STANDARDIZATION OF TECHNIQUES FOR BETTER ROOTING AND GROWTH OF ORTHOTROPIC SHOOTS IN BLACK PEPPER (*Piper nigrum* L.)

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by

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DECLARATION

I, hereby declare that this thesis entitled **"Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)"** is a record of research work done independently by Ms. Nimisha Mathews under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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NIMISHA MATHEWS

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%	Per Cent
@	At the rate of
^{0}C	Degree Celsius
CD (0.05)	Critical Difference at 5% level
cfu	Colony Forming Unit
cm	Centimeter
cm^3	Cubic centimeter
cm^2	Square centimeter
cms^{-1}	Centmeter per Second
cv.	Cultivar
DMSO	Dimethyl Sulfoxide
et al.	And co-workers/ co-authors
FAO	Food and Agricultural Organisation
Fig.	Figure
F.wt	Fresh weight
g	Gram
g $plant^{-1}$	Gram per plant
GA	Gibberellic acid
IBA	Indole-3-butyric acid
IISR	Indian Institute of Spices Research
IPC	International Pepper Community
KAU	Kerala Agricultural University
kg	Kilogram
MAP	Months After Planting
mg g^{-1}	Milligram per gram
ml	Milliliter
nm	nanometer
No. of	Number of
NS	Non Significant

pH	Negative logarithum of hydrogen ion concentration
ppm	Parts Per Million
PGPR	Plant Growth Promoting Rhizhobacteria
sp.	Species
viz	namely
µm	Micrometer

INTRODUCTION

1. INTRODUCTION

Black pepper (*Piper nigrum* L.), known as the 'King of Spices' belonging to the family Piperaceae, is one of the most important export oriented spice crops in the world. It is indigenous to the tropical evergreen forests of the Western Ghats; and the Malabar Coast of South India was involved in the cultivation and trade of black pepper since time immemorial. In the course of time, pepper plants were carried to Indonesia, Malaysia and then to other countries by traders and travellers.

Pepper alone contributes about 70 per cent of total export earnings from all spices. Popularly it is known as "Black gold" because of its unique position in the international trade. India is one of the major producer, consumer and exporter of black pepper in the world. During 2012–13, 15,363 tonnes of black pepper products worth Rs. 63,810 lakhs were exported to various countries. Black pepper is cultivated to a large extent in Kerala, Karnataka and Tamil Nadu and to a limited extent in Maharashtra, North eastern states and Andaman and Nicobar Islands. Kerala and Karnataka account for a major share of production of black pepper in the country. In India, the crop is grown in about 2, 01,381 hectares with a production of 55,000 tonnes annually (IISR, 2014).

However, in India, the productivity of black pepper is low owing to several constraints associated with its production. Among them, non-availability of vigorous disease free productive planting material (rooted cuttings) is of prime importance (Sarma, 2007). The successful establishment and better production of black pepper depends much on the quality of planting material used. This is also a strategy to check diseases affecting black pepper, which is another major production constraint.

The commercial method of propagation of pepper is by using runner shoots which develop at the base of the vine. The vines planted with runner shoots in general start flowering from third year onwards or later. In such bushes, the fruiting lateral production invariably starts at a height of two to three feet from the base of the bush. But the vines from the terminal orthotropic shoots are found to

give better growth and large number of fruit bearing lateral branches, starting right from the base of the vine which ensures full coverage of the standard with fruiting branches without leaving any gaps and enable the plant to be more productive. This habit is missing in vines raised from runner shoots. Further, the vines start flowering earlier than plants produced by other types of planting material (Sarma *et al*., 2013).

The potential of orthotropic shoots as planting material has been identified and this practice is widely adopted in majority of the pepper growing countries. However, this practice is not popular in India due to the non-availability of shoots because most plantations are on living standards and the pepper vines grow unrestricted (Sadanandan, 2000). This practice is being followed successfully in Vietnam, Indonesia and Malaysia, which are the leading pepper producing countries. Thus it has been proven beyond doubt that terminal orthotropic shoots can be used for planting after rooting in nurseries, for getting higher yield. The rooting percentage varies from 70 to 80. Once rooted, the orthotropic shoots can be further multiplied, which can be effectively used in propagating black pepper. Further, the orthotropic shoots will be totally free from soil contamination compared to runner shoots which are often contaminated with soil that might lead to *Phytophthora* infection (Sarma *et al*., 2013). This aspect has also been identified as one of the priority areas for research in black pepper. Hence the present study, "Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)" was undertaken with the following objectives:

- To standardize techniques for profuse rooting and vigorous growth of orthotropic shoots of black pepper so as to produce quality planting material.
- To produce healthy and disease free planting material which may boost up productivity of black pepper.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Black pepper (*Piper nigrum* L.) is vegetatively propagated in order to retain and fix the yield potential of the particular variety planted. In Vietnam, Indonesia and Malaysia for propagating black pepper, cuttings are taken from orthotropic (upright growing) or terminal shoots. The criteria for selecting a parent plant for taking cuttings are as follows:

- The plant must be a recommended cultivar.
- It must be healthy and vigorously growing.
- It must be free from pests, diseases and nutritional disorders.
- The plant must not be older than two years.

Before taking the cuttings from mother plants, the top 4 to 6 nodes are pruned off to accelerate growth of axillary buds. After 10-14 days, the shoot is cut off and carefully removed from the support with the help of the blunt edge of a pruning knife. Although cuttings can be planted directly during the rainy season, they are often rooted in a nursery for 4- 8 weeks for better results (IPC and FAO, 2005).

In India, farmers used to plant cuttings from runner shoots directly at the base of the live standards during June – July coinciding with South West monsoon period. But now- a-days they have switched over to rooted poly bag cuttings, which is a common practice (Sarma *et al*., 2013).

The IPC Committee report (2007) says that quality planting material (cuttings) should be sourced from healthy plants from pest and disease-free areas preferably from vines that are less than 3 years old. Terminal orthotropic shoots are preferred for planting after rooting in nurseries. Cuttings from terminal shoots bear fruits earlier and are likely to be more vigourous. 3-5 node orthotropic shoots are preferred. Cuttings may be soaked in suitable rooting agents to enhance rooting. Application of growth regulators such as, IBA, common sugar solution and bio inoculants such as Arbuscular Mycorrhizal Fungi (AMF), *Azospirillum* sp., *Psuedomonas fluorescence* etc., has been found to enhance root proliferation.

Though there are several reports about the techniques for better rooting of cuttings from runner shoots of black pepper only a few are found with orthotropic

shoots of black pepper. Hence literature on various rooting techniques using different growth regulators, bio inoculants and their effect on growth, anatomical and physiological characters of rooted orthotropic shoot cuttings of black pepper (*P. nigrum* L.) and also the techniques of disease control are specifically reviewed in this chapter. Where ever information is lacking pertinent literature on the other crops have been included.

2.1 SOIL SOLARIZATION

Soil solarization is a safe method of pest control as it is environment friendly and it depends on renewable source of energy (Katan, 1981; Yaduraj and Karma, 1997). It is a technique used by covering soil with clear polyethylene sheets, during summer months, to trap the solar radiation to heat soil (Horouwitz *et al*., 1983; Abdallah, 1991). The improvement of plant growth, using solarized soil technique, was reported by Abdallah (2000) and soil solarization also provides excellent control of soil-borne pathogens with resultant increase in growth, yield, and quality of pepper and other crop plants (Kurt and Emir, 2004; Cimen *et al*., 2010).

Solarization mediated favourable effects were observed in lime (Stapleton and Devay, 1986), onion (Adetunji, 1994), bhindi (Bawazir *et a1*., 1995), coriander (Herrera and Ramirez, 1996), chillies (Haripriya and Manivannan, 2000) and black pepper (Sainamole *et al*., 2003). Studies conducted by Thankamani *et al*. (2008) indicated the superiority of solarized potting mixture for reducing incidence of diseases besides yielding vigorous planting material in black pepper. Plants raised in solarized potting mixture had better growth than plants raised in non-solarized potting mixture (soil, sand, and farm yard manure 2:1:1proportion). The plants grown in solarized potting mixture had shown response and produced more growth namely number of leaves, root length, leaf area and bio mass. In their study temperature under polythene sheet rose up to 55^0C compared to 30^0C in the non-solarized potting mixture. The high temperature along with high moisture content might have killed the soil borne mesophilic pathogens. Majority of the pathogens including *Phytophthora capsici*

and nematode falls in the category of mesophilic microorganisms that require temperatures below 35^0C for survival in soil. This was supported by the findings of Hrender Raj *et al*. (1997) in vegetables.

Solarization has been found effective in controlling nematodes in ginger (Vilasini, 1996).

In addition to control of pathogens and pests, soil solarisation helps in increased mineralization of nutrients in soil and shifts the soil micro biota in favour of growth promoting types (Stapleton *et al*., 1985).

2.2 BIOCONTROL AGENTS

The microbial inoculants have proved to be an effective alternative to the extensive use of chemicals. Microbial antagonists are increasingly being used for the management of plant diseases including those caused by soil borne plant pathogens (Cook and Baker, 1983; Chet, 1987; Lewis and Papavizas, 1991). Soil bacteria, having antagonistic property against soil borne plant pathogens are usually used for seed bacterization and seedling dip (Harman, 1991; Mao *et al*., 1997 and Anith *et al*., 1998).They are compatible with bio fertilizers. Anith and Manomohandas (2001) reported that bacterial bio control agents can also be used for vegetatively propagated crops like black pepper and could be combined with other fungal antagonists. It was pointed out that the application of the bio control agents was found to have profound effect on the growth parameters of black pepper cuttings. Their use as bio control agents is advantageous not only from the economical, but also from the ecological point of view.

The different bio control agents used in the study are:

1. *Trichoderma* spp.
2. PGPR Mix-II

2.2.1 *Trichoderma*

Trichoderma spp. is a group of broad-spectrum antagonistic fungi. *Trichoderma harzianum* is reported to have antagonistic activity against *P. capsici* infecting pepper. *T. harzianum* is used as a successful bio control agent against *P. capsici* attacking black pepper (Sarma *et al*., 1994; Anandaraj and Sarma, 1995

and IISR, 1999). Anith and Manomohandas (2001) reported that *T. harzianum* and *Ailcaligenes* sp. strain AMB 8 applied alone or in combination significantly reduced the incidence of *P. capsici* induced nursery rot disease of black pepper. The mode of action of *T. viride* is competition, antibiosis, hyper parasitism, hyphal coiling, hyphal penetration, production of lytic enzymes, induced resistence, plant growth promotion etc. The competition for carbon and nitrogen by *T. harzianum* suppressed the infection of *Fusarium oxysporum* for sp. *melonis* (Sivan and Chet, 1989) on several crops through competition.

Trichoderma spp., though produce antibiotics and cell wall degrading enzymes, mainly act as mycoparasites on other fungi and bring about the disease control (Lewis and Papavizas,1991). Antibiotics like trichodermin, dermadin, trichoviridin, viridian etc. are produced by *Trichoderma*. Mycoparasitism relies on the production of mycoparasite for the lysis of cell walls.

Trichoderma induces plant growth like increased germination, early emergence, fresh and dry weight of roots, shoots, root length, yield and flowering. The growth promoting ability of antagonistic bacteria and *Trichoderma* spp .have been reported in many crops (Kloepper and Schroth, 1981a; Windham *et al*., 1986; Dileepkumar and Dube, 1992).

Raising the cuttings in solarized mixture fortified with *T. harzianum* and VAM is reported to produce robust disease free rooted black pepper cuttings (Sarma 2000; Anandaraj *et al*., 2001).

2.2.2 PGPR Mix-II

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and promote growth when added to seeds, roots or tubers have been termed plant-growth promoting rhizobacteria and increase plant growth and yield (Wu *et al*., 2005). Plant growth-promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Rodríguez *et al*., 2006) and have better ability to multiply and persist in varying soil conditions.

Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Vikram *et al*.,

2007), cytokinins (Arkhipova *et al*., 2005) and gibberellins (Joo *et al*., 2004) produced by rhizobacteria can influence plants growth, including root development which improve uptake of essential nutrients thus increasing plant growth (Vikram *et al*., 2007). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide, siderophores (Suresh *et al*., 2010), siderophores, protease, antimicrobials, phosphate solubilizing enzymes (Chaiharn *et al*., 2008). These may vary with the edaphic conditions (Abbas *et al*., 2009).

PGPR Mix-II is a consortium of highly compatible rhizobacteria having broad spectrum of inhibitory property with different mechanisms. PGPR Mix-II is effective against all fungal and bacterial plant pathogens of crop plants (KAU, 2011).

The consortium approach for disease management in plantation crops was suggested earlier (Sarma and Anandaraj, 1998).Mutual compatibility of fungal and bacterial antagonists' viz. *T. harzianum* and fluorescent Pseudomonads were studied in order to establish an efficient consortium for the management of foot rot of black pepper caused by *P. capsici*. The study revealed that the fungal and bacterial antagonists are compatible (Jisha *et al*., 2002).

2.3 DIFFERENT TREATMENTS

Apart from the propagating media, the beneficial effect of growth regulators for enhancing root and shoot growth of black pepper is also known (Kandiannan *et al*., 1994).

2.3.1 IBA

It is well known that exogenously applied natural or synthetic auxins favour rooting, and there is evidence that this hormone is the most effective inducer of the process (Lyndon, 1990). A number of studies have demonstrated that exogenous application of auxin accelerates the rate of rooting, increases final rooting percentage and increases the number of roots of leafy cuttings (Leakey *et al*., 1982; Leakey, 1990).

Auxin enters cuttings mostly via the cut surface (Kenney *et al*., 1969) and is rapidly taken up in cells by pH trapping (Rubery and Sheldrake, 1973) and by influx carriers (Delbarre *et al*., 1996).

However, relatively high concentrations of auxins have been reported to be inhibitory to rooting, indicating that in many species, optimal concentrations for rooting may be defined (Leakey *et al*., 1982). For general use in rooting stem cuttings of the majority of species, IBA and / or IAA are recommended (Davis and Haissing, 1990).IBA is widely used as a root-initiation promoter in agriculture (Waisel, 1991).

Dipping of nodal cuttings in 1000 ppm IBA for 45 seconds gave better rooting of black pepper in poly bags (Pillai *et al*., 1982 and Shridhar and Singh, 1990).

2.3.2 Common Sugar Solution

While treating roots of young trees with a sugar solution enhances root growth upon transplantation (Percival, 2004), no study has looked at the effects of sucrose on adventitious rooting.

Sucrose is the major product of photosynthesis and the major transport carbohydrate in plants (Koch, 2004). It has been recognized as contributing to various regulatory mechanisms in plants including growth and development, differential gene expression and stress-related responses (Wind *et al*., 2010).

Singh (1980) noticed that a high sucrose to auxin ratio led to phloem production and low sucrose to auxin ratio led to xylem which is necessary for vascularization of root primordia.

Suparman and Zaubin (1988) observed that undefoliated cuttings of black pepper cv. (Belantung) treated with 2 per cent sucrose was reported to give high rooting percentage (80 per cent).

2.3.3 AMF

Arbuscular mycorrhizal fungi (AMF) are soil fungi colonizing most of the plant roots and forming an association called endomycorrhiza. AMF commonly form beneficial associations with roots of many horticultural crops (Smith and Read, 1997).

The AM fungi inoculated to crop plants colonize the plant root system and increase the growth and yield of crop plants including pepper (Rao, 1993; Thanuja, 2002 and Durgapal *et al*., 2002). The AMF associated improvement of plant growth is attributed to various mechanisms such as increased uptake of nutrients and water, production of plant growth promoting substances, tolerance to drought and salinity and resistance to plant pathogens (Dalpe and Monreal, 2004). It seeks out nutrients particularly P from far greater soil area than the plant can access by itself. It increases the surface area of root biomass. It aids in penetrarion of small hyphae into sites too small for plant root to reach. It also increases the uptake of N, K, S, Cu and Zn. It aids in better survival during drought. It protects against heavy metals and increases the accumulation of hormones like GA and Cytokininin plants. As the fungus acts as an extension of the root, it increases the overall absorption capacity of roots due to morphological and physiological changes in the plant. AMF helps in better development of P solubilizing bacteria in the mycorhizoshere (Sivaprasad and Meena Kumari, 2005).

2.3.4 *Azospirillum*

Azospirillum is a plant growth promoter bacterium (PGPBs), and it is not known as typical biocontrol agent. Chitinase production by some *Azospirillum* strains could have a promising future for application of *Azospirillum* as biological fungicides and biological insecticides. The ability of *Azospirillum* to attain significant populations on the host root system has been shown to be a prerequisite for their beneficial effects on plant growth (Bashan, 1986). Govindan and Chandy (1985) studied the utilisation of the diazotroph *Azospirillum* for inducing rooting in pepper cuttings. Inoculation of *Azospirillum* increased the number of roots per cutting (7.4 per 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. Even though 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. The promotion of plant growth by

Azospirillum has been reported in field and nursery plants, resulting in significant changes in several characteristics of plants. The *Azospirillum* inoculation responses in non-leguminous plants are still difficult to estimate (Bashan *et a1*.1995).

2.3.5 *Pseudomonas*

They are known as plant growth promoting rhizobacteria (PGPR). Fluorescent pseudomonads are some of the effective candidates for biological control of soil borne plant pathogens owing to their rhizosphere competence (Kloepper *et al*.,1980; Kloepper and Schroth 1981b).These bacteria are termed as Plant Growth Promoting Rhizobacteria (PGPRs) because of their ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators such as gibberellins, cytokinins and indole acetic acid (Suslow and Schroth 1982). They are used as biological control agents for the suppression of soil – borne diseases by competing with pathogens for resources such as nutrients, producing antibiotics or activating host defence mechanisms. They often bring about the growth effects synergistically. Dubeikovsky *et al.* (1993) reported that fluorescent pseudomonads produce plant growth regulators and even suppress deleterious soil-borne pathogens. In case of spices several *Pseudomonas* isolates have been obtained from black pepper and ginger and screened for their ability to suppress pathogens and some were listed for their antagonistic activity on both oomycetes pathogens and nematodes. These isolates were found to enhance growth of host plants (Anandaraj and Sarma, 2003). *Pseudomonas* produces antimicrobial compounds like bacteriocins, pyrrolnitrin, pyoluteorin etc.

2.4 EFFECT OF TREATMENTS ON GROWTH CHARACTERS

2.4.1 Number of Days for Sprouting

Hormonal treatment (IBA) significantly increased sprouting in *Jatropha curcas* cuttings and sprouting in cuttings was initiated earlier (Kumari *et al*, 2010).

Kiwi fruit leafless cutting treated with IBA significantly took minimum days to sprouting (Srivastava *et al*., 2008). Similar trend was noticed by Thota (2012) in Fig (*Ficus caria*) cuttings.

2.4.2 Height of Sprouted Cutting (cm)

Ingle and Venugopal (2008) reported that, in stevia (*Stevia rebaudiana*), the maximum length (37.82 cm) of the shoot was recorded in IBA 500 ppm.

The increase in the length of the shoot with the increase in concentration of IBA was noted by Shivanna *et al*. (2006) in Jeevanthi (*Leptadenia reticulate*). In *Premna integrifolia* hard wood cuttings, maximum shoot length (33.60) was noticed in the cuttings treated with IBA 1000 ppm followed by the cuttings treated with IBA 500 ppm (Sharma, 2009).

Nath and Korla (2000) opined that biofertilizers have pronounced positive effect on plant height in ginger.

According to Kandiannan *et al*. (2000) the vine length was significantly higher in black pepper inoculated with selected species and strains of AMF and N fixing bacteria.

Ramesh *et al*, (1998) reported that biofertilizers like *Azospirillum* and Azotobacter sp. increased plant height in cashew.

Raji and Lekha (2003) observed that seed bacterization with talc based formulation of *P. fluorescence* enhanced height of rice plants.

According to Ibiene *et al*. (2012), the combination of PGPR including *Azotobacter* sp., *Nitrobacter* sp. and *Nitrosomonas* sp. had significantly increased plant height of tomato.

The association of endophytic bacterium with the plant growth hormone IBA improved plant growth in terms of increases in plant length in *Naravelia zeylanica* L. (Benson *et al*., 2014)

2.4.3 Number of Leaves

The maximum number of leaves (37.68) per cutting was recorded with IBA 500 ppm in stevia (Ingle and Venugopal, 2008). The result reported by Chalapathi *et al*, (2001) in stevia followed the similar trend.

IBA 1000 ppm resulted in maximum number of leaves in semi hardwood (4.94) and softwood cuttings (4.80) of *Camellia japonica* (Wazir, 2014) and *P. integrifolia* hard wood cuttings (Sharma, 2009).

Nath and Korla (2000) found that biofertilizers have pronounced positive effect on leaves per plant in ginger.

Varma (1995) recorded significantly higher production of new leaves in bush pepper inoculated with *Azospirillum*.

Kandiannan *et al*. (2000) reported that the number of leaves were significantly higher in plants inoculated with selected species and strains of AMF and N fixing bacteria.

2.4.4 Leaf Size

Sangeeth *et al*. (2008) reported that the *Azospirillum* isolate significantly increased leaf length in black pepper.

Highest leaf length and breadth was obtained from 1.0 mg/l each of BAP + IBA, in the in vitro growth and development of Dendrobium hybrid orchid (Khatun *et al*., 2010).

In Japanese plum cuttings, Jawada (1990) found that IBA gave maximum leaf area compared to control.

Seed inoculation of *A. brasillience* in wheat plants increased the leaf area (Panwar and Singh, 2000). Similar trend has been reported by Mahentesh *et al*. (2002) in chilli.

2.4.6 Internodal Length (cm)

In black pepper, application of *Azospirillum* isolate showed significant increase in internodal length (Sangeeth *et al*., 2008).

Ibiene *et al*. (2012) reported that application of PGPR including *Azotobacter* sp., *Nitrobacter* sp., and *Nitrosomonas* sp. had significantly increased internodal length in tomato.

2.4.9 Number of Branches

Chalapathi *et al*. (2001) reported that stevia cuttings treated with IBA 500 ppm was found to give more number of branches.

In bougainvillea cuttings, Mehraj *et al*. (2013) noticed that treatment with 1000 ppm IBA gave maximum number of branches per cutting (4.7).

According to Gupta *et al*. (2006), AMF inoculation in periwinkle enhanced the growth and number of branches.

Varma (1995) recorded significantly higher production of new branches in bush pepper inoculated with *Azospirillum*.

2.4.11 Number of Roots

Ingle and Venugopal (2008) reported that the number of roots was found to be more (49.96) in the stevia cuttings treated with IBA 500 ppm followed by IBA 400 ppm (38.20). According to Murthy *et al*. (2010), vanilla cuttings treated with IBA 500 ppm recorded the maximum number of roots per cuttings, whereas it was minimum in control.

Dipping of nodal cuttings in IBA at 1000 ppm for 45 seconds gave better rooting in black pepper (Pillai *et al*., 1982).

Increased rooting was associated with higher sucrose: starch ratios in cuttings, reflecting that an increased assimilates export are needed for rooting (Druege *et al*., 2000). Suparman and Zaubin (1988) observed that undefoliated cuttings of black pepper cv. (Belantung) treated with 2 per cent sucrose was reported to give high rooting percentage (80 per cent).

Thanuja *et al*. (2002) got a higher rooting percentage in orthotropic cuttings of black pepper on inoculation with AMF.

Govindan and Chandy (1985) studied the utilisation of the diazotroph *Azospirillum* for inducing rooting in pepper cuttings and they reported that the inoculation of *Azospirillum* increased the number of roots per cutting (7.4 per cutting) as compared to zero values for the control treatment.

The effect of inoculation of fluorescent *Pseudomonas* to enhance growth and root development of black pepper and to achieve significant reduction in foot rot disease incidence and mortality of virus has been reported by Sivaprasad *et al*. (2003). Increased feeder root production and absorptive surface area in black pepper plants due to *P. fluorescens* has been reported by Anandaraj and Sarma

(2003). Paul and Sarma (2006) observed that *P. fluorescens* strains enhanced root proliferation and fibre root production in black pepper.

All India Co-ordinated Research Project on Spices at Dapoli centre standardised a technology for rooting of orthotropic shoots in black pepper by treating 2 node orthotropic cuttings without leaves either in *P. fluorescens* 10^8 powder formulation or common sugar solution (2 per cent) for 1 minute (AICRPS, 2011).

Sumarsih and Haryanto (2012) observed that PGPR treatment on jatropa seedlings that comes from the stem cuttings can increase primary and secondary root quantity.

2.4.12 Length of the Longest Root (cm)

In *Massdevia tenacissima,* stem cuttings treated with IBA 1000 ppm recorded maximum length of the longest root over control (Pandey *et al*., 2002). Similar trend was observed by Karakurt *et al*.(2009) in hardwood stem cuttings of MM 106 semi –dwarf apple rootstock ; Murthy *et al*. (2010) in Vanilla and Mehraj *et al*.(2013) in bougainvillea cuttings.

Dewan and Subha Rao (1979) studied the effect of *Azospirillum* inoculation on rice seedling and they found that the seed inoculation enhanced length of roots at all stages of plant growth.

Raji and Lekha (2003) reported that seed bacterization with talc based formulation of *P. fluorescence* enhanced the root length of rice plants.

Application of PGPR including *Azotobacter* sp., *Nitrobacter* sp. and *Nitrosomonas* sp. had significantly increased root length in *Lycopersicum esculentus* (Ibiene *et al*., 2012). Similar trend was observed by Sumarsih and Haryanto (2012) in jatropa stem cuttings.

2.4.13 Volume of Root

The seed inoculation of *Azospirillum* increased volume of roots at all stages of plant growth in rice (Dewan and Subha Rao, 1979).

2.4.14 Percentage of Success in Establishment

Chalapathi *et al*. (2001) reported that stevia cuttings treated with IBA 500 ppm was found to be superior with respect to survival percentage and sprouting

percentage. Singh (2012) reported that hardwood cuttings of Louise Wathen variety of Bougainvillea treated with 1000 ppm IBA were found to be significantly superior with respect to establishment (190 per cent).

Plant growth promoting rhizobacteria such as fluorescent pseudomonads are known to contribute significantly towards plant establishment, growth and disease tolerance in crop plants (Suslow and Schroth, 1982).According to Baud and Pezeshki (2013) soaking cuttings of *Acer negundo* and *Salix nigra* in a dilute sucrose solution (2 per cent to 4 per cent) prior to planting enhances survival and root growth, with soaking in a 2 per cent sucrose solution giving the stronger survival response.

2.5 EFFECT OF TREATMENTS ON ANATOMICAL CHARACTERS

Application of *Azospirillum*, AMF and *Pseudomonas* recorded highest leaf cuticle thickness, number of vascular bundles in rhizome and root, stomatal frequency in Kasthuri turmeric (Nirmalatha, 2009).

2.6 EFFECT OF TREATMENTS ON PHYSIOLOGICAL CHARACTERS

2.6.1 Total Dry Matter Production

In *N. zeylanica* L., the association of endophytic bacterium with the plant growth hormone IBA improved plant growth in terms of plant dry weight (Benson *et al.*, 2014).

Venkateswarlu and Rao (1983) reported that inoculation of pearl millet with *A. brasilense* increased growth and dry matter production. Bio fertilizer mediated total dry matter production was also observed in chilli (Mahentesh *et al*., 2002) and sorghum (Patidar and Mali, 2004).

Saju *et al*. (2003) observed that *P. fluorescens* and *T. harzianum* are compatible, synergistic and enhance biomass production and disease suppression when applied to soil in black pepper.

2.6.2 Stomatal Conductance

Ponmurugan and Baby (2001) reported that stomatal conductance was highly influenced by *Pseudomonas* application in cocoa.

The stomatal conductance was increased by *Glomus fasciculatum* in unimproved genotype of maize (Aguilera – Gomez *et al*., 1998).

It was reported by Augé (2001) that the mycorrhizal symbiosis usually promotes transpiration and stomatal conductance. However, in some plants AMF colonization resulted in no effects (Syvertsen and Graham 1990) or even decrease in these parameter values (Mathur and Vyas 1995).

2.6.3 Chlorophyll Content (Chlorophyll a, b and Total Chlorophyll)

Kaur *et al*. (2002) have shown that chlorophyll a, chlorophyll b and total chlorophyll contents in leaves of grape vine stem cuttings is enhanced after IBA treatment.

Patidar and Mali (2004) observed that, use of bio fertilizers gave increased chlorophyll content in sorghum. Ramakrishan and Selvakumar (2012) observed that, *Azospirillum* treated tomato had the highest chlorophyll content. Similar results were observed in sorghum (Patidar and Mali, 2004); chilli (Selvakumar and Thamizhiniyan, 2011) and in black gram (Selvakumar *et al*., 2012).

Robinson *et al*. (2004) reported that application of *P. syringe* enhanced production of chlorophyll in sunflower.

Some researchers also reported the improved photosynthetic rate by AMF inoculation (Borkowska, 2002; Fan *et al*., 2008 and Sheng *et al*., 2008).

In a study, Jin Wu *et al*. (2011) found that higher chlorophyll contents in mycorrhizal plants, indicating that AMF indeed may stimulate photosynthesis.

Benson *et al*. (2014) reported that the association of endophytic bacterium with the plant growth hormone IBA improved chlorophyll content in *N. zeylanica L*.

Tanwar *et al*., 2013 demonstrated that single inoculation with *T. viride* increased photosynthetic rate by increasing plant chlorophyll content, both a and b in broccoli.

2.7 EFFECT OF TREATMENTS ON BIOLOGICAL PROPERTIES OF POTTING MIXTURE BEFORE AND AFTER THE EXPERIMENT

Nirmalatha (2009) observed that the total microbial load in the rhizosphere increased with application of bio inoculants. When *Trichoderma* was applied as soil amendment the cfu increased by 60 days in treated plots. Fungal population reached a maximum of 62 x 10^4 cfu g^{-1} of soil within 45 days (Prasad *et al*., 2000). Chitin added to sand soil based cultivation substrates stimulated the root colonization, growth of extra radial mycelium and production of spores of AMF fungi was observed in host plants like *Plantago lanceolata*, *Allium ampelloprasum* and *Lactuca sativa*. Stimulation of AMF sporulation was observed when autoclaved mycelium of *F. oxysporum* was used instead of chitin. Increased number of actinomycetes in the substrates as a result of chitin treatment was recorded (Gryndler *et al*., 2003). In *Pisum sativum*, *Pseudomonas* application reduced the pH. The decrease in pH increased the fungal and yeast colony. The population of introduced and total *Pseudomonas* is increased (Naseby and Lynch, 2005).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study on "Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during 2012-2014.

The details of the experimental materials used and methodology adopted for conducting the study are presented in this chapter.

3.1 LOCATION

The experiment was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram of Kerala Agricultural University. The area is situated at 8^{o} 30' North latitude and 76^{o} 54' East longitude at an altitude of 29 m above MSL.

3.2 MATERIALS

3.2.1 Planting Material and Variety

The planting material used was 2 and 3 node orthotropic cuttings of Panniyur- 1 variety of black pepper, collected from healthy, disease and pest free vines.

3.2.2 Source of Planting Material

Instructional Farm, College of Agriculture, Vellayani and surrounding areas.

3.3 METHODS

3.3.1 Design and Layout of the Experiment

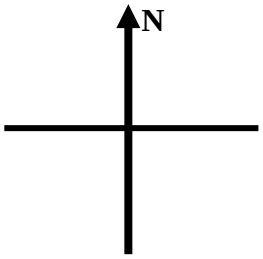
The study was conducted as pot culture experiment.

Design: CRD

Treatments: 10 (All the treatments were tried using 2 node and 3 node semi hardwood cuttings of orthotropic shoots of black pepper.)

Replication: 3

No. of pots/ treatment / replication: 3



R_1	R_2	R_3
T_8	T_7	T_8
T_7	T_{10}	T_3
T_2	T_8	T_5
T_{10}	T_6	T_9
T_5	T_1	T_7
T_1	T_4	T_4
T_6	T_2	T_6
T_4	T_9	T_1
T_9	T_3	T_{10}
T_3	T_5	T_2

Layout of the experiment

3.3.2 Potting Mixture

Potting mixture consisted of soil, sand and farm yard manure in the ratio 2:1:1. Potting mixture was solarized and enriched with *Trichoderma* to suppress soil borne fungal pathogens.

3.3.3 Solarization of Potting Mixture

Solarization is a method of hydrothermal disinfection. For solarization of potting mixture, the required type of potting mixture has been prepared as per the recommended practice. The mixture was spread on a leveled ground to a height of 15-20 cm. Moistened with water using a rose-can and covered the potting mixture with 100-150 gauge transparent polythene sheets. The edges of the sheet were sealed with soil to keep it in position in order to maintain the temperature and moisture inside the polythene mulch. Adequate care has been taken to see that the sheet is in close contact with the surface of the potting mixture to prevent the formation of air pockets between the mixture and polythene sheet (Plate 1.). The sheet was kept in this way for 30 days. After solarization, the potting mixture was used for planting. This method is found to be very effective to raise disease free pepper cuttings (KAU, 2011).

3.3.4 Multiplication of *Trichoderma*

Trichoderma spp. is a group of broad-spectrum antagonists used as bio control agent of soil borne plant pathogens. They are effective against the quick wilt of black pepper (*T. viride* T6 and *T. longibrachiatum* T2). A non- axenic system, viz. neemcake- cowdung mixture is used as food base for *Trichoderma* spp. Dry neemcake and cowdung were powdered and mixed at 1:9 ratio to get a coarse texture and then moistened by sprinkling water. The commercial preparation of *Trichoderma* spp. (available as talc based-formulation in polythene packets) was added @ 1-2 kg per 100 kg of neemcake -cowdung mixture, covered it with a moistened gunny bag and kept it in shade for 4-5 days for multiplication. Again mixed well and kept for 6 days for further multiplication. This *Trichoderma* incorporated neemcake-cowdung mixture was used in the potting mixture (KAU, 2011).

Plate 1. View of solarization of the potting mixture

3.3.5 Treatments

T_1- IBA 500ppm

T_2- IBA 1000ppm

T_3- Common sugar solution 1%

T_4- Common sugar solution 2%

T_5- Common sugar solution 3%

T_6- Arbuscular Mycorrhizal Fungi (AMF)

T_7- *Azospirillum* 15%

T_8- *Pseudomonas* 15%

T_9- Control

T_{10}- Absolute control (solarized potting mixture without bio control agent)

Commercial formulations of PGPR Mix – II, *Azospirillum*, AMF, *Trichoderma* and *Pseudomonas* developed by the Department of Microbiology, College of Agriculture, Vellayani were used.

3.3.4 Planting

All the above treatments were tried using 2 node and 3 node semi hardwood cuttings from orthotropic shoots of black pepper variety Panniyur – 1 collected during the first week of May. Two kg of the potting mixture was filled in polythene bags of 20 x 15 cm size and one cutting was planted with one node deep in the potting mixture in each bag giving following treatments (Plate 2 a and b.).

1. Hormone treatments
    a) Dipping pepper cuttings in 500 ppm aqueous solution of IBA for 45 seconds before planting.
    b) Dipping pepper cuttings in 1000 ppm aqueous solution of IBA for 45 seconds before planting
2. Common sugar treatments
    a) Dipping pepper cuttings in 1per cent common sugar solution for one minute before planting.

On the plant

Collected from the plant

Plate 2 a. Selection of orthotropic shoots

Plate 2 b. Cuttings for rooting in net house

b) Dipping pepper cuttings in 2 per cent common sugar solution for one minute before planting.

c) Dipping pepper cuttings in 3 per cent common sugar solution for one minute before planting.

3. AMF treatment

   Inoculum of AMF @ 5 g per pit was applied at the time of planting.

4. *Azospirillum* treatment

   Dipping pepper cuttings in 15 per cent slurry (15 g in 100 ml water) of WP formulation of *Azospirillum* for 15 minutes before planting.

5. *Pseudomonas* treatment

   Dipping pepper cuttings in 15 per cent slurry (15 g in 100 ml water) of WP formulation of *Pseudomonas* for 15 minutes before planting.

6. Control treatment

   Control treatment with solarized potting mixture enriched with *Trichoderma* alone.

7. Absolute control treatment

   Absolute control treatment without biocontrol agents.

PGPR Mix-II @ 2 per cent concentration was drenched uniformly in all treatments except absolute control.

After planting the cuttings were kept in net house.

3.3.7 After Cultivation

Hand weeding was done as and when needed and timely irrigation was also given.

3.3.8 Plant Protection

Pest and disease incidence was recorded periodically and timely plant protection measures were taken.

3.4 OBSERVATIONS

Observational plants per replication were taken from each treatment and the plants were tagged for taking biometric observations at monthly intervals starting

from the day of sprouting. The root characteristics, anatomical and physiological characters were recorded and biological properties of potting mixture were observed only during final month.

3.4.1. Growth Characters

3.4.1.1 Number of Days for Sprouting

Observed daily for the emergence of sprout and the duration of the sprouting was recorded as the days taken by pepper cuttings from the day of planting to sprout emergence.

3.4.1.2 Days for 50 Per Cent Sprouting

Number of days taken for 50 per cent fresh sprout development in each treatment from the date of planting was recorded.

3.4.1.3 Height of Sprouted Cutting

The height of the cutting from soil level to the tip of the top most leaf was measured and expressed in cm.

3.4.1.4 Number of Leaves

The numbers of fully opened leaves were counted and the mean was recorded.

3.4.1.5 Length of Leaf

Leaf length in cm was measured and the average was worked out.

3.4.1.6 Breadth of Leaf

Leaves used for measuring the length were used for recording the breadth of the leaves. The breadth in cm was measured at the broadest part of the leaves.

3.4.1.7 Petiole Length

Length from the node to the base of the leaf lamina was measured and expressed in cm.

3.4.1.8 Internodal Length

Length of internode was measured in between two nodes by using scale and the average was expressed in cm.

3.4.1.9 Number of Branches

The total number of branches developed per plant was counted and the mean value was recorded.

3.4.1.10 Leaf Area

Leaf area per plant was estimated using the equation,

LA = L x W x 0.61 (Ibrahim *et al*., 1985)

Where,

LA = Leaf area

L = Length of leaves

W = Width of leaves

Areas of individual leaves were added together and average was calculated and expressed in cm^2.

3.4.1.11 Number of Roots

After carefully de potting the cuttings from the poly bags, the roots were washed gently in tap water to remove all adhering soil particles and blot dried. The number of primary roots was counted.

3.4.1.12 Length of the Longest Root

The length of the longest root was measured and expressed in cm.

3.4.1.13 Volume of Root

Volume of roots per plant was estimated by displacement method and expressed in cm^3.

3.4.1.14 Percentage of Success in Establishment

Percentage of success in establishment was worked out using the following formula,

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

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

3.4.2 Anatomical Characters

3.4.2.1 Leaf Cuticle Thickness

Very thin free hand cross section of three randomly selected leaves from each replication were taken and the cuticle thickness was observed using 40X objective and 10X eyepiece and measured using micrometer and the values are expressed in micrometers (µm).

3.4.2.2 Number of Vascular Bundles in Leaf and Root

Free hand cross section of three randomly selected leaves and roots from each replication were taken and observed under 10X objective and the number of vascular bundles was recorded.

3.4.2.3 Stomatal Frequency

Stomatal frequency refers to the number of stomata per unit area of leaf. A thick mixture of thermocoal and xylene was prepared and this was smeared on both surface of leaves and allowed to dry. It was peeled gently after drying and the peels were observed under microscope and counted using a 40 X objective and 10X eye piece. The field of the microscope was measured using a stage micrometer and stomatal frequency per unit area was calculated.

Stomatal frequency = Number of stomata / Area of the microscopic field

3.4.3 Physiological Characters

3.4.3.1 Total Dry Matter Production

The leaves, petioles, stem and roots of the uprooted plants were separated and dried to a constant weight at 70^{o}C in a hot air oven. The sum of dry weights of component parts gave the total dry matter production of the plant and expressed in g $plant^{-1}$.

3.4.3.2 Stomatal Conductance

Stomatal resistance was measured directly by using a porometer (Delta T devices- Cambridge-UK).

The stomatal conductance was calculated by the formula:

$$\text{Stomatal conductance} = \frac{1}{\text{Stomatal resistance}}$$ and expressed in cm s^{-1}

3.4.3.3 Chlorophyll Content (Chlorophyll a, Chlorophyll b and Total Chlorophyll)

The leaf chlorophyll content was estimated by using DMSO method (Hiscox and Israedstam, 1979).

Leaf samples each weighing 500 mg were taken and cut into small pieces. They were put in test tubes and incubated overnight in room temperature in 10 ml DMSO: 80 per cent acetone mixture (1:1). The pigments were thus extracted in to the solution. Absorbance at 645 nm and 663 nm were recorded using spectrophotometer and total chlorophyll was estimated using the formula:

$$\text{Chlorophyll a} = (12.7\ A_{663} - 2.6\ A_{645}) \times V/\ 1000 \times 1/\ F.wt$$

$$\text{Chlorophyll b} = (22.9\ A_{645} - 4.68\ A_{663}) \times V/\ 1000 \times 1/\ F.wt$$

$$\text{Total chlorophyll (a+ b)} = (8.02\ A_{663} + 20.20 A_{645}) \times V/\ 1000 \times 1/\ F.wt$$

Where, V is the volume of extract.

The chlorophyll content was expressed in mg g^{-1}.

3.4.4 Biological Properties of Potting Mixture before and after Experiment (Percentage of Root Colonization and Spore Count)

3.4.4.1 AMF Colonization Percentage

The method of Phillips and Hayman (1970) was followed for recording mycorrhizal colonization in various root samples. For this, fresh root samples were cut into one cm length pieces. The root bits were initially washed in tap water and softened by simmering in 10 per cent KOH at 90^0 C for one hour. After cooling, the excess of alkali was removed by repeated rinsing in tap water and

then acidified with 2 per cent The KOH solution was poured off and the roots were rinsed with tap water. Then the roots were treated with 10 per cent HCl before staining with 0.05 per cent trypan blue in lactophenol at 90^0 C for three minutes. The excess stain from the root tissues was removed by clearing overnight in fresh lactophenol. Ten bits were examined at a time for the typical AMF infection under light microscope. Fresh root bit was divided into four equal segments for recording the presence or absence of Mycorrhiza. The percentage colonization by mycorrhiza in root was calculated by the formula:

$$\text{Percentage Colonization} = \frac{\text{Total no. of segments positive for mycorrhizal colonization}}{\text{Total no. of root segments observed}} \times 100$$

3.4.4.2 Spore Count of AMF

The spores of the AMF were isolated by the modified wet sieving and decanting method of Gerdemann and Nicolson (1963). For this, 250 g of rhizosphere soil was initially suspended in 1000 mi of tap water in a measuring cylinder and after the heavier particles had settled, the supernatant was passed through a set of sieves of B.S.S. No. 60 (250 microns), 150 (150 microns) and 350 (45 microns). The residue left behind in the measuring cylinder was resuspended in 1000 ml of fresh water and passed through the same set of sieves. This procedure was repeated three to four times in order to collect maximum number of spores from the soil. Finally, the material present on each sieve was transferred to 100 ml beakers in small volume of water and filtered through Watman No.1 filter paper. The content of each filter paper were carefully examined under a stereomicroscope for the typical spores of AM fungi. Spores were counted and expressed as number of spores per gram of soil.

3.4.4.3 Total Microbial Load

Total microbial load i.e., fungal, bacterial and actinomycetes population were calculated before and after the experiment. Samples were taken randomly from the potting mixture before the experiment. These values were compared with

that of the samples taken from the different treatment pots after the experimentation period.

The microbial population in the rhizosphere soil was estimated by serial dilution plate technique (Johnson and Curl, 1972). One gram of soil was taken and transferred to 100 ml of sterile water and shaken for 5-10 minutes using a rotary shaker. From this stock suspension, different dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared. The bacterial population was estimated at 10^{-6} dilution while 10^{-3} dilution was used for fungal and actinomycetes population.

The number of colony forming units (cfu) of microbes were calculated using the formula,

$$\text{cfu} = \frac{\text{Average number of colony developed x dilution factor}}{\text{Weight of soil taken (g)}}$$

3.4.4.3.1Total Bacterial Population

Nutrient Agar media with the following composition was used to enumerate the bacterial population.

Composition of Nutrient Agar

Beef extract - 3 g

Peptone - 5 g

$NaCl_2$ - 5 g

Agar - 20 g

Distilled water - 1000 ml

pH - 7.0

3.4.4.3.2 Total Fungal Population

Rose Bengal Agar media with the following composition was used to enumerate the total fungal population.

Composition of Rose Bengal Agar

Glucose - 10 g

Peptone - 5 g

K_2HPO_4 - 1 g

$MgSO_4$ $7H_2O$ - 0.5g

Streptomycin - 30 mg

Agar - 20 g

Rose Bengal - 0.035 g

Distilled water - 1000 ml

pH - 7.0

3.4.4.3.3 Total Actinomycetes Population

Ken knight's media with the following composition was used to enumerate the total Actinomycetes population.

Composition of Ken knight's media

Dextrose - 1.0 g

KH_2PO_4 - 0.10 g

$NaNO_3$ - 0.10 g

KCl - 0.10 g

$MgSO_4.7H_2O$ - 0.10g

Agar - 15.0 g

Distilled water - 1000 ml

pH - 7.0

3.5 PEST AND DISEASE INCIDENCE

Incidence of pests and diseases were noted at regular intervals and timely control measures were taken.

3.6 STATISTICAL ANALYSIS

The data was statistically analyzed as per the procedure outlined by Panse and Sukhatme (1985) for Completely Randomized Block Design.

RESULTS

4. RESULTS

The study entitled, "Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, during 2012 – 2014. The study was taken up as two experiments with 2 node and 3 node orthotropic cuttings of black pepper. The data collected were statistically analyzed and the results of two experimentations and laboratory estimations are presented in this chapter.

4.1 EFFECT OF TREATMENTS ON GROWTH CHARACTERS

4.1.1 Effect of Treatments on the Number of Days for Sprouting in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data revealed that the number of days for sprouting of the cuttings was significantly influenced by the treatments (Table 1). Early sprouting (22.50 days) was recorded by T_7 (*Azozpirillum* 15 per cent*)* in 2 node cuttings which was on par with treatments T_1 (23.11), T_2 (23.44), T_6 (23.44), T_9 (23.03) and T_8 (24.08).The treatment T_4 (Common sugar solution 2 per cent) registered the maximum number of days for sprouting (31.78) which is graphically presented in Fig. 1.

In the case of 3 node cuttings, early sprouting (24.78 days) was recorded by T_1 (IBA 500ppm) and T_2 (IBA 1000ppm) which were on par with T_8 (27.44), T_9 (25.37), and T_{10} (25.75). The treatment T_5 (Common sugar solution 3per cent) registered the maximum number of days (35.33) for sprouting (Plate 3).

4.1.2 Effect of Treatments on the Days for 50 Per Cent sprouting in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

There was significant difference among the treatments on the days for 50 per cent sprouting of the cuttings (Table 2). In 2 node cuttings, the minimum number of days (21.67) for 50 per cent sprouting was recorded by T_7 (*Azospirillum* 15 per cent) which was on par with T_1 (23.00), T_2 (22.50), T_6 (23.00), T_8 (23.53) and T_{10} (22.78). The treatment T_3 (Common sugar solution

Table 1. Effect of treatments on the number of days for sprouting in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node
T_1 (IBA 500 ppm)	23.11	24.78
T_2 (IBA1000 ppm)	23.44	24.78
T_3 (Common Sugar solution 1%)	29.44	30.44
T_4 (Common Sugar solution 2%)	31.78	32.89
T_5 (Common Sugar solution 3%)	24.89	35.33
T_6 (Arbuscular Mycorrhizal Fungi)	23.44	31.44
T_7 (Azospirillum 15%)	22.50	29.33
T_8 (Pseudomonas 15%)	24.08	27.44
T_9 (Control)	25.75	25.37
T_{10} (Absolute control)	23.03	25.75
CD (0.05)	2.22	2.95

Table 2. Effect of treatments on the days for 50% sprouting in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node
T_1 (IBA 500 ppm)	23.00	23.67
T_2 (IBA1000 ppm)	22.50	24.33
T_3 (Common Sugar solution 1%)	28.00	28.33
T_4 (Common Sugar solution 2%)	27.67	31.83
T_5 (Common Sugar solution 3%)	23.67	33.33
T_6 (Arbuscular Mycorrhizal Fungi)	23.00	30.33
T_7 (Azospirillum 15%)	21.67	25.00
T_8 (Pseudomonas 15%)	23.53	27.33
T_9 (Control)	25.75	25.37
T_{10} (Absolute control)	22.78	25.75
CD (0.05)	3.02	4.25

Plate 3. Sprouting from the cuttings

Plate 4. Growth of treated cuttings

1per cent) recorded the maximum number of days (28.00) for 50 per cent sprouting followed by T_4 (27.67) and T_9 (25.75).

In 3 node orthotropic cuttings, there was significant difference among the treatments on the days for 50 per cent sprouting of the cuttings (Fig. 2). The minimum number of days (23.67) for 50 per cent sprouting was recorded by T_1 (IBA 500 ppm) which was on par with T_2 (25.33), T_7 (25.00), T_8 (27.33), T_9 (25.37) and T_{10} (25.75). The treatment T_5 (Common sugar solution 3 per cent) recorded the maximum number of days for 50 per cent sprouting (33.33).

4.1.3 Effect of Treatments on the Height of Sprouted Cutting (cm) Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Mean values of the height of sprouted cutting are furnished in the Table 3. The data revealed that the cuttings supplied with *Azospirillum* 15 per cent (T_7) recorded the highest value (16.54 cm) which was on par with T_2 (14.52 cm) at 1st month after planting (MAP) in 2 node cuttings. During the 2nd MAP, the maximum height (19.61cm) was recorded by T_2 (IBA 1000 ppm) and was on par with T_7 (18.51 cm) and T_8 (17.59 cm). At 3 MAP, the treatment T_2 (IBA 1000 ppm) recorded the highest value (25.31cm) followed by T_7 (20.85cm).

The results revealed that there was no significant difference among the treatments on height of sprouted 3 node cutting (Table 4).

4.1.4 Effect of Treatments on the Number of Leaves in Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The number of leaves per plant was recorded at monthly intervals from 1 MAP and the data is furnished in Table 5, (Plate 4). Significant difference was observed between treatments in the number of leaves per plant in 2 node cuttings. The treatment T_2 (IBA 1000 ppm) registered the highest value at 1st, 2nd and 3rd months after planting (1.55 cm, 2.66 cm and 4.33 cm respectively) and the lowest value (1.44 cm) was recorded by T_4 (Common Sugar Solution 2 per cent).

There was no significant difference among the treatments on number of leaves in 3 node orthotropic cuttings at 1 MAP. However, significant difference was observed between treatments in the number of leaves per plant (Table 6). At 2nd and 3rd MAP, the treatment T_2 (IBA 1000 ppm) registered the highest number

Table 3. Effect of treatments on the height of sprouted cutting (cm) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Height of sprouted cutting (cm)		
	1 MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	11.99	13.65	19.37
T_2 (IBA1000 ppm)	14.52	19.61	25.13
T_3 (Common Sugar solution 1%)	11.09	12.26	15.64
T_4 (Common Sugar solution 2%)	9.56	12.13	14.73
T_5 (Common Sugar solution 3%)	9.18	10.93	12.67
T_6 (Arbuscular Mycorrhizal Fungi)	11.01	12.75	16.00
T_7 (Azospirillum 15%)	16.54	18.51	20.85
T_8 (Pseudomonas 15%)	12.27	17.59	19.41
T_9 (Control)	13.54	14.55	16.41
T_{10} (Absolute control)	13.62	14.81	17.10
CD (0.05)	2.57	3.40	4.39

Table 4. Effect of treatments on the height of sprouted cutting (cm) in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Height of sprouted cutting (cm)		
	1 MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	14.88	17.58	21.17
T_2 (IBA1000 ppm)	17.85	19.83	23.58
T_3 (Common Sugar solution 1%)	12.88	13.75	15.90
T_4 (Common Sugar solution 2%)	12.04	13.28	16.50
T_5 (Common Sugar solution 3%)	13.53	15.43	18.92
T_6 (Arbuscular Mycorrhizal Fungi)	13.77	15.45	17.33
T_7 (Azospirillum 15%)	14.28	16.45	18.76
T_8 (Pseudomonas 15%)	13.13	15.27	17.82
T_9 (Control)	16.40	17.61	20.88
T_{10} (Absolute control)	15.02	16.58	18.23
CD (0.05)	NS	NS	NS

MAP – Months After Planting

Table 5. Effect of treatments on the number of leaves in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Number of leaves		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	1.00	1.33	2.22
T_2 (IBA1000 ppm)	1.55	2.66	4.33
T_3 (Common Sugar solution 1%)	1.00	1.22	1.66
T_4 (Common Sugar solution 2%)	1.00	1.22	1.44
T_5 (Common Sugar solution 3%)	1.00	1.33	1.89
T_6 (Arbuscular Mycorrhizal Fungi)	1.00	1.33	1.66
T_7 (Azospirillum 15%)	1.00	1.22	1.78
T_8 (Pseudomonas 15%)	1.00	1.22	1.83
T_9 (Control)	1.00	1.33	1.66
T_{10} (Absolute control)	1.11	1.55	1.77
CD (0.05)	0.15	0.46	0.97

Table 6. Effect of treatments on the number of leaves in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Number of leaves		
	1MAP	2MAP	3MAP
T1 (IBA 500 ppm)	1.11	1.33	1.10
T2 (IBA1000 ppm)	1.22	2.22	3.44
T_3 (Common Sugar solution 1%)	1.00	1.11	1.44
T_4 (Common Sugar solution 2%)	1.00	1.33	1.55
T_5 (Common Sugar solution 3%)	1.00	1.11	1.50
T_6 (Arbuscular Mycorrhizal Fungi)	1.00	1.33	1.66
T7 (Azospirillum 15%)	1.00	1.33	1.66
T8 (Pseudomonas 15%)	1.00	1.11	1.66
T9 (Control)	1.00	1.33	1.89
T10 (Absolute control)	1.00	1.66	1.89
CD (0.05)	NS	0.48	0.47

MAP – Months After Planting

of leaves (2.22 and 3.44 respectively) and was found to be significantly superior to all other treatments.

4.1.5 Effect of Treatments on the Length of Leaf (cm) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on effect of treatments on the length of leaf in 2 node cuttings are presented in Table 7. The influence of treatments on length of leaf was recorded from one MAP and it was found that there was no significant difference among the treatments on the length of leaf during the 1st MAP. At 2 MAP the highest value (6.54 cm) was recorded by T_7 (*Azospirillum* 15 per cent) followed by T_4 (5.74 cm). During 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) registered the highest value (8.50 cm) which was on par with T_8 (7.93 cm) and T_{10} (7.50 cm).The lowest value (4.42 cm) was recorded by the treatment T_3 (Common Sugar Solution 1per cent).

In the case of 3node cuttings, it was found that the treatments T_2 (IBA 1000 ppm) recorded the highest value (4.32 cm) during 1st MAP which was on par with T_1 (4.29 cm), T_4 (3.64 cm), T_7 (4.18 cm), T_8 (3.67 cm) and T_{10} (3.72 cm). At 2nd MAP, the highest value (6.04 cm) was recorded by T_8 (*Pseudomonas* 15 per cent) which was on par with T_1 (5.14 cm), T_2 (5.55 cm), T_4 (5.27 cm), T_7 (5.81 cm) and T_9 (5.13 cm). During 3rd MAP, the treatment T_8 (*Pseudomonas* 15 per cent) registered the highest value (8.64 cm) which was found to be on par with T_2 (7.60 cm), T_5 (7.85 cm) and T_7 (8.14 cm). The data is presented in Table 8.

4.1.6 Effect of Treatments on the Breadth of Leaf (cm) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Table 9 shows the breadth of leaf recorded by different treatments in 2 node cuttings. It was found that there was no significant difference among the treatments on the breadth of leaf during the 1st MAP. But at 2nd MAP, the highest value (4.42 cm) was recorded by T_7 (*Azospirillum* 15 per cent) which was on par with T_4 (3.82 cm), T_5 (3.92 cm) and T_8 (3.83 cm). During 3rd MAP, the treatment T_7 registered the highest value (8.50 cm) which was found to be on par with T_5 (4.92 cm) and T_8 (4.75 cm). The lowest value (3.34 cm) was recorded by the treatment T_9 (Control).

Table 7. Effect of treatments on the length of leaf (cm) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Length of leaf (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	3.36	4.78	6.51
T_2 (IBA1000 ppm)	4.39	5.49	7.31
T_3 (Common Sugar solution 1%)	2.87	3.80	4.42
T_4 (Common Sugar solution 2%)	3.10	5.74	7.37
T_5 (Common Sugar solution 3%)	3.37	5.19	7.07
T_6 (Arbuscular Mycorrhizal Fungi)	3.04	4.62	6.87
T_7 (Azospirillum 15%)	3.97	6.54	8.50
T_8 (Pseudomonas 15%)	3.17	5.15	7.93
T_9 (Control)	3.38	4.02	5.60
T_{10} (Absolute control)	3.70	5.33	7.50
CD (0.05)	NS	0.98	1.02

Table 8. Effect of treatments on the length of leaf (cm) in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Length of leaf (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	4.29	5.14	6.42
T_2 (IBA1000 ppm)	4.32	5.55	7.60
T_3 (Common Sugar solution 1%)	2.61	4.37	5.68
T_4 (Common Sugar solution 2%)	3.64	5.27	5.62
T_5 (Common Sugar solution 3%)	3.25	4.96	7.85
T_6 (Arbuscular Mycorrhizal Fungi)	3.25	4.37	5.37
T_7 (Azospirillum 15%)	4.18	5.81	8.14
T_8 (Pseudomonas 15%)	3.67	6.04	8.64
T_9 (Control)	2.02	2.38	4.43
T_{10} (Absolute control)	3.72	5.13	6.35
CD (0.05)	0.71	0.95	1.08

MAP – Months After Planting

It was found that the treatments T_7 (*Azospirillum* 15 per cent) significantly influenced the breadth of leaf throughout the observation period in 3 node cuttings (Table 10). During the 1st MAP the treatment T_7 recorded the highest value of 2.80 cm, which was on par with T_1 (2.31 cm), T_2 (2.66 cm), T_5 (2.59 cm), T_8 (2.50 cm) and T_{10} (2.45 cm). At 2nd MAP, the treatment T_7 recorded the highest value of 3.92cm, which on par with T_2 (3.73 cm), T_5 (3.31 cm) and T_{10} (3.31 cm). During the 3rd MAP, the treatment T_7 registered the highest value (5.23 cm) and was found to be on par with T_1 (4.61 cm), T_2(4.67 cm), T_5 (5.06 cm) and T_8 (4.59 cm).

4.1.7 Effect of Treatments on the Petiole Length (cm) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data presented in Table 11 shows the effect of treatments on the petiole length in 2 node cuttings, the highest value (2.21 cm) for petiole length was recorded by T_2 (IBA 1000 ppm) which was on par with T_1 (1.10 cm), T_7 (1.72 cm) and T_8 (1.77 cm) during 1st MAP. There was no significant difference among the treatments on the petiole length during 2nd MAP. The treatment T_2 (IBA 1000 ppm) recorded the highest value for petiole length (4.34 cm) at 3rd MAP which was on par with T_1 (4.08 cm), T_6 (3.73 cm), T_7 (4.01 cm) and T_8 (3.79 cm). The lowest value (2.58 cm) was recorded by T_{10} (Absolute control).

In 3 node cuttings (Table 12), at 1st, 2nd and 3rd months after planting, the treatment T_2 (IBA 1000 ppm) recorded the highest values for petiole length (2.49cm, 4.31 cm and 4.75 cm respectively) and was found to be significantly superior to all other treatments. The lowest value (2.77 cm) was recorded by T_4 (Common Sugar Solution 2 per cent).

4.1.8 Effect of Treatments on the Internodal Length (cm) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Significant difference was observed between treatments in the internodal length at 1st and 2nd months after planting in 2 node cuttings (Table 13). The highest value (2.49 cm) was recorded by T_2 (IBA 1000 ppm) followed by T_1 (2.15 cm), T_6 (2.05 cm) and T_8 (2.30 cm) at 1MAP. At 2nd MAP, T_2 recorded the highest value (4.03 cm) and was found to be on par with T_1 (3.68 cm) and T_6 (3.22 cm).

Table 9. Effect of treatments on the breadth of leaf (cm) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Breadth of leaf (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	2.20	3.16	4.06
T_2 (IBA1000 ppm)	2.54	3.55	4.70
T_3 (Common Sugar solution 1%)	1.99	2.71	3.45
T_4 (Common Sugar solution 2%)	2.70	3.82	4.71
T_5 (Common Sugar solution 3%)	2.42	3.92	4.92
T_6 (Arbuscular Mycorrhizal Fungi)	1.66	2.82	4.32
T_7 (Azospirillum 15%)	2.89	4.42	5.84
T_8 (Pseudomonas 15%)	2.88	3.83	4.75
T_9 (Control)	2.38	2.61	3.34
T_{10} (Absolute control)	2.45	3.17	4.20
CD (0.05)	NS	0.71	1.13

Table 10. Effect of treatments on the breadth of leaf (cm) in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Breadth of leaf (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	2.31	3.21	4.61
T_2 (IBA1000 ppm)	2.66	3.73	4.67
T_3 (Common Sugar solution 1%)	1.91	2.10	3.59
T_4 (Common Sugar solution 2%)	2.06	3.20	3.66
T_5 (Common Sugar solution 3%)	2.59	3.31	5.06
T_6 (Arbuscular Mycorrhizal Fungi)	1.79	2.51	3.39
T_7 (Azospirillum 15%)	2.80	3.92	5.23
T_8 (Pseudomonas 15%)	2.50	3.23	4.59
T_9 (Control)	1.71	2.03	2.55
T_{10} (Absolute control)	2.45	3.50	3.78
CD (0.05)	0.69	0.66	0.74

MAP – Months After Planting

Table 11. Effect of treatments on the petiole length (cm) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Petiole length (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	1.10	2.62	4.08
T_2 (IBA1000 ppm)	2.21	2.95	4.34
T_3 (Common Sugar solution 1%)	1.41	2.07	3.00
T_4 (Common Sugar solution 2%)	1.27	2.19	3.16
T_5 (Common Sugar solution 3%)	1.34	2.40	3.43
T_6 (Arbuscular Mycorrhizal Fungi)	1.28	2.64	3.73
T_7 (Azospirillum 15%)	1.72	2.65	4.01
T_8 (Pseudomonas 15%)	1.77	2.48	3.79
T_9 (Control)	1.30	2.32	3.03
T_{10} (Absolute control)	1.13	1.83	2.58
CD (0.05)	0.51	NS	0.72

Table 12. Effect of treatments on the petiole length (cm) in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Petiole length (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	1.93	2.96	4.35
T_2 (IBA1000 ppm)	2.49	4.31	4.75
T_3 (Common Sugar solution 1%)	1.51	2.37	3.50
T_4 (Common Sugar solution 2%)	1.23	1.85	2.77
T_5 (Common Sugar solution 3%)	1.33	1.93	3.44
T_6 (Arbuscular Mycorrhizal Fungi)	1.45	2.10	3.69
T_7 (Azospirillum 15%)	1.50	2.36	3.12
T_8 (Pseudomonas 15%)	1.63	2.25	3.67
T_9 (Control)	1.70	2.53	3.23
T_{10} (Absolute control)	1.35	2.12	2.78
CD (0.05)	0.49	0.52	0.67

MAP – Months After Planting

However at 3rd MAP there was no significant difference among the treatments on the internodal length.

There was significant difference among the treatments on intermodal length in 3 node cuttings at 1st MAP. The treatment T_1 (IBA 500 ppm) recorded the highest value (2.98 cm) which was on par with T_2 (2.90 cm). However, no significant difference was observed between treatments in the internodal length during 2nd and 3rd months after planting (Table 14).

4.1.9 Effect of Treatments on the Number of Branches in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Table 15 shows the number of branches recorded by different treatments. It was found that there was no significant difference among treatments on the number of branches in 2 node cuttings. The same trend was noticed in the case of 3 node cuttings (Table 16); there was no significant difference among treatments on the number of branches.

4.1.10 Effect of Treatments on the Leaf Area (cm^2) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data furnished in the Table 17 indicated that in 2 node cuttings, there was no significant difference among treatments on the leaf area at 1stMAP. However there was significant difference among treatments on the leaf area during 2nd MAP and 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values of 17.62 cm^2 and 30.26 cm^2 respectively.

The data in Table 18 recorded significant variation among the treatments on leaf area in 3 node cuttings. At 1st MAP, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest value (7.24 cm^2) which was on par with T_1 (6.03 cm^2), T_2 (7.02 cm^2), T_5 (5.26 cm^2), T_8 (5.62 cm^2) and T_{10} (5.57 cm^2). At 2nd MAP, T_7 recorded the highest value of 13.91 cm^2 which was on par with T_2 (12.69 cm^2), T_8 (11.91 cm^2) and T_{10} (10.97 cm^2). At 3rd MAP, the treatment T_7 recorded the highest value (26.02 cm^2) which was on par with T_5 (24.24 cm^2) and T_8 (23.99 cm^2). The lowest value (5.99 cm^2) was recorded by the T_9 (Control).

Table 13. Effect of treatments on the internodal length (cm) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Internodal length (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	2.15	3.68	4.63
T_2 (IBA1000 ppm)	2.49	4.03	5.24
T_3 (Common Sugar solution 1%)	1.72	2.59	4.29
T_4 (Common Sugar solution 2%)	1.64	2.68	3.64
T_5 (Common Sugar solution 3%)	1.01	2.60	4.05
T_6 (Arbuscular Mycorrhizal Fungi)	2.05	3.22	4.87
T_7 (Azospirillum 15%)	1.30	2.97	4.33
T_8 (Pseudomonas 15%)	2.30	2.98	4.06
T_9 (Control)	1.43	2.29	3.14
T_{10} (Absolute control)	1.47	2.80	4.32
CD (0.05)	0.70	0.85	NS

Table 14. Effect of treatments on the internodal length (cm) in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Internodal length (cm)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	2.98	4.20	6.21
T_2 (IBA1000 ppm)	2.90	3.93	5.44
T_3 (Common Sugar solution 1%)	1.71	2.78	4.32
T_4 (Common Sugar solution 2%)	1.42	3.01	4.83
T_5 (Common Sugar solution 3%)	1.96	2.83	4.27
T_6 (Arbuscular Mycorrhizal Fungi)	1.91	2.97	4.05
T_7 (Azospirillum 15%)	2.02	3.25	4.82
T_8 (Pseudomonas 15%)	2.10	3.41	5.09
T_9 (Control)	2.13	2.80	4.20
T_{10} (Absolute control)	2.05	2.90	4.28
CD (0.05)	0.78	NS	NS

MAP – Months After Planting

Table 15. Effect of treatments on the number of branches in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Number of branches		
	1 MAP	2 MAP	3 MAP
T_1 (IBA 500 ppm)	1.00	1.00	1.00
T_2 (IBA1000 ppm)	1.00	1.00	1.00
T_3 (Common Sugar solution 1%)	1.00	1.00	1.00
T_4 (Common Sugar solution 2%)	1.00	1.00	1.00
T_5 (Common Sugar solution 3%)	1.00	1.11	1.11
T_6 (Arbuscular Mycorrhizal Fungi)	1.00	1.00	1.00
T_7 (Azospirillum 15%)	1.00	1.00	1.00
T_8 (Pseudomonas 15%)	1.00	1.11	1.11
T_9 (Control)	1.00	1.00	1.00
T_{10} (Absolute control)	1.00	1.00	1.00
CD (0.05)	NS	NS	NS

Table 16. Effect of treatments on the number of branches in 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Number of branches		
	1 MAP	2 MAP	3 MAP
T_1 (IBA 500 ppm)	1.00	1.00	1.00
T_2 (IBA1000 ppm)	1.00	1.00	1.00
T_3 (Common Sugar solution 1%)	1.00	1.00	1.00
T_4 (Common Sugar solution 2%)	1.00	1.00	1.00
T_5 (Common Sugar solution 3%)	1.00	1.00	1.00
T_6 (Arbuscular Mycorrhizal Fungi)	1.00	1.00	1.00
T_7 (Azospirillum 15%)	1.00	1.00	1.00
T_8 (Pseudomonas 15%)	1.00	1.00	1.00
T_9 (Control)	1.00	1.00	1.00
T_{10} (Absolute control)	1.00	1.00	1.00
CD (0.05)	NS	NS	NS

MAP – Months After Planting

Table 17. Effect of treatments on the leaf area (cm^2) in 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Leaf area (cm^2)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	4.53	9.28	16.13
T_2 (IBA1000 ppm)	6.95	11.89	20.99
T_3 (Common Sugar solution 1%)	3.48	6.26	9.28
T_4 (Common Sugar solution 2%)	6.51	13.38	21.17
T_5 (Common Sugar solution 3%)	5.01	12.39	20.10
T_6 (Arbuscular Mycorrhizal Fungi)	3.07	7.75	17.84
T_7 (Azospirillum 15%)	7.40	17.62	30.26
T_8 (Pseudomonas 15%)	5.47	11.10	22.88
T_9 (Control)	4.94	6.39	12.96
T_{10} (Absolute control)	5.65	10.83	18.43
CD (0.05)	NS	3.67	4.07

Table 18. Effect of treatments on the leaf area (cm^2) in 3node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Leaf area (cm^2)		
	1MAP	2MAP	3MAP
T_1 (IBA 500 ppm)	6.03	10.03	18.01
T_2 (IBA1000 ppm)	7.02	12.69	21.61
T_3 (Common Sugar solution 1%)	3.10	8.06	12.41
T_4 (Common Sugar solution 2%)	4.65	10.40	12.48
T_5 (Common Sugar solution 3%)	5.26	10.04	24.24
T_6 (Arbuscular Mycorrhizal Fungi)	3.57	6.82	11.20
T_7 (Azospirillum 15%)	7.24	13.91	26.02
T_8 (Pseudomonas 15%)	5.62	11.91	23.99
T_9 (Control)	2.14	2.99	5.99
T_{10} (Absolute control)	5.57	10.97	14.48
CD (0.05)	2.28	3.19	3.38

MAP – Months After Planting

4.1.11 Effect of Treatments on the Number of Roots in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

At 3 MAP (Table 19), analysis of data indicated significant difference among treatments on the number of roots of the plants. The treatment T_2 (IBA 1000 ppm) was found to be significantly different from all other treatments both in the case of 2 and 3 node orthotropic cuttings (Fig. 3).

The treatment T_2 (IBA 1000 ppm) recorded highest value (7.27) in 2 node cuttings and the lowest value was recorded by T_4 (Common Sugar Solution 2 per cent). In 3 node cuttings, with highest value recorded by T_2 (IBA 1000 ppm) was 6.83. Whereas, the lowest value (1.22) was recorded by T_4 (Common Sugar Solution 2 per cent) and T_9 (Control) (Plate 5 and 6).

4.1.12 Effect of Treatments on Length of the Longest Root (cm) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

There was significant difference among the treatments on the length of longest root in both types of cuttings (Table 20). In 2 node cuttings, the length of root was significantly higher (14.44 cm) in T_8 (*Pseudomonas* 15 per cent) which was on par with T_7 (12.52 cm) at 3 MAP (Fig.4). Here the lowest value (3.47 cm) was recorded by the treatment T_6 (AMF).

While in 3 node cuttings, the length of root was significantly higher (17.24 cm) in T_1 (IBA 500 ppm) which was on par with T_2 (15.13 cm) at 3 MAP. The lowest value (2.03 cm) was recorded by the treatment T_3 (Common Sugar Solution 1 per cent).

4.1.13 Effect of Treatments on Volume of Root (cm^3) in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The volume of root showed significant difference among the treatments (Table 21). In 2 node cuttings, the highest value (3.55 cm^3) was recorded by T_2 (IBA 500 ppm).The lowest value (0.39 cm^3) was recorded by T_9 (Control).

In the case of 3 node cuttings, the highest value (1.90) was recorded by T_2 (IBA 500 ppm) which was on par with T_1 (1.83 cm^3), T_3 (1.51 cm^3), T_7 (1.75 cm^3), T_8 (1.39 cm^3) and T_{10} (1.44 cm^3). Here, the lowest value (0.67 cm^3) was recorded by T_9 (Control).

Table 19. Effect of treatments on the number of roots in orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node
T_1 (IBA 500 ppm)	4.17	3.83
T_2 (IBA1000 ppm)	7.27	6.83
T_3 (Common Sugar solution 1%)	1.11	1.50
T_4 (Common Sugar solution 2%)	1.00	1.22
T_5 (Common Sugar solution 3%)	1.50	1.67
T_6 (Arbuscular Mycorrhizal Fungi)	1.67	1.83
T_7 (Azospirillum 15%)	2.17	1.67
T_8 (Pseudomonas 15%)	1.66	3.33
T_9 (Control)	1.33	1.22
T_{10} (Absolute control)	1.11	1.33
CD (0.05)	1.31	1.48

Table 20. Effect of treatments on len oot (cm) in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

Table 1

Trea	2 node	3 node
T_1 (IBA 500 ppr	9.88	17.24
T_2 (IBA1000 ppm)	10.58	15.13
T_3 (Common Sugar solution 1%)	6.32	2.03
T_4 (Common Sugar solution 2%)	3.98	2.10
T_5 (Common Sugar solution 3%)	6.27	6.27
T_6 (Arbuscular Mycorrhizal Fungi)	3.47	9.70
T_7 (Azospirillum 15%)	12.52	10.37
T_8 (Pseudomonas 15%)	14.44	10.77
T_9 (Control)	3.93	2.92
T_{10} (Absolute control)	4.46	1.47
CD (0.05)	3.10	4.39

4.1.14 Effect of Treatments on Percentage Success in Establishment in the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The percentage success in establishment was recorded at 3 months after planting (Table 22). There was significant influence among treatments on percentage success in establishment of the plants (Fig. 5). In 2 node cuttings, the highest value (77.77 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) which was on par with T_1 (IBA 500 ppm) and T_2 (IBA 1000 ppm) and they recorded 66.66 per cent (Plate. 7). The lowest value (33.33 per cent) was recorded by the treatments T_3 (Common Sugar Solution 1 per cent), T_4 (Common Sugar Solution 2 per cent), T_5 (Common Sugar Solution 3 per cent) and T_{10} (Absolute Control).

The highest value (88.89 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) in 3 node cuttings and was found to be on par with T_1 (77.77 per cent). The lowest value was recorded by the treatments T_3, T_4, T_5 and T_{10} (33.33 per cent).

The pooled analysis of the data revealed that the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest percentage success (83.33 per cent) in establishment of cuttings followed by T_1 (72.23 per cent) and T_2 (66.66 per cent) (Table 23 and Fig.6).

4.2 EFFECT OF TREATMENTS ON ANATOMICAL CHARACTERS

4.2.1 Effect of Treatments on Leaf Cuticle Thickness of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on leaf cuticle thickness revealed that there was significant influence among treatments on the leaf cuticle thickness (Table 24). In 2 node cuttings, the leaf cuticle thickness was the highest (1.71 µm) in T_5 (Common Sugar Solution 3per cent) and was on par with T_6 (1.54 µm). The lowest value (0.94 µm) was recorded by T_{10} (Absolute Control).

There was significant influence among treatments on the leaf cuticle thickness (Table 25) in 3 node cuttings. The leaf cuticle thickness was the highest (1.74 µm) in T_6 (AMF). The lowest value (0.84 µm) was recorded by T_9 (Control).

Table 21. Effect of treatments on volume of root (cm^3) in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node
T_1 (IBA 500 ppm)	0.96	1.83
T_2 (IBA1000 ppm)	3.55	1.90
T_3 (Common Sugar solution 1%)	0.70	1.51
T_4 (Common Sugar solution 2%)	0.44	1.12
T_5 (Common Sugar solution 3%)	0.46	0.68
T_6 (Arbuscular Mycorrhizal Fungi)	1.90	0.99
T_7 (Azospirillum 15%)	0.86	1.75
T_8 (Pseudomonas 15%)	1.23	1.39
T_9 (Control)	0.39	0.67
T_{10} (Absolute control)	1.33	1.44
CD (0.05)	0.45	0.65

Table 22. Effect of treatments on percentage success in establishment in orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node
T_1 (IBA 500 ppm)	66.66	77.77
T_2 (IBA1000 ppm)	66.66	66.66
T_3 (Common Sugar solution 1%)	33.33	33.33
T_4 (Common Sugar solution 2%)	33.33	33.33
T_5 (Common Sugar solution 3%)	33.33	33.33
T_6 (Arbuscular Mycorrhizal Fungi)	55.55	44.44
T_7 (Azospirillum 15%)	77.77	88.89
T_8 (Pseudomonas 15%)	55.55	66.66
T_9 (Control)	44.44	44.44
T_{10} (Absolute control)	33.33	33.33
CD (0.05)	20.73	20.73

T_7 – *Azospirillum* 15%

T_1– IBA 500 ppm

T_2 – IBA 1000 ppm

Plate 7. The best treatments with highest percentage success in the establishment of orthotropic cuttings of black pepper.

Table 23. Effect of treatments on the % success in establishment of 2 node cuttings, 3 node cuttings and their combined effect on orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	2 node	3 node	Combined
T_1 (IBA 500 ppm)	66.66	77.77	72.23
T_2 (IBA1000 ppm)	66.66	66.66	66.66
T_3 (Common Sugar solution 1%)	33.33	33.33	33.33
T_4 (Common Sugar solution 2%)	33.33	33.33	33.33
T_5 (Common Sugar solution 3%)	33.33	33.33	33.33
T_6 (Arbuscular Mycorrhizal Fungi)	55.55	44.44	49.10
T_7 (Azospirillum 15%)	77.77	88.89	83.33
T_8 (Pseudomonas 15%)	55.55	66.66	61.11
T_9 (Control)	33.33	33.33	33.33
T_{10} (Absolute control)	44.44	44.44	44.44
Mean	49.10	52.22	
CD (0.05) cutting	6.35		
CD (0.05) treatment	14.20		
CD (0.05) cutting x treatment	20.09		

4.2.2 Effect of Treatments on Number of Vascular Bundles in Leaf of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Data furnished in table 23 revealed that, in 2 node cuttings the highest number of vascular bundles in leaf (6.28) was recorded by T_2 (IBA 1000 ppm) which was on par with T_5 (5.00) and T_6 (5.00). The lowest value (2.55) was recorded by T_8 (*Pseudomonas* 15 per cent) (Plate.8).

The highest number of vascular bundles in leaf (7.50) was recorded by T_2 (IBA 1000 ppm) in 3 node cuttings and was significantly different from other treatments (Table 25). The lowest value (2.17) was recorded by T_8 (*Pseudomonas* 15 per cent) (Plate 9).

4. 2.3 Effect of Treatments on Number of Vascular Bundles in Root of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

There was significant difference among the treatments on number of vascular bundles in root of 2 node cuttings (Table 24). The highest value (7.89) was recorded by T_1 (IBA 500 ppm) and was significant different from other treatments. The lowest value (4.44) was recorded by T_{10} (Absolute Control).

The highest value (7.39) for vascular bundles in root was recorded by T_1 (IBA 500 ppm) in 3 node cuttings (Table 25) and was found to be on par with T_5 (6.77) and T_6 (6.33). The lowest value (4.66) was recorded by T_9 (Control).

4.2.4 Effect of Treatments on Stomatal Frequency of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The stomatal frequency showed significant difference among the treatments (Table 24). The stomatal frequency of the plant was significantly higher in T_7 (*Azospirillum* 15 per cent) in 2 node cuttings (285.89).The lowest value (137.78) was recorded by T_6 (AMF).

The stomatal frequency of the plant was significantly higher in T_7 (207.10) and was on par with T_3 (205.94) in the case of 3 node cuttings (Table 25).The lowest value (134.67) was observed in T_6 (AMF).

4.3 EFFECT OF TREATMENTS ON PHYSIOLOGICAL CHARACTERS

4.3.1 Effect of Treatments on Total Dry Matter Production (g plant^{-1}) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

Data presented in Table 26 revealed that in 2 node cuttings, the total dry matter production varied significantly among the treatments. At 3 MAP the highest total dry matter production (6.22 g plant^{-1}) was registered by T_8 (*Pseudomonas*) which was on par with T_1 (5.33 g plant^{-1}). The lowest value (1.85 g plant^{-1}) was recorded by T_5 (Common Sugar Solution 3 per cent).

In the case of 3 node cuttings, the highest total dry matter production (5.21 g plant^{-1}) was registered by T_1 (IBA 500 ppm) and was on par with T_8 (4.62 g plant^{-1}) at 3 MAP (Table 27). The lowest value (1.90 g plant^{-1}) was recorded by T_3 (Common Sugar Solution 1per cent).

4.3.2 Effect of Treatments on Stomatal Conductance (cms^{-1}) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on effect of treatments on stomatal conductance is presented in Table 25. It was found that there was no significant difference among treatments on stomatal conductance at 3 MAP. Same trend was noticed in the case of 3 node cuttings (Table 27).

4.3.3 Effect of Treatments on Chlorophyll a (mg g^{-1}) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on the effect of treatments on leaf chlorophyll a content is presented in Table 26, taken at 3 MAP. There was significant difference among treatments on chlorophyll a content in 2 node cuttings. The treatment T_5 (Common Sugar Solution 3per cent) recorded the highest value (0.63 mg g^{-1}) which was on par with all other treatments except T_2 (0.56 mg g^{-1}) and T_3 (0.52 mg g^{-1}).

In 3 node cuttings also, there was significant difference among treatments on chlorophyll a content (Table 27). The treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.63 mg g^{-1}) which was on par with T_1 (0.62 mg g^{-1}), T_5 (0.61 mg g^{-1}) and T_6 (0.61 mg g^{-1}).

Table 24. Effect of treatments on anatomical characters of 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Leaf cuticle thickness (µm)	No. of vascular bundles in leaf	No. of vascular bundles in root	Stomatal frequency
T_1	1.24	4.83	7.89	160.00
T_2	1.34	6.28	6.44	166.67
T_3	1.25	3.17	5.66	217.11
T_4	1.11	3.83	5.77	180.00
T_5	1.71	5.00	5.10	169.78
T_6	1.54	5.00	6.11	137.78
T_7	1.37	4.22	5.66	285.89
T_8	1.34	2.55	6.33	172.33
T_9	1.05	2.72	4.78	142.67
T_{10}	0.94	2.67	4.44	157.67
CD (0.05)	0.21	1.38	0.88	6.27

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

Table 25. Effect of treatments on anatomical characters of 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Leaf cuticle thickness (µm)	No. of vascular bundles in leaf	No. of vascular bundles in root	Stomatal frequency
T_1	1.35	5.50	7.39	159.22
T_2	1.44	7.50	5.50	179.11
T_3	1.15	2.83	5.89	205.94
T_4	0.93	4.00	6.11	179.78
T_5	1.02	4.00	6.77	177.33
T_6	1.74	3.17	6.33	134.67
T_7	1.14	2.67	5.22	207.10
T_8	1.44	2.17	5.77	179.59
T_9	0.84	4.33	4.66	158.22
T_{10}	0.87	5.00	4.78	150.67
CD (0.05)	0.19	1.76	1.07	5.72

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

4.3.4 Effect of Treatments on Chlorophyll b (mg g^{-1}) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on the effect of treatments on leaf chlorophyll b content is presented in Table 26, taken at 3 MAP. There was significant difference among treatments on chlorophyll b content in 2 node cuttings. The treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.48 mg g^{-1}) which was on par with T_1 (0.42 mg g^{-1}), T_5 (0.47 mg g^{-1}), T_7 (0.46 mg g^{-1}) and T_9 (0.48 mg g^{-1}).

There was significant difference among treatments on chlorophyll b content in 3 node cuttings (Table 27). The treatment T_1 (IBA 500 ppm) recorded the highest value (0.48 mg g^{-1}) which was on par with T_8 (0.41 mg g^{-1}), T_9 (0.37 mg g^{-1}) and T_{10} (0.37 mg g^{-1}).

4.3.5 Effect of Treatments on Total Chlorophyll (mg g^{-1}) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on the effect of treatments on total leaf chlorophyll content is presented in Table 26, taken at 3 MAP. The total chlorophyll content was significantly higher (1.09 mg g^{-1}) in treatment T_7 (*Azospirillum* 15 per cent) which was on par with T_1 (1.07 mg g^{-1}), T_5 (1.05 mg g^{-1}), T_6 (1.03 mg g^{-1}), T_9 (1.03 mg g^{-1}) and T_{10} (1.03 mg g^{-1}) in 2 node cuttings.

It was observed that there was no significant difference among treatments on total chlorophyll content in 3 node orthotropic cuttings of black pepper (Table 27).

4.4 EFFECT OF TREATMENTS ON BIOLOGICAL PROPERTIES OF POTTING MIXTURE BEFORE AND AFTER THE EXPERIMENT.

4.4.1 Effect of Treatments on AMF Colonization (Percentage) and Spore Count (no. of spores g^{-1} of soil) of the Orthotropic Cuttings of Black Pepper (*P. nigrum* L.)

The data on the effect of treatments on AMF colonization and spore count taken before and after the experiment revealed that the plants treated with AMF (T_6) alone recorded 46.33 per cent colonization and spore count of 5 g^{-1} of soil in 2 node cuttings and in the case of 3 node cuttings the colonization was 50 per cent

Table 26. Effect of treatments on physiological characters of 2 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Total dry matter production (g)	Stomatal conductance (cm s^{-1})	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
T_1	5.33	0.05	0.63	0.42	1.07
T_2	3.13	0.05	0.56	0.24	0.72
T_3	1.96	0.10	0.52	0.27	0.76
T_4	2.21	0.10	0.61	0.34	0.90
T_5	1.85	0.02	0.63	0.47	1.05
T_6	4.38	0.05	0.61	0.38	1.03
T_7	2.90	0.05	0.63	0.46	1.09
T_8	6.22	0.06	0.58	0.48	0.91
T_9	2.66	0.10	0.61	0.48	1.03
T_{10}	4.19	0.04	0.59	0.34	1.03
CD (0.05)	1.38	NS	0.006	0.009	0.15

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

Table 27. Effect of treatments on physiological characters of 3 node orthotropic cuttings of black pepper (*Piper nigrum* L.)

Treatments	Total dry matter production (g)	Stomatal conductance (cm S^{-1})	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
T_1	5.21	0.06	0.62	0.48	0.84
T_2	4.00	0.04	0.58	0.27	0.83
T_3	1.90	0.05	0.53	0.26	0.80
T_4	2.10	0.07	0.59	0.28	0.87
T_5	2.30	0.08	0.61	0.28	0.88
T_6	2.53	0.07	0.61	0.30	0.90
T_7	3.20	0.03	0.58	0.22	0.87
T_8	4.62	0.05	0.63	0.41	1.36
T_9	3.87	0.07	0.59	0.37	0.92
T_{10}	3.10	0.08	0.63	0.37	1.00
CD (0.05)	1.19	NS	0.003	0.12	NS

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

and spore count was 6 g^{-1} of soil. Initially there was no spore present in the potting mixture and the AMF colonization in roots of the cutting occurred only after the inoculation of the culture.

4.4.2 Effect of Treatments on Total Bacterial Population in the Potting Mixture before and after the Experiment.

Effects of treatments on total microbial population (bacterial, fungal and actinomycetes) of potting mixture are presented in Table 28. The initial bacterial population in the potting mixture was $2x10^6$. Due to the application of microbial inoculants the microbial population in the potting mixture increased after the experimental period (Plate. 10). In 2 node cuttings, the bacterial population was found highest (186.00 x 10^6) in treatment T_1 (IBA 500 ppm) which was on par with T_2 (171.33 x 10^6), T_6 (124.67 x 10^6), T_7 (182.22 x 10^6)and T_8 (152.00 x 10^6).The lowest value (17.78 x 10^6) was recorded in T_{10} (Absolute Control).

The bacterial population in 3 node cuttings (Table 29) was found highest (141.67 $x10^6$) in treatment T_8 (*Pseudomonas* 15 per cent) which was on par with all other treatments except T_4 (77.55 $x10^6$), T_6 (65.33 $x10^6$) and T_{10} (25.33 $x10^6$).The lowest value was recorded in T_{10} (Absolute Control) (Fig.8, Plate 11).

4.4.3 Effect of Treatments on Total Fungal Population in the Potting Mixture Before and After the Experiment.

The total fungal population in potting mixture before the experiment was $3x10^3$. After the experiment, the treatment T_6 (AMF) recorded the fungal load of 13.55 $x10^3$, which was found to be significantly superior in 2 node cuttings (Table 28). This was followed by T_1 (11.78 $x10^3$) and T_8 (9.33 $x10^3$).The lowest value (3.11 $x10^3$) was recorded by T_7 (*Azospirillum* 15 per cent) (Fig.7).

In 3 node cuttings, the treatment T_1 (IBA 500 ppm) recorded the fungal load of 13.67 $x10^3$, which was found to be significantly superior (Table 29). This was followed by T_5 (9.78 $x10^3$), T_6 (13.11 $x10^3$) and T_8 (10.43 $x10^3$).The lowest value (3.61 $x10^3$) was recorded by T_7 (*Azospirillum* 15 per cent) (Fig.8).

Table 28. Effect of treatments on biological properties of potting mixture before and after experiment in 2 node cuttings

Treatments	Total microbial population					
	Bacteria x (10^6)		Fungi x (10^3)		Actinomycetes x (10^3)	
	Before	After	Before	After	Before	After
T_1	2	186.00	3	11.78	4.5	7.55
T_2	2	171.33	3	6.10	4.5	4.55
T_3	2	102.92	3	5.55	4.5	2.89
T_4	2	79.08	3	6.22	4.5	4.44
T_5	2	80.00	3	5.55	4.5	6.00
T_6	2	124.67	3	13.55	4.5	2.78
T_7	2	182.22	3	3.11	4.5	16.67
T_8	2	152.00	3	9.33	4.5	5.00
T_9	2	83.00	3	4.33	4.5	7.00
T_{10}	2	17.78	3	4.11	4.5	4.22
CD(0.05)	-	79.39	-	4.48	-	5.91

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

Table 29. Effect of treatments on biological properties of potting mixture before and after experiment in 3node cuttings

Treatments	Total microbial population					
	Bacteria x (10^6)		Fungi x (10^3)		Actinomycetes x (10^3)	
	Before	After	Before	After	Before	After
T_1	2	110.77	3	13.67	4.5	5.67
T_2	2	128.00	3	6.66	4.5	3.67
T_3	2	117.50	3	4.33	4.5	3.44
T_4	2	77.55	3	3.55	4.5	3.33
T_5	2	97.33	3	9.78	4.5	5.78
T_6	2	65.33	3	13.11	4.5	2.44
T_7	2	131.67	3	3.61	4.5	13.33
T_8	2	141.67	3	10.43	4.5	8.22
T_9	2	94.33	3	3.99	4.5	8.44
T_{10}	2	25.33	3	4.777	4.5	4.33
CD (0.05)	-	60.02	-	5.30	-	6.28

T_1 (IBA 500 ppm)
T_2 (IBA1000 ppm)
T_3 (Common Sugar solution 1%)
T_4 (Common Sugar solution 2%)
T_5 (Common Sugar solution 3%)
T_6 (Arbuscular Mycorrhizal Fungi)
T_7 (Azospirillum 15%)
T_8 (Pseudomonas 15%)
T_9 (Control)
T_{10} (Absolute control)

4.4.4 Effect of Treatments on Total Actinomycetes Population in the Potting Mixture before and after the Experiment.

The total actinomycetes population in potting mixture before the experiment was 4.5 x 10^3. The highest actinomycetes population (16.67 x 10^3), in potting mixture after the experiment was observed in T_7 (*Azospirillum* 15 per cent) in 2 node cuttings and was significantly superior to all other treatments. The treatment T_6 (AMF) recorded the lowest value (2.78 x 10^3).The data is shown in the table 28, Fig.7.

The highest actinomycetes population (13.33 x 10^3) in 3 node cuttings was observed in T_7 (*Azospirillum* 15 per cent) which was on par with T_8 (8.22 x 10^3) and T_9 (8.44 x 10^3). The treatment T_6 (AMF) recorded the lowest value (2.44 x 10^3). The data is presented in the Table 29, Fig.8.

4.5 INCIDENCE OF PEST AND DISEASES

No pest and disease incidence was noticed during the study period.

DISCUSSION

5. DISCUSSION

The results of the investigation "Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram, during 2012 – 2014 are discussed below.

5.1 GROWTH CHARACTERS

A significant effect was exhibited by different treatments on number of days taken for sprouting and 50 per cent sprouting of the cuttings after planting. The minimum number of days (22.50) for sprouting was recorded by T_7 (*Azospirillum* 15 per cent) in 2 node cuttings (Fig.1). Subramanian *et al*., (2003) inferred that *Azospirillum* application help to fix nitrogen in soil and also produce growth promoting substances like GA and cytokinin and antifungal substances, which could be the reason for enhanced vegetative growth of the cuttings under the treatment T_7. In the case of 3 node cuttings, the minimum number of days (24.78) for sprouting was recorded by T_1 (IBA 500 ppm) and T_2 (IBA 1000 ppm). This result derives support from the works of Kumari *et al*. (2010) who observed that hormonal treatment (IBA) significantly increased sprouting in *J. curcas* cuttings. This is because auxins positively influence cell enlargement, bud formation and root initiation and also promote the production of other hormone in conjunction with cytokinins (Osborne and McManus, 2005). Similar trend was noticed by Srivastava *et al*. (2008) in kiwi fruit leafless cuttings and Thota (2012) in fig (*F. caria*) cuttings.

The treatment T_7 (*Azospirillum* 15 per cent) also recorded minimum days for 50per cent sprouting (21.67) in 2 node cuttings. This could be due to the ability of *Azospirillum* inoculated plants to absorb nutrients from soil solution at faster rate than uninoculated plants (Okon, 1985). The minimum number of days for 50per cent sprouting (23.67) was recorded by T_1 (IBA 500 ppm) in 3 node cuttings (Fig. 2). This result is in conformity with that of Kumari *et al*. (2010) in

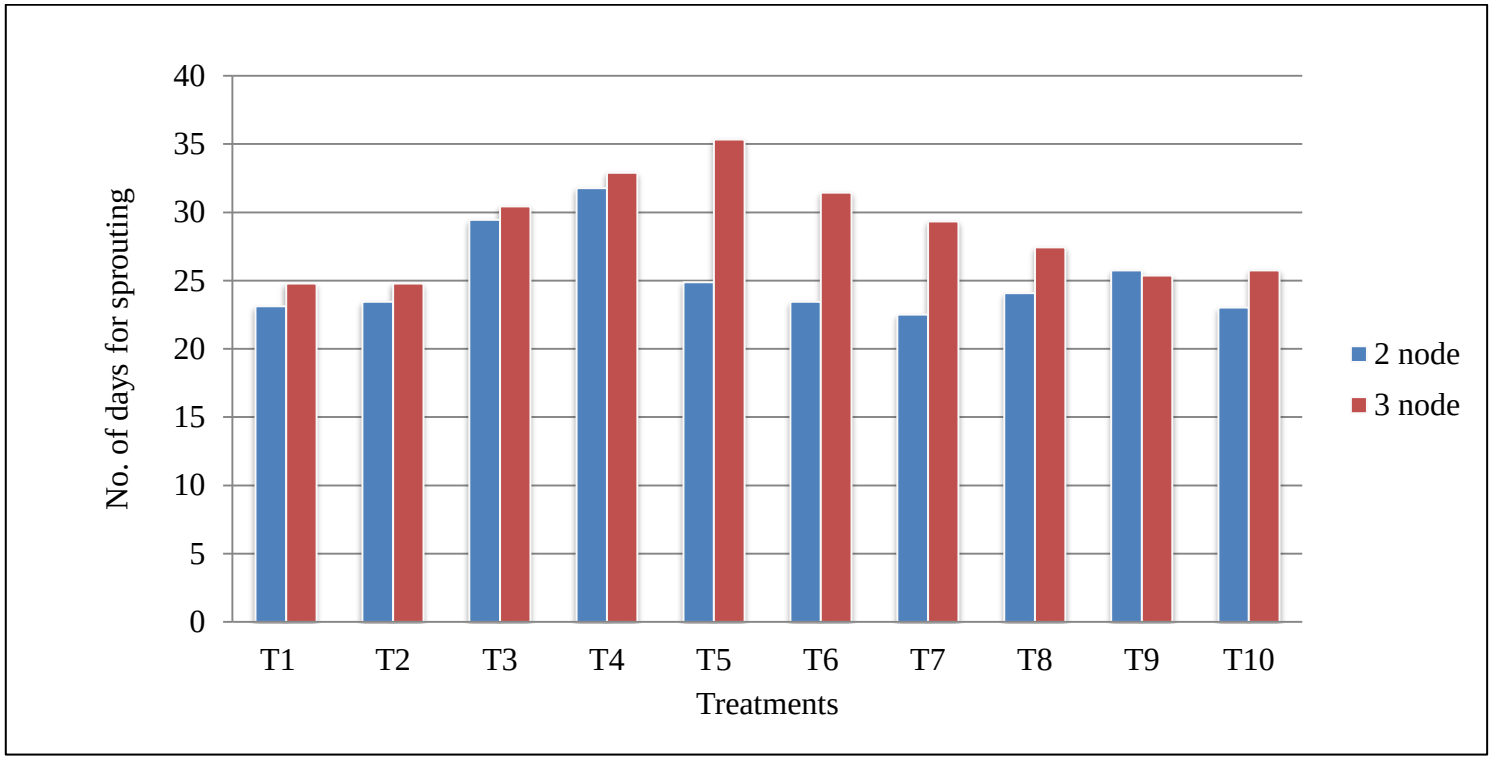


Fig.1. Effect of treatments on the number of days for sprouting in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

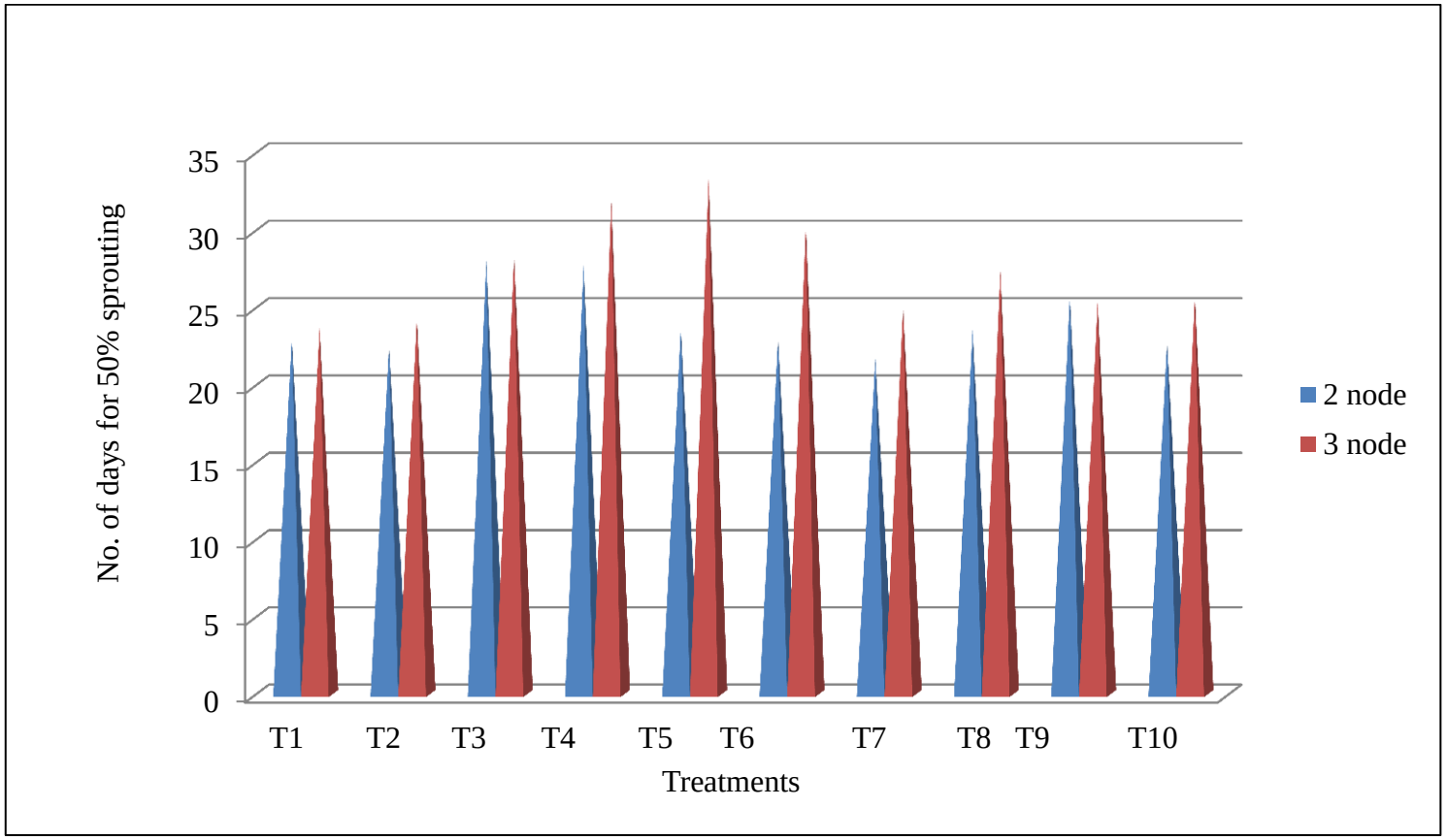


Fig.2. Effect of treatments on the days for 50% sprouting in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

J. curcus. According to Srivastava *et al*. (2008), sprouting might also be due to favorable condition in humidity-chamber which thus minimizes the water loss from their surface.

At one month after planting, the treatment T_7 (*Azospirillum* 15 per cent) recorded the maximum height of 16.54 cm in 2 node cuttings. This is in agreement with the earlier findings that *Azospirillum* inoculation could improve ion uptake and contributed to the significant elevation of plant growth (Lin *et a1*., l983; Nath and Korla, 2000 and Molla *et al*., 2001). During 2^{nd} and 3^{rd} MAP, the treatment T_2 (IBA 1000 ppm) recorded the maximum height of 19.61cm and 25.31cm respectively which is supported by earlier findings of Sharma (2009) in *P. integrifolia* hard wood cuttings. The increase in the length of the shoot with the increase in concentration of IBA was also noted by Shivanna *et al*. (2006) in Jeevanthi (*L. reticulate*). There was no significant difference among the treatments on height of sprouted cutting in 3 node cuttings.

Significant difference was observed between treatments in the number of leaves per plant in 2 node cuttings (Table 4). The treatment T_2 (IBA 1000 ppm) registered the highest values (1.55 cm, 2.66 cm and 4.33 cm) at 1^{st}, 2^{nd} and 3^{rd} MAP respectively. In the case of 3 node cuttings, there was no significant difference among the treatments on leaf number at 1 MAP but the treatment T_2 (IBA 1000 ppm) registered the highest value at 2^{nd} and 3^{rd} MAP (2.22 and 3.44 respectively) which was found to be significantly superior to all other treatments. These results are in conformity with the findings of Ingle and Venugopal (2008); Chalapathi *et al*. (2001) in stevia; Wazir, (2014) in *C. japonica* and Sharma (2009) in *P. integrifolia* hard wood cuttings. This may be due to more shoot length observed with same treatment.

At 1MAP, there was no significant difference among the treatments on the length of leaf. At 2^{nd} and 3^{rd} MAP, the treatmentT_7 (*Azospirillum*15 per cent) recorded the highest leaf length (6.54cm and 8.50cm respectively) in 2 node cuttings. This result finds cognizance with earlier work of Sangeeth *et al*. (2008) in black pepper. In 3 node cuttings, the treatments T_2 (IBA 1000 ppm) recorded the highest value (4.32 cm) at the 1^{st} MAP. During 2^{nd} and 3^{rd} MAP, T_8

(*Pseudomons* 15 per cent) recorded the highest value (6.04 cm and 8.64cm respectively).

At 1st MAP, there was no significant difference among the treatments on the breadth of leaf. At 2nd and 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values (4.42 cm and 8.50 cm respectively). It was found that the treatment T_7 (*Azospirillum* 15 per cent) significantly influenced the breadth of leaf throughout the observation period in 3 node cutting. At 1st, 2nd and 3rd MAP, T_7 exhibited highest values for breadth of leaf (2.80 cm, 3.92 cm and 5.23 cm respectively)

The treatment T_2 (IBA 1000 ppm) recorded the highest value for petiole length throughout the growing period except 2nd MAP in 2 node cuttings. At 1st MAP, T_2 registered 2.21cm and during 3rd MAP T_2 recorded the highest petiole length of 4.34cm. In the case of 3 node cuttings, during 1st, 2nd and 3rd MAP, T_2 recorded the highest values for petiole length (2.49 cm, 4.31cm and 4.75 cm respectively).

Significant difference was observed between treatments in the internodal length at 1st and 2nd month after planting, the treatment T_2 (IBA 1000 ppm) recorded the highest values of 2.49 cm and 4.03 cm respectively. There was significant difference among the treatments on internodal length in 3 node orthotropic cuttings at 1 MAP. The treatment T_1 (IBA 500 ppm) recorded the highest value (2.98) and was on par with T_2 (IBA 500 ppm) which recorded 2.90 cm. However, no significant difference was observed between treatments in the internodal length at 2nd and 3rd month after planting. The possible reason for such increase may be due to the activation of shoot growth which probably increased the internodal length.

It was found that there was no significant difference among treatments on the number of branches in both 2 and 3 node cuttings.

The data on leaf area revealed that in 2 node cuttings, there was no significant difference among treatments on the leaf area during 1MAP. However there was significant difference among treatments on the leaf area during 2nd and 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values

(17.62 cm^2 and 30.26 cm^2). In 3 node cuttings, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values throughout the observation period (7.24cm^2, 13.91 cm^2 and 26.02 cm^2 respectively).This result finds resonance in the works of Panwar and Singh, (2000) in rice and Mahentesh *et al*. (2002) in chilli. It is thus evident that application of *Azospirillum* promotes leaf area.

The treatment T_2 (IBA 1000 ppm) was found to be significantly different from all other treatments on number of roots produced in both 2 and 3 node cuttings at 3 MAP (Fig. 3, Plate 5 and 6). In 2 node cuttings, T_2 recorded the highest value of 7.27 and in 3 node cuttings the number of roots recorded by T_2 was 6.83.This is in agreement with the findings of Pillai *et al*. (1982). It has been widely documented that auxins promote adventitious root development of stem cuttings, through their ability to promote the initiation of lateral root primordia and to enhance transport of carbohydrates to the cutting base (Leakey *et al*., 1982; Hartmann *et al*., 1990).

There was significant difference among the treatments on the length of longest root (Fig.4). In 2 node cuttings, the length of root was significantly higher (14.44 cm) in T_8 (*Pseudomonas* 15 per cent).This was supported by the findings of Raji and Lekha (2003) in rice. The length of root was significantly higher (17.24 cm) in the treatment T_1 (IBA 500 ppm), in the case of 3 node cuttings. The increase in length of the roots might be due to an early initiation of roots at higher concentrations of IBA and more utilization of the food materials due to early formation of the roots. Similar trend has been reported by Chalapathi *et al*. (2001) and Debnath (2008) in stevia. It is also attributed to the action of auxin activity which might have caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings and resulted in accelerated cell elongation and cell division in suitable environment (Singh *et al*., 2003).

The volume of root showed significant difference among the treatments. The highest value was recorded by T_2 (IBA 1000 ppm) in both 2 and 3 node cuttings. In 2 node cuttings, T_2 recorded the highest root volume (3.55 cm^3) and in 3 node cuttings the highest root volume recorded by the treatment T_2 was 1.90

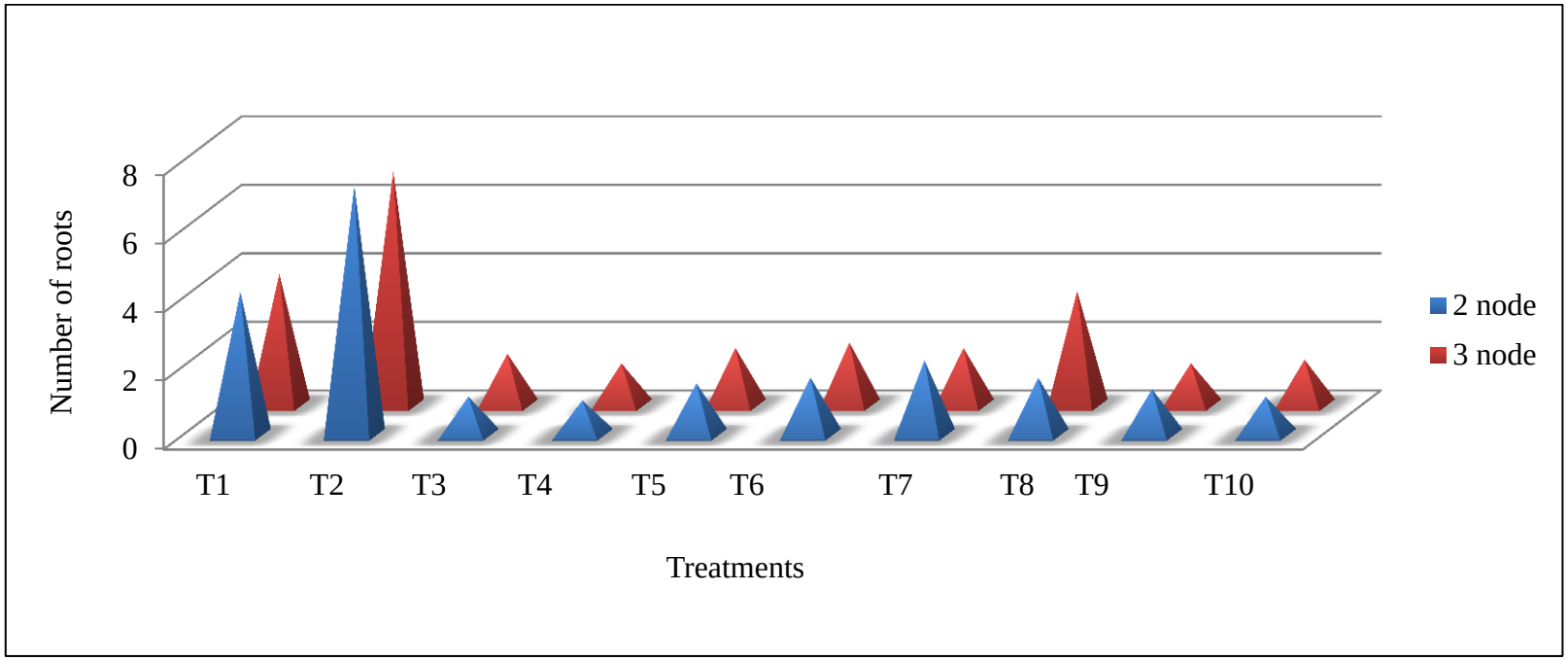


Fig.3. Effect of treatments on the number of roots in orthotropic cuttings of black pepper (*Piper nigrum* L.)

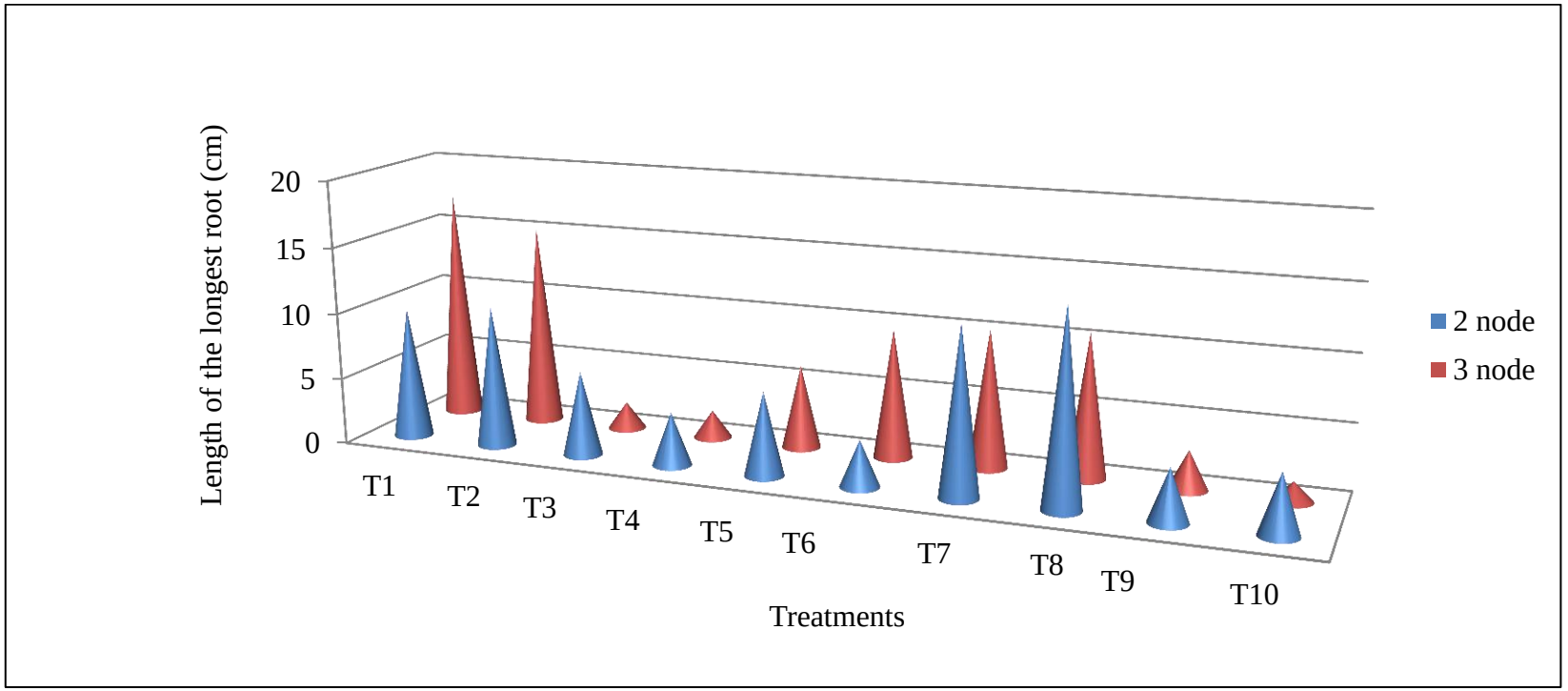


Fig.4. Effect of treatments on length of the longest root (cm) in the orthotropic cuttings of black pepper (*Piper nigrum* L.)

Plate 5. Growth of root and shoot in T_2 treated 2 node cutting.

Plate 6. Growth of root and shoot in T_2 treated 3 node cutting.

cm^3. It may be due to the increase in number of roots which resulted by this treatment.

The percentage success in establishment showed significant influence among treatments at 3 months after planting (Fig. 5). The highest value (77.77 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) and was found to be on par with T_1 (IBA 500 ppm) and T_2 (IBA 1000 ppm) with 66.66 per cent success in establishment. In the case of 3 node cuttings the highest value (88.89 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) and was found to be on par with T_1 (IBA 500 ppm) with 77.77 per cent success in establishment. The pooled analysis of the data revealed that the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest percentage success (83.33 per cent) in establishment of cuttings followed by T_1 (72.23 per cent) and T_2 (66.66 per cent) (Fig.6, Plate 7). The promotion of plant growth by *Azospirillum* has been reported in field and nursery plants, resulting in significant changes in several characteristics of plants. The ability of *Azospirillum* to attain significant populations on the host root system has been shown to be a prerequisite for their beneficial effects on plant growth (Bashan, 1986). Govindan and Chandy (1985) reported that bacterial inoculation favoured the production of more healthy and strong roots, a trait desirable for better establishment of rooted pepper cuttings than IBA. Enhanced mineral uptake (Nair and Chandran, 2001) and phyto- hormone producing ability of *Azospirillum* (Bashan and Levanony, 1987) might have enhanced growth and establishment of rooted cuttings (Table 23).

The promontory effect of IBA on rooting and shooting of stem cuttings has been reported by several workers. Chalapathi *et al*. (2001) reported superior survival percentage and sprouting percentage in stevia cuttings treated with IBA 500 ppm. Singh (2012) reported significantly superior establishment hardwood cuttings of Louise Wathen variety of Bougainvillea treated when with 1000 ppm IBA.

Plant growth promoting rhizobacteria also might have contributed significantly towards plant establishment. This is in accordance with the findings of Suslow and Schroth (1982).

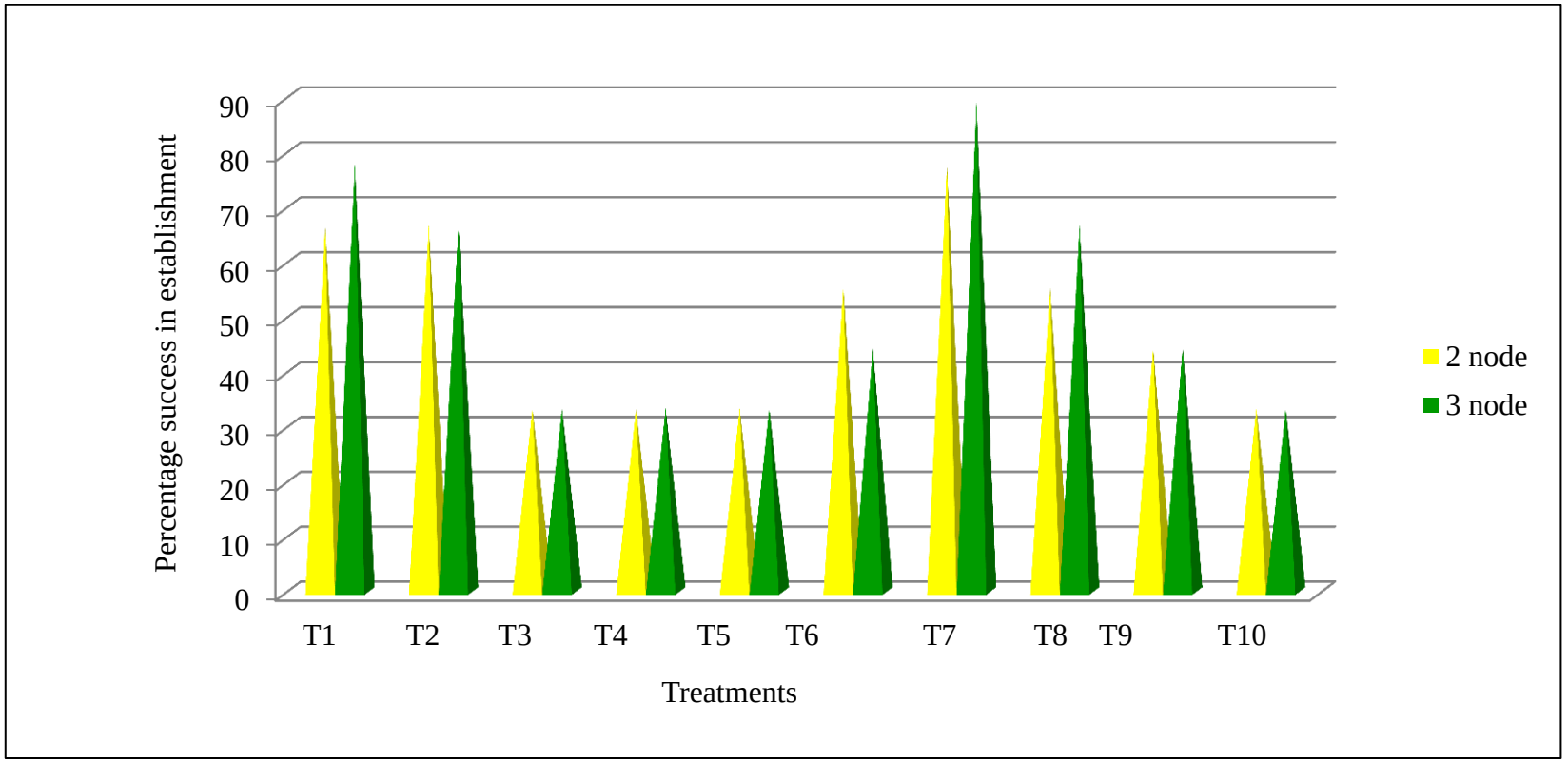


Fig.5. Effect of treatments on percentage success in establishment in orthotropic cuttings of black pepper (*Piper nigrum* L.)

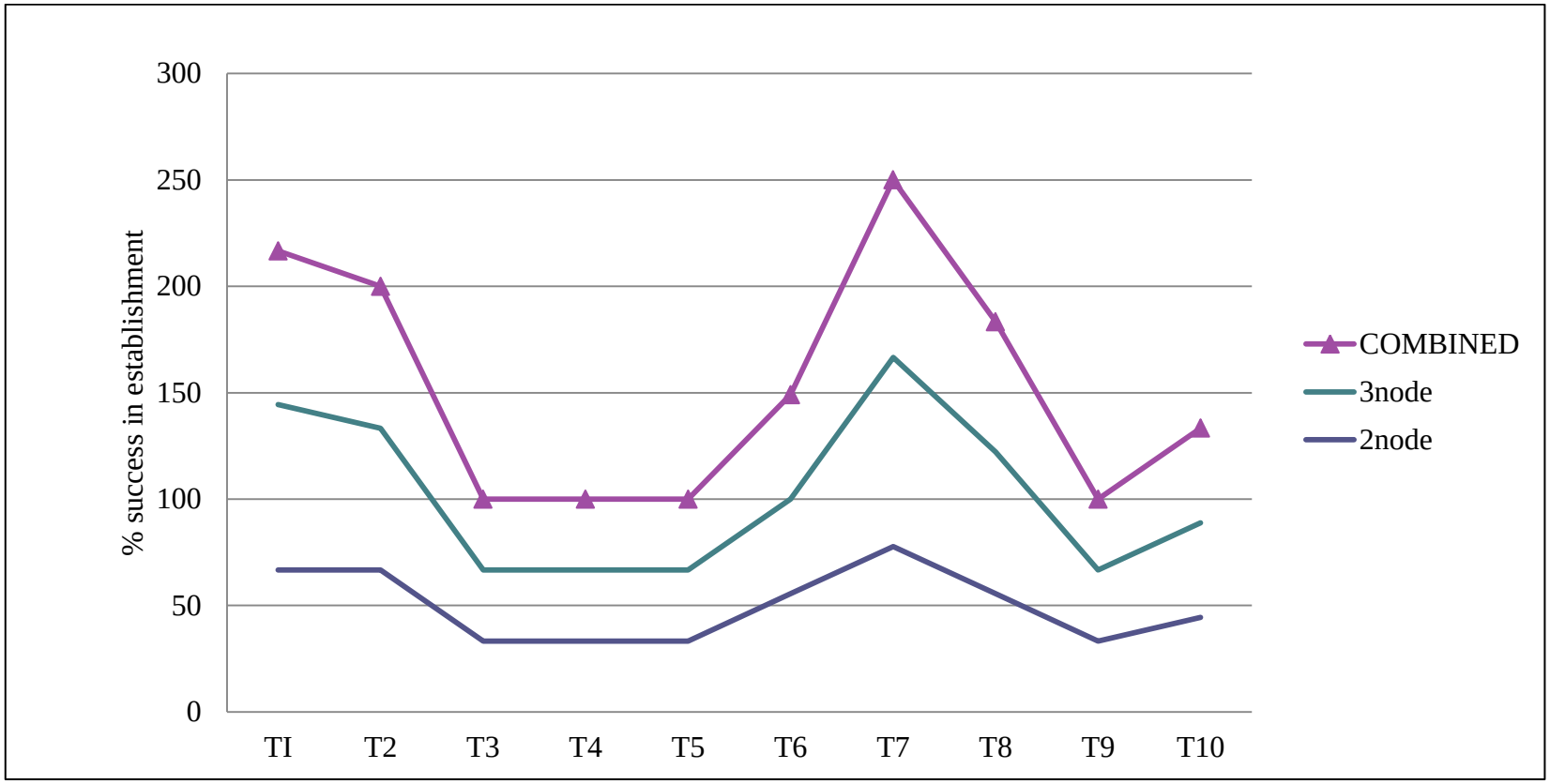


Fig.6. Effect of treatments on the percentage success in establishment of 2 node cuttings, 3 node cuttings and their combined effect.

5.2 ANATOMICAL CHARACTERS

The data on leaf cuticle thickness revealed that there was significant influence among treatments on the leaf cuticle thickness. The leaf cuticle thickness was the highest (1.71 µm) in T_5 (Common Sugar Solution 3per cent) followed by T_6 (AMF) with leaf cuticle thickness of 1.54 µm. In the case of 3 node cuttings, the leaf cuticle thickness was highest (1.74 µm) in T_6 (AMF).

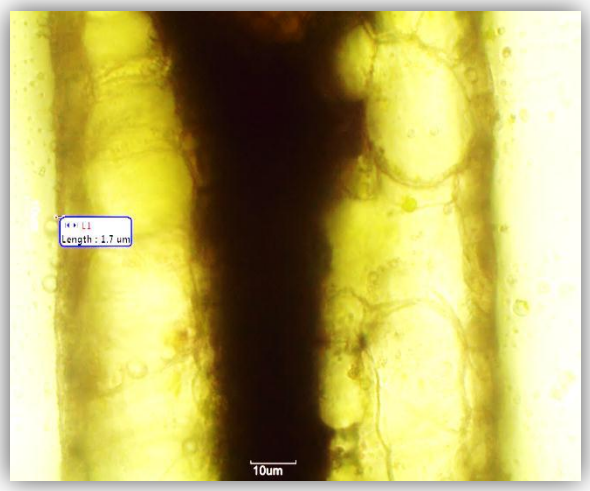
In 2 node cuttings, the highest number of vascular bundles in leaf (6.28) was recorded by T_2 (IBA 1000 ppm) which was on par with T_5 (Common Sugar Solution 3per cent) and T_6 (AMF) of value 5.00 (Plate 8). In the case of 3 node cuttings, the highest value (7.50) was recorded by T_1 (IBA 500 ppm) which was significantly different from other treatments (Plate 9).

There was significant difference among the treatments on number of vascular bundles in root of 2 node cuttings (Table 24). The highest value (7.89) was recorded by T_1 (IBA 500 ppm) and was significant different from other treatments. The lowest value (4.44) was recorded by T_{10} (Absolute Control).

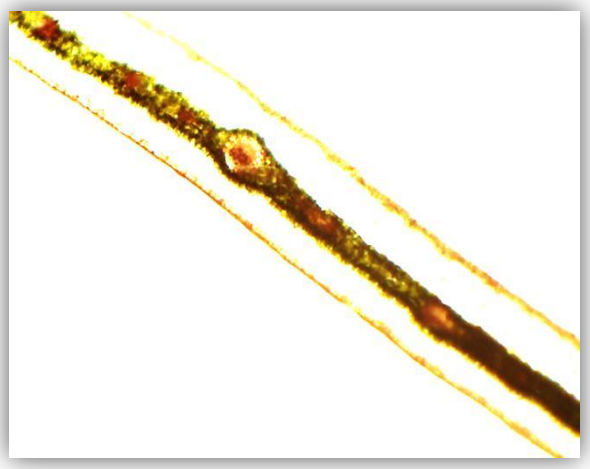
The highest value (7.39) for vascular bundles in root was recorded by T_1 (IBA 500 ppm) in 3 node cuttings and was found to be on par with T_5 (6.77) and T_6 (6.33). The lowest value (4.66) was recorded by T_9 (Control).The data is presented in the Table 25.

The stomatal frequency showed significant difference among the treatments both in 2 node and 3 node cuttings. The stomatal frequency of the plant was significantly higher in T_7 (*Azospirillum* 15 per cent) in 2 node cuttings (285.89). In the case of 3 node cuttings the stomatal frequency of the plant was significantly higher in T_7 (207.10) and was on par with T_3 (205.94).

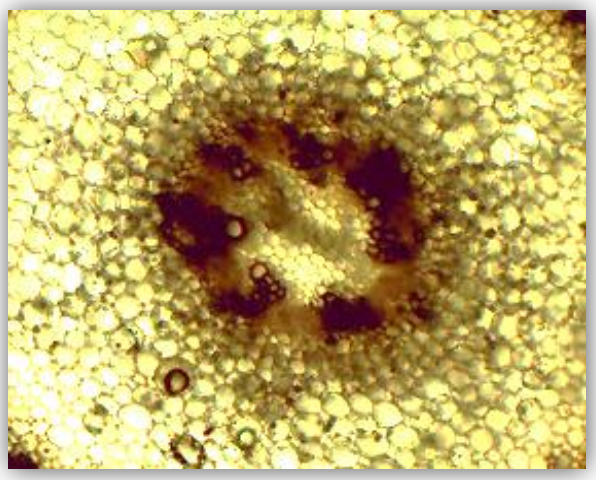
The above results were supported by the findings of Nirmalatha (2009) in Karthuri turmeric. The application of growth regulators and bio inoculants with their fast release of growth hormones, nutrient availability and rich nutrient use efficiency produced healthy plants, with healthy leaves and roots, which might have contributed to better anatomical characters.



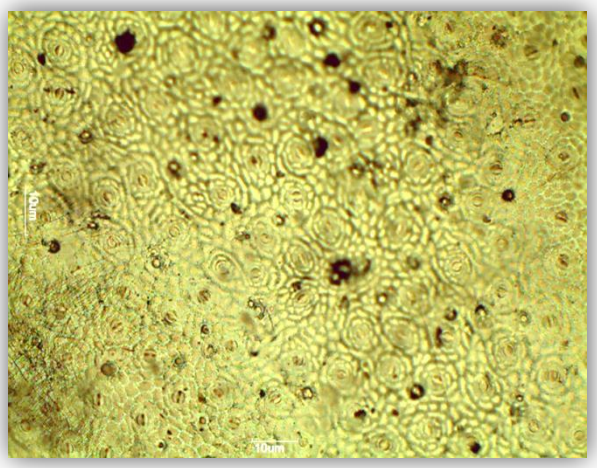
Leaf cuticle thickness
(T_5 – Common Sugar Solution 3%)



No. of vascular bundles in root
(T_1 – IBA 500 ppm)

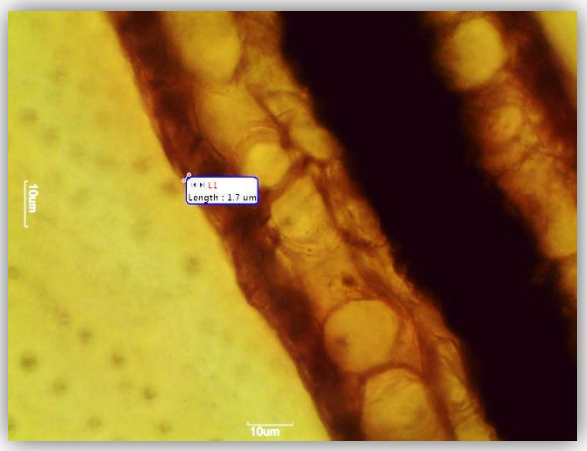


No. of vascular bundles in leaf
(T_2 – IBA 1000 ppm)

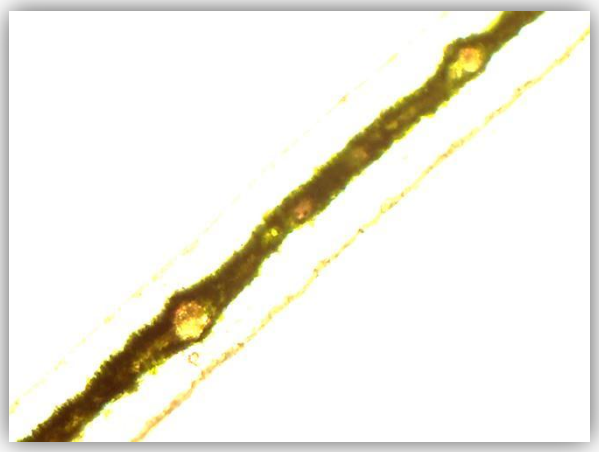


Stomatal count
(T_7 – *Azospirillum* 15%)

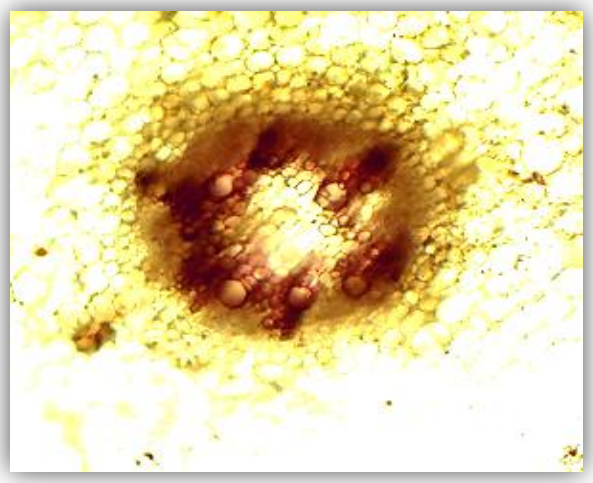
Plate 8. Effect of treatments on anatomical characters in 2 node rooted cuttings of black pepper



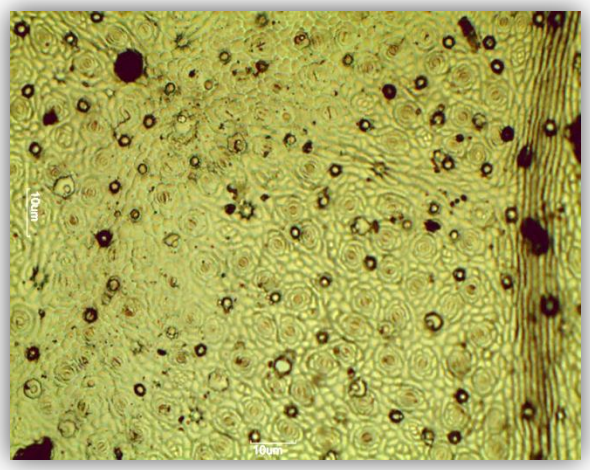
Leaf cuticle thickness (T_6 - AMF)



No. of vascular bundles in leaf
(T_2 – IBA 1000 ppm)



No. of vascular bundles in root
(T_1 – IBA 500 ppm)



Stomatal count
(T_7 – *Azospirillum*15%)

Plate 9. Effect of treatments on anatomical characters in 3 node rooted orthotropic cuttings of black pepper

5.3 PHYSIOLOGICAL CHARACTERS

Data revealed that in 2 node cuttings, the total dry matter production varied significantly among the treatments. At 3 MAP the highest total dry matter production (6.22 g plant^{-1}) was registered by T_8 (*Pseudomonas* 15 per cent) in 2 node cuttings. The increased dry matter production could be attributed to the increased plant height, which is one of the main contributing factors for shoot biomass. The increased vegetative growth is the resultant of the increased nutrient uptake by plant as a result of enhanced microbial activity observed in this treatment. This is in agreement with the finding of Burr *et al*. (1978) who observed that, *P. fluorescens* has the ability to produce plant growth promoting substances and some secondary metabolites which enhance nutrient uptake and plant growth. Saju *et al*. (2003) also observed that *P. fluorescens* and *T. harzianum* are compatible, synergistic and enhance biomass production and disease suppression when applied to soil. In the case of 3 node cuttings, the highest total dry matter production (5.21 g plant^{-1}) was registered by T_1 (IBA 500 ppm) and was on par with T_8 (4.62 g plant^{-1}) at 3 MAP. This result is supported by the findings Benson *et al*. (2014) in *N. zeylanica.*

The data on effect of treatments on stomatal conductance revealed that there was no significant difference among treatments on stomatal conductance in both 2 and 3 node cuttings.

The data on the effect of treatments on leaf chlorophyll a content revealed that, there was significant difference among treatments on chlorophyll a content in 2 node cuttings. The treatment T_5 (Common Sugar Solution 3per cent) recorded the highest value (0.63 mg g^{-1}). In 3 node cuttings also, there was significant difference among treatments on chlorophyll a content. The treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.63 mg g^{-1}). The application of microbial inoculants produced healthy plants with healthy green leaves which might have increased the chlorophyll content in plants. This is supported by Nihad (2005) in *Plumbago rosea*.

The data on the effect of treatments on leaf chlorophyll b content revealed that there was significant difference among treatments on chlorophyll b content in

2 node cuttings. The treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.48 mg g^{-1}). This result is supported by the work of Robinson *et al*. (2004) in sunflower. The treatment T_1 (IBA 500 ppm) recorded the highest value (0.48 mg g^{-1}) of chlorophyll b content in 3 node cuttings. The result of Kaur *et al*. (2002) in grapevine stem cuttings was in agreement with the above finding. Tanwar *et al*. (2013) also reported that single inoculation with *T. viride* increased photosynthetic rate by increasing plant chlorophyll content, both a and b.

The data on the effect of treatments on total leaf chlorophyll content revealed that the total chlorophyll content was significantly higher (1.09 mg g^{-1}) in treatment T_7 (*Azospirillum* 15 per cent) in 2 node cutngs. This is in conformity with finding of Ramakrishan and Selvakumar (2012).They observed that *Azospirillum* treated tomato had the highest chlorophyll content. Some scientists also observed that the biofertilizers significantly improved the chlorophyll content in crops (Patidar and Mali, 2004 in sorghum; Selvakumar and Thamizhiniyan, 2011 in chilli and Selvakumar *et al*., 2012 in black gram). There was no significant difference among treatments on total chlorophyll content in 3 node orthotropic cuttings.

5.4 BIOLOGICAL PROPERTIES OF POTTING MIXTURE BEFORE AND AFTER THE EXPERIMENT

The data on the effect of treatments on AMF colonization and spore count taken before and after the experiment revealed that the plants treated with AMF (T_6) alone recorded 46.33 per cent colonization and spore count of 5 g^{-1} of soil) in 2 node cuttings and in the case of 3 node cuttings the colonization was 50 per cent and spore count of 6 g^{-1} of soil). Initially there was no spore present in the potting mixture and the AMF colonization in roots of the cutting occurred only after the inoculation of the culture. Mycorrhizae are highly evolved symbiotic associations formed between soil fungi and plant roots. *G. mosseae*, an AM fungus, forms large asexual chlamydospores at the hyphal tips, usually one per tip, which is highly infective to wide range of plants in a wide range of conditions. It has a high reproduction ability mediated through the production of spores. At maturity, the

spore contents are separated from the attached hypha by a septum or by occlusion with deposits of wall material. Spores are borne singly in soil and also formed in the root cortex or in sporocarps (James and Schenck, 1984). The *G. mosseae* can form mycorrhizal associations with many plant species with significant effects (Xioutang, 1994). The AMF inoculated to crop plants colonize the plant root system and increase the growth and yield of crop plants including pepper (Rao, 1993; Thanuja, 2002, Durgapal *et al*., 2002). Dalpe and Monreal, (2004) reported that the AM associated improvement of plant growth is attributed to various mechanisms such as increased uptake of nutrients and water, production of plant growth promoting substances, tolerance to drought and salinity and resistance to plant pathogens.

Effect of treatments on total microbial population (bacterial, fungal and actinomycetes) of potting mixture are presented in Table 24. The microbes in the potting mixture increased from the initial uninoculated level (Fig. 7 & 8, Plate.10 & 11).

In 2 node cuttings, the bacterial load was found highest (186.00 x 10^6) in treatment T_1 (IBA 500 ppm) and the lowest value (17.78 x 10^6) was recorded in T_{10} (Absolute Control). The bacterial load in 3 node cuttings was found highest (141.67 x10^6) in treatment T_8 (*Pseudomonas* 15 per cent) .The lowest value was recorded in T_{10} (Absolute Control).

The initial value of total fungal load in potting mixture before the experiment was (3 x 10^3). After the experiment, the treatment T_6 (AMF) recorded the fungal load of 13.55 x10^3.In 3 node cuttings, the treatment T_1 (IBA 500 ppm) recorded the fungal load of 13.67 x10^3, which was found to be significantly superior.

The application of microbial inoculants in 2 node cuttings was found to increase actinomycetes load significantly from the initial value (4.5 x 103). The highest actinomycetes load (16.67 x 10^3), in potting mixture after the experiment was observed in T_7 (*Azospirillum* 15 per cent) which was significantly superior to all other treatments.

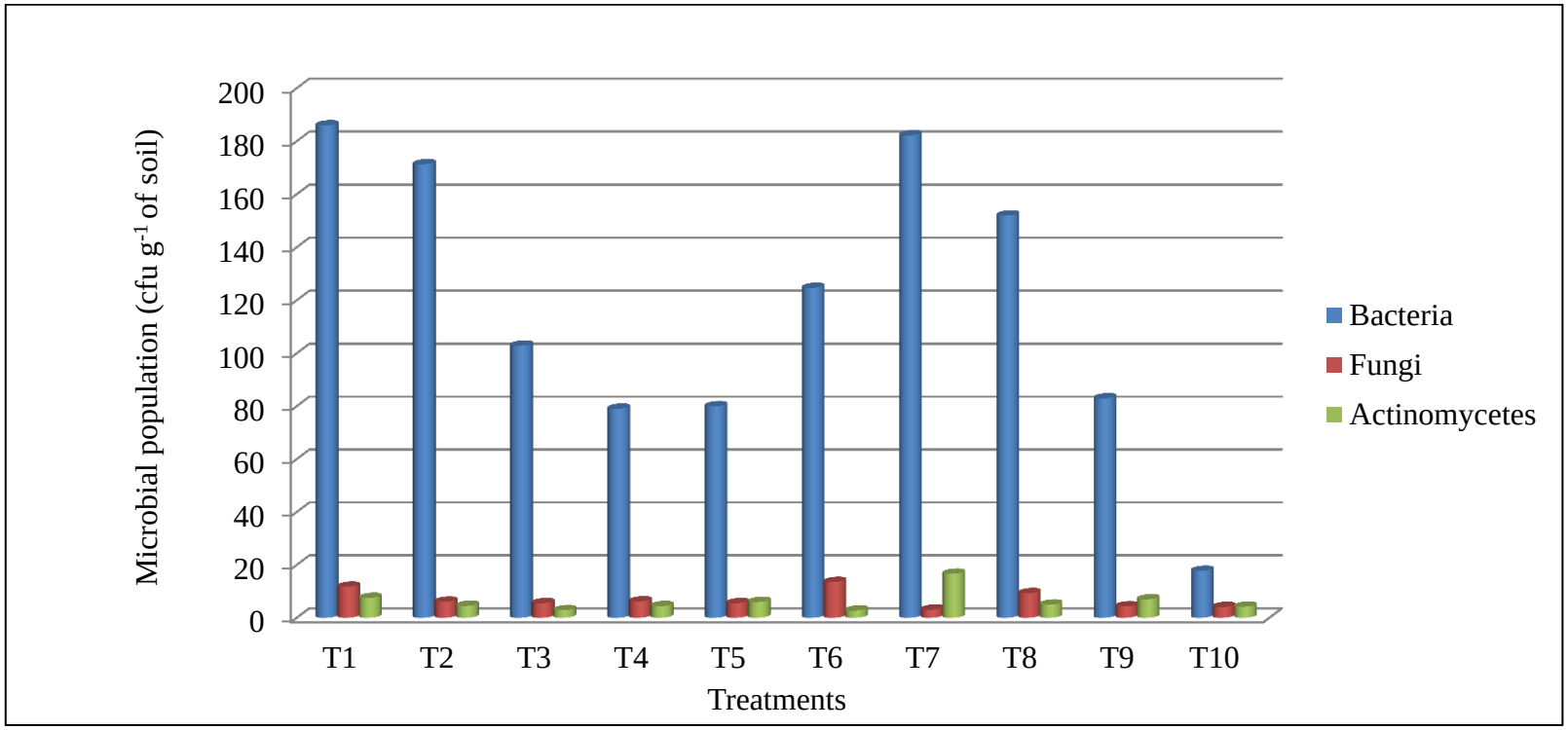


Fig.7. Effect of treatments on microbial population of potting mixture after experiment in 2 node cuttings

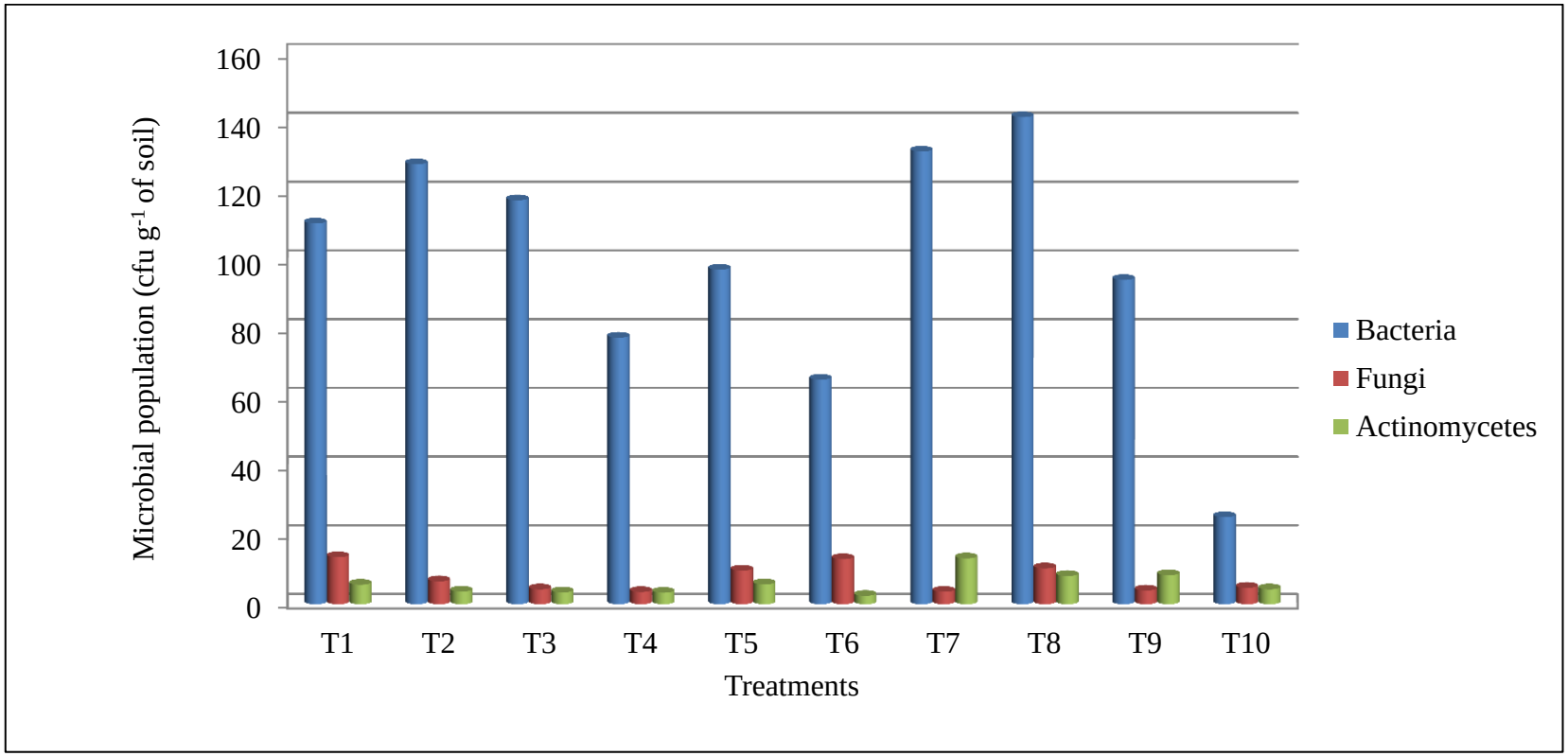
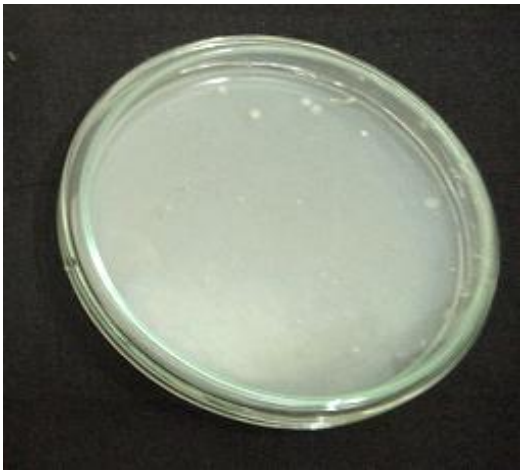
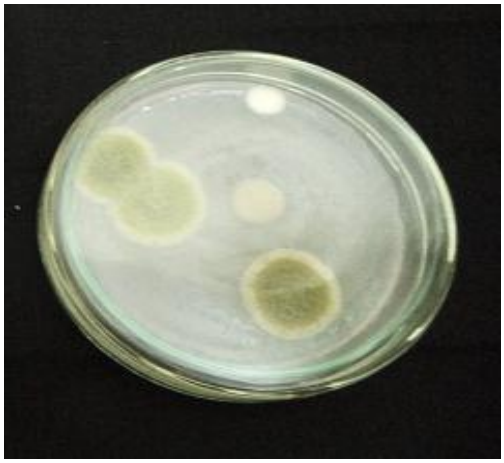


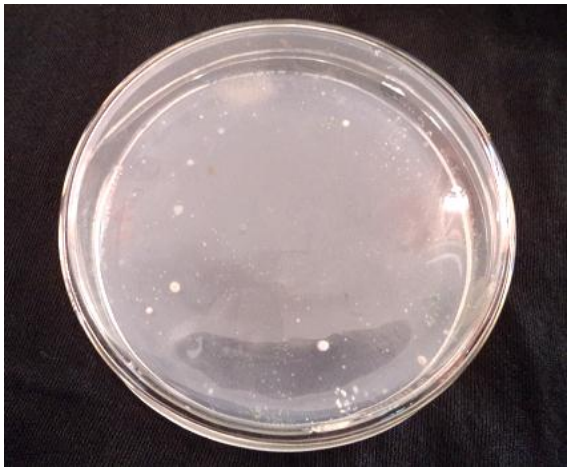
Fig.8.Effect of treatments on microbial population of potting mixture after the experiment in 3node cuttings



Bacteria x 10^6

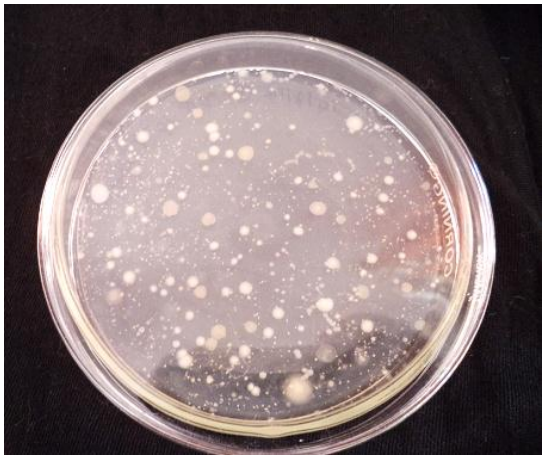


Fungi x 10^3

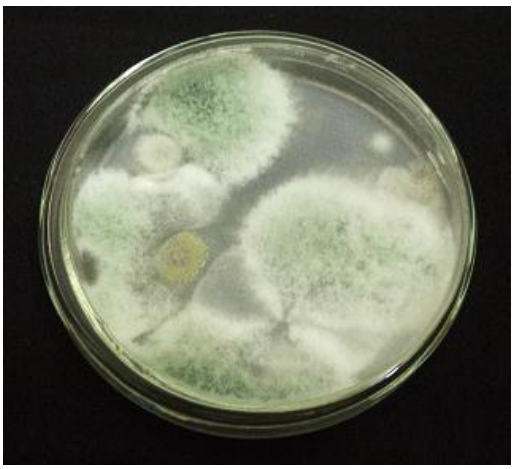


Actinomycetes x 10^3

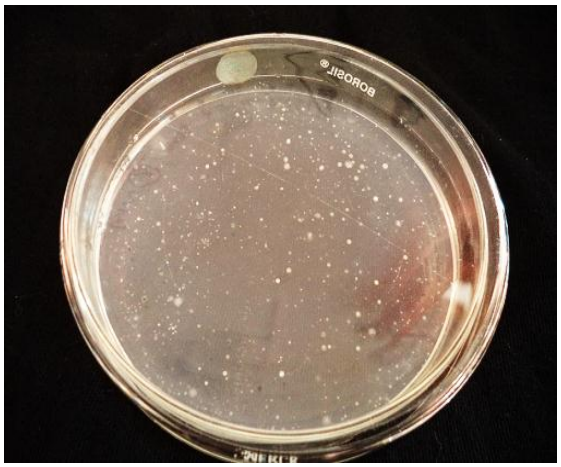
Plate 10. Microbial population in the potting mixture used for planting the cuttings before the experiment



Bacteria x 10^6



Fungi x 10^3



Actinomycetes x 10^3

Plate 11. Microbial population in the potting mixture after the experiment

The highest actinomycetes load (13.33 x 10^3) in 3 node cuttings was observed in T_7 (*Azospirillum* 15 per cent) which was on par with T_8 (8.22 x 10^3) and T_9 (8.44 x 10^3). This result is in corroborance with that of Nirmalatha (2009) in Kasthuri turmeric, who observed that the total microbial load in the rhizosphere increased with application of bio inoculants.

5.5 INCIDENCE OF PEST AND DISEASES

No pest and diseases were observed throughout the period of investigation. Soil solarization might have provided excellent control of soil-borne pathogens with resultant increase in growth, yield, and quality of pepper (Sainamole *et al*. 2003 and Kurt and Emir, 2004). This result also finds support in the works conducted by Thankamani *et al*. (2008) in black pepper and Vilasini (1996) in ginger. The high temperature along with high moisture content might have killed the soil borne mesophilic pathogens. Majority of the pathogens including *P. capsici* and nematode falls in the category of mesophilic microorganisms that require temperatures below 35°C for survival in soil. This was supported by the findings of Hrender Raj *et al*. (1997) in vegetables.

Application of bio control agents such as *Trichoderma* and PGPR Mix –II might have suppressed the soil pathogens. Though they produce antibiotics and cell wall degrading enzymes, mainly act as mycoparasites on other fungi and bring about the disease control (Lewis and Papavizas, 1991). *T. harzianum* is reported to have antagonistic activity against *P. capsici* infecting black pepper (Anandaraj and Sarma, 1994; Ganeshan, 2000). AMF may play a role in protecting plants from soil- borne pathogens either through direct competition for root occupancy or by modifying the microbial community in the rhizosphere (Paulitz and Linderman, 1991).

FUTURE LINE OF WORK

The bioinoculant, *Azospirillum* was found to be superior in enhancing the rooting efficiency and growth of orthotropic shoots of black pepper. Hence, other bio inoculants such as *Azotobacter*, PGPR Mix – I, PGPR Mix – II etc. could also

be tried, standardized and taken as a technology for the production of vigorous, disease free rooted orthotropic cuttings for supplying as quality planting material especially in organic black pepper production.

SUMMARY

6. SUMMARY

Investigation for standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.) was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, during 2012-2014. The experiment was undertaken to standardize techniques for profuse rooting and vigorous growth of orthotropic shoots of black pepper so as to produce quality planting material.

The salient findings of the above study are summarized in this chapter.

1. Early sprouting (22.50 days) was recorded by T_7 (*Azozpirillum* 15 per cent*)* in 2 node cuttings. The treatment T_4 (Common sugar solution 2 per cent) registered the maximum number of days for sprouting (31.78). With 3 node cuttings, the minimum number of days (24.78) for sprouting was recorded by T_1 (IBA 500ppm) and T_2 (IBA 1000ppm). The treatment T_5 (Common sugar solution 3 per cent) registered the maximum number of days (35.33) for sprouting.
2. In 2 node cuttings, the minimum number of days (21.67) for 50 per cent sprouting was recorded by T_7 (*Azospirillum* 15 per cent). In 3 node orthotropic cuttings, the minimum number of days (23.67) for 50 per cent sprouting was recorded by T_1 (IBA 500 ppm).
3. The cuttings supplied with *Azospirillum* 15 per cent (T_7) recorded the highest value (16.54 cm) for height of 2 node sprouted cutting at 1st month after planting (MAP) in 2 node cuttings. At 2nd MAP, the maximum height (19.61cm) was recorded by T_2 (IBA 1000 ppm) and at 3rd MAP the treatment T_2 (IBA 1000 ppm) recorded the highest value (25.31cm).There was no significant difference among the treatments on height of sprouted 3 node cutting.
4. Significant difference was observed between treatments in the number of leaves per plant in 2 node cuttings. The treatment T_2 (IBA 1000 ppm) registered the highest values of 1.55 cm, 2.66 cm and 4.33 cm at 1st, 2nd and 3rd months after planting respectively. There was no significant difference among the treatments on number of leaves in 3 node orthotropic

cuttings at 1 MAP. However, significant difference was observed between treatments in the number of leaves per plant. At 2nd and 3rd MAP, the treatment T_2 (IBA 1000 ppm) registered the highest number of leaves (2.22 and 3.44 respectively) and was found to be significantly superior to all other treatments.

5. It was found that there was no significant difference among the treatments on the length of leaf at the 1st MAP. At 2 MAP the highest value (6.54 cm) was recorded by T_7 (*Azospirillum* 15 per cent).During 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) registered the highest value (8.50 cm) in 2 node cuttings. In the case of 3node cuttings, it was found that the treatments T_2 (IBA 1000 ppm) recorded the highest value (4.32 cm) during 1st MAP. At 2nd MAP, the highest value (6.04 cm) was recorded by T_8 (*Pseudomonas* 15 per cent) and at 3rd MAP, the treatment T_8 (*Pseudomonas* 15 per cent) registered the highest value (8.64cm).
6. It was found that there was no significant difference among the treatments on the breadth of leaf during the 1st MAP in 2 node cuttings. The treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values of 4.42 cm and 8.50cm during 2nd MAP and 3rd MAP respectively. In 3 node cuttings, the treatments T_7 (*Azospirillum* 15 per cent) significantly influenced the breadth of leaf throughout the observation period. The treatment, T_7 recorded the highest value of 2.80cm, 3.92cm and 5.23cm during 1st, 2nd and 3rd MAP.
7. In 2 node orthotropic cuttings, the highest value (2.21cm) for petiole length was recorded by T_2 (IBA 1000 ppm) during 1st MAP. There was no significant difference among the treatments on the petiole length during 2nd MAP. The treatment T_2 (IBA 1000 ppm) recorded the highest value for petiole length (4.34cm) at 3rd MAP.
8. In 3 node cuttings, the treatment T_2 (IBA 1000 ppm) recorded the highest values for petiole length of 2.49cm, 4.31 cm and 4.75cm at 1st, 2nd and 3rd months after planting respectively and was found to be significantly superior to all other treatments.

9. Significant difference was observed between treatments in the internodal length at 1st and 2nd months after planting in 2 node cuttings. The highest value (2.49cm) was recorded by T_2 (IBA 1000 ppm) at 1MAP. During 2nd MAP, T_2 recorded the highest value of 4.03. However at 3rd MAP there was no significant difference among the treatments on the internodal length. There was significant difference among the treatments on intermodal length in 3 node cuttings at 1st MAP. The treatment T_1 (IBA 500 ppm) recorded the highest value (2.98cm). However, no significant difference was observed between treatments in the internodal length during 2nd and 3rd months after planting.
10. It was found that there was no significant difference among treatments on the number of branches in 2 node cuttings. The same trend was noticed in the case of 3 node cuttings.
11. There was no significant difference among treatments on the leaf area during 1stMAP in 2 node cuttings. However there was significant difference among treatments on the leaf area during 2nd MAP and 3rd MAP, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest values of 17.62 cm^2 and 30.26 cm^2 respectively. In 3 node cuttings, the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest value of 7.24cm^2, 13.91 cm^2 and 26.02 cm^2 at 1st, 2nd and 3rd MAP respectively.
12. The treatment T_2 (IBA 1000 ppm) recorded highest number of roots (7.27) in 2 node cuttings and the lowest value was recorded by T_4 (Common Sugar Solution 2 per cent). In 3 node cuttings, with highest value recorded by T_2 (IBA 1000 ppm) was 6.83.Whereas, the lowest value (1.22) was recorded by T_4 (Common Sugar Solution 2 per cent) and T_9 (Control).
13. The length of root was significantly higher (14.44cm) in T_8 (*Pseudomonas* 15 per cent) which was on par with T_7 (12.52cm) at 3 MAP in 2 node cuttings. Here the lowest value (3.47cm) was recorded by the treatment T_6 (AMF).While in 3 node cuttings, the length of root was significantly higher (17.24cm) in T_1 (IBA 500 ppm). The lowest value (2.03cm) was recorded by the treatment T_3 (Common Sugar Solution 1 per cent).

14. The volume of root showed significant difference among the treatments in 2 node cuttings. The highest value (3.55 cm^3) was recorded by T_2 (IBA 500 ppm).The lowest value (0.39 cm^3) was recorded by T_9 (Control).In 3 node cuttings, the highest value (1.90) was recorded by T_2 (IBA 500 ppm).Here, the lowest value (0.67 cm^3) was recorded by T_9 (Control).
15. There was significant influence among treatments on percentage success in establishment of the plants. In 2 node cuttings, the highest value (77.77 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) followed by T_1 (IBA 500 ppm) and T_2 (IBA 1000 ppm) and they recorded 66.66 per cent. The lowest value (33.33 per cent) was recorded by the treatments T_3 (Common Sugar Solution 1 per cent), T_4 (Common Sugar Solution 2 per cent), T_5 (Common Sugar Solution 3 per cent) and T_{10} (Absolute Control).The highest value (88.89 per cent) was recorded by T_7 (*Azospirillum* 15 per cent) in 3 node cuttings followed by T_1 (77.77 per cent).The lowest value was recorded by the treatments T_3, T_4, T_5 and T_{10} (33.33 per cent).The pooled analysis of the data revealed that the treatment T_7 (*Azospirillum* 15 per cent) recorded the highest percentage success (83.33 per cent) in establishment of cuttings followed by T_1 (72.23 per cent) and T_2 (66.66 per cent).
16. In 2 node cuttings, the leaf cuticle thickness was the highest (1.71 µm) in T_5 (Common Sugar Solution 3 per cent) and the lowest (0.94 µm) in T_{10} (Absolute Control). In 3 node cuttings, the leaf cuticle thickness was the highest (1.74 µm) in T_6 (AMF). The lowest value (0.84 µm) was recorded by T_9 (Control).
17. In 2 node cuttings, the highest number of vascular bundles in leaf (6.28) was recorded by T_2 (IBA 1000 ppm) same was in the case of 3 node cuttings, the highest value (7.50) was recorded by T_2 (IBA 1000 ppm) and was significantly different from other treatments (Table 24).
18. There was significant difference among the treatments on number of vascular bundles in root of 2 node cuttings. The highest value (7.89) was recorded by T_1 (IBA 500 ppm) and was significant different from other

treatments. The lowest value (4.44) was recorded by T_{10} (Absolute Control).The highest value (7.39) for vascular bundles in root was recorded by T_1 (IBA 500 ppm) in 3 node cuttings. The lowest value (4.66) was recorded by T_9 (Control).

19. The stomatal frequency of the plant was significantly higher in T_7 (*Azospirillum* 15 per cent) in 2 node cuttings (285.89) and 3 node cuttings (207.10).
20. At 3 MAP the highest total dry matter production (6.22 g plant^{-1}) was registered by T_8 (*Pseudomonas* 15 per cent) in 2 node cuttings. In the case of 3 node cuttings, the highest total dry matter production (5.21 g plant^{-1}) was registered by T_1 (IBA 500 ppm).
21. It was found that there was no significant difference among treatments on stomatal conductance at 3 MAP in both 2 node and 3 node cuttings.
22. There was significant difference among treatments on chlorophyll a content in 2 node cuttings. The treatment T_5 (Common Sugar Solution 3 per cent) recorded the highest value (0.63 mg g^{-1}).In 3 node cuttings also, the treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.63 mg g^{-1}) for chlorophyll a content.
23. There was significant difference among treatments on chlorophyll b content in 2 node cuttings. The treatment T_8 (*Pseudomonas* 15 per cent) recorded the highest value (0.48 mg g^{-1}). In 3 node cuttings, the treatment T_1 (IBA 500 ppm) recorded the highest value (0.48 mg g^{-1}).
24. The total chlorophyll content was significantly higher (1.09 mg g^{-1}) in treatment T_7 (*Azospirillum* 15 per cent) in 2 node cuttings. There was no significant difference among treatments on total chlorophyll content in 3 node orthotropic cuttings of black pepper
25. The plants treated with AMF (T_6) alone recorded 46.33 per cent colonization and spore count was of 5 g^{-1} of soil in 2 node cuttings and in the case of 3 node cuttings the colonization was 50 per cent and spore count was of 6 g^{-1} of soil) compared to the initial value zero.

26. The microbes in the potting mixture increased from the initial bacterial population (2 x 10^6) due to the application of microbial inoculants. In 2 node cuttings, the bacterial population was found highest (186.00 x 10^6) in treatment T_1 (IBA 500 ppm) .The lowest value (17.78 x 10^6) was recorded in T_{10} (Absolute Control).The bacterial population in 3 node cuttings was found highest (141.67 x10^6) in treatment T_8 (*Pseudomonas* 15 per cent) The lowest value was recorded in T_{10} (Absolute Control).
27. The initial value of total fungal population in potting mixture before the experiment was 3 x 10^3.After the experiment, the treatment T_6 (AMF) recorded the fungal population of 13.55 x10^3, which was found to be significantly superior in 2 node cutting. The lowest value (3.11 x10^3) was recorded by T_7 (*Azospirillum* 15 per cent). In 3 node cuttings, the treatment T_1 (IBA 500 ppm) recorded the fungal population of 13.67 x10^3, which was found to be significantly superior. The lowest value (3.61 x10^3) was recorded by T_7 (*Azospirillum* 15 per cent).
28. The effect of microbial inoculants was found significant and increased actinomycetes population from the initial value of 4.5 x 10^3. The highest actinomycetes population (16.67 x 10^3), in potting mixture after the experiment was observed in T_7 (*Azospirillum* 15 per cent) and was significantly superior to all other treatments in 2 node cuttings. The treatment T_6 (AMF) recorded the lowest value (2.78 x 10^3). The highest actinomycetes population (13.33 x 10^3) in 3 node cuttings was observed in T_7 (*Azospirillum* 15 per cent) .The treatment T_6 (AMF) recorded the lowest value (2.44 x 10^3).
29. No pest and diseases were noted.
30. Both 2 node and 3 node cuttings were found to be good for the production of quality and healthy planting material. However, 2 node cuttings can be recommended since the availability of orthotropic shoots in large number is a limitation.

31. The cuttings treated with *Azospirillum* 15 per cent or IBA (1000 ppm and 500 ppm) were the best treatments for better rooting and growth of orthotropic shoots in black pepper (*P. nigrum* L.)
32. Solarized potting mixture with biocontrol agents suppressed the incidence of diseases and helped in the production of healthy planting material on which the vigour, longevity and productivity of pepper depends.

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7. REFERENCES

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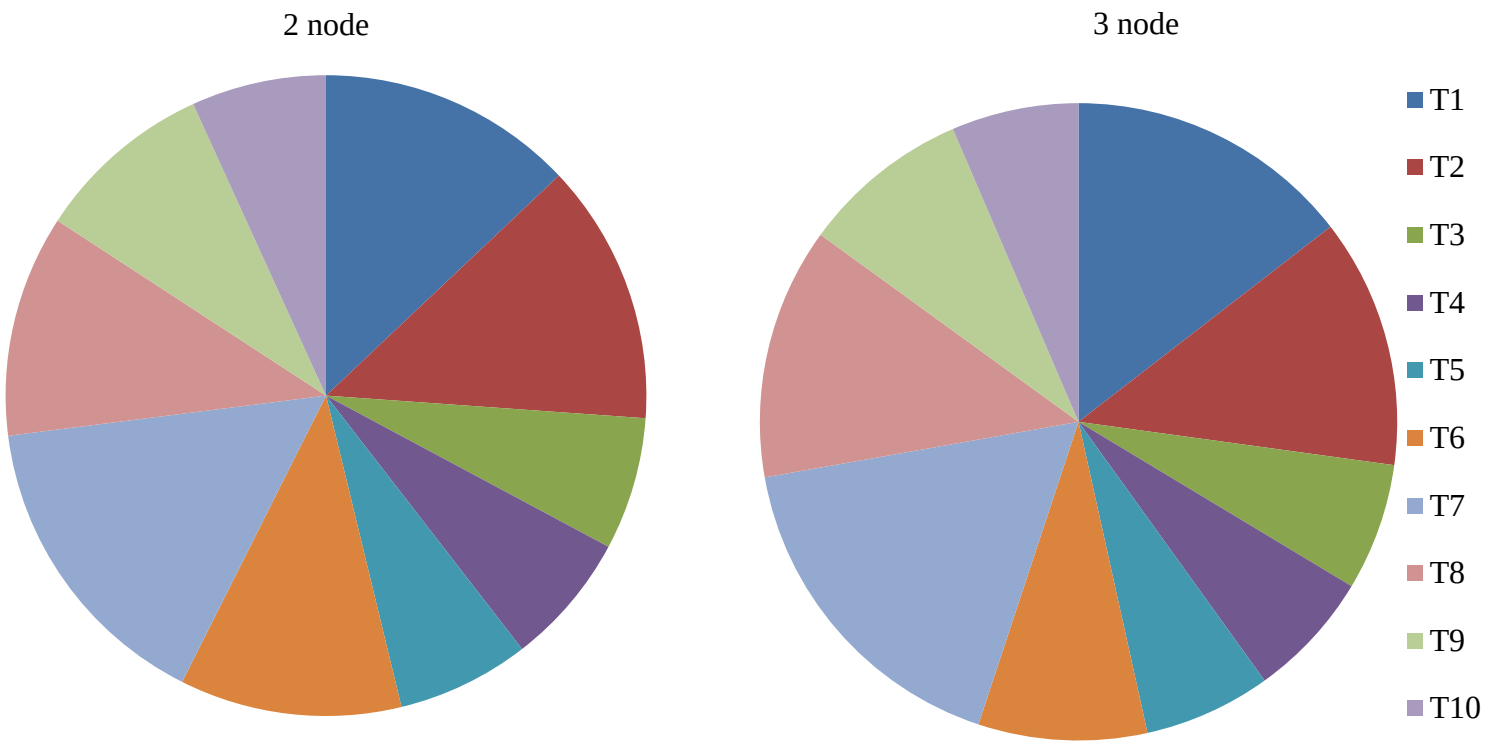
APPENDIX

APPENDIX I



Effect of treatments on the B: C ratio of 2 node and 3 node cuttings

STANDARDIZATION OF TECHNIQUES FOR BETTER ROOTING AND GROWTH OF ORTHOTROPIC SHOOTS IN BLACK PEPPER (*Piper nigrum* L.)

by

NIMISHA MATHEWS

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Abstract of the thesis

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ABSTRACT

The present study on "Standardization of techniques for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.)" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during 2012- 2014. The objective of the study was to standardize techniques for profuse rooting and vigorous growth of orthotropic shoots of black pepper so as to produce quality planting material. The experiment was laid out in completely randomized design consisting of 10 treatments with 3 replications. All the treatments were tried using 2 node and 3 node semi hardwood cuttings of orthotropic shoots of black pepper. The treatments consisted of IBA (500 ppm and 1000 ppm), common sugar solution (1 per cent, 2 per cent and 3 per cent), AMF, *Azospirillum* 15 per cent and *Psuedomonas* 15 per cent. Solarized potting mixture enriched with *Trichoderma* was used for all the treatments including control and PGPR Mix -II was drenched uniformly in all treatments except absolute control.

In the experiment with 2 node cuttings, T_7 (*Azospirillum* 15 per cent) was found to be superior for most of the growth characters like minimum number of days for sprouting and number of days for 50 per cent sprouting . The same treatment recorded the highest value for height of sprouted cutting, length of leaf, breadth of leaf and leaf area. But the maximum values for number of leaves, petiole length, internodal length, root volume and number of roots was recorded in T_2 (IBA 1000 ppm). In experiment with 3 node cuttings, T_2 registered minimum number of days for sprouting, number of days for 50 per cent sprouting, highest values for height of sprouted cutting, number of leaves, petiole length, number of roots and root volume.

The anatomical characters when studied, leaf cuticle thickness was highest in 2 node cuttings treated with T_5 (common sugar solution 3 per cent) and in 3 node cuttings treated with T_6 (AMF). The number of vascular bundles in leaf was found highest in T_2 (IBA 1000 ppm) for both 2 node and 3 node cuttings. But the number of vascular bundles in root was highest in T_1 (IBA 500 ppm), both in the

case of 2 node and 3 node cuttings. For stomatal frequency, T_7 showed maximum value in both the cases. The treatment T_8 (*Psuedomonas* 15 per cent) recorded highest value for total dry matter production in both the experiments. The biological properties of potting mixture (after the experiment) showed that, AMF colonization and spore count was maximum in T_6 for 2 node and 3 node cuttings .The observation on total actinomycetes load was highest in T_7 in both the experiments. The treatment, T_7 showed the highest percentage of success in the establishment of 2 node orthotropic cuttings which was on par with T_1 and T_2. With 3 node cuttings also T_7 and T_1 showed highest percentage success in the establishment of cuttings.

From the results, it can be concluded that 2 or 3 node cuttings with *Azospirillum* 15 per cent or IBA 1000 ppm or IBA 500 ppm were found to be the best treatments for better rooting and growth of orthotropic shoots in black pepper (*Piper nigrum* L.) when planted in solarized potting mixture enriched with *Trichoderma*. However, 2 node cuttings are preferred because the availability of orthotropic shoots are scarce in black pepper.