	Number of leaves	Fresh weight of shoot (g)	Dry weight of shoot (g)
G.1 - RI with Azospirillum				
25g Azo 34 + F	80	20.2	19.38	8.62
25g Azo 34 - F	100	18.8	20.77	9.45
100g Azo 34 + F	80	11.2	16.36	7.19
100g Azo 34 - F	100	13.2	20.66	9.66
G.2 - RI with IBA				
25g Azo 34 + F	80	14.2	15.04	6.54
25g Azo 34 - F	80	16.8	21.63	9.76
100g Azo 34 + F	80	13.8	15.23	6.75
100g Azo 34 - F	100	21.4	20.16	9.14
G.3 - Control treatment				
25g Azo 34 + F	80	7.6	15.22	6.92
25g Azo 34 - F	100	14.4	16.93	7.49
100g Azo 34 + F	80	9.0	17.20	7.67
100g Azo 34 - F	80	13.6	14.98	6.62
CD (0.05)		12.9	11.28	5.03

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

Fig.9 – Effect of Azospirillum and fertilizer application on leaf production in bush pepper

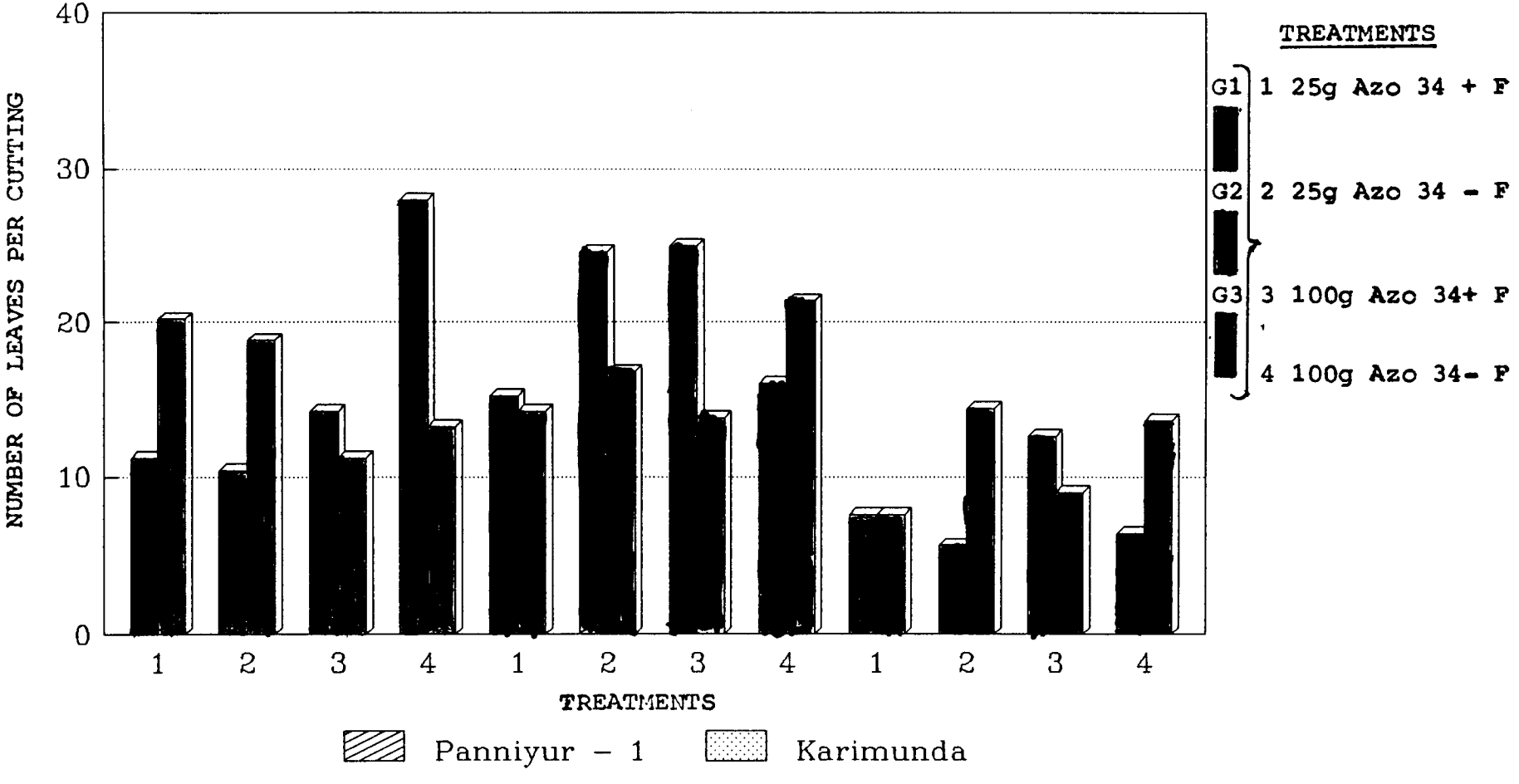
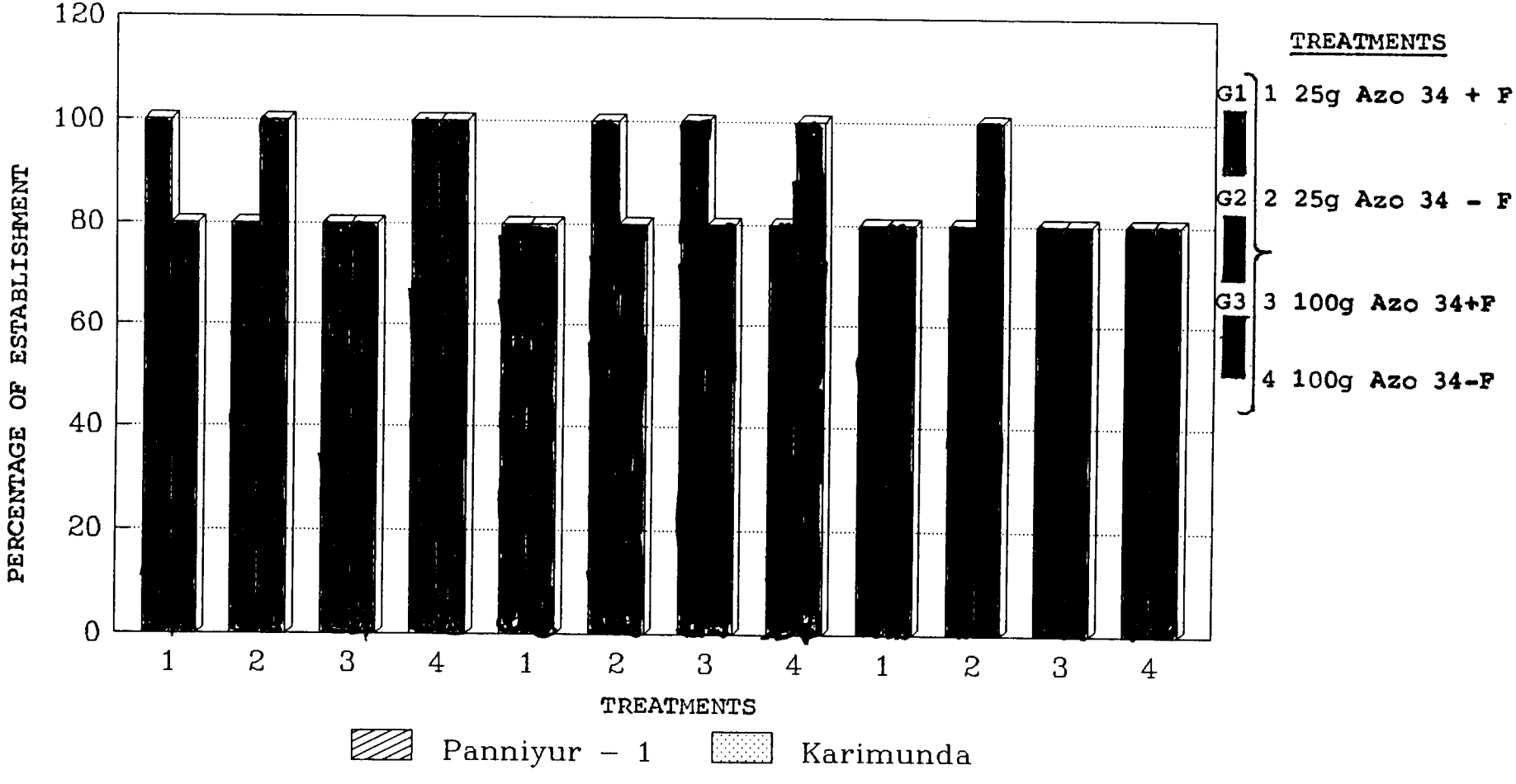


Fig.10 – Effect of Azospirillum and fertilizer application on establishment in bush pepper



In Panniyur-1, the mean number of leaves produced per cutting, 28.0, fresh and dry weight of shoot 34.07 g and 15.49 g respectively, were maximum in pepper cuttings pretreated with Azospirillum for initial root induction and grown subsequently in potting mixture supplemented with 100g Azospirillum culture alone. A similar effect was observed for IBA treated cuttings when raised in potting mixture supplemented with 25g Azospirillum culture alone and in the 100g Azo 34+F treatment for fresh and dry weight of shoot. In these treatments, the number of leaves produced per cutting, fresh and dry weight of shoot were 24.6 g, 29.10 g and 13.48 g and 34.44 g and 15.06 g respectively (Table 13, Figure 9) The number of leaves produced in treatments such as 100g Azo 34+F of group I 25g Azo 34+F and 100g Azo 34+F of group 2 plants were statistically on per with the best treatment of 100g Azo 34 - F of group I pepper cuttings. Similarly, the fresh and dry weight of shoot in the treatments of 25g Azo 34-F of group I and 25 g Azo 34+F and 100g Azo 34-F of group 2 plants were statistically on par with best treatment of 100g Azo 34+F of group 2, for fresh weight of shoot and 100g Azo 34-F of group I for dry weight of shoot.

In Karimunda, there were no significant differences between treatments in the number of leaves produced per cutting and in the fresh and dry weight of

shoot (Table 14, Figure 9). The leaf number was maximum in pepper cuttings pretreated with IBA for initial root induction and grown subsequently in potting mixture supplemented with 100g Azospirillum 34 carrier based inoculum alone. At the same time, the fresh and dry weight of shoot were higher in the 25g Azo 34-F of group 2 plants. These were 21.4 leaves per cutting and 21.63g and 9.76g respectively for fresh and dry weight of shoot. Lastly between the two varieties of bush pepper, statistically significant treatment effects were obtained only in Panniyur-1 in terms of number of leaves produced per cutting and in fresh and dry weight of shoot at 180 days of plant growth.

6.3 Effect of Azospirillum and fertilizer application on branching in bush pepper

In Panniyur-1, the average length of branches, produced, 56.4 cm, fresh and dry weight of branches, 41.80 g and 18.31g were maximum in pepper cuttings pretreated with Azospirillum for initial root induction and grown subsequently in potting mixture supplemented with 100g Azospirillum isolate 34 carrier based inoculum, alone (Table 15). A similar effect was also obtained for IBA treated cuttings when raised in potting mixture supplemented with 25g Azospirillum culture alone. In this treatment, the

Table 15. *Effect of Azospirillum and fertilizer application on branching in bush pepper variety - Panniyur 1

Treatments in potting mixture	Length of branch (cm)	Fresh weight of branch (g)	Dry weight of branch (g)
G.1 - RI with Azospirillum			
25g Azo 34 + F	21.8	18.36	8.43
25g Azo 34 - F	21.2	16.38	7.15
100g Azo 34 + F	29.0	22.98	10.27
100g Azo 34 - F	56.4	41.80	18.31
G.2 - RI with IBA			
25g Azo 34 + F	33.4	24.96	11.07
25g Azo 34 - F	50.0	37.76	17.50
100g Azo 34 + F	46.8	35.28	15.84
100g Azo 34 - F	29.6	21.86	9.59
G.3 - Control treatment			
25g Azo 34 + F	17.2	12.73	5.95
25g Azo 34 - F	12.4	9.59	4.33
100g Azo 34 + F	25.0	19.52	8.91
100g Azo 34 - F	13.4	10.11	4.60
CD (0.05)	29.9	21.79	9.61

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

average length of branches produced, fresh and dry weight of branches were 50.0 cm, 37.76 and 17.50g respectively. The above two treatments were statistically significant when compared to corresponding control. The average length of branches and its fresh and dry weight in treatments such as 100g Azo 34+F in group 1, 25g Azo 34+F and 100g 34+F of group 2 plants were statistically on par with best treatment of 100g Azo 34-F of group I pepper cuttings. The addition of chemical fertilizer in the form of vegetable mixture did not have a significant effect on branching in Panniyur-1 variety of bush pepper.

In Karimunda, there were no significant differences between treatments in the average length of branches produced. The length of branch 46.2 cm was maximum in the 100g Azo 34-F treatment of group 2 plants. However, significant increase in fresh and dry weights of branches were obtained in the 25g Azo 34+F treatment of group I pepper cuttings when compared to corresponding control. (Table 16). The increase in fresh and dry weight of branches, in most of the remaining cases were statistically on par with the above treatment. Lastly between the two varieties of bush pepper, Panniyur-1 had a desirable response to branching characters due to various treatments.

Table 16. *Effect of Azospirillum and fertilizer application on branching in bush pepper variety - Karimunda

Treatments in potting mixture	Length of branch (cm)	Fresh weight of branch (g)	Dry weight of branch (g)
G.1 - RI with Azospirillum			
25g Azo 34 + F	43.4	39.47	18.07
25g Azo 34 - F	41.0	37.50	16.55
100g Azo 34 + F	21.8	18.98	8.62
100g Azo 34 - F	28.8	28.25	12.43
G.2 - RI with IBA			
25g Azo 34 + F	29.6	26.95	12.07
25g Azo 34 - F	34.0	31.14	13.79
100g Azo 34 + F	29.2	26.59	12.13
100g Azo 34 - F	46.2	42.04	19.98
G.3 - Control treatment			
25g Azo 34 + F	17.0	14.63	6.69
25g Azo 34 - F	29.2	25.66	11.21
100g Azo 34 + F	18.6	16.70	7.26
100g Azo 34 - F	29.4	26.75	11.94
CD (0.05)	26.8	24.34	11.00

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

6.4. Effect of *Azospirillum* inoculation on establishment and growth of bush pepper

The survival and growth of pepper cuttings in fresh potting mixture was taken as the criterion for establishment of bush pepper. In both Panniyur-1 and Karimunda varieties the percentage of establishment varied from 80-100 percent. In treatments such as 25g Azo 34-F and 100g Azo 34-F of Panniyur-1 and 25g Azo 34-F and 100g Azo 34-F of Karimunda of group I plants, 25g Azo 34-F and 100g Azo 34+F of Panniyur-1 and 100g Azo 34-F of Karimunda of group 2 and 25g Azo 34-F of Karimunda of group 3 plants, 100 percent establishment were obtained. The use of chemical fertilizer in the form of vegetable mixture did not have a uniform effect on the establishment particularly in Karimunda, where 100 percent establishment of pepper cuttings were achieved without any chemical fertilizer application.

In Panniyur-1 a significant increase in shoot to root ratio was obtained in 100g Azo 34+F of group 2 plants. In this treatment, the shoot to root ratio was 5.4 (Table 17). Significant increase in total fresh and dry weight of pepper cuttings were obtained in 100g Azo 34-F of group I and 25g Azo 34-F treatments of group 2 pepper cuttings. The total dry weight in the treatment of 100g Azo 34-F of group I, the total fresh and dry weight in treatments such as

Table 17. *Effect of Azospirillum and fertilizer application on establishment and growth of bush pepper variety - Panniyur I

Treatments in potting mixture	percentage of establishment	Shoot to root ratio	Total fresh weight (g)	Total dry weight (g)
G.1 - RI with Azospirillum				
25g Azo 34 + F	100	3.8	52.52	22.67
25g Azo 34 - F	80	3.2	51.38	21.99
100g Azo 34 + F	80	2.6	54.23	23.50
100g Azo 34 - F	100	3.7	97.45	41.15
G.2 - RI with IBA				
25g Azo 34 + F	80	2.7	63.02	26.45
25g Azo 34 - F	100	4.1	83.60	36.23
100g Azo 34 + F	100	5.4	85.81	36.42
100g Azo 34 - F	80	2.8	54.08	22.89
G.3 - Control treatment				
25g Azo 34 + F	80	3.5	36.97	15.84
25g Azo 34 - F	80	5.4	28.29	12.11
100g Azo 34 + F	80	2.6	49.66	21.02
100g Azo 34 - F	80	3.1	31.28	13.56
CD (0.05)		2.7	41.94	17.84

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

25g Azo 34+F and 100g Azo 34+F of group 2 plants were statistically on par with the best treatment of 100g Azo 34-F of group I pepper cuttings (Table 17, Figures 11, 12).

In Karimunda, there were no significant differences between treatments with regard to shoot to root ratio, total fresh and dry weight of pepper cuttings. These were maximum in the 25g Azo 34-F of group 3 for shoot to root ratio and 100g Azo 34-F of group 2 plants for total fresh and dry weight (Table 18, Figures 11, 12). The use of chemical fertilizer in the form of vegetable mixture did not have a significant effect on shoot to root ratio, total fresh and dry weight in both Panniyur-1 and Karimunda varieties of bush pepper.

The effect of incorporating Azospirillum culture in potting mixture had a definite but varying growth response in Panniyur-1 depending on the initial treatment for root induction. Thus in pepper cuttings pretreated with Azospirillum isolate 34 carrier based inoculum for root induction, the growth response was significant for all the characters studied when the same culture was also incorporated in the potting mixture, at the rate of 100g per kg of potting mixture. In this treatment of 100g Azo 34-F, the increase in the number of roots produced per cutting, fresh and dry weight of roots, number of leaves per cuttings, fresh and dry weight of shoot, length of branch

Table 18. *Effect of Azospirillum and fertilizer application on establishment and growth of bush pepper variety - Karimunda

Treatments in potting mixture	percentage of establishment	Shoot to root ratio	Total fresh weight (g)	Total dry weight (g)
G.1 - RI with Azospirillum				
25g Azo 34 + F	80	3.5	74.57	32.14
25g Azo 34 - F	100	4.2	73.31	31.22
100g Azo 34 + F	80	3.2	44.80	19.24
100g Azo 34 - F	100	3.9	62.23	26.72
G.2 - RI with IBA				
25g Azo 34 + F	80	3.3	53.76	22.71
25g Azo 34 - F	80	4.4	64.98	27.80
100g Azo 34 + F	80	3.6	53.27	22.84
100g Azo 34 - F	100	4.6	77.75	34.48
G.3 - Control treatment				
25g Azo 34 + F	80	5.7	34.47	15.21
25g Azo 34 - F	100	7.1	49.37	21.01
100g Azo 34 + F	80	4.6	39.79	16.98
100g Azo 34 - F	80	3.3	52.67	22.32
CD (0.05)		3.1	43.20	18.35

*Mean of 5 replications.

RI - Root induction.

G1, G2, G3 - Group 1, Group 2 and Group 3 plants.

Fig.11 – Effect of Azospirillum and fertilizer application on total fresh weight in bush pepper

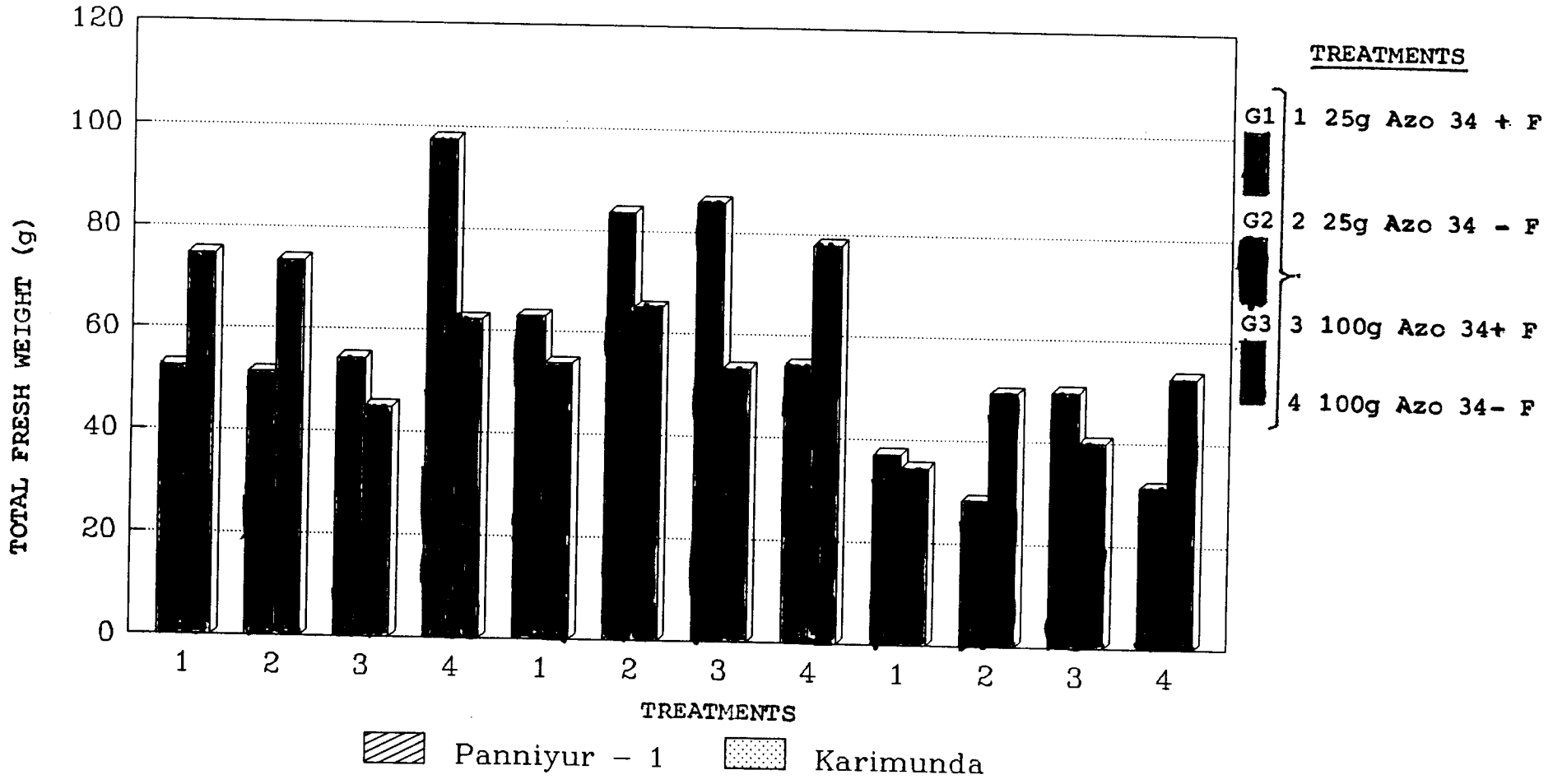


Fig.12 – Effect of Azospirillum and fertilizer application on total dry weight in bush pepper

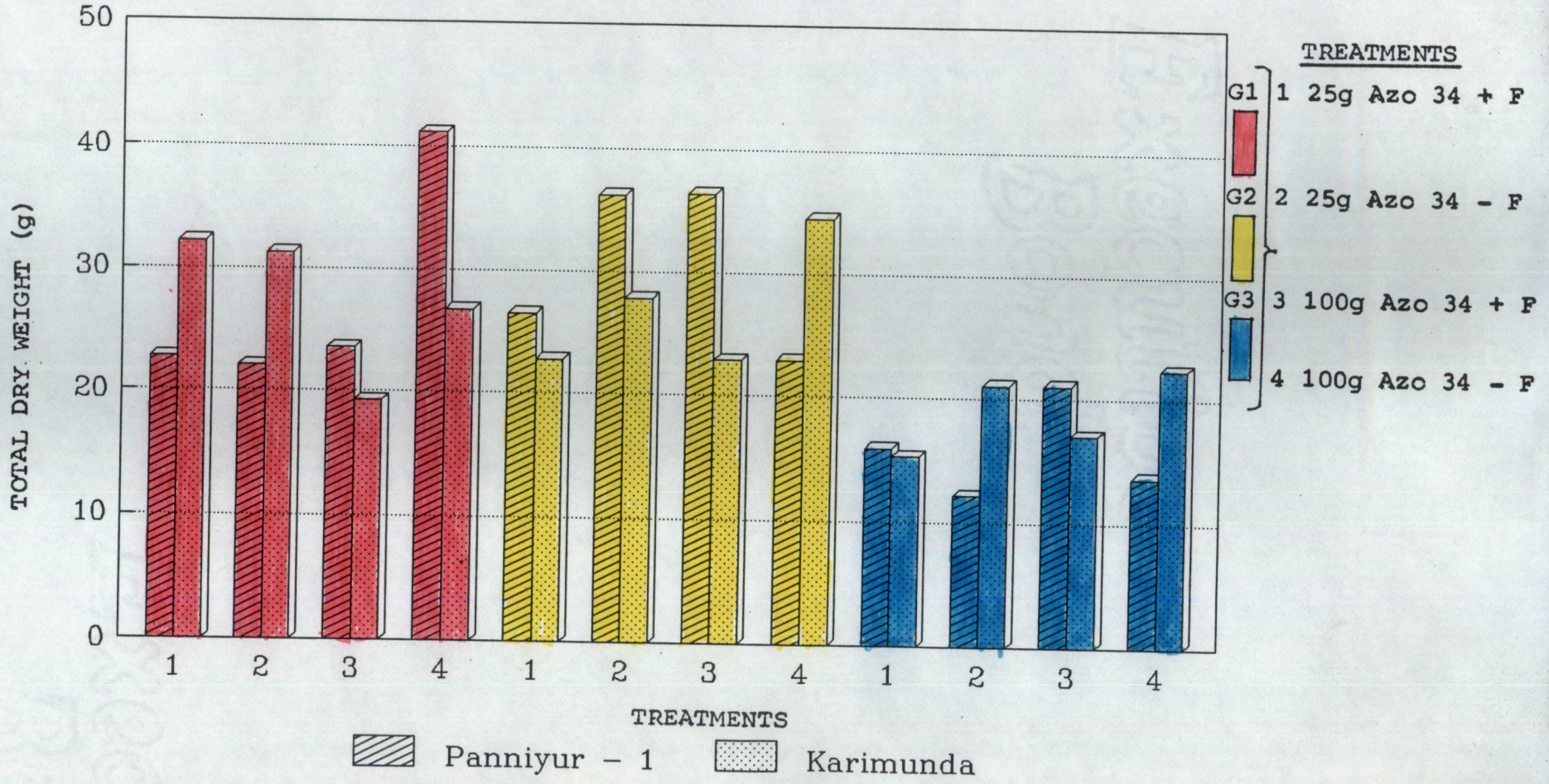


Plate 7

Growth and establishment of Panniyur - 1 after inoculation with 25 g Azospirillum culture.

34 - Root induction with Azospirillum isolate 34.

C - Root induction without Azospirillum and hormone treatments.

F^- - Without fertilizer application.

Plate 8

Growth and establishment of Panniyur- 1 after inoculation with 25 g Azospirillum culture and fertilizer application.

34 - Root induction with Azospirillum isolate 34

C - Root induction without Azospirillum and hormone treatments.

F^+ - With fertilizer application.

Plate 7

Plate 8

Plate 9

Growth and establishment of Panniyur - 1 after inoculation with 100 g Azospirillum culture.

34 - Root induction with Azospirillum culture

C - Unsterilized potting mixture.

F^- - Without fertilizer application.

Plate 10

Growth and establishment of Panniyur - 1 after inoculation with 100 g Azospirillum culture and fertilizer application.

34 - Root induction with Azospirillum isolate 34.

C - Unsterilized potting mixture.

F^+ - With fertilizer application.

Plate 9

Plate 10

Plate 11

Growth and establishment of Panniyur-1 after inoculation with 100 g *Azospirillum* culture.

34 - Root induction with *Azospirillum* isolate 34.

F^+ - With fertilizer application.

F^- - Without fertilizer application.

Plate 12

Growth and establishment of Karimunda after inoculation with 100 g *Azospirillum* culture.

34 - Root induction with *Azospirillum* isolate 34.

F^+ - With fertilizer application.

F^- - Without fertilizer application.

Plate 11

Plate 12

Plate 13

Rooting pattern of bush pepper variety Panniyur - 1 during establishment.

34 - Root induction with Azospirillum isolate 34.

100 A - Post treatment of isolate 34 @ 100g/kg of potting mixture.

F^+ - With fertilizer application.

F^- - Without fertilizer application.

Plate 14

Rooting pattern of bush pepper variety Karimunda during establishment.

34 - Root induction with Azospirillum isolate 34.

100 A - Post treatment of isolate 34 @ 100 g/kg of potting mixture.

F^+ - With fertilizer application.

F^- - Without fertilizer application.

Plate 13

Plate 14

produced, fresh and dry weight of branches, total fresh and dry weight of the cuttings were statistically significant when compared to control treatment. In IBA treated cuttings of Panniyur-1 a similar growth response was observed with the use of 25g Azospirillum culture alone in potting mixture. In both the above treatments there was no addition of chemical fertilizers. The increase in fresh and dry weight of shoot and shoot to root ratio were however significant with 100g Azo 34+F cuttings pretreated with IBA for root induction.

In Karimunda, a uniform growth response was absent for the Azospirillum and chemical fertilizer application in the potting mixture. The number of roots produced per cutting, fresh and dry weight of roots, fresh and dry weight of branches were significantly higher in the 25g Azo 34+F plants pretreated with Azospirillum for root induction. However the fresh and dry weight of shoots, leaf number and total fresh and dry weight of pepper cuttings were higher in the 25g Azo 34-F and 100g Azo 34-F of plants pretreated with IBA for root induction.

Due to random nature of berry formation in different pepper cuttings, the observations in yield were not taken during this experiment.

DISCUSSION

Discussion

Kerala is the land of spices in India. Among the different spices grown here, pepper is cultivated in 1,69,670 hectares with an annual production of 41,560 metric tonnes. Traditionally it is cultivated as a climber by using runner shoots produced at the base of mother plants. However, it is also possible to use flowering branches for vegetative propagation (Menon, 1949). Root induction in such cuttings are usually done with IBA or Ceradix treatment. But this could also be achieved by using specific phytohormone producing bacteria. The present investigation was done to study the possibility of using Azospirillum for root induction, establishment and growth of Panniyur-1 and Karimunda varieties of bush pepper.

Initial isolation of Azospirillum was done from 25 different cultivars of pepper by using nitrogen free semisolid malate agar medium. Sixteen different isolates of Azospirillum were obtained (Table 2). All these were gram negative and were maintained on malate agar medium supplemented with 0.3 percent ammonium chloride for various studies. The association of Azospirillum with a number of crop plants have been observed earlier by Dobereiner et al (1976) in field grass, Lakshmikumari et al (1976) in maize, sorghum, sugarcane, and forage grasses, Kavimandan et al

(1978) and Padshetty et al (1986) in wheat. Dewan and Subba Rao (1979), Purushothaman (1988) and Khan and Hossain (1990) in rice and by Lukin et al (1992) in barley. The presence of Azospirillum in the rhizosphere of black pepper has also been reported by Govindan and Chandy (1985) and Bopaiah and Khader (1989).

The different isolates of Azospirillum were initially screened for the production of phytohormones like indole acetic acid and gibberellins under in vitro conditions. A culture obtained from Tamil Nadu Agricultural University (TN culture) was used as a standard. Six native isolates, such as 34, 39, 41, 55, 60 and 64 and TN culture produced IAA in malate broth supplemented with L-Tryptophan. (Table 3, Figure 2). The production of IAA by Azospirillum has been reported earlier by Reynders and Vlassak (1979), Tien et al (1979), Hartmann et al (1983), Govindan and Purushothaman (1984), Kolb and Martin (1985) and Crozier et al (1988). However, the fact that only 6 out of the 16 native isolates alone produced IAA indicated that there existed certain variations in the ability of Azospirillum to produce this phytohormone. Further, among the positive culture also, there were differences in the quantity of IAA produced. Thus while Azospirillum isolate 34 from pepper cultivar karimunda produced maximum amount of 69 µg/ml of culture broth, the isolate 39 from Balankotta produced only

17 µg of IAA under *in vitro* conditions. The production of this phytohormone by native isolate 60 from the cultivar Chumala and exotic isolate TN culture were also relatively higher. (Table 3, Figure 2). This sort of variations in the amount of IAA produced by different isolates of *Azospirillum* has been reported by Mascarua *et al* (1988). They observed that while strains of *Azospirillum brasilense* produced comparatively higher amount of 36.5-77 µg of IAA, strains of *Azospirillum lipoferum* produced only 6.5-17.5 µg of IAA.

The period of culture growth also had an effect on the quantity of IAA produced by various isolates of *Azospirillum*. Thus in isolate 34, the production of this phytohormone reached a peak level during second week of culture incubation. The amount of IAA produced during this period was 69 µg/ml of growth medium (Table 3, Figure 2). But afterwards, the quantity of IAA produced declined gradually and reached a low level of 26 µg after incubation for 5 weeks. This reduction could be due to a partial oxidation of IAA taking place during the prolonged period of culture incubation. The possibility of NO_2 production in the growth medium from ammonium chloride supplemented as a nutrient additive could also be a factor responsible for this oxidation process. Eventhough, the production of gibberellins by *Azospirillum* has been reported by Tien *et al* (1979), Govindan and Purushothaman (1984) and Bottini *et al*

(1989) none of the cultures screened during this investigation tested positive for the production of this phytohormone.

The study on the requirement of biotin for growth showed that while the native isolate of Azospirillum 34 did not require biotin, the growth of TN culture was dependent on the availability of this vitamin. The requirement of biotin is usually taken as an important criterion for distinguishing between Azospirillum brasilense and Azospirillum lipoferum species (Dobereiner et al 1976; Okon et al 1976; Tarrand et al 1978; Dobereiner, 1983). Based on this, Azospirillum isolate 34 was tentatively identified as A. brasilense and TN culture as A. lipoferum. This TN culture was also capable of using sucrose as a carbon source (Table 4). Dobereiner et al (1976), Okon et al (1976) and Tarrand et al (1978) reported the ability of A. lipoferum to use glucose as a carbon source and the inability of A. brasilense to use the same for growth in nitrogen free medium.

The pH sensitivity of Azospirillum isolate 34 and TN cultures were tested under in vitro conditions by using malate broth initially adjusted to different pH of 4.0, 5.0, 6.0, 7.0 and 8.0. The growth of isolate 34 was maximum at pH 6.0 with an optical density of 0.689 after incubation for 120 hours (Table 5, Figure 3). The growth of TN culture

was however, more at pH 8.0. (Table 6, Figure 4). This observation particularly with respect to the native isolate was not in agreement with that of Dobereiner (1983) who while summarising the characters of A. brasilense and A. lipoferum reported that these species grew well only at pH 6.8. However, the natural occurrence of Azospirillum in acidic soils with pH 3.2-4.8 has been reported earlier by Dobereiner et al (1976) and Beerkeem and Bohlool (1980). This indicated that Azospirillum was capable of growing at low pH. Therefore, the ability of isolate 34 to grow at pH 6.0 could very well be due to the fact that, this culture was originally isolated from an acidic soil sample. By the same reasoning one can also explain the better growth of TN culture at pH 8.0 which might have been originally isolated from a neutral or slightly alkaline soil sample. The occurrence of Azospirillum in alkaline soils with pH 8-8.8 in Tamil Nadu had been reported by Purushothaman and Oblisami (1985). But neither the native isolate nor the exotic isolate tested for pH sensitivity grew at pH below 5.0.

The mass production of isolate 34 and TN culture were done in modified malate broth which was supplemented with L-Tryptophan. This was done to induce the production of IAA by these cultures and thereby enhance root induction in bush pepper. This was significantly high in both Panniyur-1

and Karimunda varieties after treatment with the carrier based inoculum of Azospirillum and 1000 ppm IBA. The percentage of rooted cuttings obtained due to these treatments varied from 23.3-26.8 in Panniyur-1 and from 17.5-17.9 in Karimunda. (Tables 7, 8, Figure 5). Eventhough in Panniyur-1, IBA treatment was superior in terms of percentage of rooted cuttings produced, the average number of roots formed per cutting as well as root dry weight were maximum in bacterial treatment. These were 3.5 and 0.68 g respectively when compared to 3.3 and 0.59 g in hormone treatment. But in Karimunda, treatment with carrier based inoculum of Azospirillum was superior for all the three rooting parameters studied. (Table 8, Figure 5). The positive influence of Azospirillum inoculation in enhancing root biomass has been reported earlier in several crop plants like rice (Dewan and Subba Rao, 1979 and Purushothaman, 1988), Pearl millet (Venkateswarlu and Rao,1983), wheat (Kapulnik et al 1985 and Okon and Kapulnik, 1986)tomato (Hadas and Okon, 1987) and maize (Fallik et al 1988; Fallik and Okon, 1989; Lee et al 1989; Zaady et al 1993). Such an effect of Azospirillum treatment on root induction was also observed in pepper by Govindan and Chandy (1985) and Bopaiah and Khader (1989). Govindan and Chandy (1985) further reported that Azospirillum treatment was more beneficial as it induced the formation of higher

number of healthy and strong roots in pepper cuttings. The beneficial effect of Azospirillum inoculation on root formation has been mainly attributed to the production of phytohormones by this bacterium. (Tien et al 1979; Inbal and Feldman, 1982; Hartmann et al 1983; Govindan and Purushothaman, 1984; Morgenstern and Okon, 1988). This indicated that the use of IBA for root induction in bush pepper could effectively be substituted with an efficient phytohormone producing strain of Azospirillum. Another observation made during this study was that between the broth and carrier based inocula of Azospirillum and IBA and Ceradix treatments, the carrier based inoculum and IBA resulted in better root induction in bush pepper. The relative ineffectiveness of Ceradix treatment may be due to its low hormone content as compared to a pure sample of IBA.

The formation of new leaf primordia by way of fresh sprout formation was taken as the criterion for germination. But there were no significant differences between treatment in Panniyur-1 and Karimunda varieties. (Tables 9, 10, Figure 6). This could be due to the fact that the observation on germination was taken only after 90 days of plant growth. But the increase in fresh weight of shoot and root in Panniyur-1 were statistically significant in both Azospirillum 34 carrier based inoculum and IBA

treatments. These were 3.39, 0.71 g respectively for bacterial treatment and 3.63, 0.62 g for IBA treatment (Table 9). In Karimunda, on the other hand the fresh and dry weight of shoot and root were more on Azospirillum broth inoculum and carrier based inoculum treatments respectively (Table 10). In both Panniyur-1 and Karimunda varieties, the shoot to ratio were maximum in the hormone treatment (Tables 9, 10, Figure 7). The comparatively low fresh weight of root in IBA treated cuttings might be a reason for such an observation.

The study on establishment and growth of bush pepper was conducted by using 3 sets of rooted cuttings of Panniyur-1 and Karimunda varieties. In group 1 and 2 plants, root induction was done by using a carrier based inoculum of Azospirillum isolate 34 and 1000 ppm IBA respectively. However, in the third group, no bacterial or hormone treatments were given for initial root induction. These cuttings were grown separately in polypropylene bags containing potting mixture supplemented with either 25 or 100 g. Azospirillum culture and with or without chemical fertilizer application, at the rate of 0.5 g of vegetable mixture (10:10 (5): 10) per kg of potting mixture. These treatments were given to study the effects of Azospirillum and chemical fertilizer application on establishment and growth of bush pepper. The observation on number of roots

and leaves formed, length of branches, fresh and dry weight of branches and shoot, total fresh and dry weight of pepper cuttings, percentage of establishment, percentage of germination and shoot to root ratio were taken at 180 days of growth after transplanting to fresh potting mixture.

The survival of pepper cuttings in all the 5 replications taken as the criterion for 100 percent establishment. This was found related to some extent with the rooting parameters studied at the time of final observation. For example, in Panniyur-1 in treatments such as 100 g Azo 34-F of group 1 plants, 25 g Azo 34-F and 100 g Azo 34+F of group 2 plants where 100 percent establishment was obtained, the number of roots produced per cutting and fresh and dry weight of roots were either significantly high or on par with each other. (Table 11, Figure 8). Similarly in Karimunda, in treatments such as 25 g Azo 34-F and 100 g Azo 34-F of group 1 plants, 100 g Azo 34-F of group 2 and 25 g Azo 34-F of group 3 plants where 100 percent establishment was obtained, the number of roots produced per cutting, fresh and dry weight of roots were statistically on par with the best treatment of 25 g Azo 34 + F of group 1 plants (Table 12, Figure 8). However, in the latter treatment, full establishment of pepper cuttings was not there. This was also observed in 25 g Azo 34 +F of group 1 plants of Panniyur-1 variety. This might be due to the

reason that since only rooted cuttings were used for initial transplanting, the presence of absence of healthy rootlets in such cuttings was more critical for their survival. The subsequent production of fresh roots will have a positive influence only on later growth and yield of bush pepper.

The data on the production of new leaves, branches, fresh and dry weight of branches and shoots were used as criteria for growth of bush pepper. The production of fresh leaves in all the 5 replications of different treatments was again taken as the criterion for 100 percent germination. Interestingly in all those treatments were 100 percent establishment was achieved there were also 100 percent germination (Tables 13 and 14). In the remaining treatments these were about 80 percent. The production of new leaves, branches, fresh and dry weights of shoot were significant only in Panniyur-1 variety of bush pepper. In this variety, the increase in number of leaves produced, branch length, fresh and dry weight of branches and shoots were statistically significant in the 100 g Azo 34-F of group 1 and 25 g Azo 34-F of group 2 plants. In these treatments, the number of leaves produced were 28 and 24.6 respectively. The length of branches were 56.4 and 50.0 cm. The fresh and dry weight of branches were 41.80 and 18.31 g and 37.76 and 17.50 g respectively while the fresh and dry

weight of shoot were 34.07, 15.49 and 29.10 and 13.48 g respectively. (Tables 13 and 15). But in Karimunda, no such treatments effects were observed except for the fresh and dry weight of branches which were significant in the 25 g Azo 34 + F of group 2 plants. The number of leaves produced and fresh and dry weight of shoot were maximum in the 100 g Azo 34-F and 25 g Azo 34-F of group 2 plants (Table 14). The increase in total fresh and dry weight of plants were also significant in the 100 g Azo 34-F of group 1 and 25 g Azo 34-F of group 2 plants of Panniyur-1 (Table 17, figure 11 and 12). However, the shoot to root ratio of 5.4 was maximum in the 100 g Azo 34 + F of group 2 plants (Table 17). As in the case of earlier observations, no significant treatment effects were obtained for the above growth parameters in Karimunda. The total fresh and dry weights were maximum in 100 g Azo 34-F of group 2 plants. The shoot to root ratio of 7.1 was maximum in 25 g Azo 34-F of group 3 plants.

Treatments such as 100 g Azo 34-F of group 1, 25 g Azo 34-F and 100 g Azo 34+F of group 2 plants of Panniyur-1 and 25 g Azo 34+F of group 1 and 25 g Azo 34-F and 100 g Azo 34-F of group 2 plants of Karimunda varieties were found move beneficial to bush pepper. In most of these treatments, there were not only 100 percent establishment, but also the production of more number of roots, leaves and branches. Interestingly, in majority of these treatments, the application of chemical fertilizer did not have a

significant effect on various growth parameters. This might be due to the fact that the above observations were made at a comparatively early stage of plant growth and that the supply of additional nutrients become essential only at a later stage to support better vegetative growth and spike formation. This indicated that for the early phase of bush pepper growth, particularly during the first year, Azospirillum inoculation alone will be sufficient to meet the nitrogen requirement of this crop. The beneficial effects of Azospirillum inoculation on growth and yield has been well documented in many crop plants like wheat (Kapulnik et al 1985; Bashan, 1986), rice (Jeyaraman and Ramaiah (1986); Jeyaraman and Purushothaman, 1988; Mahapatra and Sharma, 1988; Purushothaman, 1988), maize (Fallik et al 1988; Fallik and Okon, 1989; Lee et al 1989; Zaady et al 1993), sorghum (Morgenstern and Okon, 1988), tomato (Hadas and Okon, 1987) and black pepper (Bopaiah and Khader, 1989). But in order to make a specific recommendation of Azospirillum inoculation, further studies on a large scale using different levels of Azospirillum inoculum will have to be conducted. It will also be desirable to develop a separate package of practices recommendation for bush pepper preferably based on the use of organic manures and biofertilizers. This will be of great advantage since at present, bush pepper is mainly cultivated as a homestead crop under pot culture conditions.

SUMMARY

Summary

The study on the effect of *Azospirillum* inoculation on root induction, establishment and growth of bush pepper was conducted at College of Agriculture, Vellayani, Trivandrum during 1992-94.

Twenty five different cultivars of pepper were used for the initial isolation of *Azospirillum* by enrichment culture technique using nitrogen free semi solid malate agar medium. Sixteen isolates of *Azospirillum* were obtained.

All the native isolates of *Azospirillum* and a culture from TNAU which was used as standard, were screened for the production of IAA and gibberellins under *in vitro* conditions. The isolate 34 produced maximum quantity of IAA equivalent to 69 µg/ml of culture broth while TN culture produced 46 µg/ml under identical growth conditions. The period of culture incubation also had an effect on the amount of IAA produced by different isolates. It's production was maximum during second week of culture growth. However none of these isolates were found capable of producing gibberellins.

Based on the requirement of biotin for growth *Azospirillum* isolate 34 was tentatively identified as *Azospirillum brasilense* and TN culture as *Azospirillum lipoferum*. The native isolate was capable of growing at pH

6.0 while the exotic isolate had maximum growth at pH 8.0. The isolate 34 and TN culture was selected for root induction studies in Panniyur-1 and Karimunda varieties of bush pepper. The percentage of rooted cuttings obtained varied from 23.3 - 26.8 percent in Panniyur-1 and 17.5-17.9 in Karimunda after treatment with a carrier based inoculum of Azospirillum and 1000 ppm IBA. However, in Panniyur-1 the average number of roots formed per cutting and the dry weight of roots were maximum in bacterial treatment. Between the two varieties of pepper root induction was better in Panniyur-1.

The study on establishment and growth of bush pepper was conducted by using 3 sets of rooted cuttings of Panniyur-1 and Karimunda varieties where root induction was done by using either carrier based inoculum of Azospirillum and 1000 ppm IBA or without any bacterial or hormone treatment. These were grown in potting mixture supplemented with 25 or 100 g Azospirillum culture and with or without chemical fertilizer application. The survival of pepper cuttings was found to be related to some extent with the rooting parameters studied after 180 days of plant growth. Thus in Panniyur-1 in treatments such as 100g Azo 34-F of group 1 plants, 25g Azo 34-F and 100g Azo 34+F of group 2 plants where 100 percent establishment was achieved, the number of roots produced per cutting,and the fresh and dry

weight of roots were either significantly high or on par with each other. Similarly, in Karimunda, in treatments such as 25g Azo 34-F and 100g Azo 34-F of group 1 plants,100g Azo 34-F of group 2 and 25g Azo 34-F of group 3 plants were 100 percent establishment was obtained, the number of roots produced per cutting and fresh and dry weight of roots were statistically on par with best treatment of 25g Azo 34+F of group 1 plants. In all these treatments where 100 percent establishment was achieved, there were also 100 percent germination of pepper cuttings.

The production of new leaves, branches and fresh and dry weight of shoot were significant only in Panniyur-1 variety of bush pepper. In this variety, the increase in number of leaves produced, length of branches, fresh and dry weight of branches and shoots were statistically significant in the 100g Azo 34-F of group 1 and 25g Azo 34-F of group 2 plants. In these treatments the number of leaves produced were 28.0 and 24.6 respectively. The length of branches were 56.4 cm and 50.0 cm, and the fresh and dry weight of branches were 41.80 and 18.31g and 37.76 and 17.50g respectively. The fresh and dry weight of shoots were 34.07, 15.49g and 21.10, 13.48g respectively. But in Karimunda, no such treatment effects were observed except for the fresh and dry weight of branches which were significant only in the 25g Azo 34 + F of group 1 plants.

The increase in total fresh and dry weight of plants were also significant in the 100g Azo 34 - F of group 1 and 25g Azo 34-F of group 2 plants of Panniyur-1 variety . However, the shoot to root ratio of 5.4 was maximum in 100g Azo 34+F of group 2 plants. As in the case of earlier observations, no significant treatment effects were obtained for these growth parameters in Karimunda. The total fresh and dry weight were maximum in 100g Azo 34-F of group 2 plants while the shoot to root ratio was maximum in the 25g Azo 34-F of group 3 plants. In most of the treatments where significant results were obtained, the use of chemical fertilizer had no significant effect on various growth parameters. Further, between the two varieties of pepper, the establishment and growth were better in Panniyur-1 variety of bush pepper.

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* Originals not seen.

APPENDICES

APPENDIX I

Analysis of Variance Table

Effect of *Azospirillum* inoculation on root induction in bush pepper variety - Panniyur - 1

Source	df.	Mean Square		
		Percentage of rooted cuttings	Number of roots per cutting	Dry weight of roots(g)
Total	79			
Treatments	7	972. 9999 **	19.2857 **	0.6436 **
Error	72	278.0388	4.8583	0.2047

** Significant at 0.01 level

APPENDIX 2

Analysis of Variance Table

Effect of Azospirillum inoculation on root induction in bush pepper variety - Karimunda

Source	df.	mean Square		
		Percentage of rooted cuttings	Number of roots per cutting	Dry weight of roots(g)
Total	79			
Treatments	7	694.0261 **	7.5714 *	0.4675
Error	72	231.0785	2.9444	0.1612

* Significant at 0.05 level

** Significant at 0.01 level

APPENDIX 3

Analysis of Variance Table

Effect of Azospirillum inoculation on germination and growth of bush pepper variety - Panniyur-1

Source	df	Mean Square			
		Percentage of germinated cuttings	Fresh weight of shoot (g)	Fresh weight of root(g)	shoot to root ratio
Total	79				
Treatments	7	157.0988	10.9807	0.7036**	14.916**
Error	72	253.235	3.1693	0.2237	3.4005

** Significant at 0.01 level

APPENDIX 4

Analysis of Variance Table

Effect of Azospirillum inoculation on germination and growth of bush pepper variety - Karimunda

Source	df	Mean Square			
		Percentage of germinated cuttings	Fresh weight of shoot (g)	Fresh weight of root(g)	shoot to root ratio
Total	79				
Treatments	7	179.8916	3.7129	0.6067**	3.6890**
Error	72	209.7111	3.1261	0.1936	1.2273

** Significant at 0.01 level

APPENDIX 5

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on root growth in bush pepper variety - Panniyur-1

Source	df.	Mean Square		
		Number of roots	Fresh Weight of roots (g)	Dry weight of roots(g)
Total	59			
Treatments	11	65.7575	106.9613 *	12.1763 *
Error	48	37.3666	49.6955	5.9629

* Significant at 0.05 level

APPENDIX 6

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on root growth in bush pepper variety- Karimunda

Source	df.	Mean Square		
		Number of roots	Fresh weight of roots(g)	Dry weight of roots(g)
Total	59			
Treatments	11	47.7500	70.1855	8.4290
Error	48	43.4666	70.2719	8.4483

APPENDIX 7

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on germination and shoot growth in bush Pepper variety - Panniyur-1

Source	df.	Mean Square		
		Number of leaves	Fresh weight of shoot(g)	Dry weight of shoot(g)
Total	59			
Treatments	11	282.0122 *	249.2321	50.6254
Error	48	130.075	141.0029	28.8613

* Significant at 0.05 level

APPENDIX 8

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on germination and shoot growth in bush pepper variety - Karimunda

Source	df	Mean Square		
		Number of leaves	Fresh weight of shoot(g)	Dry weight of shoot(g)
Total	59			
Treatment	11	88.9620	32.6514	7.9373
Error	48	103.3833	79.4904	15.7724

APPENDIX 9

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on branching in bush pepper variety - Panniyur-1

Source	df	Mean Square		
		Length of branch (cm)	Fresh weight of branch (g)	Dry weight ofbranch(g)
Total	59			
Treatments	11	1049.7990	570.2719	113.5786
Error	48	557.9416	296.3118	57.6130

APPENDIX 10

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on branching in bush pepper variety - Karimunda

Source	df	Mean Square		
		Length of branch (cm)	Fresh weight of branch (g)	Dry weight ofbranch(g)
Total	59			
Treatments	11	428.5622	375.8672	83.4644
Error	48	447.6417	369.7706	75.5072

APPENDIX 11

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on establishment and growth of bush pepper variety - Panniyur-1

Source	df	Mean Square		
		Shoot to root ratio	Total fresh weight(g)	Total dry weight (g)
Total	59			
Treatments	11	4.8509	2371.6020 *	425.4230
Error	48	4.7012	1097.8870	198.7977

* Significat at 0.05 level

APPENDIX 12

Analysis of Variance Table

Effect of Azospirillum and fertilizer application on establishment and growth of bush pepper variety - Karimunda

Source	df	Mean Square		
		Shoot to root ratio	Total fresh weight(g)	Total Dry weight (g)
Total	59			
Treatments	11	6.6306	976.9304	187.3818
Error	48	5.8193	1165.2660	210.2928

ABSTRACT

EFFECT OF *AZOSPIRILLUM* INOCULATION ON ESTABLISHMENT AND GROWTH OF BUSH PEPPER

BY

YAMINI VARMA C. K.

ABSTRACT OF THESIS SUBMITTED
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VELLAYANI, TRIVANDRUM

1995

Abstract

The study on the effect of *Azospirillum* inoculation on root induction, establishment and growth of bush pepper was conducted at College of Agriculture, Vellayani, Trivandrum during 1992-94.

Out of the 25 different cultivars of pepper used for initial isolation, *Azospirillum* was isolated from 16 cultivars. These 16 isolates along with a culture from TNAU were screened for the production of IAA and gibberellins under *in vitro* conditions. The native isolate 34 produced maximum quantity of IAA equivalent to 69 µg/ml of culture broth. The production of this phytohormone was maximum during the second week of culture growth. However, none of these isolates produced gibberellins under *in vitro* conditions.

Based on the requirement of biotin for growth isolate 34 and TN culture were tentatively identified as *Azospirillum brasilense* and *A. lipoferum* respectively. The isolate 34 and TN culture had their maximum growth at pH 6.0 and 8.0 respectively.

Azospirillum isolate 34 and TN culture were selected for root induction studies in Panniyur-1 and Karimunda varieties of bush pepper. The percentage of rooted cuttings varied from 23.3-26.8 percent in Panniyur-1 and

17.5-17.9 in Karimunda. However, the average number of roots per cuttings and root dry weight were maximum in bacterial treatment. Between the two varieties of bush pepper, root induction was better in Panniyur-1 variety.

The study on the establishment and growth of bush pepper was conducted by using 3 sets of rooted cuttings of Panniyur-1 and Karimunda varieties where root induction was done by using carrier a based inoculum of Azospirillum and 1000 ppm IBA or without any of the above treatments. These were grown in potting mixture supplemented with 25 g or 100g of isolate 34 and with or without chemical fertilizer application.

After 180 days of plant growth, 100 percent establishment was obtained in Panniyur-1 in treatments such as 100 g Azo 34-F of group 1, 25 g Azo 34-F and 100 g Azo 34+F of group 2 plants. In these treatments the number of roots produced per cutting, fresh and dry weight of roots were significantly high or on par with each other. In Karimunda, also, in treatments such as 25 g Azo 34-F and 100 g Azo 34-F of group 1, 100 g Azo 34-F of group 2 and 25 g Azo 34-F of group 3 plants where 100 percent establishment was obtained, the number of roots produced per cuttings, fresh and dry weights of roots were statistically on par with best treatment for these parameters. The production of new leaves, branches and fresh and dry weight of shoot were

significantly high only in Panniyur-1 and these were in the treatments 100 g Azo 34-F of group 1 and 25 g Azo 34-F of group 2 plants. But in Karimunda, no such treatment effects were noticed except for fresh and dry weight of branches. In Panniyur-1 the total fresh and dry weight of plants were significant also in 100 g Azo 34-F of group 1 and 25 g Azo 34-F of group 2 plants.

In most of the treatments where significant results were obtained, chemical fertilizer application had no significant effect on different growth parameters studied. Further, between the two varieties, Panniyur-1 responded better than Karimunda in rooting, establishment and growth of bush pepper.