SURVEY OF ARBUSCULAR MYCORRHIZAL FUNGI AND THEIR APPLICATION AS BIO-FERTILIZER IN *Mangifera indica*

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Submitted by

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STATEMENT BY THE CANDIDATE

As required by the University ordinance 770, I wish to state that the work embodied in this thesis titled 'SURVEY OF ARBUSCULAR MYCORRHIZAL FUNGI AND THEIR APPLICATION AS BIO-FERTILIZER IN *Mangifera indica'* forms my own contribution to the research work carried out under the guidance of Dr. Ravindra G. Bagool, at the Department of Botany, D.U.B.S.S.College, Dapoli. This work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and is included in the bibliography.

Gauri S. Phadke

Certified by

Dr. R. G. Bagool.

CERTIFICATE SUBMITTED UNDER ORDINANCE 80 AND REGULATION 104

I certify that the thesis submitted by Ms. Gauri S. Phadke represents her original work, which was carried out by her, at the Department of Botany, Dapoli Urban Bank Senior Science College, Dapoli, Ratnagiri, 415712; from the period 2009 to 2013.

I further certify that I am of the opinion there is a prima facie case for consideration of the thesis.

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INTRODUCTION

Chapter-I

INTRODUCTION

Maharashtra is the third largest state of India. Although, it is one of the most industrialized states, agriculture continues to be the only occupation of major part of the population. Total irrigated area under cultivation is about 33,500 km^2 (that counts to be 10% of the total area) of which remarkable part is used exclusively for cultivation of fruit crops. Though the tropical climate prevails in Maharashtra, the topographical variations result in climatic and soil type differences in various parts of the state. The soil types and climate are the key factors that enable the farmers to cultivate variety of crops in this state of India. The major fruit crops that have become speciality of Maharashtra include oranges from Nagpur, grapes from Nasik and Sangli, strawberries from cold weather regions like Mahabaleshwar, chikoo from Thane region of Konkan and mangoes, cashews and coconut from rest of Konkan.

Konkan region of Maharashtra state is located in between the Arabian Sea and the Western Ghat as a narrow belt of about 50 km width. The region enjoys heavy rain fall of South-west monsoon and hot and moist climate during rest of the year. The agro-climatic conditions in Konkan belt are more suitable for horticultural crops.

Mango (*Mangifera indica* L., family *Anacardiaceae*), the native of India, is the most celebrated fruit worldwide. It is known to have been cultivated in India since ancient times. It is an evergreen plant occurring abundantly in forests as well as in cultivated lands. It is adaptable to a wide range of soil and climatic conditions and grows well right from Assam to the southern limits of the country and from sea level up to about 1500

meters. It withstands both, fairly dry conditions and heavy rainfall, provided severe and recurring frosts in winter do not endanger the young trees (Randhawa, 1991).

Out of total world production of mangoes, 39 percent is contributed by India (Singh, 2008). Thus, with respect to mango production and export, India is the major competitor in the international market. However, the yield is actually at its lowest level. Among many reasons leading to low yield of mangoes, two important reasons are lack of education about correct agronomic practices and poor management of orchards (Venkatraman, 2002). In recent years uncertainty of weather has emerged as the third major factor affecting the mango yield, however it is beyond anybodies control.

For cultivation of any crop, in general, majority of the farmers depend more on chemical fertilizers than any other source due to their faster effect. Chemical fertilizers ensure faster growth of the crop in the initial years; however, they leave a major and long term impact on soil health. Excessive use of these fertilizers leads to their accumulation in the soil, forming insoluble complexes. As a result, their availability to the crop goes on decreasing rendering the soil infertile.

Targeted research and technology helps to overcome these problems. The researchers were attracted towards development of better alternatives to the traditional chemical fertilizers. A concept of 'Bio-fertilizers' emerged from the extensive research carried out throughout the world. 'Bio-fertilizers' are the indigenous micro-flora of soil that fixes, solubilises and mobilizes the nutrients and enhances growth of the plants. The term 'Bio-fertilizers' encompasses nitrogen fixing bacteria (NFB), phosphate solubilising bacteria (PSB), bio-control agents conferring disease resistance and endophytes that synthesize PGRs.

Various classes of these soil microorganisms have been screened for their efficacy in enhancing plant growth. In recent years, another group of fungal microorganisms namely, 'Arbuscular Mycorrhiza' (AM), has grabbed attention of microbiologists and mycologists working on interaction between various crops and bio-fertilizers.

MYCORRHIZA

Vittadini, in 1842, proposed that 'tree rootlets are nourished by certain fungal mycelia which mantle them'. Frank coined the term 'Mycorrhiza' in 1885. The concept of fungus-root symbiosis is since been a subject of research (Bagyaraj and Verma, 1988). Literally, mycorrhiza means 'fungus roots'. It is a mutually beneficial association between fungus (mycobiont) and root system of plants. This is a natural association of universal occurrence.

1.1.1. Classification

The earlier classification of mycorrhiza was based on the position of the fungal partner within the root system of host. Broadly, it included three types namely ectomycorrhiza, endomycorrhiza and ect-endomycorrhiza. The detailed study of mycorrhizal association in plants led to the discovery of newer types, particularly, based on the host plant. Harley and Smith (1983), proposed more elaborate classification of mycorrhizae as below –

- **Ectomycorrhiza:**

  The fungus grows superficially forming mantle of mycelia that covers the root surface. The hyphae also span the epidermal layer, forming meshwork between

cortical and epidermal cells. This characteristic structure is termed as 'Hartig net'. Members of *Basidiomycetes*, *Ascomycetes and Zygomycetes* are common fungi forming ectomycorrhiza. Host plants mostly belong to *Pinaceae, Betulaceae and Myrtaceae* families.

- **Ect-endo mycorrhiza:**

  This is an intermediate type showing characteristics of both ecto- and endomycorrhiza. Members from the genera *Pinus* and *Larix* harbour this type of mycorrhiza.

- **Vesicular Arbuscular mycorrhiza (now termed as Arbuscular Mycorrhiza):**

  This type is most abundant among the plants. AM is an obligate endomycorrhiza that can be multiplied only within the root system of living plant. They form vesicle and arbuscules within cortical cells of root. Vesicles are the storage organelles while the arbuscules help in transfer of nutrients to the root cell by providing greater surface area. The fungal species belong to *Glomeromycota;* earlier known as *Endogonales* of *Zygomycetes.* Though AM association is a very common phenomenon in the nature, it is absent in the plants belonging to *Pinaceae, Betulaceae, Fumariaceae, Commalinaceae, Urticaceae and Ericaceae families.* Also the plants from the family *Crucifereae, Chenopodiaceae, Polygonaceae and Cyperaceae* rarely from AM association.

- **Orchidaceous mycorrhiza:**

  Fungi from *Ascomycetes* and *Basidiomycetes* form association exclusively with members of *Orchidaceae* family; hence, the name orchidaceous mycorrhiza. The orchid embryo gets infected with mycorrhizal fungi at few cells stage failing which the embryo stops growing further.

- **Ericoid mycorrhiza:**

  Its occurrence is restricted to the members of Ericales. The fungus forms coiled and branched structures inside the cortical cells.

- **Monotropoid mycorrhiza**:

  This association is formed between plants belonging to the family Monotropaceae and Basidiomycetous fungi. The host lacks chlorophyll. The fungal partner forms Hartig net as well as pegs closely associated with cortical cells.

- **Arbutoid mycorrhiza** :

  Plants belonging to two families namely, Arbutoideae and Pyrrolaceae produce this association with Basidiomycetous fungi. The fungal partner forms outer sheath and well developed Hartig net. Also the fungal hyphae penetrate within the cortical cells forming intracellular coils.

1.2. ARBUSCULAR MYCORRHIZA: A promising mycorrhizal type

Due to their inherent properties, AM fungi gained much attention than other mycorrhizal types. AM fungi are ubiquitous in wild as well as cultivated plants. They possess remarkable ability of mobilizing insoluble phosphates and capacity to absorb various macro- and micro-nutrients. They also enhance ability of host plant to produce PGRs, to withstand water stress and they provide protection against soil borne pathogens (Manoharachary, 2009).

1.2.1. Morphology

AM association shows structures like hyphae and spores which are typical features of fungi and also specialized structure like 'arbuscules' and 'vesicles' that contribute to the function of nutrient absorption and their transfer to the host plant. Various morphological characters of AM are as follows-

- **Hyphae:**

  AM fungi produce non-septate hyphae surrounding the root surface. The hyphal network penetrates the root cortex and extends into the intercellular spaces and also within the cortical cells. Their role appears to be nutrient absorption. In nutrient deficient soils, hyphae are more efficient in absorbing nutrients than the root hairs. This is, particularly, evident in phosphate deficient soil (Garret, 1981).

- **Subtending hyphae:**

  They are the spore bearing (Sporogenous) hyphae. The width of hypha varies considerably within different genera and species of AM fungi. Thus they are of great taxonomic significance. They may be simple or recurved or sometimes swollen or constricted at the point of attachment to the spore in *Golmus* species. They are bulbous in case of spores of genera *Gigaspora, Scutellospora, Racocetra, Cetraspora, Dentiscutata, Fuscutata* and *Quatunica.* In some genera like *Acaulospora* and *Entrophospora,* the spores are sessile or may bear a small pedicel.

- **Spores:**

  Spores may be produced terminally, laterally on subtending hyphae or on a single suspensor like cell. Spore colour, shape and size may vary considerably depending on the developmental stages and environmental conditions.

  Spore colour varies from hyaline- white to yellow, red, brown and black.

  Intra-radical spores are mainly globose, sub-globose to ellipsoidal while extra-radical spores may be globose, sub-globose, ellipsoidal, oblong or ovate to highly irregular shaped.

- **Sporocarp:**

  It is a structure enclosing spore/spores observed in some species of AM fungi. It may be formed in soil, root, empty seed coat, insect carcasses or rhizome. External Sporocarp colour ranges from white to brown, while the internal Sporocarp colour ranges from white to black and brown.

- **Spore wall:**

  A spore wall has been defined as the first individual structure formed, originating from the wall of sporogenous hypha and differentiating into phenotypically distinctive layers (Morton *et al.*, 1995). Thus, spore wall characteristics are universally accepted as more stable, reliable criteria than other spore features (Mehrotra, 1997). Every spore, irrespective of species, forms spore wall (Morton, 2002).

  The different wall types encountered in AM fungal spores (Rodrigues and Muthukumar, 2009) are as follows

1) Amorphous**:** It is a formless, flexible wall whose elasticity is affected by mountant. It appears rigid in water and glycerol; in acidic mountants (pH <2.0) it is plastic and tends to collapse partially. The shape is maintained when attached to a more rigid wall.
2) Coriaceous: These are robust, tough but flexible inner walls which turn leathery in appearance in hypertonic solutions.
3) Germinal: It is the innermost layer in *Gigaspora* species from which the germ tube arises. It frequently bears papillae which project distally from innermost surface.
4) Evanescent: It is an outermost ephemeral one to multilayered wall, which is sloughed off as spore matures. It is seen only in pot culture and rarely in field.
5) Laminated: This is generally outer many layered wall. The layers increase as the spore ages.
6) Membranous: It is generally inner, very thin, frequently wrinkled, flexible wall that frequently collapse in hypertonic solutions.
7) Hyphal Peridium: These are tightly adherent hyphal layers around the spore or spores.
8) Unit: It is outer, single, rigid, non-layered wall sheathings like brittle plastic on crushing.
9) Expanding: It is a unit wall which expands when placed in lactic acid or polyvinyl alcohol.

- **Arbuscules and Vesicles:**

As the hyphae penetrate cortical cells, it initiates series of changes in the hyphal ends.

Intracellularly, the fungal hyphae produce short, repeatedly dichotomous branches. The branches appear like a tree and they completely occupy the cortical cells. These structures are called 'Arbuscules'. Their wide surface area forms the site of nutrient transfer between fungal symbionts and host plant.

Another unique sac like structure is 'Vesicle'. It is formed either at the tip of intercellular hypha or intercallery in position. Harley and Smith (1983) suggested that vesicles might perform the function of storage because lipids and glycolipids are the most abundant substances in them. Biermann and Lindermann (1983) thought that intraradical vesicles in some species of AM fungi act as propagules and contribute significantly to the colonization of other roots.

1.2.2. Taxonomy

The soil borne AM fungi were previously classified in the division Zygomycotina due to presence of coenocytic hyphae, chitinous cell wall and formation of Chlamydospores.

Another classification based on morphological and spore characteristics was given by Morton and Benny (1990). They divided AM fungi into two suborders *viz.* Glominae and Gigasporinae belonging to order Glomales of class Zygomycetes. Suborder Glominae included family Glomaceae comprising two genera namely *Glomus* and *Sclerocystis.* However, based on molecular evidences, species of genus *Sclerocystis* were transferred to the genus *Glomus* by Redecker *et al* (2000); thereby, eliminating the genus *Sclerocystis* from the kingdom fungi. The second suborder Gigasporinae comprised of a single family Gigasporaceae

which in turn was divided into two genera i.e. *Gigaspora* and *Scutellospora* (Manoharachary, 2006).

Recently, the AM fungal classification was revised on the basis of molecular, morphological and biochemical data. In this classification system, given by Schulβler *et al* (2001), all the AM species are placed in four orders i.e. Archaeosporales, Diversisporales, Glomerales and Paraglomerales which comprise of 13 families and19 genera that belong to class Glomeromycetes of phylum Glomeromycota. The family Geosiphonaceae with a single taxon *Geosiphon pyriformis* is placed under the order Archaeosporales and does not form arbuscular mycorrhizae. It forms endocytosymbiosis with cyanobacteria (*Nostoc* sp.). It is placed under phylum Glomeromycota due to its close molecular relationship.

1.2.3. Occurrence and Distribution:

AM fungi occur in almost all terrestrial habitats (Mosse and Hayman, 1971). AM association is a common phenomenon in the nature wherein, over 90% of the plants harbour AM fungi in their root tissues. AM fungi show diverse host range including agricultural and horticultural crops, herbs, shrubs and some tropical and temperate tree species. Plant roots readily favour colonization by AM fungi, especially, in nutrient deficient soil types (Bagyaraj and Verma, 1988). In nature, different soil types influence density and distribution of AM fungi associated with host plants (Vogeti *et al*., 2008).

In India, there is great variation in soil texture and composition as well as climatic conditions. Several soil types, particularly, sand, coal mine dumps, iron

ore mine dumps, waste lands, coastal sand dunes etc. have been surveyed for their AM status. Efforts are also being made to investigate occurrence and distribution of AM fungal species in various states of India. This is evident from the surveys carried out by many workers in different parts of India (Rekha Rani *et al.,* 1987; Khade and Rodrigues, 2003; Katdare and Bagool, 2004; Hasan and Khan, 2005). However, there are very few reports on AM survey in the state of Maharashtra.

1.2.4. AM AS BIO-FERTILIZER

AM fungi fulfil almost all criteria to be used as bio-fertilizers.

- They possess ability to absorb macro-nutrients like P, S, Ca, K and micro-nutrients like Zn, Cu, Fe, Cl, Br etc. and transferring them to the host plant.
- AM fungi help in fixing atmospheric nitrogen as well as deriving it from organic and inorganic components of soil.
- Improved uptake of nutrients in AM associated plants leads to enlargement of biomass.
- They enhance production of Plant Growth Hormones by host plant
- Association with arbuscular mycorrhizal fungi induces disease resistance in the plants, particularly, against soil borne pathogens. Thus, AM fungi exhibit characteristics of bio-control agents.
- AM symbionts impart ability to withstand water stress in their host plants, thus making them drought resistant.

1.2.5.Present Work:

The status of AM association in mango is not yet been studied, though it is an economically important horticultural crop of Maharashtra, particularly, of Konkan region. Therefore, there is a scope to survey occurrence and distribution of AM fungi in mango plants in this region.

In India, the commercially grown varieties of mango have arisen through seedling selection made for different characters like colour, taste, flavour, size etc. Later, these varieties have been vegetatively propagated and cultivated in wide area (Mukherjee, 1972). Commonly, monoembryonic types are used for cultivation. Polyembryonic types are grown only in southern India, especially in coastal parts of Kerala, Karnataka and Goa. (Ravishankar *et al,* 2004).

One step towards improving mango productivity can be application of AM bio-fertilizer from the early stages of the growth of seedlings. To confirm the effect of AM fungi on early stages of mango plant, various morphological and physiological parameters can be studied. AM fungi as bio-fertilizer may prove to be the best alternative to chemical fertilizers since they have the ability to enhance nutrient uptake of the plant and are eco-friendly, being the part of indigenous soil micro-flora.

AIMS AND OBJECTIVES

- To collect rhizosphere soil and root samples of different mango cultivars from different soil types and different localities in Konkan region.
- To isolate, identify and quantify the AM fungal spores from collected samples.

- To perform physico-chemical analysis of soil samples.

- To carry out multiplication of AM spores and preparation of inoculum.

- To study the effect of AM inoculum on growth parameters of mango seedlings of a known variety.

CHAPTER – II

REVIEW OF LITERATURE

Chapter-II

Review of Literature

2.1 General:

Exhaustive research in mycorrhiza has taken place in 20th century.

The first major step in mycorrhizal studies, since establishment of relationship between plant roots and soil fungi by Frank (1885), was the finding of a method to isolate the spores of these ubiquitous soil fungi. Gerdemann and Nicolson (1963) successfully extracted spores of *Endogone sp.* from soil by 'Wet sieving and decanting' method. This invention allowed separation and collection of AM fungal spore in pure cultures and their use in further experimentation.

Phillips and Hayman (1970) developed an improved procedure of staining the roots for rapid assessment of infection by AM fungi. However, this procedure was under continuous revision so as to replace hazardous stain, Trypan blue, used in it. A safer staining procedure involving use of aniline blue or methyl blue in glycerol was suggested by Koske (1989) and Grace and Stribley (1991).

Abbot and Robson (1979) observed that addition of phosphate to the soil, extensively eliminated vesicle formation; however, phosphorus supply had no effect on the formation of arbuscules, density of hyphae within the infected root and the branching morphology.

It was Warner and Mosse (1980) who reported that, germinating spores or infected root pieces did show limited growth, but, such growth ceased when the emerging hyphae were separated from parent spore or when the root died. This revealed that

mycorrhizal fungi are obligate bio-trophs and cannot be grown in axenic cultures. In 1987, Loius and Scott described an *in vitro* technique for observing mycorrhizal synthesis in root organ culture of *Shorea roxburghii* under axenic conditions.

Experiments carried out by Bagyaraj and Verma (1988) proved that crop plants respond to VA mycorrhizal inoculation in un-sterile soil, in India. Their work disproved the belief that the plants growing in un-sterile soil may not respond to VAM inocula, since the soil naturally harbours VAM.

Singh (1989), developed techniques for mass production of arbuscular mycorrhiza free from plant pathogens and standardised conditioning models for different forms of inocula. After studying the effectiveness of inocula at the host plant level, he standardised the methodology for large scale production of AM inocula. This was an outcome of a series of experiments carried out at Bhartiya Agro Industries Foundation (BAIF), Pune. The final mass production was achieved through scaling up, starting from monocultures, primary multiplication, green house up to nursery trials and quality control.

A modification in the method for estimation of VAM spores was suggested by Gaur and Adholeya (1994). The modified method using Whatmann filter paper No.1., in their opinion, gives rapid and more accurate estimation of spore.

2.2. Taxonomy:

The pioneering work of recognizing AM fungi as *Endogone* sp. is credited to Peyronel (1924). Thaxter (1922), described all the AM species known at that time.

The first identification key for AM fungal species was developed by Mosse and Bowan, in 1968.

Gerdemann and Trappe (1974) made changes in then-existing classification and introduced *Glomus* as a separate genus in the family Endogonaceae. They moved several species from *Endogone* to *Glomus.* Thus *Endogone* was left with only 11 species which were either ecto-mycorrhizal or saprophytic.

Later, all the arbuscule forming soil borne fungi were placed in the new order Glomales by Morton and Banny (1990). It was further divided into two suborders *viz.* Glominae and Gigasporinae.

Mehrotra (1997), studied the problems associated with morphological taxonomy of AM fungi.

Based on molecular evidences, species of genus *Sclerocystis* were transferred to genus *Glomus* by Redecker *et al.,* (2000) eliminating the genus *Sclerocystis* from kingdom fungi.

The present classification system was given by Schulβler, in 2001. This classification is based on molecular, morphological and biochemical properties of AM fungal species.

2.3. Occurrence and Survey:

Invention of various mycorrhizal techniques for their isolation, visualization and identification, provoked many researchers to explore the unseen community of AM fungi. This initiated a new field in mycorrhizal research in terms of 'Survey'.

AM fungi are commonly present in the top 15-30 cm soil (Redhead, 1977). Because they are soil-borne, their active dispersal occurs usually through propagules like mycelia and spores which can be moved by biotic and abiotic agents. Dispersal of spores over greater distances is dependent on passive mode i.e by wind and water; especially, in arid environments. Bagyaraj (1991), suggested dissemination of AM propagules by

invertebrates such as earthworms, millipedes, wasps and ants through ingestion of VAM propagules with feed or by bringing VAM propagules on the soil surface.

AM fungi are important part of indigenous microflora of the soil throughout the world. Occurrence of AM species is, therefore, influenced by edaphic factors as well as physiological status of the host plant.

According to Hayman (1970), maximum root colonization and sporulation occurs in soils of low fertility.

A more elaborate view regarding interaction between mycorrhizal fungi and plant roots, in relation to phosphatic nutrients, was given by Menge *et. al* (1978). As per their observations, internal concentration of phosphorus in the roots rather than the external concentration in the soil controls root colonization by AM fungi.

Vesicular Arbuscular fungi vary considerably in their reaction to different plant and soil conditions (Rekha Rani and Mukerji, 1987). A country report given by Adholeya (1991) underlines high dependency of tree vegetation of desert soils on VAM. It also records substantial effect of seasonal variation on VAM spore population in forest soils.

Chandra and Jamaluddin (1999) studied occurrence and distribution of AM fungi in different tree species growing in coal mine soils. Their work reported presence of AM association in all the plant species under study.

An attempt was made to study the VAM association in tree species planted in sludge garden at the Ballarpur Paper Mills, Maharashtra, by Jamaluddin *et. al.*(2002). The studies found the prevalence of *Glomus* spp along with few species of *Acaulospora.*

Radhika and Rodrigues (2007), reported AM association with aquatic and marshy plant species while Rodrigues and Jaiswal (2008) observed occurrence of AM fungi in the rhizosphere soil of coastal sand dune vegetation of Goa.

AM fungal diversity and distribution around natural salt Lake of Lonar, Maharashtra, India, was studied by Deotare and Wankhede (2010).

Although of rare occurrence, the presence of mycorrhizal fungi has been detected in parts other than roots *viz.* leaves of *Salvinia* (Bagyaraj *et. al.,* 1979), senescent leaves of *Fumaria hygrometrica* (Park and Linderman, 1980), in decaying peanut leaves and rhizomatous tissue of *ZIngiber officinale* (Taber and Trappe, 1982), leaf sheath, submerged petiole and scale leaves of *Colocasia esculanta* (Ram Rao *et, al.,*1987), scale leaves and leaf bases of *Curcuma longa* (Sampath and Sillia, 1992), corms of *Amorphophylus commutatus* (Rodrigues, 1995), tuber of *Pueraria tuberosa* (Rodrigues, 1996) and tubers of *Gloriosa superba* (Khade and Rodrigues, 2003).

Many scientists have carried out surveys of AM association in variety of host plants and also in varied regions of India. Some of the remarkable ones include those carried out by Rekha Rani and Mukerji (1987), Manoharachary *et. al*.(1987), Kehari *et. al*. (1987), Arul and Vivekanand (1994), Dalal and Hippalgaonkar (1995), Mohan and Mishra (1998), Kunwar *et. al.* (1999), Vogeti *et. al.* (2008), Narilona *et. al.* (2008) and Toman and Jha (2011).

There are few examples of surveys carried out in the state of Maharashtra, particularly, Western Ghats and west coast.

Khade and Rodrigues (2002) selected commonly occurring pteridophytes of *Western Ghats* for survey of AM association. They observed fairly good diversity of AM fungi in

the rhizospheres of pteridophytes of this region. Total of 17 AMF species were recorded from this survey. Later, in 2003, they also surveyed 25 different tree species of the same region. They found that the degree of AM colonization varied with different families and genera of hosts.

Katdare and Bagool (2004), carried out a survey of arbuscular mycorrhizal association in some parts of *Western Ghats*. They reported AM association in over 75% of the wild plants collected during survey.

Riju *et. al.* (2006) carried out a survey of medicinal plants of *Western Ghats.* Total five host plants growing in natural ecosystems were selected for the survey. Observations indicated that genus *Glomus* was dominant over other genera. About 75% of the AM species isolated were belonging to this genus.

In 2007, a survey of AM association in plants near Mumbai and surrounding area was carried out by Katdare and Bagool. The randomly selected host plants showed co-existence of two AM species from different genera. Also, the predominance of *Glomus* species was evident from this survey.

2.3.1. Survey in Mango-

Survey of AM association in mango plants has been rarely attempted.

Bhattarai (1991), while studying status of AM fungi in Nepal, had selected mango and 13 other perennials for screening. She reported highest percentage of root colonization (93%) in mango than in any other selected perennial crop.

In 2005, Abul Hasan and Khan, had undertaken the study of arbuscular mycorrhizal status in mango. They surveyed AM association in mango in six districts of Uttar Pradesh.

There are no reports, till date, of mycorrhizal survey of mango plants in *Konkan* belt of Maharashtra state which is a major contributor to the total mango production of India.

2.4. Role of AM fungi in symbiosis:

AM fungi are obligate symbionts and hence they depend on host plant for survival and multiplication. In return, they perform many physiological activities favourable to their host plant.

Allen *et. al.* (1981), pointed out that association of plants with AM fungi greatly improves soil-plant absorption of water and nutrients, mainly, phosphorated compounds. A number of researchers have emphasized the involvement of AM fungi in absorption of other macro- and micro-nutrients. The mycorrhizal roots showed improved uptake of Fe, Cu, Zn, S, Cl, Br etc. (Tinker, 1982; Bagyaraj, 1984). Clark (1997), noticed enhanced up take of mineral nutrients, particularly, Ca, Mg and K, in acidic soils.

According to the observations of Azcon (1994), mycorrhizal plants could derive nitrogen from organic as well as inorganic sources. In another research (Azcon, 1995), he revealed the role of AM fungi in increasing drought resistance in the host. Various mechanisms such as increased root hydraulic conductivity, stomatal regulation, hyphal water uptake and osmotic adjustments have been proposed through this work.

Bano (1987), suggested role of fungal partner in improving plant growth and root nodulation in legumes through production of phytohormones. An account of several

studies indicating positive role of AM fungi in production of plant growth regulators like auxins, cytokinins, gibberellins and abscissic acid has been given by Sujan Singh (2006).

Fungal hyphae, during some stages of colonization, cover the root surface. Naturally, AM fungi might provide physical protection to the root system up to some extent. AM association also induces some physiological changes in the roots. Jalali (1987), has thrown light on this aspect of AM fungi. He indicated that, AM colonization could provide effective protection to the host roots against infections by soil borne pathogens. Sharma and Bhargava (1993), found AM as a cost effective, potential means of controlling nematodes in tomato. Experiments carried out by Srivastava *et. al.* (2001) indicated that mycorrhiza can be an effective tool in the wilt disease management in guava.

2.5. Practical use of AM inocula as bio-fertilizer:

Positive effect of AM fungi on health and growth of host plant were suggestive of their future application as bio-fertilizer. Menge (1983) talked about the commercial use of VAM as an alternative to reduce the cost of fertilizer. Utilization and commercialization of mycorrhizal fungi was also emphasized by Bagyaraj (1991).

Vegetable and cereal crops:

Azcon and Ocampo (1980), studied the factors affecting AM infection and mycorrhizal dependency of thirteen cultivars of wheat. Influence of arbuscular mycorrhizal fungi on vegetative growth of wheat plants has also been reported by Katdare and Bagool (2006). They claimed that the vigour in vegetative growth could eventually transform into yield.

Gupta and Ali (1998) reported beneficial effect of VAM association on yield of '*Kranti*' variety of rice under water logged conditions. It was the first report related to rice variety. In a unique experiment, Maiti *et. al.* (2008), transplanted the rice crop raised in dry seed bed into a land previously grown with fodder crops susceptible to colonization of VAM fungi. High root colonization along with higher 'P' acquisition, dry matter production and grain yield was observed in these plants.

Krishna *et. al.*(1982), Mathew and Johari (1989), Hazarika *et. al.*(1999), Gautam and Mehmood (2002), Mali *et. al.*(2004) have carried out experiments to assess effect of AM on variety of cereal crops.

Remarkable work has been carried out to check effect of mycorrhizal inoculation on various aspects of vegetable crops. Krishna and Bagyaraj (1982) on lady's fingers, Rai (1990) on potato, Narayan Reddi and Gudige (1999) on carrot, Prabhu *et. al.* (2002) on coriander, Jadhav *et al.* (2010) on spinach beet have shown positive influence of AM inocula on the test plants.

Aromatic and Medicinal plants:

Gupta *et al.* (1991) studied the effect of VAM fungus on the growth of palmrosa in green house experiment using steam sterilized soil inoculated with *Glomus aggregatum*. In citronella java, Kothari and Singh (1996) obtained positive effect on nutrient uptake due to VAM inoculation. Nelson *et. al.* (2000) observed enhancement of various vegetative parameters, while working on *Santalum album* seedlings. In a field experiment on patchouli and ashwagandha, Jadhav (2011) reported significant influence on growth and nutrient uptake as well as desirable effect on total oil content (patchouli) and alkaloids (ashwagandha).

Fruit crops:

Kennedy and Rangarajan (2001), Manjunatha *et. al.* (2002), Shivaputra *et. al.* (2004) studied different aspects of papaya inoculated with AM fungi.

Effect of mycorrhizal inoculation on mineral status of apple was assessed by Thakur *et. al.* (2005), in two types of soils and with different soil amendments.

Sabarad *et. al.* (2007), recorded growth and yield enhancement in banana. Similar effect of AM inoculation in grapes has been reported by Borde *et. al.* (2009).

Devachandra *et. al.* (2008) studied synergistic effect of AM fungi and other bio-formulations on grafting in jamun (*Syzygium cuminii* Skeels).

Santosh (2004), carried out a nursery experiment to study the response of mango to bio-formulations. Germination of mango stones was found to be fastened due to treatment with various AM species. They also reported enhancement in vigour and growth of root stocks which were inoculated with different AM species as compared to un-inoculated control.

In similar experiment, Bassangowda (2005) assessed effect of AMF in combination with bio-formulations on germination, graft-take, growth and yield of mango.

CHAPTER – III

MATERIALS AND METHODS

Chapter III

Materials and Methods

3A. Survey

Status of Arbuscular Mycorrhizal (AM) fungi associated with Mango plants (*Mangifera indica Linn.*) was studied in this part of the work. Occurrence and distribution of AM fungal species associated with mango plants of different ages, varieties and growing in different soil types/ localities were surveyed during this experiment. Materials and methods used for survey and the following analyses are included in this part of the chapter.

3A. 1. Selection of Sites for sample collection:

Mango orchards from 15 different localities in three districts *viz.* Raigad, Ratnagiri and Sindhudurg of Konkan region of Maharashtra State were selected as sites for sample collection. The selection of sites was done so as to get variation in soil types. Number of samples collected varied, depending up on the total area of the collection site. Approximate age and varieties of selected mango plants from every site were recorded.

3A. 2. Sample collection:

The samples were collected in the form of rhizosphere soil and the secondary roots and were brought to the laboratory in polythene bags. The roots and soil were sampled from 15-20 cm depth from the surface and near the line of fertilizer application. Various parameters related to soils of the selected sites (such as colour, texture, topography etc.) were recorded for every site of collection.

3A. 3. Season of sample collection:

Samples from selected plants in each site were surveyed during two different seasons *viz.* Pre-monsoon (i.e. April-May) and Post-monsoon (i.e. December-February). The pre-monsoon collection was carried out during a period between 15th April and 20th May 2009 and post-monsoon collection between 29th December and 10th February 2010. The selected plants were marked at the time of first sampling so the collection could be done from the same in the second season.

Table No. 01: **Name of the collection site, No. of samples collected and Sample code**

Site No.	Name of the site	No. of samples collected	Sample code
1.	Math (Vengurla)	02	SdMM1
			SdMM2
2.	Wadatal (Deogad)	05	SdWJ1
			SdWJ2
			SdWJ3
			SdWJ4
			SdWJ5
3.	Wada-Padel	05	SdWP1
			SdWP2
			SdWP3
			SdWP4
			SdWP5
4.	Pangari (Rajapur)	05	RtPK1

			RtPK2
			RtPK3
			RtPK4
			RtPK5
5.	Purnagad	04	RtPT1
			RtPT2
			RtPT3
			RtPT4
6.	Kasop	05	RtKJ1
			RtKJ2
			RtKJ3
			RtKJ4
			RtKJ5
7.	Nevare	03	RtNJ1
			RtNJ2
			RtNJ3
8.	Malgund	04	RtMK1
			RtMK2
			RtMK3
			RtMK4
9.	Palshet	03	RtPO1
			RtPO2
			RtPO3
10.	Devghar	03	RtDJ1

			RtDJ2
			RtDJ3
11.	Sakhloli	06	RtSB1
			RtSB2
			RtSB3
			RtSB4
			RtSB5
			RtSB6
12.	Rohale (Kelshi)	02	RtRV1
			RtRV2
13.	Kelshi	03	RtKP1
			RtKP2
			RtKP3
14.	Velas	07	RtVJ1
			RtVJ2
			RtVJ3
			RtVJ4
			RtVJ5
			RtVJ6
			RtVJ7
15.	Hashiware	02	RgHM1
			RgHM2
16.	Gothghar	02	RgGP1
			RgGP2

3A. 4. Mycorrhizal studies:

Presence of Mycorrhizal association in the samples was confirmed by investigating AM colonization of roots and determining the density of AM spores in the rhizosphere soils.

3A. 4. 1. Root colonization-

Young roots from each sample were thoroughly washed under running tap water and subjected to a staining procedure given by Koske (1989) and modified by Grace and Stribley (1991). Per-cent colonization was, then, calculated using following Nicolson's (1963) formula–

$$\% \textbf{ Root colonization} = \frac{\boldsymbol{No.\ of\ roots\ showing\ AM\ colonization}}{\boldsymbol{Total\ no.\ of\ roots\ observed}}$$

The staining procedure involved following steps.

1. Fixation and Preservation :-

   Washed root samples were fixed and stored in 50% ethanol.

2. Clearing of tissues :-

   Fixed roots were washed with distilled water and then treated with 10% potassium hydroxide (KOH) solution. The roots were kept in the oven at 90^0C for 3 to 4 hours. The duration of this step was increased than that prescribed in the standard procedure for the hard and deeply coloured roots of mango.

3. Rinsing and Bleaching :-

   After cooling, the root samples were washed several times with tap water. To remove excess colouration, roots were bleached with 3% hydrogen

peroxide (H_2O_2) solution for 20 minutes followed by several changes of water.

4. Acidification :-

   For neutralizing the effect of KOH, roots were kept overnight in 2N hydrochloric acid (HCl).

5. Staining :-

   Acidified roots were thoroughly washed with tap water to remove acid traces. They were kept in 0.05% aniline blue solution in acidic glycerol. Excess stain was drained off and root pieces were mounted on glass slide in glycerol for microscopic observation.

3A. 4. 2. Isolation and Quantification of AM spores-

AM spores from rhizosphere soil samples were isolated and quantified by 'Wet sieving and Decanting' method given by Gerdemann and Nicolson (1963). Ten gram of soil sample was weighed and thoroughly mixed with water by stirring with the glass rod. The mixture was allowed to stand till soil particles settled down. The suspension was then passed through series of sieves stacked in descending order of their mesh sizes *viz.* 375 µm, 125µm, 75µm and 37µm from top to bottom. The sieves were washed with the running tap water and washings were collected in a beaker.

Washings were filtered through Watmann filter paper No.1 with 1cmX1cm grid marked on it. This procedure was repeated several times in order to collect maximum spores present in the soil. After filtration, the filter paper was observed under stereo binocular microscope (Leica make, E57) to count and isolate the AM spores.

The above procedure was performed in triplicate for every soil sample and number of AM spores per 10g of rhizosphere soil was averaged.

3A. 4. 3. Identification of AM spores-

AM spores isolated from soil samples were collected separately on the basis of their morphological features like colour, shape, hyphal attachments etc. Intact as well as broken spores were mounted on microscopic slides using glycerine and observed by putting cover slip over it. Identification of AM species was done using manual given by Schenk and Perez (1989). Identification was confirmed by referring to Dr. V. Mohan of IFGTB, Coimbatore, Tamil Nadu.

3A. 5. Multiplication of AM spores-

A mixture of sand: soil (1:1) was sterilized by autoclaving and was filled in polythene bags up to $3/4^{th}$. AM spores isolated from samples were spread on top of the potting mixture and over layered with a thin layer of sand: soil mixture again. Maize seeds were sown in these polythene bags and were watered every day. At 30 days, few roots of the maize seedlings were collected and stained to confirm AM colonization. After 60 days the seedlings were cut off from soil and roots were mixed with potting mixture after chopping into pieces. This mixture was stored in the refrigerator as inoculum for further studies.

3A. 6. Physicochemical studies of rhizosphere soil samples

Standard procedures were followed to study the physical properties of the soil samples and their chemical analyses.

3A. 6. 1. Determination of soil pH-

For determination of soil pH, 1:2 suspension of soil was prepared by mixing 20g of soil in 40 ml of distilled water thoroughly. The pH of the supernatant was measured using combined pH electrode (Equiptronics make) and recorded for each sample.

3A. 6. 2. Determination of per-cent moisture-

Pre-weighed soil samples were kept at 60^0C in hot air oven till constant reduced weights (g) were obtained. Difference in weight before and after oven drying was calculated and percentage was worked out for each sample.

3A. 6. 3. Chemical analysis-

Concentrations of macro- and micro-nutrients were determined for rhizosphere soil samples collected in both the seasons using standard chemical analysis methods given in the table below (Jackson, 1971).

Organic Carbon	Walkley and Black method (Wet oxidation method)
Total Nitrogen	Kjedahl Method
Phosphorus	Bray and Kurtz Method
Sodium & Potassium	Flame photometric method
Micronutrients (Cu^{+2}, Zn^{+2}, Fe^{+2}, Mn^{+2})	Atomic Absorption Spectrophotometry

3B. Nursery Experiment

An experiment was conducted to study the effect of AM bio-fertilizer, in comparison with chemical fertilizer and its different combinations with AM inoculum on vegetative growth of young seedlings of Mango (*Mangifera indica*). The details of materials and methods used for this experiment are included in this chapter.

3B.1. Experimental variety:

Test plant: *Mangifera indica;* Variety: *Villaicolumban*

It is an Indian polyembryonic, homozygous variety of mango. The seedlings of the variety are commonly used as rootstock for grafting.

3B. 2. Germination of mango stones:

Mango stones required for the experiment were obtained by separating them from ripened fruits. Raw fruits were procured from Regional Fruit Research Centre of Dr. Balasaheb Savant Konkan Krishi Vidyapeeth, Vengurla, District- Sindhudurg, in the month of May. After the fruits ripened, stones were separated, washed and sown in the potting mixture of garden soil and cow dung (3:1) prepared in polybags. The seeds were watered daily.

3B. 3. Separation of multiple seedlings:

The seeds were observed daily for germination. Germinated mango stones were removed from polybags and opened up manually to separate out multiple embryos showing sprouting and developing root system. Each germinated stone possessed two types of seedlings *viz.* a zygotic seedling and a few nucellar seedlings. They differed from each other in phenotypic characters such as colour and texture of

leaves and general appearance. The zygotic seedlings were rejected as they are not true to the type (maternal genotype). The nucellar seedlings are true to type and hence were selected for the experiment. They were transferred to polybags containing soil, sand and treatment mixtures such a way, that each treatment contained seedlings of all sizes/ stages.

3B. 4. Treatment details and Experimental Design:

3B. 4.1. Potting mixture:

Soil and cow dung mixture (3:1) was thoroughly mixed with sand (1:1) and the same was filled in the polybags as the main potting mixture. The physical and chemical properties listed in Table No. 2. were recorded for the soil used in potting mixture.

Table No.2: Physico-chemical properties of potting mixture

1.	pH	**6.**	Sodium
2.	Organic carbon	**7.**	Iron
3.	Total Nitrogen	**8.**	Manganese
4.	Phosphorus	**9.**	Copper
5.	Potassium	**10.**	Zinc

3B. 4.2. Chemical fertilizer:

It is a common practice to use single superphosphate (SSP) as the inorganic source of phosphorus. Hence, the same was used as a chemical fertilizer for the experiment. It was added in to the polybags in the proportions given in

Table No. 3.

3B. 4. 3 AM Inoculum :

A commercial AM bio-fertilizer was procured from Tamil Nadu Agriculture University, Coimbatore, India and was used for the experiment. It was added in to the polybags in the proportions given in Table No. 3.

Quantities of both AM inoculum and chemical fertilizer as per the requirement of the treatment were thoroughly mixed in 4 kg of potting mixture and filled in polybags tagged with corresponding treatment symbol. Three replications each with five seedlings were maintained per treatment.

Two types of controls *viz.* Control without Chemical fertilizer as well as AM bio-fertilizer (T1) and Control with chemical fertilizer alone (T2) were maintained along with other test combinations.

Table No. 3: **Details of the fertilizer treatments**

Sr. No.	**Treatment Symbol**	**Composition**
1.	**T1**	Un-inoculated Control
2.	**T2**	100% dose of SSP* (Chemical fertilizer Control)
3.	**T3**	50% dose of AM inoculum
4.	**T4**	100% dose of AM inoculum**
5.	**T5**	50% dose of AM inoculum + 50% dose of SSP
6.	**T6**	50% dose of AM inoculum + 100% dose of SSP
7.	**T7**	100% dose of AM inoculum + 50% dose of SSP
8.	**T8**	100% dose of AM inoculum + 100% dose of SSP

[*100% dose of SSP- 20g/ 4kg soil; **100% dose of AM inoculum **-** 40/ 4kg soil**]**

3B. 5. Duration of Experiment:

The nursery experiment was conducted for the period of 12 months extending from September 2010 to October 2011.

3B. 6. Growth parameters selected for study:

Two seedlings per replication of each treatment were marked to record effect on following parameters @ 30days interval.

i) Height of seedling (cm)-

Total height of main growing shoot from ground level up to the tip was measured in centimetres. Average height of plant was calculated by taking mean of the readings for all three replications (2 seedlings/ replication) i.e. total six seedlings per treatment.

ii) Number of leaves –

Total number of leaves per plant was counted for the marked seedlings and the average number of leaves per treatment was calculated.

iii) Stem girth –

Stem girths at collar region and first node were measured in centimetres for each seedling with the help of thread. The stem girth at collar region and first node were recorded and are presented separately which are mean values of three replications per treatment.

Following parameters were recorded @ 180 days and 360 days by carefully uprooting the seedlings other than the marked ones from potting mixture for each treatment.

i) Root length (cm) –

Length of main (primary) root was measured in centimetres per replication per treatment. Mean root length was determined by calculating the average of three replications.

ii) Root and shoot fresh weights (g) –

Root and shoot parts of up-rooted seedlings were cut apart and their fresh weights were taken separately. Average root and shoot fresh weights were then calculated for all the treatments.

iii) Root and shoot dry weights (g) –

Following fresh weight determination, root and shoot parts of the seedlings were subjected to oven drying at 60˚C till constant reduced weights were observed. Mean values of root and shoot dry weights were worked out for each treatment.

iv) Mycorrhizal studies-

1. Root colonization:

   Roots of up-rooted seedlings were washed thoroughly, under tap water and were processed using staining method given by Carol and Stribley (1991) to assess per-cent root colonization by AM fungi.

2. AM spore density:

   Density of AM fungal spores in rhizosphere soil, collected from each treatment, was determined by 'Wet sieving and decanting' method (Gerdemann and Nicolson, 1963).

CHAPTER – IV

OBSERVATIONS AND RESULTS

Chapter-IV

Observations and Results

4A. Survey

4A.1. Sample collection

The localities for sample collection were selected from three major mango producing districts of Konkan region of Maharashtra state *viz.* Raigad (Rg), Ratnagiri (Rt) and Sindhudurg (Sd). Samples were collected from sixteen sites including 02 from Raigad, 11 from Ratnagiri and 03 from Sindhudurg district. Names of the collection sites, number of samples collected, age and variety of sampled mango plants are presented in the Table No.4.

Table No.4: Sample code, Name of the site, Variety and Age of the sample plant.

Site No.	Name of the site	Sample code	Variety	Age of the plant (yrs)
1.	Math (Vengurla)	SdMM1	Alphonso	15
		SdMM2	Pairi	>75
2.	Wadatal (Deogad)	SdWJ1	Alphonso	>50
		SdWJ2	Alphonso	>50
		SdWJ3	Alphonso	>50
		SdWJ4	Alphonso	10
		SdWJ5	Alphonso	>50
3.	Wada-Padel	SdWP1	Alphonso	60
		SdWP2	Pairi	60
		SdWP3	Alphonso	60
		SdWP4	Alphonso	10

		SdWP5	Alphonso	>100
4.	Pangari (Rajapur)	RtPK1	Alphonso	>100
		RtPK2	Alphonso	>100
		RtPK3	Ratamba	>55
		RtPK4	Alphonso	5
		RtPK5	Pairi	40-45
5.	Purnagad	RtPT1	Alphonso	>90
		RtPT2	Alphonso	15
		RtPT3	Kesar	15-20
		RtPT4	Raiwal	>90
6.	Kasop	RtKJ1	Alphonso	>90
		RtKJ2	Alphonso	50
		RtKJ3	Alphonso	50
		RtKJ4	Alphonso	50
		RtKJ5	Alphonso	50
7.	Nevare	RtNJ1	Alphonso	15-20
		RtNJ2	Alphonso	15-20
		RtNJ3	Alphonso	15-20
8.	Malgund	RtMK1	Alphonso	15
		RtMK2	Alphonso	15
		RtMK3	Alphonso	15
		RtMK4	Alphonso	15
9.	Palshet	RtPO1	Alphonso	15-20
		RtPO2	Alphonso	15-20

		RtPO3	Alphonso	15-20
10.	Devghar	RtDJ1	Alphonso	>100
		RtDJ2	Alphonso	40-50
		RtDJ3	Alphonso	15
11.	Sakhloli	RtSB1	Alphonso	10
		RtSB2	Kesar	10
		RtSB3	Alphonso	10
		RtSB4	Alphonso	5
		RtSB5	Alphonso	5
		RtSB6	Alphonso	5
12.	Rohale (Kelshi)	RtRV1	Alphonso	>40
		RtRV2	Alphonso	30
13.	Kelshi	RtKP1	Alphonso	>70
		RtKP2	Alphonso	>70
		RtKP3	Alphonso	>70
14.	Velas	RtVJ1	Alphonso	15-20
		RtVJ2	Alphonso	15-20
		RtVJ3	Alphonso	15-20
		RtVJ4	Alphonso	15-20
		RtVJ5	Alphonso	15-20
		RtVJ6	Alphonso	15-20
		RtVJ7	Alphonso	15-20
15.	Hashiware	RgHM1	Pairi	>100
		RgHM2	Alphonso	>100

16.	Gothghar	RgGP1	Alphonso	50
		RgGP2	Alphonso	15

4A.2. Mycorrhizal studies:

The sampled plants collected from various localities (as listed in Table No.4) during two seasons were screened for per-cent root colonization by arbuscular mycorrhizal fungi and density of AM spore in their rhizosphere. The site wise data regarding mycorrhizal status and its correlation with soil pH and moisture are presented in Table No. 5

Site 1: Math (Vengurle), District- Sindhudurg.

Two samples (**SdMM1 & SdMM2**), one each from *Alphonso* and *Pairi* variety were collected from this site. The observations of both the collections are given in Table No. 5. During both the samplings SdMM2 showed greater root colonization (31.25% and 28.06% respectively) than that of SdMM1 (2.73% and 2.19% respectively).

The same trend was observed in case of AM spore density. The spore count recorded in Sample SdMM2 was 342 spores/ 10g of soil in season I and 37 spores/ 10g soil in season II. Spore count in SdMM1 was found to be 222 spore/10g of soil and 27spore/10g soil in season I and II, respectively.

Overall, root colonization by AM fungi as well as spore count in rhizosphere soil, both were higher in pre-monsoon season than the post monsoon period. Decrease in pH from 6.82 (season I) to 6.03 (season II) was noticed during the collections.

Site 2: Wadatal (Deogad), District- Sindhudurg.

Total five samples (**SdWJ 1 to SdWJ 5**) were screened from this site. Root colonization was highest in SdWJ 4 in both, season I (62.73%) as well as season II (88.23%) followed by SdWJ 2 (39.68% and 64.74%), SdWJ 1 (23.2% and 15.38%) and SdWJ 5 (20.09% and 23.08%). Samples SdWJ 3 exhibited very poor colonization of roots in both the seasons (3.47% and zero). Exceptionally, samples SdWJ 1 and SdWJ 3 showed more colonization during first season than second which is contrary to the other three samples.

AM spore density in case of all the five samples was found to be more during first season than that observed in second season. Sample SdWJ 4 showed spore count of 452 in season I and 24 in season II. The spore density in sample SdWJ 3 was 451 and 37 in season I and II respectively. Sample SdWJ 1 showed 241 and 39 spores/10g of soil, SdWJ5 showed 236 and 56 spores/10g of soil and SdWJ2 showed 109 and 43 AM spores/10g of soil during season I and II, respectively. Soil pH decreased from 7.33 (season I) to 6.49 (season II).

Site 3: Wada- Padel, District- Sindhudurg.

Five samples (**SdWP1 to SdWP5**) were collected from this site. In both the seasons, highest root colonization was observed in SdWP3 (37.5% and 36% respectively). Samples SdWP4 (13.33% & 31.67%), SdWP2 (20% & 29.05%) and SdWP1 (8.33% & 12.68%) showed more colonization by AM fungi in season II than season I. Least colonized was sample SdWP5 (4.16% & zero), in both the collection seasons.

All the samples showed higher AM spore count in pre-monsoon season compared to the post-monsoon season. The highest spore density in season I was assessed in SdWP3 (132 spores/10g soil); however, it showed lower count (54 spores/10g soil) in Season II. Following were sample SdWP2 (127 & 76 spores/10g soil respectively), SdWP4 (98 & 37 spores/10g soil), SdWP1 (73 & 62 spores/10g soil). Sample SdWP5 showed a different trend with almost similar spore density in season I (49 spores/10g soil) and season II (51 spores/10g soil). The mean soil pH showed decrease by almost one unit from season I (7.08) to season II (6.17).

Site 4 : Pangari (Rajapur), District- Ratnagiri.

Total five samples (**RtPK1 to RtPK5**) were collected from this site. Three samples (RtPK1, RtPK2 & RtPK4) belonged of *Alphonso* variety, RtPK3 was a local hybrid variety *Ratamba and* RtPK5 was from the variety *Pairi*. Root samples assessed for AM association, in season I, revealed that the spore density and root colonization were inversely proportional. Maximum colonization was seen in RtPK4 (57.14 %) followed by RtPK5 (54.76%), RtPK2 (40%) and RtPK1 (25%) while RtPK3 exhibited least root colonization (12 %). However, the same pattern did not continue in the second season of collection. In season II, RtPK1 showed maximum colonization (36%) followed by RtPK2 (22%), RtPK4 (17.6%), RtPK5 (13%) and RtPK3 (3.7%).

Highest spore count was recorded in RtPK3 (344 spores/10g soil) during season I, however, it reduced to 49 spores/10g soil during second season of sample collection. The observations in Alphonso variety samples showed spore densities as RtPK1 76 & 46 spores/10g soil, RtPK2 41 & 70 spores/10g soil, RtPK4 53 & 121 spores/10g soil

and RtPK5 23 & 117 spores/10g soil in season I and season II, respectively. Soil pH was slightly acidic during seasons I (5.96) and season II (5.53) both.

Site 5: Purnagad, District- Ratnagiri

Four samples (**RtPT1 to RtPT4**) were collected from this site. Two of them were of *Alphonso* variety (RtPT1 and RtPT2), one of variety '*Kesar*' (RtPT3) and one from '*Raiwal*' variety (RtPT4). Percentage of root colonization by AM fungi was assessed for both the collection seasons. It was found to be maximum (83.72%) in RtPT3 during season I and (56.52%) in RtPT4 during season II. AM colonization in RtPT1 was 33.34% and 12.24%, RtPT2 was 33.92% and 21.25%, RtPT3 was 83.72% and 20.75% and in RtPT4 was 19.64% and 56.52% in season I and season II, respectively.

The spore count/ 10g of all the soil samples indicated that the spore density was more in first season than that in second season. Sample RtPT2 (254) and RtPT3 (66) showed highest density in season I and season II, respectively. AM spore counts for all the samples were recorded as follows, RtPT1- 142 and 12, RtPT2- 254 and 32, RtPT3- 206 and 66 and RtPT4- 119 and 6 per 10g soil. Soil pH showed decrease from season I (7.04) to season II (6.21).

Site 6: Kasop, District: Ratnagiri

All the five samples (**RtKJ1 to RtKJ5**) collected from this site were of *Alphonso* variety. Root colonization percentage was found to be more in post monsoon season (season II) than that in pre-monsoon season (season I). Sample RtKJ4 showed

maximum (68%) colonization followed by RtKJ1 (52.4%), RtKJ5 (51%), RtKJ3 (49.65%) and RtKJ2 (36.25%) during first sampling. At the time of second sampling, RtKJ1 (72.25%) exhibited greater degree of colonization. It was followed by RtKJ4 (71%), RtKJ5 (67.4%), RtKJ3 (52.33%) and RtKJ2 (40%). On the whole, RtKJ2 exhibited lowest degree of root colonization during both the collections.

In contrast to root colonization, a decrease was noticed in the AM spore density from pre-monsoon to post-monsoon period. During both the samplings, RtKJ2 showed highest spore density (204 and 113 respectively). It was followed by RtKJ1 (165 and 92), RtKJ3 (142 and 106), RtKJ5 (139 and 94) and RtKJ4 (131 and 87). Drop in soil pH was noticed from season I (6.34) to season II (5.6).

Site 7: Nevare, District: Ratnagiri

Three samples (**RtNJ1 to RtNJ3**) of *Alphonso* variety were collected from this site. Observations from the Table No. 5 revealed that, AM status of the plants was high during season I than that in season II.

In case of root colonization, RtNJ3 (71.1% and 28.33% respectively) showed greater degree of colonization in both the collection seasons. It was followed by RtNJ2 (70% and 26%) and RtNJ1 (64 % and 19.23%).

AM spore density per 10g of soil was recorded to be highest in RtNJ1 (102 and 83) during both samplings. Sample RtNJ2 showed moderate spore densities (91 and 78) while RtNJ3 exhibited lowest spore counts (57 and 52) in season I and II**.** The mean pH of the soil decreased from 6.9 (season I) to 5.72 (season II).

Site 8: Malgund, District: Ratnagiri

Four samples (**RtMK1 to RtMK4**) were collected from this site. All the samples were of the same variety namely, *Alphonso*. AM colonization was found to be more in sample RtMK2 (56.25% and 33.41%) during both the collection seasons followed by RtMK3 (21.05% and 20.8%) and RtMK1 (21.8% and 18.42%). Exceptionally, sample RtMK4 showed more colonization in season II (23.7%) than season I (16.12%).

The same trend was also observed for AM spore density in soil samples where it was more in season I than season II. Highest AM spore density was recorded in sample RtMK1 during both seasons (210 & 121/ 10g soil) followed by RtMK4 (116 & 102 spores/ 10g soil) and RtMK3 (61 & 34 spores/ 10g soil) in descending order of spore densities. Surprisingly, no seasonal variation was recorded in spore densities in case of RtMK2 (43 & 41 spores/ 10 g soil). Soil pH was observed to be 6.65 and 6.1 during season I and II, respectively.

Site 9: Palshet, District: Ratnagiri

All the three samples (**RtPO1 to RtPO3**) collected from this site belonged to *Alphonso* variety**.** Samples exhibited AM association during both collection periods. Root colonization per-cent was found to be more during first sampling than that in second sampling with the exception RtPO2. Maximum colonization was recorded in RtPO2 (47.5% and 51%) followed by RtPO1 (39.13% and 21.33%) and RtPO3 (24% and 19.25%) in season I and II, respectively.

AM spore count per 10g of soil was found to be highest in RtPO1 (161 and 114) in both seasons. It was followed by RtPO3 (134 and 78) and RtPO2 (89 and 56). Reduction in soil pH value was noticed in season II (6.08) than season I (6.49).

Site 10: Devghar, District: Ratnagiri

All the three samples (**RtDJ1 to RtDJ3**) collected from this site were of *Alphonso* variety. Sample RtDJ2 (47.33%) exhibited maximum degree of root colonization followed by RtDJ3 (41%) during season I. In season II, RtDJ3 (61.25%) showed more colonization than that in RtDJ2 (52%). The observations revealed that (Table No.5), RtDJ1 (16 % and 14.25%) showed lowest per-cent colonization during both the collections.

AM spore densities (per 10g soil) as observed in season I and II, revealed that, RtDJ2 exhibited highest values (163 and 94 respectively) followed by RtDJ3 (130 and 90 respectively) and the least was shown by RtDJ1 (88 and 48 respectively). Overall, RtDJ1 exhibited low AM status compared to the other two samples.

Site 11: Sakhloli, District- Ratnagiri

Total six samples (**RtSB1 to RtSB6**) were collected from this site including five of Alphonso and one of 'Kesar' variety (RtSB2). In season I, root colonization was maximum in RtSB2 (77.8%) followed by RtSB3 (71%), RtSB6 (61%), RtSB4 (48%), RtSB1 (47%) and least was recorded in RtSB5 (32%). However, during second season the pattern of colonization varied. Sample RtSB5 showed maximum (87.87%)

colonization by AM fungi followed by RtSB4 (84%), RtSB6 (58%), RtSB3 (50%), RtSB1 (38.23%) and least was shown by RtSB2 (23.07%).

In case of AM spore density, highest density was shown by sample RtSB1 (1329 spores/ 10g soil) in season I and RtSB4 (211 spores/10g soil) in season II. Spore densities for rest of the samples per 10 g of soil were as follows: RtSB2 - 780 and 56, RtSB3- 308 and 197, RtSB5- 622 and 173 and RtSB6 - 287 and 89 in season I and season II, respectively. Soil pH dropped from 7.43 (season I) to 6.89 (season II).

Site 12 : Rohale (Kelshi), District- Ratnagiri

Two samples (**RtRV1 and RtRV2**) of *Alphonso* variety were collected from this site. Per-cent root colonization during season I was slightly higher in RtRV2 (14%) than that of RtRV1 (12.35%). Colonization percentage in RtRV1 (30.76%) increased more than double, where as that of RtRV2 (6.25%) reduced to half in season II.

Though colonization decreased, there was remarkable increase in spore density (per 10g soil) of RtRV2 (72 and 130) from season I to season II. On the other hand the same decreased in case of sample RtRV1 (51 and 29) between the two collection seasons.

The mean soil pH decreased from season I (6.46) to season II (5.93).

Site 13: Kelshi, District- Ratnagiri

Collection from this site comprised three samples (**RtKP1 to RtKP3**) of *Alphonso* variety. An increase in root colonization percent and decrease in spore density was noticed between the two collections (Table No. 5).

Maximum root colonization was observed in RtKP1 (27%) followed by RtKP3 (18.25%) and RtKP2 (16.35%) in season I. During second season, RtKP3 (59.85%) showed higher degree of colonization than that in RtKP1 (42.1%) and RtKP2 (33.33%).

Spore density (per 10g soil) reported in RtKP1 (104) was highest in season I. It was followed by RtKP3 (90) and RtKP2 (62). In case of second collection, spore density of RtKP3 (84) was found to be maximum than that in RtKP2 (73) and RtKP1 (69). A decrease in soil pH was observed in season II (6.08) than season I (6.49).

Site 14: Velas, District- Ratnagiri

Total seven samples (**RtVJ1 to RtVJ7**), all belonging to 'Alphonso' variety were collected from this site. The spore count per 10g of soil ranged between 98 and 168 in season I and between 06 and 82 during season II. Maximum spore density in season I was recorded in sample RtVJ6 (168 spores/10g soil) followed by RtVJ7 (157), RtVJ1 (123), RtVJ4 (116), RtVJ2 (102) and RtVJ3 (100). Sample RtVJ5 exhibited least spore density of 98 spores/10g of soil.

In second season the overall spore count of this site was found to be lower than the first season. Sample RtVJ5 showed highest spore count (82) followed by RtVJ7 (79), RtVJ6 (60), RtVJ4 (51), RtVJ2 (35), RtVJ1 (34) and the least was found in sample RtVJ3 (06) per 10g of soil.

Root colonization, during season I and season II was RtVJ1- 20.4% and 5.26 %, RtVJ2- 28% and 5.12%, RtVJ4- 18.5% and 20%, RtVJ5- 50% and 19.29%, respectively. Sample RtVJ7 showed maximum colonization (66%) during season I. Root

colonization for samples RtVJ3 and RtVJ6 in both seasons and RtVJ7 in season II could not be assessed due to difficulty in staining the harder roots. The mean soil pH was recorded to be 7.16 and 6.84 during season I and II, respectively.

Site 15: Hashiware, District- Raigad

Two samples including one each of *Payari* (**RgHM1**) and *Alphonso* (**RgHM2**) variety were collected from this site. Colonization of roots by AM fungi was more during first season; however, AM spore density was greater in second season (Table No. 5).

Extent of root colonization in both the samples was nearly equal in season I as well as season II. It was observed to be 36.25% (RgHM1) and 39.5% (RgHM2) during season I, whereas, in season II it was 60.25% (RgHM1) and 58.5% (RgHM2).

AM spore count per 10g soil from sample RgHM2 (300 and 92) was greater than RgHM1 (93 and 47) during both the collections. The mean soil pH varied from 6.96 (season I) to 6.43 (season II) during both the collections.

Site 16: Gothghar, District- Raigad

Samples collected from this site (**RgGP1 and RgGP2**) were of *Alphonso* variety.

Higher root colonization was recorded in sample RgGP1 (20.28%) for season I as well as season II (44.25%). Sample RgGP2 showed colonization percentage of 13.09 and 29.33 during season I and II, respectively.

Sample RgGP1 also recorded maximum spore density (per 10g soil) during both the collection seasons. It was found to be 99 and 76 respectively in season I and II. Spore

count (per 10g soil) of RgGP2 as observed in season I and II was 46 and 49, respectively.

Soil pH during collections was recorded to be 7.01 (season I) and 6.72 (season II).

4A. 3. Identification of AM species

Identification of isolated species of AM fungi was based on the spore morphology and was carried out by referring to the keys given by Schenk and Perez (1990). Total eighteen different AM species were recorded from samples of both collection seasons. The isolates included 12 species belonging to *Glomus,* 05 species from genus *Acaulospora* and one species of genus *Scutellospora* (Table No. 07).

Various species of AM fungi isolated from the rhizosphere soil samples (Plate No. 06 to 09) were as described below.

1. *Glomus fasciculatum*

   Description-Globose to sub-globose spores are pale yellow to pale yellow-brown in colour. The spore size ranged between 60-110 µm. Three layered spore wall was observed. Subtending hypha was cylindrical to slightly flared.

   Occurrence- It was the most abundant species. It was isolated from Site1 (Math-Vengurle), Site 3(Wada-Padel), Site 4 (Pangari-Rajapur), Site 6 (Nevare), site 12 (Rohale) and site (Kelshi) during both collection seasons, while from site 8 (Malgund) in only season I and from site 9 (Palshet), site 10 (Deoghar) and site 11(Sakhloli) during season II.

2. *Glomus aggregatum*

Description-Hyaline to pale yellow coloured spores, globose to ovate in shape with diameter ranging between 40-120 µm were isolated. Spore wall consisted of two layers. Spores were observed in loose cluster without a peridium.

Occurrence- In pre- monsoon season, this species was isolated from site 2(Wadatal-Deogad) and site 10 (Deoghar), while in post-monsoon season it was from site 1 (Math-Vengurle) and site 7 (Kasop).

3. *Glomus claroidem*

Description-Cream to light yellow coloured spores, globose to subglobose in shape with size ranging between 80 to 160 µm. Spore wall comprised of four layers of which first two are present mostly in all juvenile spores. Subtending hyphae are cylindrical to slightly flare with the width of 6-8 µm.

Occurrence- It was isolated from the soil sample of site 2 (Wadatal-Deogad) during pre-monsoon season.

4. *Glomus macrocarpum*

Description-The spores were embedded in the hypha. Yellow to yellowish brown coloured spores were 30-45 µm in diameter and globose in shape. There was a smooth single layered spore wall.

Occurrence- Presence of this species was observed in soil of site 5 (Purnagad) during both seasons, site 4 (Pangari-Rajapur) in post-monsoon period and site 7 (Kasop) in pre-monsoon period.

5. *Glomus albida*

   Discription- Chlamydospores were globose with approximately 15-20 µm diameter and blackish brown Sporocarp. Spore showed subtending hyphal attachment.

   Occurrence- It was reported at site 6 (Nevare) in season I and site 4 (Pangari) during season II.

6. *Glomus taiwanensis* **(Wu and Chen)**

   Description-Chlamydospores were cylindro-clavate with approximately 40-85 x 22-30 µm in diameter and reddish brown sporocarp. Spore wall was thick, laminate or single. The spore wall was thickest at the apex. Spores with or without a septum at the base, were tightly packed radially around a central plexus.

   Occurrence- It was observed at site 14 (Velas) and site 15 (Hashiware) during both collections.

7. *Glomus clavisporum* **(Trappe)**

   Description-Brown coloured chlamydospores with clavate to sub cylindrical shape and approximately 105 x 36 µm in size were isolated. Spore wall was thick and became thickest at the apex. Spores were formed radially around the central plexus

   Occurrence- It was found only at one locations i.e. site 15 (Hashiware) during both the season.

8. *Glomus sinosum* (Gerdemann and Bakshi) Almeida and Schenck

Description-Brown coloured chlamydospores with yellow to dark brown sporocarp and cylindro-clavate, elongated shape without thickened apex were observed. The size of the spore was approximately 85-200 x 32-55 µm in diameter. The chlamydospores were radially arranged on the central plexus of hyphae

Occurrence- This species found to be occurring at two sites *viz.* site 11 (Sakhloli) and site 16 (Gothghar) in pre- and post-monsoon period.

9. *Glomus coremiodes* (Berk. And Broome)

Description-The chlamydospores were 65-100 µm x 40-75 µm in size with obvoid- ellipsoid to oblongellipsoid in shape and brown in colour. The spores were separated from the subtending hyphae by a septum at the base of the spores. Chlamydospores were arranged in a single layer and were tightly grouped in a hemisphere around the central plexus.

Occurrence- The species was restricted to site 14 (Velas) but occurred during both collections.

10. *Glomus multicaulae* (Gerdemann and Bakshi)

Description-Spores were isolated singly from the rhizosphere soil. Sub-globose shaped spores were approximately 149-249 x 124-162 µm in size. They showed reddish brown to dark brown colour. Multiple hyphal attachments, commonly situated at opposite ends were observed. Rounded projections were noticed throughout the surface of spore wall.

Occurrence- It was exclusively found at site 14 (Velas) during both collection seasons.

11. *Acaulospora scrobiculata* **(Trappe)**

Description-Globose to ellipsoid spores were observed with olive to light brown colour and 100-240 x 100-220 µm in size. Small depressions were observed throughout the surface of the spore. The spore wall is four layered.

Occurrence- It was of rare occurrence. It was found only during pre-monsoon season at site 15 (Hashiware).

12. *Acaulospora laevis*

Description- Spores with pale orange-brown colour and globose to subglobose shape were observed. The size ranged between 140 and 240 µm (mean= 198 µm). The spore wall showed three layers and germinal wall showed two layers.

Occurrence- This was moderately occurring species. In pre-monsoon season, it was recorded at site 3 (Wada-Padel) and site 8 (Malgund), while it was reported at site 5 (Purnagad) during post-monsoon period.

13. *Scutellospora calospora*

Description-Subglobose to oblong spores with 120 to 220 µm diameter (mean= 165 µm) were recorded. The colour of the spores ranged from pale yellow to yellow- brown.

Occurrence- This species was limited to site 11 (Sakhloli) where it was reported in both the seasons.

4A. 4. Physicochemical studies of rhizosphere soil samples

Rhizosphere soil samples were analysed for various macro- and micro- nutrients. Concentrations of nutrients namely K^{+}, Cu^{+2}, Zn^{+2}, Fe^{+2} and Mn^{+2} were determined spectrophotometrically. Analyses of four major macro-nutrients *viz.* Carbon, Nitrogen, Sulphur and Phosphorus were carried out using chemical estimation methods. The results presented in Table No. 06 are the means of the values obtained for samples collected from each site.

1. Organic carbon-

   Adequate range of organic carbon in soil for mango crop is between 1 to 5 %. Organic carbon content of soil during both collection seasons, as estimated by Walkley and Black method, revealed that it was slightly more during season II than in season I. All the soil samples showed the same pattern. The organic carbon content during season I ranged between 1.16 % and 5.13% while that of season II was between 1.66% and 5.42%. Soil sample RtKJ1 showed highest carbon content (5.13%) during season I while sample RtKV1 (5.42%) was highest during season II.

2. Nitrogen-

   Mango crop requires approximately 289- 496 kg/ha of total nitrogen in the soil. During the pre-monsoon season (season I), nitrogen content of the soil samples was estimated and was found to be in the range from 109.4 kg/ha to 499 kg/ha. Maximum content was found to be in sample RtKJ (499 kg/ha) followed by RtMK (428.48 kg/ha), RtVJ (374.61 kg/ha), RtPO (369.7 kg/ha), RtNJ (368.53 kg/ha), RtPK (362.24 kg/ha), RtSB (326.23 kg/ha) and RtPT (321.84 kg/ha). Remaining

seven sites showed nitrogen content significantly less than the above sites. Nitrogen content of site RtKP (109.4kg/ha) was recorded to be lowest during first season.

In season II i.e. post- monsoon season, nitrogen content of all the sites showed increase over that during season I except sample RtKJ (407.88 kg/ha) and RtNJ (331.17 kg/ha). Highest content of nitrogen was recorded in soil sample RtKV (607.7 kg/ha). The lowest nitrogen content was recorded for site RtKP (176.1 kg/ha).

3. Phosphorus-

Phosphorus content in the range of 14 to 27 kg/ha is optimum for cultivation of mango crop. The phosphorus levels of rhizosphere soil samples were found to be high during season II in comparison to that estimated during season I. During season I, phosphorus content ranged between 3.13 kg/ha and 18.98 kg/ha. Out of fifteen sites phosphorus content of ten sites ranged between 3.13 kg/ha and 8.24 kg/ha. However, four sites showed higher phosphorus content (above 10kg/ha). Highest phosphorus content was recorded in sample SdWJ (18.98 kg/ha) and lowest in RgHM (3.13kg/ha).

In season II, highest phosphorus content was recorded in sample RtPO1 (44.39 kg/ha) followed by SdWP (43.74kg/ha). Among remaining sites, it ranged between13. 91 kg/ha and 28.1 kg/ha for nine sites while it was remarkably low (between 5.89 and 10.77 kg/ha) for four samples *viz.* RtKV, RgHM, RtPK and RtPT.

4. Potassium-

Potassium content of soil within a range from 13 to 20 ppm is adequate for mango cultivation.

During season I, the potassium contents of all the sites ranged between 17.9 ppm and 24.9 ppm with the only exception of site SDWJ that showed low potassium content of 10.34 ppm. Maximum potassium content was observed in sample RtMK (24.9 ppm) followed by RtKJ (24.3 ppm), RgGP (23.55 ppm), RtSB (23.48 ppm), RtNJ (22.83 ppm), RtPO (20.5 ppm) and RtDJ (20.23 ppm). The concentrations of potassium in sample RtPT (19.4 ppm), RtKP (19.4 ppm), SdWP (19.28ppm), RtKV (18.75 ppm), RtPK (18.32ppm), RtVJ (18.3 ppm) and SdMM (18.03 ppm) were found to be almost equal. Sample RgHM showed low potassium content (17.9 ppm).

In season II, potassium content of the soil samples increased with the exception of sample RtDJ which showed slight decrease in concentration in comparison to season I. The concentrations ranged from 18.76 ppm (RtPK) to 31.7 ppm (SdWJ). The highest 'K' content in SdWJ (31.7 ppm) was followed by RtMK (29 ppm), SdWP (28.86 ppm), RgGP (27.93 ppm), RtPO (27.6 ppm), RtKJ (26.9 ppm), RtVJ (25.83 ppm), SdMM (25.55 ppm), RtSB (24.83 ppm), RtKP (23.55 ppm), RtKV (23.25 ppm) and RgHM (22.6 ppm). Potassium contents of RtPT (19.46 ppm), RtDJ (19.18 ppm) and RtPK (18.76 ppm) were low compared to other samples.

5. Iron

The optimum concentration of iron for cultivation of mango crop is between 6 and 15ppm. Concentrations of iron (Fe) during both collection seasons were estimated spectrophotometrically and are represented in Table no. 6.

Iron content of soil samples during season I ranged between 12.9 ppm and 31.75 ppm with highest being in RgGP () and the lowest was recorded in sample RtPO(). In second season, iron content of the soils was found to be from 13.13 ppm to 35.31 ppm.

Iron content increased significantly during season II except the five sites *viz.* RtKJ, RtMK, RtDJ, RtKP and RtSB which showed less iron content in second season than that in the first season.

6. Manganese

The amount of manganese required by mango crop is from 2 to 8 ppm. All the soil samples were analysed for their Manganese content in both the collection seasons.

Concentration of manganese during season I ranged from 5.59 ppm (SdMM) to 11.45 ppm (RtKV). It was followed by sample RtPT (11.33 ppm), RtPK (10.91 ppm), RtMK (10.82 ppm), RtDJ (10.26 ppm) and RtNJ (9.64 ppm). The remaining samples also showed manganese content falling in very close range *viz*. RtKP (8.58 ppm), RtSB (7.83 ppm), RtKJ (7.41 ppm), SdWP (7.37 ppm), RtVJ (7.34 ppm), RtPO (7.28 ppm), SdWJ (7.06 ppm) and RgGP(6.91 ppm).

A decrease in Manganese content was observed for six of the collection sites during second season. The sites included SdWP (6.64 ppm), RtPK (8.42 ppm), RtPT (8.69 ppm), RtNJ (7.05 ppm), RtMK (6.72 ppm) and RtKV (10.82 ppm). Maximum content was recorded in RtDJ (11.5ppm) followed by RgHM (11.4 ppm) and RtKV (10.82 ppm). Lowest manganese content was recorded in RtMK (6.72 ppm).

7. Copper

Concentrations of copper during two collection seasons for all the soil samples were estimated spectrophotometrically. Mango crop requires 2 to 4 ppm of copper in the soil.

Copper ion concentration in first season was found to be between 0.47 ppm and 1.36 ppm with sample SdWP showing lowest and sample RgHM showing highest concentration. Sample RtMK (1.08 ppm) and SdWJ (1.05 ppm) also showed copper content above 1ppm. Among rest of the sites, ten sites *viz.* RtDJ and RgGP (0.84ppm), RtPK (0.79 ppm), RTVJ (0.78 ppm), RtSB and RtKV (0.75 ppm), RtPO (0.71 ppm), RtKP (0.67 ppm), SdMM (0.55 ppm) and RtNJ (0.5 ppm) had copper content ranging between 0.5 ppm and 1ppm. However, copper contents of three sites *viz*. RtKJ, RtPT and SdWP were below 0.5 ppm which was lower compared to the above sites.

During season II, copper concentration was found to be increased for half of the sites; however, half showed concentrations less than those in season I. Maximum copper content was recorded in RgHM (1.82 ppm), followed by RgGP (1.12 ppm), RtDJ (1.064 ppm), RtVJ (0.82 ppm), SdWP (0.79 ppm), RtMK (0.67 ppm), RtPT (0.64 ppm), RtKJ (0.63 ppm), SdMM and RtPK (0.56 ppm), SdWJ, RtPO and RtSB (0.53 ppm) and RtKV (0.47 ppm). The copper content estimated in sample RtKP (0.33 ppm) was the overall lowest i.e. of both the collection seasons.

8. Zinc

It is also an essential micro-nutrient for mango crop which is required in the range of 2-4 ppm. Zinc content was estimated (in ppm) by spectrophotometric method for soil samples of both seasons.

The zinc content during first season ranged from 0.12 ppm to 0.25 ppm. The highest was recorded in RgGP (0.25 ppm) followed by RtNJ (0.23 ppm), RtVJ (0.22 ppm), RtPK (0.19 ppm), SdWJ and RtPO (0.18 ppm), RtKJ, RgHM and RtKV (0.17 ppm), SdMM, RtKP and RtDJ (0.16 ppm), RtPT (0.15 ppm), SdWP and RtMK (0.14 ppm) and RtSB (0.12 ppm).

In season II, zinc content was found to be increased with the exception of five sites *viz*. SdWJ (0.16 ppm), SdWP (0.097 ppm), RtPK (0.15 ppm), RtKJ (0.16 ppm) and RtPO (0.14 ppm). Highest content was observed in RtMK (0.35 ppm) while sample SdWP showed lowest content of 0.097 ppm. It was the lowest of all for both the seasons.

4B. Nursery Experiment

Details of treatments used in Nursery experiment:-

As mentioned in Chapter II, a nursery experiment was designed using eight treatments detailed in the Table No. 02.

4B. 1. Effect on plant height:

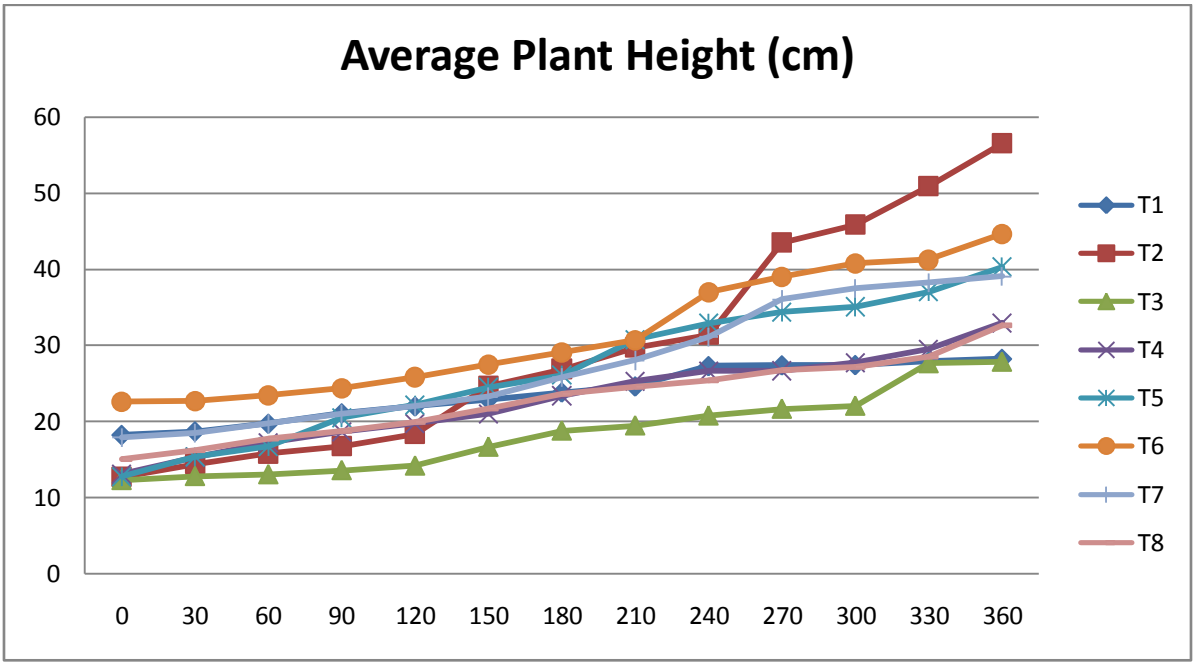
Heights (in cm) of the seedlings from all the replications of each treatment were recorded at 30 days interval and the mean heights are represented in Table No. 08 and Figure No.02.

The initial height of seedlings (zero DAS) in control treatments T1 and T2 was recorded to be 18.26cm and 12.74cm, respectively. The mean heights of seedlings from experimental treatments *viz.* T3, T4, T5, T6, T7 and T8 were 12.30cm, 13.10cm, 12.78cm, 22.60cm, 17.90cm and 15.06cm, respectively, as recorded at the beginning of the experiment.

Table No. 08: Effect of various AM treatments (T) on the height of mango seedlings (in cm)

T	No. of Days												
	0	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**	**330**	**360**
T1	18.26	18.72	19.73	21.1	22.01	22.91	23.84	**24.63**	**27.3**	27.4	27.44	27.89	28.25
T2	12.74	14.46	15.81	16.74	18.4	24.68	27.75	29.7	31.43	43.5	45.85	50.9	56.55
T3	12.3	12.83	13.06	13.6	14.2	16.68	18.22	19.48	20.78	21.67	22.07	27.65	27.85
T4	3.1	15.32	17.2	18.65	19.73	21	23.01	25.28	26.6	26.68	27.76	29.52	32.95
T5	12.78	15.38	16.7	20.5	22.2	24.5	27.4	30.75	32.93	34.37	35.06	37.03	40.3
T6	22.6	22.65	23.4	24.33	25.83	27.48	29.63	30.7	37	39.05	40.75	41.25	44.65
T7	17.9	18.48	19.76	21.02	22.04	23.24	25.08	28.03	31.1	36.05	37.5	38.25	39.08
T8	15.06	16.2	17.72	18.76	19.92	21.7	23.84	24.58	25.4	26.73	27.17	28.47	32.63
F test							**S**						**S**
C.D. (0.5%)							**13.25**						**10.89**

Figure No.1: Average height (in cm) of the seedlings in all the treatments at 30 DAS interval



Seedlings in treatment T1 (un-inoculated control) showed slow but consistent increase in the height at every 30 days interval up to 180 days. During an interval between 210 days and 240 days the increase was more (almost 2.6 cm) than rest all the intervals which slowed down remarkably till the end of the experiment.

On the other hand, chemical fertilizer control exhibited enhanced effect on height of the seedlings. In first six months there was rise in height from 12.74cm (zero days) to 27.75 cm. An increase (6.28 cm) during fifth month of observations (120days to 150 days) was greater. Height of T2 seedlings was found to shoot up between 8th and 9th month (12.07 cm) once again which further increased consistently till the end.

Seedlings in treatment T3 showed slow but steady increase in height up to ten months which increased suddenly in the 11th month by 5.58 cm with no more change till the end of the experiment. Similar observations were recorded for T4 seedlings.

Maximum rise in height was reported during the last month of the experiment (3.43cm).

Treatment T5 exhibited a different trend. The increase was more around 6th month (2.9cm and 3.35 cm) and also during the last month of observations (3.27cm). Treatments T6 and T7 exhibited greater effect in 8th (6.3cm) and 9th (4.95cm) month of the experimental period, respectively. Seedlings in treatment T8 showed gradual increase throughout the observation period.

4B. 2. Effect on number of leaves:

The data pertaining to effect of treatments on number of leaves is given in Table No. 09 and the same is depicted in figure No. 03.

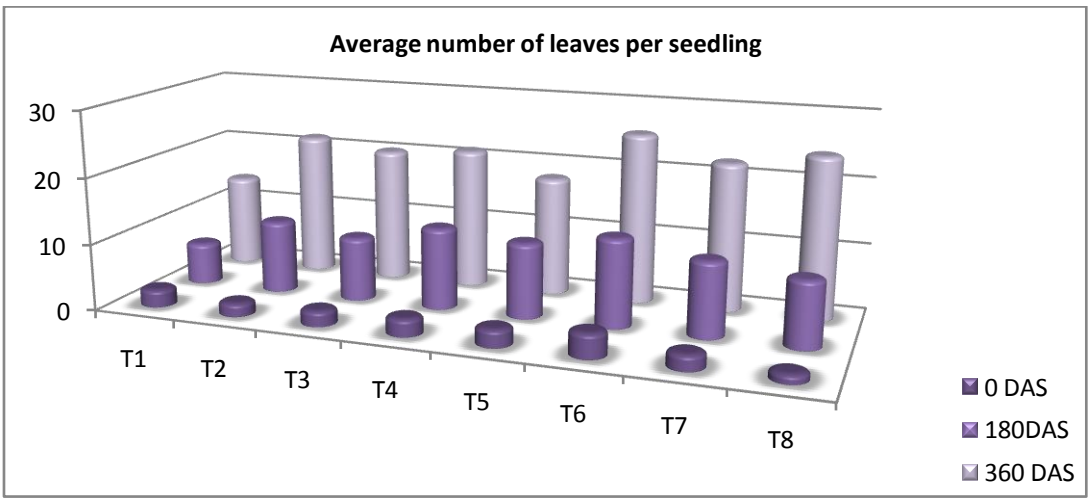
The initial mean number (zero DAS) of leaves per seedlings was almost similar with that of control T1 and T2 being 1.33 and 2, respectively. The mean number of leaves per plant of seedlings in AM treatments *viz.* T3, T4, T5, T6, T7 and T8 were 2.33, 2.67, 2.67, 3.67, 2.33 and 1.33, respectively. There was a consistent increase in number of leaves per plant over the period of the experiment.

Effect of some of the AM treatments was significant at 180 DAS. The mean leaf number in the chemical control T2 (11) was remarkably more than the un-inoculated control T1 (6.33). AM treatment T6 (13.33) produced maximum number of leaves followed by T4 (12.33), T5 and T7 (11.33). These treatments were statistically at par with chemical control but superior to un-inoculated control T1. Treatments T3 (9.67) and T8 (10) were at par with the control T1 though they were slightly less than control T2.

Table No. 09: Effect of various nutrient treatments on number of leaves per seedling.

Treatments	No. of leaves per seedling		
	Zero DAS	180 DAS	360 DAS
T1	1.33	6.33	14
T2	2	11	21.67
T3	2.33	9.67	20.33
T4	2.67	12.33	21.33
T5	2.67	11.33	18
T6	3.67	13.33	25.67
T7	2.33	11.33	22.33
T8	1.33	10	24.33
F -test	**S**	**S**	**S**
C.D.(0.5%)	1.66	4.5571	2.80474

Fig. No. 02: Average number of leaves per seedling per treatment at Zero DAS, 180 DAS and 360 DAS interval.



At 360 DAS, all the treatments were significantly superior to un-inoculated control T1 (12) in increasing mean number of leaves per seedling. However, mean number of leaves per seedling in chemical control T2 (21.67) exceeded that in control T1 as well as some of the AM treatments. Among AM treated seedlings, T8 (100% AM + 100%

CF) seedlings exhibited greater influence on mean number of leaves in second half of the experiment i.e. between 180 DAS and 360 DAS (24.33). Treatments T7 (22.33), T4 (21.33) and T3 (20.33) were at par with control T2. Seedlings in T6 showed more number of leaves (25.67) which was statistically superior over rest all the treatments except treatment T8.

4B. 3. Effect on stem girth:

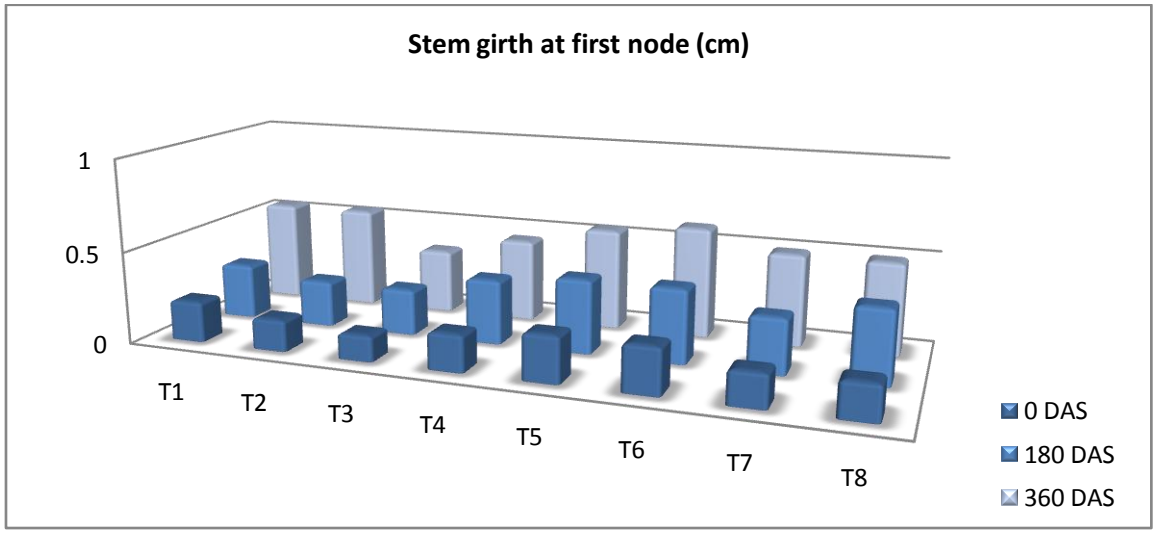
Stem girth of the seedlings from three replications of each treatment were recorded. Girths at the first node and collar region were measured and averaged separately for all the treatments. The mean values are represented in Table No. 10 and figure no 04 and 05.

i) Stem girth at first node:-

At the beginning of the experiment (Zero DAS), all the treated seedlings showed average stem girth at first node ranging between 0.14 cm and 0.25 cm. The average stem girths of un-inoculated control T_1 and chemical control T_2 were 0.22 cm and 0.17 cm, respectively. Initial node stem girths of the seedlings in experimental treatments were as follows- T3 (0.14 cm), T4 (0.20 cm), T5 and T6 (0.25 cm), T7 (0.18 cm) and T8 (0.19 cm).

At 180 days interval, three of the AM treatments had significant effect on mean stem girth at the node of the seedlings. In control treatments they were recorded to be 0.30 cm in T1 and 0.25 cm in T2. Maximum stem girth (0.40 cm) was observed in Treatment T5, T6 and T8 which was significantly superior over both the controls. It was followed by treatment T4 (0.35 cm), T7 (0.30 cm) and T3 (0.25 cm). These were at par with the control treatments.

Fig. No. 03: Average stem girth at first node of seedlings of experimental treatments at Zero DAS, 180 DAS and 360 DAS.



At 360 days, both the controls T1 and T2 showed the stem girth of 0.55 cm. Among AM treatments, T6 showed highest stem girth (0.60 cm) which was slightly greater than that observed in T5 (0.55 cm). Thus both were statistically at par with the controls. Following were treatments T7, T8 (0.50 cm) and T4 (0.45 cm). Least reading for mean stem girth was recorded in T3 (0.35 cm) which was significantly less than the control treatments.

Table No. 10: Effect of various nutrient treatments on Mean stem girth (cm) at collar and first node region of the seedlings

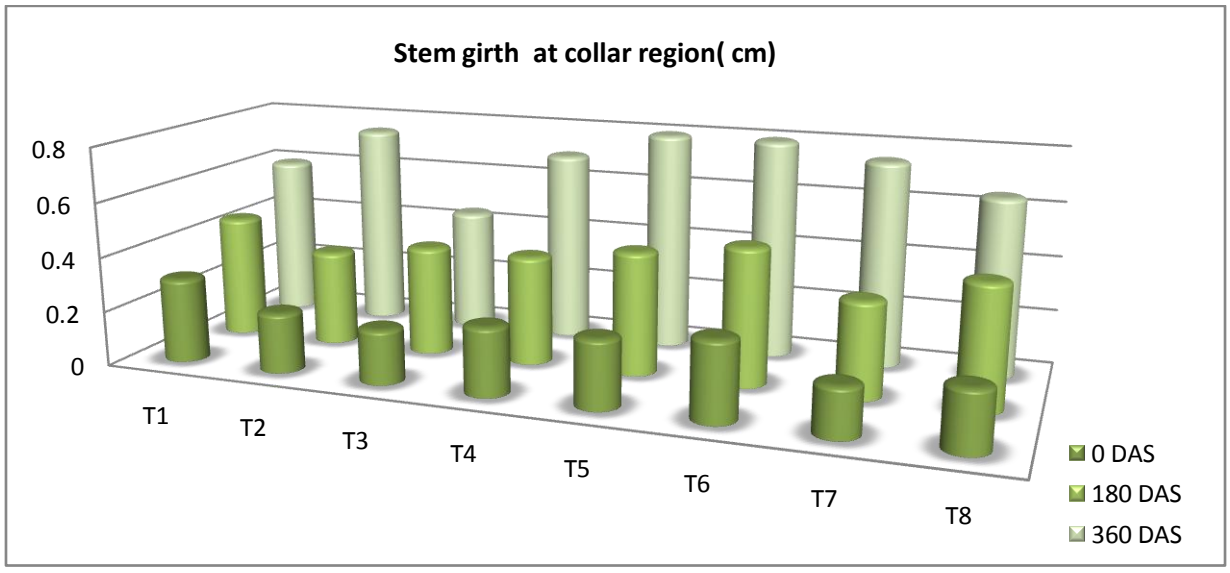
	First node			Collar region		
Treatment	**Zero DAS**	**180 DAS**	**360 DAS**	**Zero DAS**	**180 DAS**	**360 DAS**
T1	0.22	0.30	0.55	0.31	0.45	0.6
T2	0.17	0.25	0.55	0.22	0.35	0.75
T3	0.14	0.25	0.35	0.2	0.40	0.45
T4	0.2	0.35	0.45	0.25	0.40	0.70
T5	0.25	0.40	0.55	0.25	0.45	0.80
T6	0.25	0.40	0.60	0.29	0.50	0.80
T7	0.18	0.30	0.50	0.18	0.35	0.75
T8	0.19	0.40	0.50	0.22	0.45	0.65
F-test		**S**	**S**		**S**	**S**
C.D. (0.5%)		0.06299	0.0839		0.06299	0.11972

ii) Stem girth at collar region:-

Average stem girth at collar region of the seedlings (zero DAS) in all the treatments ranged between 0.18 cm and 0.31 cm. The stem girths of un-inoculated control (T1) and chemical control (T2) initially, were recorded to be 0.31 cm and 0.22 cm, respectively. In case of the AM treatments, the initial mean stem girth were in the order- T3 (0.20 cm), T4 and T5 (0.25 cm), T6 (0.29 cm), T7 (0.18 cm) and T8 (0.22 cm).

At 180 days, mean stem girth of control T1 increased to 0.45cm which was greater than that observed in chemical control T2 (0.35cm). AM treatments did not exhibit significant effect on collar stem girth at 180 DAS. Among these, treatment T6 (0.50cm), T5 and T8 (0.45 cm), T3 and T4 (0.40 cm) were at par with the control T1 but significantly effective over control T2. Remaining treatment *viz.* T7 (0.35 cm) was equally effective as control T2 though not as much as control T1.

Fig. No. 04: Average stem girth at collar region of the seedlings of control and experimental treatments.



At the end of the experiment (360 DAS), the effect of various experimental treatments was not that significant compared to that of chemical control T2 (0.75cm); however, it was better over the un-inoculated control T1 (0.60cm). The treatments T5 and T6 (0.80 cm) recorded greater collar stem girth which was slightly more than that in control T2. Mean stem girth observed in T7 (0.75 cm) and T4 (0.70 cm) were at par; however, T8 (0.65 cm) and T3 (0.45 cm) showed stem girth which was least of all the treatments.

4B. 4. Effect on root length:-

With reference to this parameter the observations were recorded after uprooting the selected seedlings. Table No. 11 and figure No. 06 represent the observations of root lengths at zero DAS, 180 DAS and 360 DAS intervals.

At the beginning (Zero DAS) the mean root length of un-inoculated seedlings (T1) was 7.8 cm and that of T2 seedlings was 7.23 cm. The initial root lengths of other experimental treatments were as follows- T3- 6.23 cm, T4- 6.47 cm, T5-6.63 cm, T6-6.13 cm, T7- 5.73 cm and T8- 5.2 cm.

At 180 days, root length in un-inoculated control (18.05cm) was at par with the chemical control treatment T2 (16.0cm). Among the AM treatments, root length recorded in T6 (26.45cm) was significantly greater as compared to rest of the treatments. It was followed by T4 (20.0cm), T8 (19.7cm), T5 (16.95cm), T7 (16.1cm) and T3 (15.6cm). These treatments were at par with the controls T1 and T2.

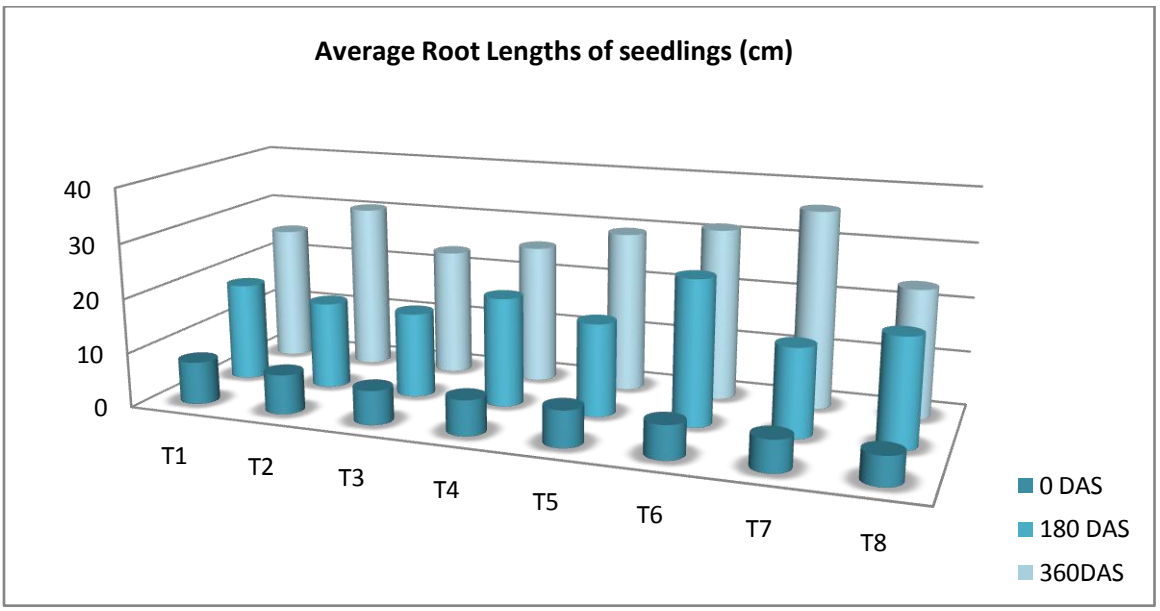
At the end of 360 days the chemical control treatment T2 (30.6cm) exhibited greater influence on root length than the un-inoculated control T1 (25.15cm). In case of AM treatments, treatment T7 (36.0cm) was superior to all other treatments including

controls, significantly. It was followed by T6 (31.45cm) and T5 (29.45cm) which were at par with control T2 and significantly superior over control T1, T4(25.6cm), T8(23.5cm) and T3(23.4cm) .

Table No. 11: Effect of various nutrient treatments on Mean root length (cm) of the experimental seedlings

	Root length (cm)		
Treatment	**0 DAS**	**180 DAS**	**360DAS**
T1	7.8	18.05	25.15
T2	7.23	16	30.6
T3	6.23	15.6	23.4
T4	6.47	20	25.6
T5	6.63	16.95	29.45
T6	6.13	26.45	31.45
T7	5.73	16.1	36
T8	5.2	19.7	23.5
F-test	**NS**	**S**	**S**
C.D. (0.5%)	3.907497	5.83	3.05

Fig. No. 05: Average root lengths of the seedlings in control and experimental treatments at zero DAS, 180 DAS and 360 DAS.

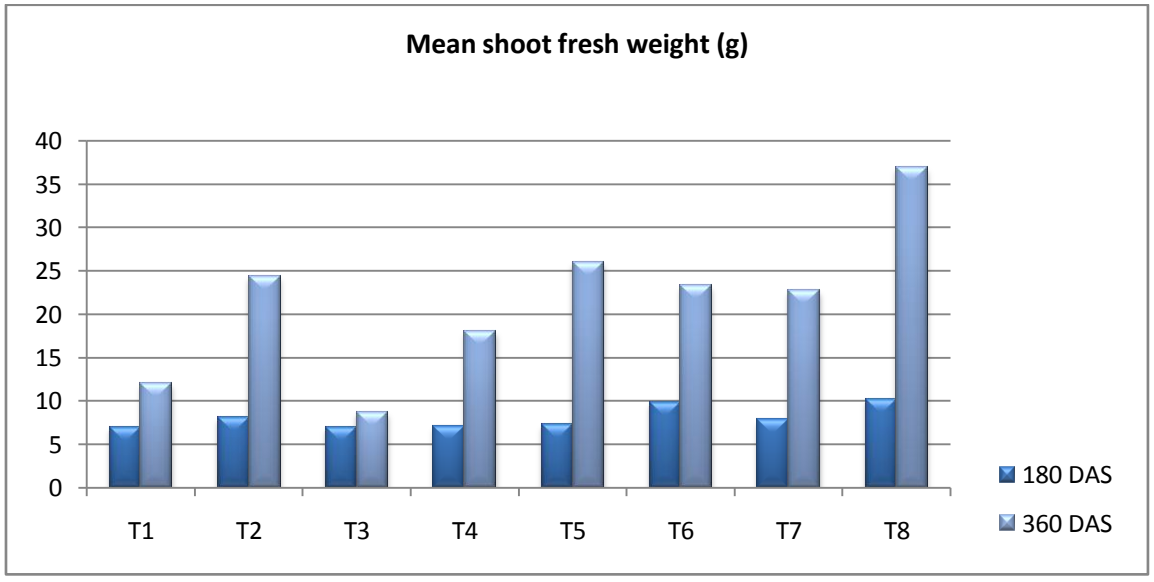


4B. 5. Effect on Fresh Weight of shoot:

Effect of various doses of AM inoculum, its combinations with chemical fertilizer and chemical fertilizer alone on fresh weight of experimental seedlings was studied. The observations of fresh weight of shoot as recorded at 180 day and 360 days are represented in Table No. 12 and Figure No. 07.

The data regarding shoot fresh weights at 180 days indicated no significant effect of any of the treatments. Shoot fresh weight in control treatments T1 and T2 was found to be 7.0g and 8.1g, respectively. At 180 DAS, all the AM treatments except T8 were at par with the controls T1 and T2. Among AM treatments, shoot fresh weight of T8 seedlings (11.2g) was statistically superior over un-inoculated control T1 but at par with the chemical control T2. It was followed by T6 (9.85g), T7 (7.83g), T5 (7.3g), T4 (7.13g) and T3 (7.0g).

Fig. No. 06: Mean Shoot fresh weight of the seedlings in control and experimental treatments at 180 DAS and 360 DAS.



At 360 DAS, un-inoculated control T1 and chemical control T2 exhibited shoot fresh weight of 12.08g and 24.4g respectively. In case of AM treatments, highest fresh weight was recorded in T8 (36.98g) which was significantly superior over both control T2. Treatment T5 (26.07g), T6 (23.38g), T7 (22.81g) and T4 (18.09g) were significantly superior over un-inoculated control (T1) while less effective than the chemical control T2.

Table No. 12: Effect of various nutrient treatments on Shoot Fresh and Dry weights (g) of the seedlings

	Fresh wt (g)		Dry wt. (g)	
Treatment	**180 DAS**	**360 DAS**	**180 DAS**	**360 DAS**
T1	7	12.08	2.34	4.04
T2	8.1	**24.4**	3.46	14.7
T3	7	8.74	2.81	3.51
T4	7.13	18.09	2.98	7.9
T5	7.3	26.07	2.83	10.11
T6	9.85	23.38	5.34	12.68
T7	7.83	22.81	3.15	9.18
T8	11.2	36.98	2.78	9.18
F-test	**S**	**S**	**S**	**S**
C.D. (0.5%)	3.53133	4.06682	2.134849	1.28864

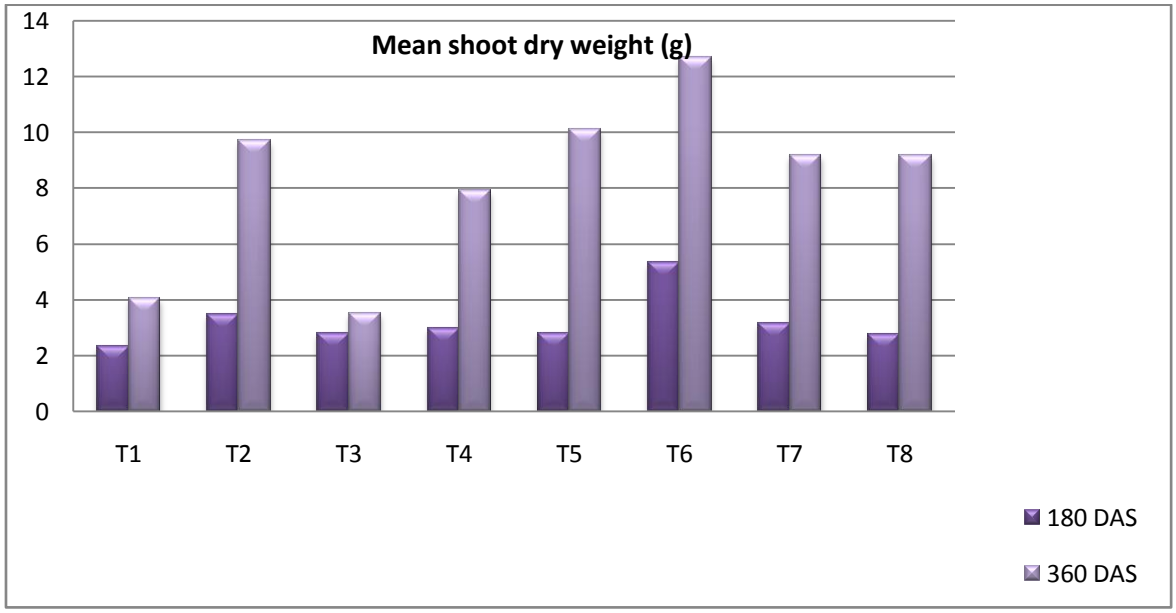
4B. 6. Effect on Dry Weight of shoot:

Dry weights (g) of the oven dried shoots of seedlings from all the treatments were recorded and compared to study the influence of AM inocula, chemical fertilizer alone and their combinations. Table No. 12 and Figure No.08 represent the observations regarding shoot dry weights.

At 180 days, all the AM treatments, except T6, were at par with the control T1 (2.34g) and T2 (3.46g). Treatment T6 was successful in producing highest dry weight (5.34g)

which was significantly superior over other treatments except T2. The other treatments T7 (3.15g), T4 (2.98g), T5 (2.83g), T3 (2.81g) and T8 (2.78g) were at par with T1 and T2.

Fig. No. 07: Mean shoot dry weight (g) of seedlings in control and experimental treatments at 180 DAS and 360 DAS.



At the end of the 360 days, all the treatments showed significantly superior effect over un-inoculated control T1 (4.04g) with the exception of T3 (3.51g). At the end of the experiment, chemical control treatment (T2) recorded shoot dry weight (14.7 g) notably greater than treatment T6 (12.68g). Remaining treatments T5 (10.11g), T7 & T8 (9.18g) and T4 (7.9g) were significantly superior over T1 but significantly inferior to T2.

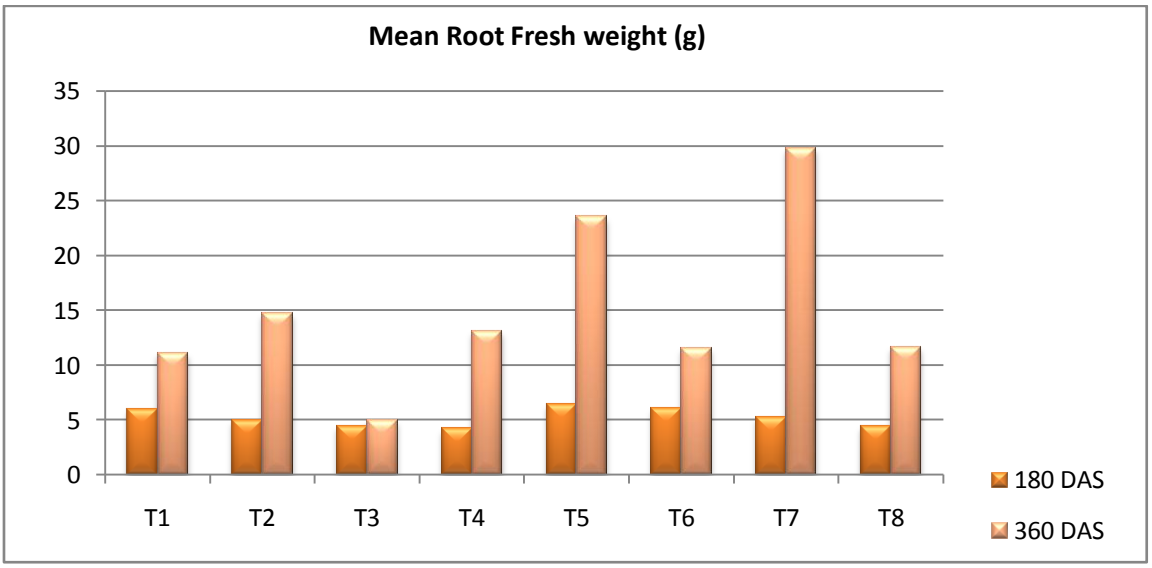
4B. 7. Effect on Root fresh weight (g):

Observations regarding root fresh weight were recorded at two intervals viz. 180 days i.e. at the middle of the experiment and 360 days i.e. at the end. Table no. 13 and

Figure no. 09 represents effect of various experimental treatments on root fresh weight at both time intervals.

Till 180 DAS, effect of the AM treatments was not worth considering. The root fresh weight of un-inoculated control T1 (6.0g) was slightly but not significantly more than that of the chemical control T2 (5.0g). Treatments T5 and T6 showed root fresh weight of 6.4g and 6.1g, respectively followed by T7 (5.25g), T8 & T3 (4.5g) and T4 (4.2g). In all, the experimental treatments were at par with each other as well as the controls.

Fig. No. 08: Mean fresh weight of root (g) of seedlings in control and experimental treatments at 180 DAS and 360 DAS.



At the end of the experiment (360 days), control treatments T1 and T2 showed root fresh weights of 11.16g and 14.74g, respectively. Among AM treatments, T7 (29.78g) and T5 (23.54g) showed significantly higher root fresh weights than both the controls. The remaining treatments *viz.* T4 (13.11g), T8 (11.68g) and T6 (11.56g) were at par with both the controls. Least weight was observed in T3 (4.97g).

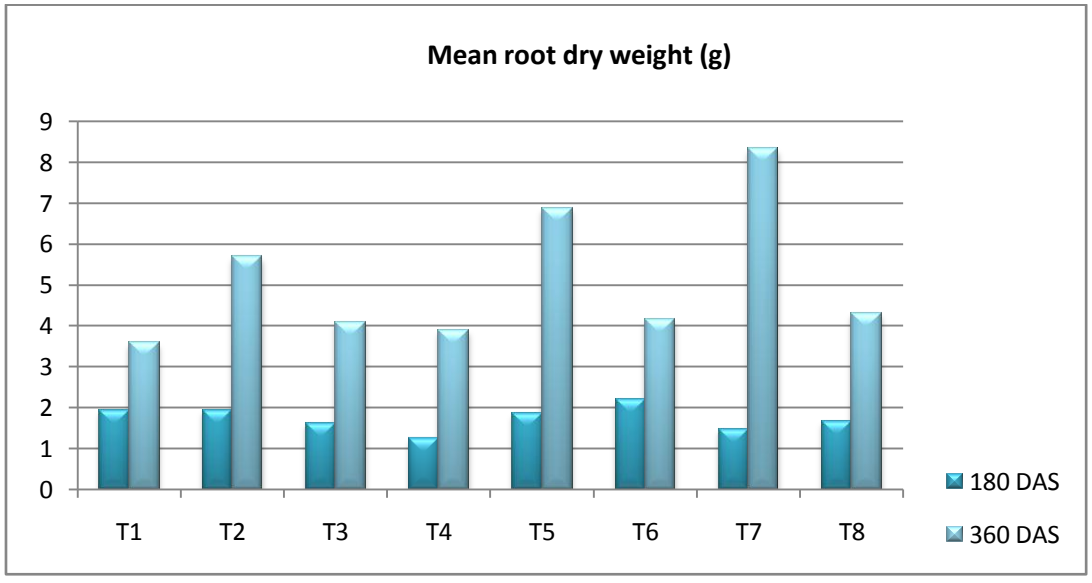
Table No. 13: Effect of various nutrient treatments on Root fresh and Dry weight (g) of the seedlings

	Fresh wt (g)		Dry wt (g)	
Treatment	**180 DAS**	**360 DAS**	**180 DAS**	**360 DAS**
T1	6	11.16	1.93	3.59
T2	5	14.74	1.93	5.69
T3	4.5	4.97	1.61	1.78
T4	4.2	13.11	1.25	3.9
T5	6.4	23.54	1.87	6.88
T6	6.1	11.56	2.19	4.15
T7	5.25	29.78	1.47	8.34
T8	4.5	11.68	1.66	4.31
F-test	**S**	**S**	**S**	**S**
C.D. (0.5%)	2.86498	4.909231	1.3177	0.9207

4B. 8. Effect on root dry weight (g):

Table No. 13 and Figure no. 10 represent effect of various treatments on root dry weight.

Fig. No. 09: Mean Dry weight of roots (g) of the seedlings in control and experimental treatments at 180 DAS and 360 DAS.



At 180 DAS, all the treatments were at par with each other as well as both the controls. Root dry weight noted in both control treatments *viz.* T1 and T2 was 1.93g. Treatment T6 showed highest root dry weight (2.19g), though not statistically superior over controls. Rest of the treatments namely, T3 (1.61g), T4 (1.25g), T5 (1.87g), T7 (1.47g) and T8 (1.66g) were at par with the controls.

Comparison between various AM treatments revealed that, only few had notable effect on root dry weight at 360 DAS. The control treatments T1 and T2 showed the root dry weight of 3.59g and 5.69g, respectively. Treatment T7 (8.34g) and T5 (6.88g) were significantly superior to both the control treatments. These were followed by T8 (4.31g), T6 (4.15g) and T4 (3.9g) which were at par with both the controls. Treatment T3 exhibited least root dry weight of 1.78g, which was remarkably lesser than both the controls.

4B. 9. Mycorrhizal status of nursery seedlings

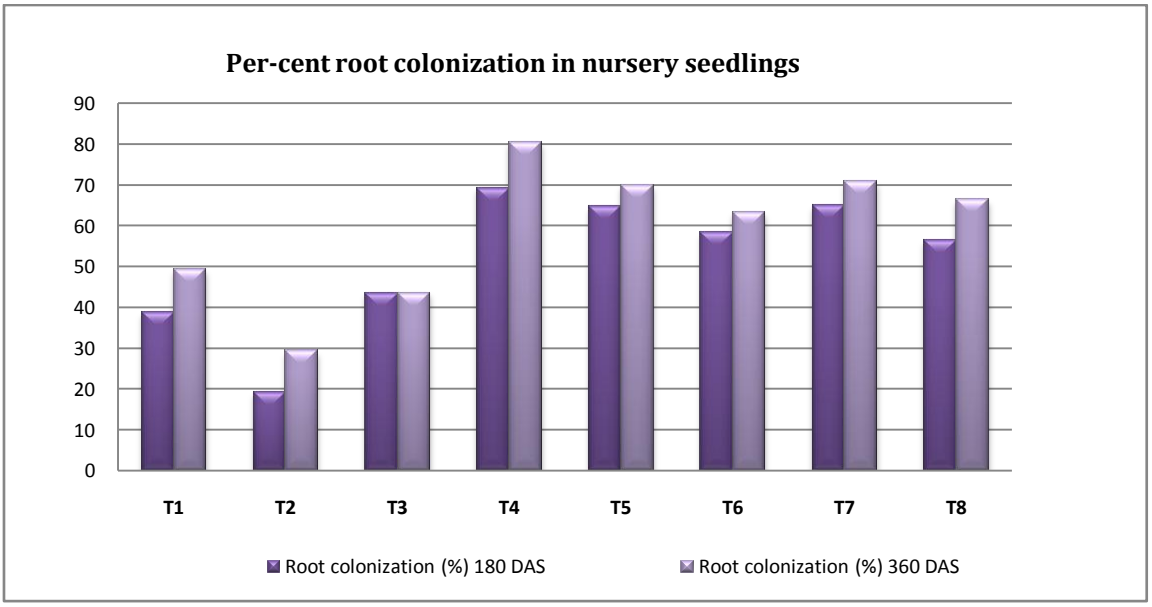
Status of Mycorrhizal inoculum, in terms of root colonization and spore density in rhizosphere soil, in un-inoculated and inoculated nursery seedlings, was assessed at six months interval i.e. 180 DAS and 360 DAS. Mean values of the same are represented in Table No. 14. And Figure No. 11 & 12.

i) Root colonization (Per-cent)

Percentage of root colonization was assessed by staining the root samples of the uprooted seedlings from all treatments and the mean was worked out for each treatment.

At 180 DAS, AM colonization in un-inoculated control and chemical control seedlings was found to be 38.93% and 19.2%, respectively. Plants from treatment T4 (69.33%) showed the highest AM colonization. Following were treatment T7 (64.9%), T5 (64.82%), T6 (58.5 %), T8 (56.5%) and T3 (43.67%). All the treatments were significantly superior over the un-inoculated control (T1) as well as chemical control (T2).

Fig. No. 10: percentage of root colonization in nursery seedlings recorded at 180 DAS and 360 DAS intervals



There was an overall increase in the extent of colonization at 360 DAS and all the treatments were significantly superior to both the controls. The un-inoculated control T1 and chemical control T2 showed colonization of 49.42% and 29.33%, respectively. Among the AM treatments, treatment T4 (80.48%) showed highest per-cent of colonization followed by T7 (70.83%), T5 (69.83%), T8 (66.47%), T6 (63.28%) and T3 (53.67%).

Table No. 14: Effect of various nutrient treatments on Per-cent root colonization and Spore density in rhizosphere of the seedlings.

Treatments	Root colonization (%)		Spore count (per 10 g soil)	
	180 DAS	360 DAS	180 DAS	360 DAS
T1	38.93	49.42	74.67	90.67
T2	19.2	29.33	52.33	66.33
T3	43.67	53.67	87	101.67
T4	69.33	80.48	111	140.33
T5	64.82	69.83	114.33	157.67
T6	58.5	63.28	80	107
T7	64.9	70.83	134.33	158.67
T8	56.5	66.47	97.33	112.33
S.E.	3.08	2.90	11.36	9.57
F-test	**S**	**S**	**S**	**S**
C.D.(0.5%)	**9.03**	**9.28468**	**33.29**	**28.08**

ii) Spore density (per 10g soil)

The initial spore densities (per 10 g soil) of garden soil used for potting mixture and the AM inoculum were recorded. They were found to be 67 spores and 251 spores, respectively.

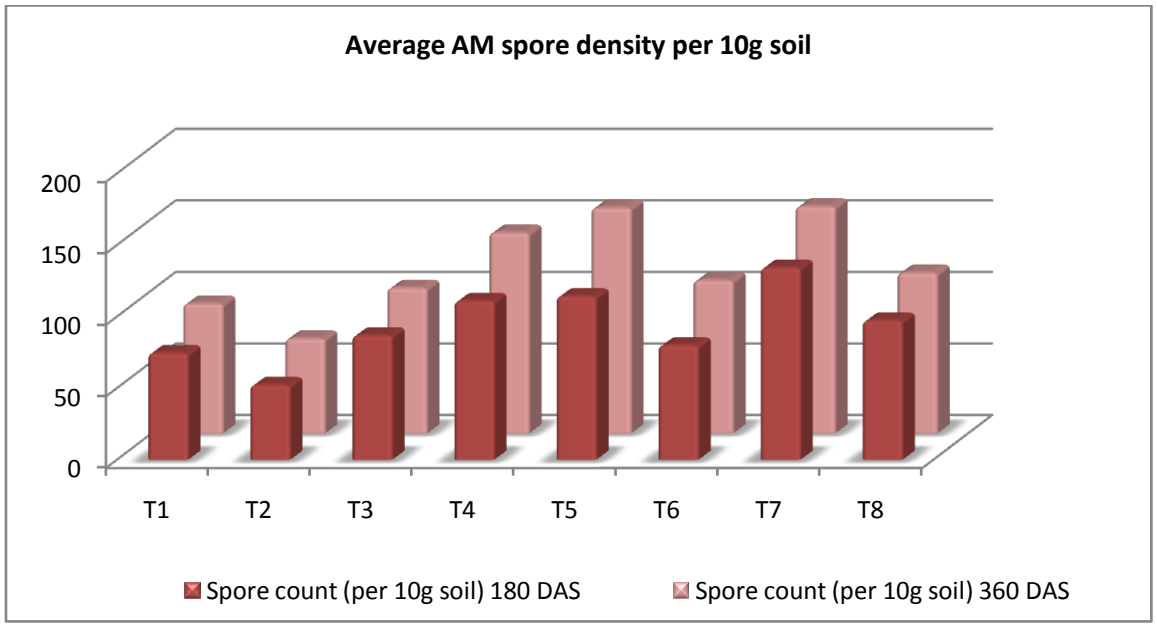
Spore density of the rhizosphere was determined at 180 days and 360 days interval for all the treatments.

At 180 DAS, spore density estimated in un-inoculated control (T1) was 74.67 per 10g soil and that in chemical control (T2) was 52.33 per 10g soil. Amongst AM treatments, T7 (134.33) showed highest spore count (per 10g soil) which was followed by T5 (114.33), T4 (111), T8 (97.33), T3 (87) and T6 (80). Treatments T7, T5 and T4 were significantly superior over both the controls T1 and T2.

Spore count increased significantly at the end of the experiment (360 DAS). In control plants, spores density was found to be 90.67 spores and 66.33 spores per 10g soil for T1 and T2, respectively. Treatments T7 (158.67 spores), T5 (157.67 spores), T4 (140.33 spores), T8 (112.33 spores) exhibited remarkably high spore count than

both the controls. While T6 (107 spores) and T3 (101.67 spores) were found to be at par with the control T1. All the treatments were significantly superior over chemical control T2.

Figure No. 11: Spore densities (per 10 g soil) in rhizosphere soils of nursery seedlings at 180DAS and 360 DAS interval.



CHAPTER – V

DISCUSSION

Chapter-V

DISCUSSION

5A. Survey:

5A. 1. Selection of sites for sample collection:

Amongst various fruit crops, mango is the major horticultural crop of India that attracts international market. India is top ranked with contribution of 39% to the total world production of mangoes (Singh, 2008). It is cultivated in many states of India as it is adaptable to the wide range of soil and climatic conditions. However, the varieties grown in the Konkan region of Maharashtra state have more demand in national as well as international market because of the characteristic flavour and attractive colour. Therefore, the sites were selected from Konkan region so as to survey the status of AM fungi associated with this crop.

5A. 2. Sample collection:

Sixteen sites were located from three districts (Raigad, Ratnagiri and Sindhudurg) in Konkan region and total of 58 samples, comprising rhizosphere soil and the young roots, were collected from selected mango plants. The mango plants selected for sampling from various localities were of different ages ranging from 5 years up to about 100 years.

5A. 3. Season of sample collection:

Change in season causes change in the microbial activities of the soil which leads to change in the physico-chemical properties of soil. In turn, it may influence the AM status i.e. density, diversity and root colonization ability of AM fungi. Therefore, the

survey was carried out in two seasons *viz.* Pre-monsoon (i.e. April-May) and Post-monsoon (i.e. December- February). As far as Mango plants are concerned, in pre-monsoon season the fruit almost reaches maturity and hence it is a period for harvest. Thus, nutritional requirement of plants is reduced.

Monsoon is the most important season of India. The *Konkan* region (West coast of Maharashtra state) receives heavy rain fall (above 2500 mm per annum). It may cause leaching of minerals and have dilution effect on soil micro-flora. Post-monsoon period is very crucial for mango plants, since it is the beginning of the flowering process. The requirement for nutrients, particularly, phosphorus is high during this period. Therefore, the chances of formation of AM association increase.

In order to find the seasonal variations in AM status, if any, the two seasons were selected.

5A. 4. Mycorrhizal status:

Assessment of roots and spore density in rhizosphere soils revealed that, all the mango plants of various varieties, irrespective of the age groups possessed AM association.

5A. 4.1. Root colonization and age of host plant:

The old plants (age approximately 75-100 yrs) showed varied degrees of root colonization. Percent root colonization values determined for these plants ranged from as low as 4.16% and zero% (SdWP5- 100 yrs) to as high as 52.4% and 72.25% (RtKJ1- 90 yrs) in season I and season II, respectively. Similarly, in younger plants (age approximately 5-25 years) the lowest degree of colonization was found to be 2.73% & 2.19% (SdMM1) and the highest of 83.72% (RtPT3) & 88.23% (SdWJ4),

respectively, in season I and season II. The plants of moderate age (approximately between 40 and 70 years) exhibited the root colonization ranging between 3.47% & 68% in season I and between zero % (SdWJ3) and 71% (RtKJ4) in season II. This shows that, the extent of root colonization by AM fungi was not dependent on the age of the host plant.

5A. 4. 2. Soil factors and AM status:

The samples were collected twice during different time periods; particularly, during pre-monsoon and post-monsoon seasons. The data revealed that, the pH of the soil decreased slightly, invariably at all the collection sites during second season. A post-monsoon increase in moisture content was noticed in all the samples. The increased moisture content well supported soil microbial activity, as a result the soil pH showed shift towards acidic side.

The first collection site (Math, Vengurla, Dist.- Sindhudurg) was situated on a plateau consisting of basaltic rock with very thin soil cover. The samples showed decrease in soil pH from season I (6.82) to season II (6.03). On the other hand, the moisture level remained almost same in both the seasons for all the samples collected (SdMM1- 1.71 & 1.78 and SdMM2- 1.08 & 1.33). The site was rich in Iron (Fe-17.1 ppm & 25.5ppm) but deficient in other micronutrients (Mn: 2 and 8ppm, Zn: 0.16 and 0.17ppm, Cu: 0.55 and 0.56ppm, K: 18.03 and 25.55kg/h) with respect to the requirements of mango plants (Mn: 2- 8ppm, Zn: 2- 4ppm, Cu: 2- 4ppm, K: 150-230kg/h). Also, the organic carbon content and concentration of fixed nitrogen during first season (1.91% and 196.75 kg/h, respectively) were slightly less than that estimated during

second season (2.41% and 274.85 kg/h) but it was adequate*** for the mango plants. Mycorrhizal fungi are mainly involved in absorption of phosphatic compounds. The analysis of soil for phosphorus revealed that its concentration increased from season I (9.05 kg/h) to season II (23.07 kg/h). It is a common practice of mango farmers to apply phosphatic fertilizer prior to monsoon. This might have resulted in diffusion of the fertilizer in the rhizosphere and hence the increased concentration in post-monsoon season.

Both the samples showed higher spore density in season I (SdMM1- 222 and SdMM2- 342 spores/10g soil) which decreased during the next season (SdMM1- 27 and SdMM2- 37 spores/10g soil). In case of root colonization there was slight decrease from pre-monsoon (2.73 % and 31.25%) to post-monsoon (2.19% and 28.02%) season. Mycorrhizal studies of both the seasons clearly indicated that, spore density and degree of root colonization were more when moisture content of the soil was low, particularly, in pre-monsoon season.

The second collection site was situated on the banks of Deogad creek along the slope of 15-20^{o}. The site comprised of basaltic rock; however, each tree was raised on soil bed of almost 2-3 feet and was protected from side by brick wall to prevent the displacement of soil due to water run-off and also leaching of nutrients. It is clear from the data obtained by chemical analysis the soil exhibited optimum amounts of organic carbon, nitrogen, potassium and all the micro-nutrients analysed during both the collection seasons. Screening of soil samples showed increase in soil moisture content from 2.11% (season I) to 5.75% (season II) while the pH dropped from 7.33 to 6.49 between the two seasons. The AM spore density during season I was high in all the five samples (ranging from 109 to 452) compared to that in season II (ranging

from 24 to 56 spores/10g soil). The decrease in spore density might be due to the drop in pH (7.33 to 6.49). Surprisingly, variation in degree of root colonization was random and did not depend on the age and variety of the host plant. With the exception of SdWJ2, the remaining samples exhibited influence of soil phosphorus on degree of root colonization. As expected, the per-cent root colonization was more under low phosphorus conditions and the same decreased with rise in phosphorus concentration.

Third site from district Sindhudurg namely Wada-Padel (SdWP) was also situated along a hill slope showing basaltic rock with very thin soil cover. The plantation was made by drilling pits in the rock bed which were filled with soil. A change in soil pH was recorded from neutral (season I- 7.08) to slightly acidic (season II- 6.17) between the two collection periods. There was obvious increase in moisture level in post monsoon period (5.4%) from that in pre-monsoon period (1.42%). The soil showed adequate levels of macro- and micro- nutrients except for Potassium, Copper and Zinc which were deficient in both the seasons. Due to application of fertilizer before post monsoon period, the phosphorus content of all the five samples was very high in season II (43.74kg/ha) than that recorded during season I (4.88 kg/ha). Surprisingly, the colonization process was not dependent on the availability of the phosphorus nutrients. The soil pH and moisture influenced the spore density in the rhizosphere such that it was more during summer and reduced during winter. Sample SdWP5 (4.16% and zero) exhibited clear correlation with the P content of the rhizosphere and they were inversely proportional. Sample SdWP5 was very old plant (> 100 yrs) amongst the plants selected from this site and that justifies the poor degree of root colonization by AM fungi.

Next site, Pangari (Rajapur, Dist.- Ratnagiri) was an orchard situated along the slope of a small hill with thick top soil layer. The soil was acidic and the average pH of the soil further decreased from 5.96 to 5.53 from season I to season II. On the other hand, there was expected rise in soil moisture content in season II (2.57%) though it was just slightly more than that in season I (1.53%). Similarly the nutrient status of the soil increased during season II. The data indicates that external population of AM propagules decreased when degree of invasion was more and vice versa. Samples RtPK1 exhibited 25% root colonization when spore density was 76 spores/10g soil (season I) and it increased to 36% as the spore density decreased to 46 spores/10g soil (season II). Similar pattern was reported in RtPK2 and RtPK4. Overall, the AM status of this site varied in response to the soil pH and moisture rather than the nutrients.

Fifth site Purnagad (dist.- Ratnagiri) was along a very low hill slope having thick soil cover. The soil analysis revealed shift in pH towards acidic range (7.04 to 6.21) but increase in percentage moisture (3.75% to 7.99%) from pre-monsoon to post-monsoon period. Naturally, the nutrient status i.e. concentrations of organic carbon, nitrogen and phosphorus improved in post-monsoon season. The collective effect of all the above factors reflected in the extent of mycorrhizal association which was greater in pre-monsoon period (low moisture & nutrients) and lowered during post-monsoon collection (improved nutrient and moisture levels).

Site 6 (Nevare- RtNJ) was situated on a plateau with significant soil layer. Data pertaining to the soil characteristics clearly revealed that moisture content during season I (1.87%) and low levels of available phosphorus (RtNJ1-3.86, RtNJ2-3.73 and RtNJ3- 10.74) favoured AM colonization (spore density: 102, 91 & 57 spores/10g

soil and Root colonization: 64%, 70% and 71.1%, respectively) in all the three sample plants during the pre-monsoon season. On the other hand, a decrease in the AM status was noticed mainly, with increase in available concentration of phosphorus and the soil moisture in the post-monsoon period.

At site 7, Kasop (Ratnagiri), the plantation was along the hill slope with thick soil cover. Change in soil pH from 6.34 (season I) to 5.6 (season II) was observed. However, moisture levels during the two seasons (1.22 % and 1.42%) did not show any significant change. The chemical analysis of the soil indicated optimum amounts of organic carbon, nitrogen, phosphorus and other macro- and micro-nutrients except Cu (0.48 ppm and 0.64 ppm) and Zn (0.17 ppm) in both seasons. In pre-monsoon season the AM spore density (per 10g soil) ranged between 131 and 204 spores which decrease to 87 and 113 spores during post- monsoon season. In contrast, an increase in root colonization was recorded from season I (36.25% to 68%) to season II (40% to 72.25%). The soil moisture levels remained unchanged but low during both seasons. It could be the reason that plants in this site showed moderate range of AM association naturally. Another soil factor that commonly influences AM association i.e. available phosphorus (11.95 kg/ha and 26.78 kg/ha), was also present in optimum concentration. Thus the changes taking place in AM status were not in relation to soil nutrient as well as edaphic factors.

At the following site, Malgund (RtMK), the plantation was along a hill slope mainly consisting of rock with poor soil cover. The data pertaining to physico-chemical analysis revealed no correlation between AM association and soil properties and its nutrient levels.. Also the degree of root colonization and the age of the sample plant did not correlate.

The collection site at Palshet (RtPO, Dist.- Ratnagiri) was situated at the base of a hill slope along the ground level. The results obtained for the samples from this site followed the same trend as recorded for most of the samples. Slight increase in soil moisture (1.29% & 3.11%), acidic nature (6.49 & 6.08) as well as phosphorus content (8.24 & 44.39 kg/ha) were observed between the two seasons. Above factors remarkably influenced the mycorrhizal association in all the sample plants. The AM spore density (ranging between 89 and 161) as well as degree of root colonization (24 % to 47%) was greater during pre-monsoon season which decreased subsequently in post-monsoon period (spore density: from 56 to 114 and root colonization: 19.25% to 21.33%). The change in AM status was in accordance with the changes in soil characteristics and the phosphorus levels.

Site Devghar, Dist.- Ratnagiri (RtDJ) was situated on ground level and showed very thick soil cover. A significant change from 0.94% to 2.27% in moisture level of soil was observed during two collection seasons. On the other, hand pH value decreased from 7.04 (season I) to 6.38 (season II). The soil showed optimum nutrient levels with the only exception of phosphorus, as compared to the levels required by mango crop. Though the soil was deficient in phosphorus, the concentration reported in season II (17.65 kg/ha) was better than that seen in season I (5.24kg/ha). AM spore density was found to decrease (season I: 88, 163 & 130) with increase in phosphorus content in season II (48, 94 & 90), in all the three samples. However, root colonization was more during post-monsoon season (52% & 61.25%) as compared to pre-monsoon period (47.33% & 41%) with the exception of sample RtDJ1 (16% and 14.25%) which showed almost same extent of colonization.

Sakhloli (RtSB: Site 11, Dist.- Ratnagiri), one more site that was situated along a hill slope with thick top soil layer. The soil samples showed drop in pH from 7.43 in season I to 6.89 in season II. Also the average moisture content varied between the seasons from 1.21% to 5.21% along with increase in almost all the nutrient concentrations from pre-monsoon to post-monsoon seasons, with hardly few exceptions. Unlike other sites, the phosphorus content (season I- 14.37kg/ha and season II- 14.35 kg/ha) remained same in both the seasons. The mycorrhizal screening once again revealed the variation in spore density/10g soil and the per-cent root colonization (both high in season I and low in season II, with few exceptions) was as per the pH and moisture conditions of the soil.

Two sites (RtKP & RtRV) from the village Kelshi (Dist.- Ratnagiri) were studied to know the AM status of mango plants.

The site RtKP was near the sea shore, hence, the soil was sandy and was along the ground level. The pH observed was slightly acidic (6.49 & 6.08) with low moisture content (1.08% & 1.92%) of the soil and both remained almost unchanged over the two collection periods. The chemical analysis revealed that the nutrient status improved slightly during post-monsoon period over that observed in the pre-monsoon season, except for the phosphorus. However, the soil, overall, was deficient in organic carbon (season I-1.16 & Season II- 1.57%), total nitrogen (season I-109.4 & Season II- 176.1kg/ha) and phosphorus content (season I-5.83 & Season II- 5.32) in comparison with all the other sites. This reflected in the degree of root colonization by AM fungi. For all the three samples, colonization was more in 2nd season (42.1%, 33.33% & 59.85%, respectively) than that during the first season (27%, 16.35% & 18.25%, respectively). On the other hand, the spore density per 10g of soil was found

to be almost the same during both seasons (RtKP2: 62 & 73 and RtKP3: 90 & 84) with the exception of RtKP1 (104 & 69) which showed decrease in spore density with increase in soil moisture content.

The second site (RtRV) from Kelshi (Rohale) was situated on the hill slope away from the sea shore. Soil showed very low percentage of moisture (0.71%) in pre- monsoon season which slightly increased (2%) post- monsoon. The pH values during both seasons (6.46 and 5.93) revealed that the soil was acidic. The overall nutrient status of the soil was optimum according to the requirement of mango plants. The rhizosphere soil of both the samples (RtRV1 & RtRV2) showed almost the same nutrient levels during both the collection seasons still there was less AM spore density (51 & 29) but greater colonization (12.35% & 30.76%) in RtRV1 as compared to the higher spore density (72 & 130) but poor colonization (14% & 6.25%) in RtRV2. Topographically, RtRV2 was at higher level than RtRV1 which might have contributed to difference in moisture content of the rhizosphere. This could be the reason behind greater spore density in RtRV2.

Velas (RtVJ: site14, Ratnagiri), was the most wide spread orchard, developed on the hill slope. Soil cover was very thick. Like many other sites, the trend of pH and moisture change was inversely proportional to each other. Soil pH showed shift towards slightly acidic value (7.16 to 6.84) from season I to season II. Moisture content of the soil was less in season I (2.26%) than that in season II (4.11%). The concentrations of nutrients of the site also exhibited slight increase during post-monsoon season. Amounts of organic carbon, nitrogen and other macro- and micro-nutrients were optimum as per required by mango crop. However, the soil chemical analysis indicated deficiency of Cu (0.78 and 0.82ppm) and Zn (0.22 and 0.22ppm)

which did not change in either collection seasons. The AM spore density (per 10 g soil) recorded during season I was between 98 and 168 which showed significant decrease (between 6 and 82) in post-monsoon collection. Root colonization also decreased during post-monsoon period in all the sample plants except RtVJ4 (18.5% and 20%). On comparison, it was seen that, when concentration of available phosphorus (7.35 kg/ha -season I and 18.75 kg/ha -season II), other nutrients as well as moisture content increased, the AM spore density and root colonization decreased.

Site 15 (Hashiware, RgHM) was an old orchard (75-100 years) situated along the ground level with thick soil cover. Naturally, the soil was very slightly acidic which is evident from the nearly same pH (6.96 and 6.43) in season I and season II both. The soil moisture content varied from season I (1.43%) to season II (3.11%) showing increase. The soil was rich in organic and mineral nutrients except Cu and Zn, as observed during both the collections. Phosphorus was also an exception, since its concentration was very low (3.13 kg/ha) in season I but showed increment (16.81 kg/ha) in second season. AM spore density and percentage of root colonization were in inverse proportion. The spore density (93 and 300- season I and 47 and 92- season II) was more in pre-monsoon period than that in post-monsoon season. In contrast, root colonization in pre-monsoon period (36.25% and 39.5%) was less than that recorded in post-monsoon period (60.25% and 58.5%). Overall, it can be said that the spore density was influenced by soil moisture levels. However, post-monsoon increase in root colonization was not in correlation with any of the edaphic or nutritional factor.

The second site from Raigad district, Gothghar (site 16) was a young orchard situated along the ground level and was having thick soil cover. The pH value exhibited a

decrease from 7.01 (season I) to 6.72 (season II) which was in contrast to soil moisture content that showed slight increase (1 %- season I and 2.5%- season II). Nutritional status of the site was optimum during both the seasons except, Cu (0. 84 ppm and 1.25 ppm) and Zn (0.25 ppm and 0.305ppm) which were low in concentration. Spore density in soil sample RdGP1 decreased (99 and 47) while that in RdGP2 (46 and 49) remained unchanged. In case of root colonization, increase was recorded in season II (44.25% and 29.33%) over season I (20.28% and 13.09%). No significant correlation could be established between changes in soil characteristics and AM association of the plants in this site.

5A. 4. 3. Identification, Occurrence and Distribution of AM isolates:

Observations pertaining to identification revealed that total of eighteen diverse AM species were isolated from mango plants of Konkan belt (Table No 07 and 15). The collection comprised of ten identified and two unidentified *Glomus species,* two identified and three unidentified *Acaulospora species* and one species belonging to genus *Scutellospora.*

Genus *Glomus* exhibited wide range in terms of types as well as distribution. Survey of AM association in wild plants of 'Western Ghats' also revealed predominance of *Glomus spp.* (Katdare and Bagool, 2004). In Uttar Pradesh, Hasan and Khan (2008) recorded presence of *Glomus* sp. more than other genera in association with mango plants.

Glomus fasciculatum and *Glomus sp.* (1) were found to be distributed over southern parts i.e. in Ratnagiri and Sindhudurg districts. Their occurrence in northern parts of

study area *viz.* site 14 (Velas), site 15 (Hashiware) and site 16 (Gothghar) was not reported.

On the other hand, some AM species were found to be present only in the soil samples of northern extremes of Ratnagiri and Raigad district. *Glomus taiwanensis* (site 14 and 15)*, Glomus clavisporum* (site 15), *Glomus sinosum* (site 11 and site 16), *Glomus coremiodes and Glomus multicaulae* (site 14) *and Acaulospora sp* (3) (site 15 and 16) were exclusively distributed over this area.

Some of the AM species were of moderate occurrence, where in they appeared in soils of more than one site but the sites varied in pre- and post-monsoon season. *Glomus aggregatum* was isolated from sites 2 and 10 during first seasons and site 1 and 7 at the time of second season. Similarly, *Glomus albidum (site 6 and sites 4 &12), Acaulospora laevis (sites 3 & 8 and site 5), Acaulospora sp (1) (site 4 and site 2 & 3) and Acaulospora sp (2) (sites 2 & 5 and site 8)* varied in their site of occurrence during season I and season II, respectively.

Occurrence of two species namely *Glomus claroideum* (Site 2) *and Acaulospora scrobiculata* (site 15) was rare. They were found only during pre-monsoon season and at single collection site.

Only one species namely *Scutellospora calospora,* of this genus was isolated from the rhizosphere soil. Surprisingly, it was restricted to site 11 (Sakhloli). It could be isolated in both the collection seasons. This may be indicative of this species being native of this site.

5B. Nursery Experiment

5B.1. Effect on plant height:

The statistical analysis shows significant effect of the treatments on the height of seedlings, in comparison with the un-inoculated control. The increase in plant height was slow and irregular during the four trimesters of the experimental period. The per-cent increase in height of seedlings in all the experimental treatments is represented in Table No. 16 and Figure No. 14.

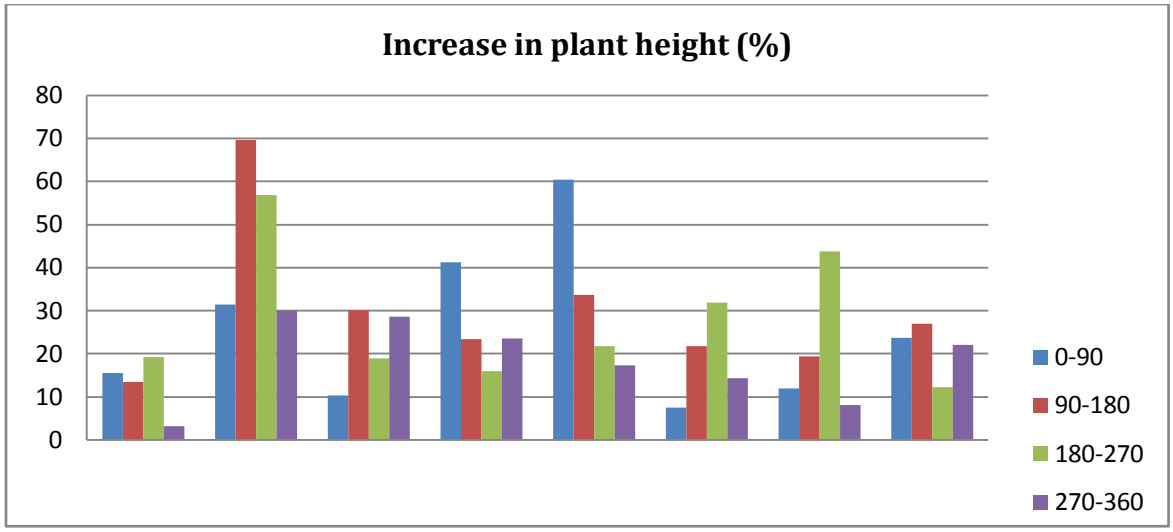
Table No. 16: Comparison between effects of experimental treatments on height of the seedlings in terms of per-cent increase.

Treatment	Increase in height (cm)				Increase in height (%)				Total Increase (%)
	0-90	90-180	180-270	270-360	0-90	90-180	180-270	270-360	
T1	2.83	2.82	4.56	0.85	15.50	13.36	19.12	3.10	**54.71**
T2	4.00	11.64	15.75	13.00	31.39	**69.53**	**56.75**	**29.88**	**343.88**
T3	1.27	4.08	3.45	6.18	10.32	30.00	18.93	**28.51**	**126.44**
T4	5.40	4.36	3.67	6.27	41.22	23.37	15.94	23.50	**151.53**
T5	7.72	6.90	5.97	5.93	**60.40**	33.65	21.78	17.25	**215.37**
T6	1.69	5.30	9.42	5.60	7.47	21.78	31.79	14.34	**97.57**
T7	2.12	4.06	10.97	3.03	11.84	19.31	**43.74**	8.04	**118.32**
T8	3.68	5.06	2.89	5.90	23.58	26.97	12.12	22.07	**116.67**

Un-inoculated control T1 (9.99cm) showed least increase in plant height over initial height which was estimated to be 54.71%. After observing the results trimester wise, it was clear that the un-inoculated seedlings had almost equal increase in first three trimesters (15.5%, 13.36% and 19.12%) which declined drastically (3.1%) during the last trimester of the experimental period. On the other hand, total increase in plant height of seedlings in T2, measured at the end of the experiment (360 day) was 43.81cm. It was calculated to be 343.88% increase over its own initial height. The seedlings showed gradual increase in 1st trimester (31.39%). It almost doubled

(69.53%) during second trimester and was maintained during 3rd (56.75%). However, there was drastic decease in 4th (29.88%) trimester.

Figure No. 12: Trimester wise per-cent increase in height of the seedlings per treatment.



Among combination treatments, T5 (50% AM + 50% SSP - 27.52 cm) was found to be more successful with total increase of 215.37% over its initial height. Seedlings under treatment T5 exhibited highest increase during 1st three months (60.4%) which was almost twice and thrice the increase recorded during 2nd (33.65%) and 3rd trimester (21.78%), respectively. In the last trimester the seedlings did not show much increase in height (17.25%). Other combinations of AM bio-fertilizer and SSP were less effective compared to T5 in increasing plant height (T7- 118.32%, T8- 116.67%, T6- 97.57%). Major increment in height of T5 treated seedlings occurred during first trimester (60.4%) while seedlings under chemical control T2 took 180 days to reach the same height (69.53%) as T5. This indicates the boosting effect of the AM supplement in the uptake of phosphorus which reflected in increase in height

at an earlier date. In similar experiment, Jadhav (2011) has reported that maximum height was attained with 3/4th dose of AM in combination with recommended dose of chemical fertilizer in *Withania somnifera* and with full dose of AM inoculum and chemical fertilizer in patchouli

Treatment T6 (31.79%) and T7 (43.74%) both exhibited greater increase between 180 DAS and 270 DAS (i.e. 3rd trimester) which contributed largely, to the total increase in the height throughout the experiment. Seedlings inoculated solely with AM inoculum, viz. T3 (50% dose of AM) and T4 (100% dose of AM) showed total increase in height as 126.44% and 151.53%, respectively. The major rise in height can be seen during 2nd trimester for T3 (30%) and 1st trimester for T4 (41.22%). Over all, the effect of only AM treatments was slightly better than some of the treatments in combination (T6, T7 and T8).

In terms of per-cent increase over control (T1) all the treatments were found to be significantly superior. The increase calculated for all the treatments reveals that, chemical control (T2- 100.28%) proved to be better. Seedlings treated with 50% AM+ 100% SSP exhibited greater effect on height (T6- 58.05%) than those treated with 50% AM+ 50% SSP (T5- 42.65%). Treatments involving 100% AM in combination with SSP showed less influence on height of the seedlings. Per-cent increase in the height of seedlings in T7 (100% AM + 50% SSP) and T8 (100% AM + 100% SSP) was calculated to be 38.34% and 15.5%, respectively. Increase observed in only AM treated plants i.e. T4 (100% AM) was 16.64% over control T1.

Effect of chemical fertilizer on height of seedlings was drastically greater when used alone; however, in combinations with AM inoculum the effect was moderate.

5B.2. Effect on number of leaves:-

The leaves are the site of fixation of atmospheric CO_2 and photosynthesis. Hence, it was selected as a parameter to observe the effect of various treatments. As expected, gradual increase in number of leaves was observed which was statistically significant. It is evident from the increase in number of leaves over control as well as total per-cent increase over initial number of leaves per seedling worked out for all the treatments at 360 days. Table No. 17 and Figure No. 15 represent per-cent increase in number of leaves during first (zero to 180 DAS) and second half (180 DAS to 360 DAS) of the experiment.

Table No. 17: Per-cent increase in number of leaves per seedling per treatment at 180 DAS and 360 DAS interval.

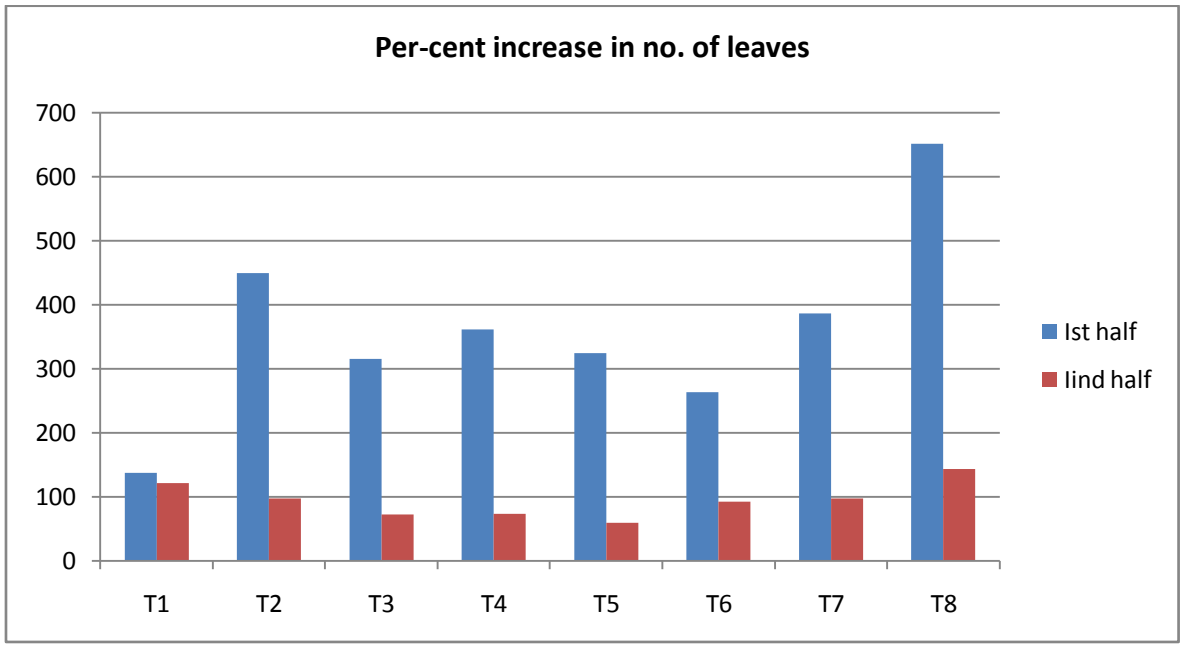
Treatment	Initial No. of leaves	Increase in No. of leaves		Final No. of leaves	Per-cent increase		Total increase (%)
		Zero-180DAS	180-360DAS		Zero-180DAS	180-360DAS	
T1	1.33	5.00	6.00	12	137.07	121.16	424.35
T2	2	9.00	10.67	21.67	450.00	97.00	983.50
T3	2.33	7.33	10.67	20.33	315.02	72.38	772.53
T4	2.67	10.66	9.00	21.33	361.79	72.99	698.88
T5	2.67	8.66	6.67	18	324.34	58.87	574.15
T6	3.67	9.66	12.33	25.67	263.21	92.57	599.45
T7	2.33	9.00	10.00	22.33	386.26	97.08	858.36
T8	1.33	9.67	14.33	24.33	651.88	143.30	1729.32

As calculated for 1st and 2nd half of the experimental period, the per-cent increase in number of leaves per seedling was significantly higher during the 1st half (T2- 450%, T3-315.02%, T4- 361.79%, T5- 324.34%, T6- 263.21%, T7- 386.26% and T8- 651.88%). However, the increase in number of leaves slowed down drastically in the 2nd half (T2- 97%, T3-72.38%, T4- 72.99%, T5- 58.87%, T6- 92.57%, T7- 97.08% and T8- 143.3%). The un-inoculated seedlings (control) showed almost equal increase in

no. of leaves during both, 1st (137.07%) and the 2nd half (121.16%). Acquisition of extra nutrients through application of chemical fertilizer and/ or their uptake mediated by AM bio-fertilizer might have influenced this growth parameter in all the treated seedlings. Results obtained by Santosh (2004) support the present observations.

Santosh (2004) observed significant effect of AM species on number of leaves in nursery seedlings of mango. Seedlings inoculated with *Glomus fasciculatum, Gigaspora margarita, Acaulospora laevis* and *Glomus monosporum* exhibited greater increase in height over that of un-inoculated seedlings.

Figure No. 13: Comparison between per-cent increase in No. of leaves per seedling per treatment after 180 DAS and 360 DAS.



Comparison between the total per-cent increase in number of leaves per plant under various treatments and the control T1 (424.35%) revealed that, the mean leaf number increased significantly in the seedlings receiving AM bio-fertilizer solely (T3- 772.53% & T4- 698.88%). However, most effective treatment to increase the number

of leaves was the one, with 100% AM inoculum + 100% SSP (T8- 1729.32%) which was high above the chemical fertilizer alone (T2- 983.5%). Other combinations of AM and SSP exhibited positive effect but not as much of the above ones.

The seedlings treated with combinations of AM bio-fertilizer and SSP showed noteworthy increment in number of leaves over un-inoculated control T1. The data reveal that the combination treatments are much better than the chemical fertilizer alone as far as this parameter is concerned. Of the combination treatments, particularly, the T6 (50% AM+ 100% SSP) showed almost 1.5 times the number of leaves(85.71%) as compared to SSP alone (57.14%). Almost the same effect (78.57%) is seen with T8 (both 100%). This is significant because of the fact that more the number of leaves greater will be the photosynthesis.

5B.3. Effect on stem girth at first node:-

The experiment was conducted using seedlings of *Villaicolumban* variety which is commonly used as a rootstock for grafting. Thus stem girth becomes an essential parameter for such seedlings to check their suitability and strength to take up the graft. The comparative data pertaining to periodic increase in stem girth at the node of seedlings per treatment is given in Table No. 18 and Figure No. 16.

The un-inoculated control, T1 showed 150% increase in stem girth, in 360 DAS.

Overall, in case of stem girth at first node, all the treatments with AM inoculum alone or its combinations with SSP exhibited positive effect right from the beginning. It is evident from the greater per-cent increase in the 1st half than in the 2nd half. Treatment T8 showed higher increase in girth at first node in 1st half (110.5%);

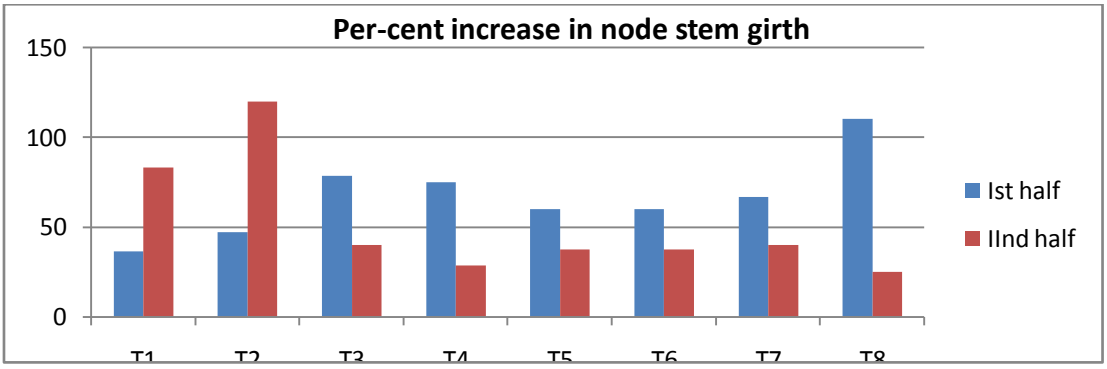
however, it slowed down drastically to 25% in second half. Increase in stem girth at first node of the seedlings in treatments T3 (78.57% & 40%), T5 (60% & 37.5%), T6 (60% & 37.5%), T7 (66.67% & 40%) and T4 (75% & 28.57%) also slowed down during 2nd half, considerably.

However, rate of increase (%) in comparison with un-inoculated control (T1) worked out for all the treatments evidently showed that 50% AM inoculum + 100% SSP combination (T6) only had positive effect on stem girth at node, while all other treatments including chemical control T2 were unsuccessful in increasing node stem girth.

Table No. 18: Comparison between effect of various treatments on stem girth at first node of the seedlings after 180DAS and 360 DAS (%).

Treatment	Initial Stem Girth (cm)	Increase in Node stem Girth (cm)		Final Stem girth at node (cm)	Per-cent increase		Total increase (%)
		Zero-180DAS	180-360DAS		Zero-180DAS	180-360DAS	
T1	0.22	0.08	0.25	0.55	36.36	83.33	150.00
T2	0.17	0.08	**0.30**	0.55	47.06	**120.00**	**223.53**
T3	0.14	0.11	0.10	0.35	78.57	40.00	**150.00**
T4	0.20	0.15	0.10	0.45	75.00	28.57	125.00
T5	0.25	0.15	0.15	0.55	60.00	37.50	120.00
T6	0.25	0.15	**0.20**	0.60	60.00	37.50	140.00
T7	0.18	0.12	**0.20**	0.50	66.67	40.00	**177.78**
T8	0.19	**0.21**	0.10	0.50	**110.52**	25.00	**163.16**

Figure No. 14: Comparison between effect of various treatments on stem girth at first node of the seedlings after 180DAS and 360 DAS (%).



5B.4. Effect on stem girth at collar region:-

In control treatments T1 and T2, there was slow increase in stem girth at the collar region during first half while it drastically shot up in the 2nd half. Increase observed in T2 was 1.5 times greater than that in T1. Considerable enhancement of girth occurred during the initial growth while it continued to increase further but with slower rate in the second half of the experiment, in almost all the combination treatments.

The per-cent increase in stem girth was equal in seedlings receiving T2 (100% SSP) and T7 (100% AM+ 50% SSP) treatments, in 2nd half. It is interesting to note that, the overall increase in T7 was far more as compared to T2. This shows that the AM fertilizer can partly substitute the chemical fertilizer to achieve the results.

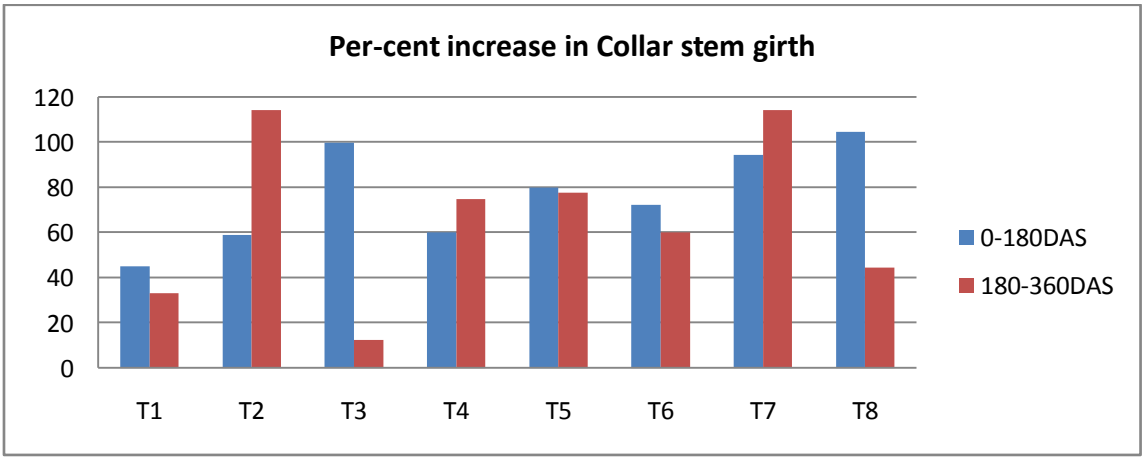
On the other hand, there was visible influence of all the AM and SSP combination treatments, AM inoculum alone as well as SSP alone on stem girth as compared to the control T1. Once again 50% AM+ 50% SSP combination (T5- 33.34%) and 50% AM+ 100% SSP in combination (T6- 33.34%) proved to be successful in increasing stem girth than SSP alone (T2- 25%). Treatments T5 and T6 both comprised of 50% dose of AM inoculum with different proportions of chemical fertilizer. It is noteworthy that treatment T5 with 50% chemical fertilizer exhibited same effect as T6 with 100% dose of chemical fertilizer. Half dose of AM inoculum alone (T3) failed to show any effect on collar stem girth but, AM inoculum in full dose (T4) worked better (16.67%) giving increase as much as half of that by T6 & T5. Treatment T7 (25%) was at par with chemical control T2.

Table No. 19: Comparison between effect of various treatments on stem girth at collar region of the seedlings after 180DAS and 360 DAS (%).

Treatment	Initial stem girth (cm)	Increase in collar stem Girth (cm)		Final stem girth at collar (cm)	Per-cent increase		Total increase (%)
		Zero-180DAS	180-360DAS		Zero-180DAS	180-360DAS	
T1	0.31	0.14	0.15	0.60	45.16	33.33	93.00
T2	0.22	0.13	0.40	0.75	59.09	114.28	240.91
T3	0.2	0.20	0.05	0.45	100.00	12.50	125.00
T4	0.25	0.15	0.30	0.70	60.00	75.00	180.00
T5	0.25	0.20	0.35	0.80	80.00	77.78	220.00
T6	0.29	0.21	0.30	0.80	72.41	60.00	175.00
T7	0.18	0.17	0.40	0.75	94.44	114.28	316.67
T8	0.22	0.23	0.20	0.65	104.54	44.44	195.45

The percent increase in stem girth over un-inoculated control (T1) also indicated the superiority of treatments T5 and T6 (both showing 33.34% increase). It was also greater than that exhibited by T2 (25%). Rest of the treatments except T3 *viz*. T4 (16.67%), T7 (25%) and T8 (8.34%) had poorly positive effect on stem girth over control (T1).

Figure No. 15: Comparison between effect of various treatments on stem girth at collar region of the seedlings after 180DAS and 360 DAS (%).



5B. 5. Effect on Root length:

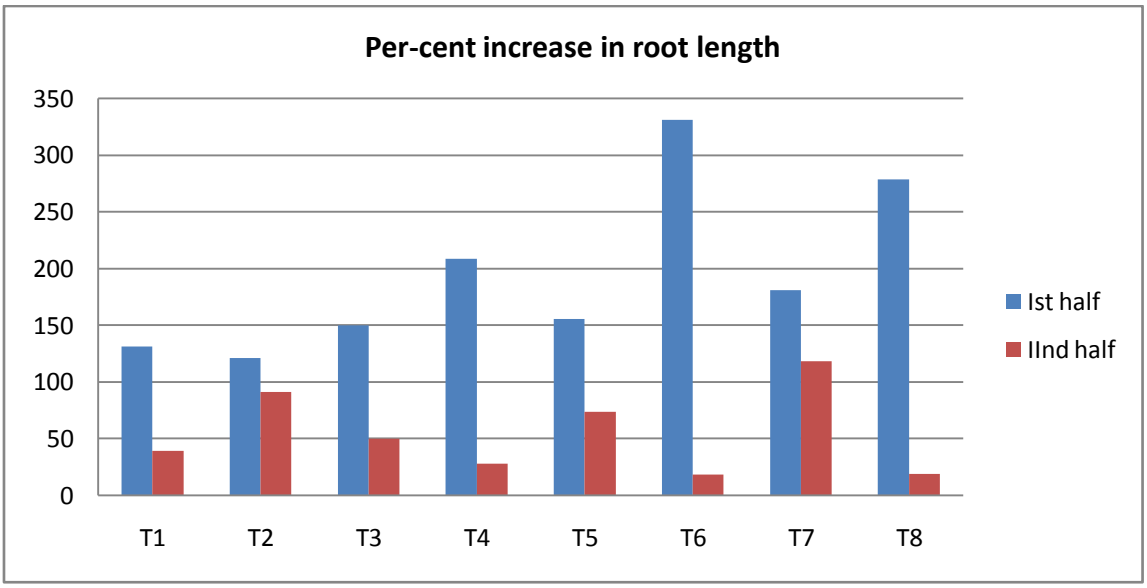
At th end of 180 DAS (1st half), the increase in root lengths of the seedlings of all the treatments were significantly superior over both the controls (T1 and T2). Surprisingly, the increase in root length of T2 seedlings was significantly less than that of T1 seedlings. Per cent increase in root length of seedlings under treatments T3 and T5 were at par with each other. The maximum was achieved in treatment T6 (2.5 times) followed by T8 (two times) and T7 (about 1.5 times) as compared to T1.

In the second half of the experiment, the maximum increase (%) in root length was observed in T7 seedlings (118.63%). It was followed by T2 (91.25%), T5 (73.74%) and t3 (50%). These treatments were significantly superior over rest of the treatments. In the remaining treatments viz. T4 (28%), T8 (19.28%) and T6 (18.90%), the increase was less than uninoculated control T1 (39.33%). The maximum overall increase in root length was seen in T7 which was followed by T6, T5 and T2. All the above treatments were found to be significantly superior over T1 when the final root lengths were considered.

Table No. 20: Comparison between effect of various treatments on root length of the seedlings after 180DAS and 360 DAS (%).

Treatment	Initial root length (cm)	Final root length (cm)	Per-cent increase	
			Zero-180DAS	180-360DAS
T1	7.80	25.15	131.41	39.33
T2	7.23	30.60	121.30	**91.25**
T3	6.23	23.40	150.40	50.00
T4	6.47	25.60	**209.11**	28.00
T5	6.63	29.45	155.66	**73.74**
T6	6.13	31.45	**331.38**	18.90
T7	5.73	36.00	**180.97**	**118.63**
T8	5.20	23.50	**278.85**	19.28

Figure No. 16: Comparison between effect of various treatments on root length of the seedlings after 180DAS and 360 DAS (%).



In comparison with the total increase in root length of control seedlings, maximum increase was noticed in T7 (39.17 %) followed by T6 (25.05%). These values are higher than the increase seen in chemical control (T2- 21.67%). Treatment T2 received only full dose of SSP and no bio-fertilizer while T6 received full dose of SSP along with AM bio-fertilizer in half dose. The data show that T6 treated seedlings exhibited better results than T2 seedlings. In treatment T7, seedlings were treated with SSP in half dose along with AM bio-fertilizer in full dose. Surprisingly, the per-cent increase in root length was almost doubled in T7 than that in T2. It might be due to AM bio-fertilizer.

The above results are in confirmation with those obtained by Singh (1999) while working on *Cacao* seedlings, Tholkappian *et . al.* (2000) on *Manihot esculenta* and Katdare and Bagool (2006) on wheat.

5B.6. Effect on root fresh weight and dry weight

Similar to root length, no significant effect of AM treatments was observed on the fresh as well as dry weights of the roots till 180 days after transplanting the seedlings. This may be due to the time taken for establishment and development of the root system and acclimatization with the rhizosphere after transferring the seedlings into the treatment mixtures. Table No. 21 and Figure No. 19 and 20, represent per-cent increase in root fresh weight of seedlings under various treatments.

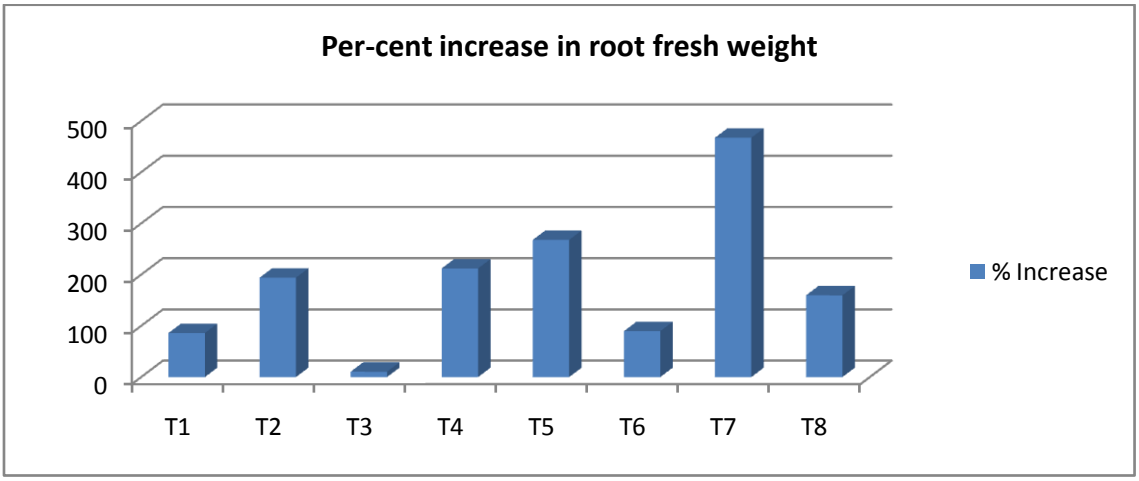
In connection with the per-cent increase over initial fresh weights, the data reveal that, T2 (194%), T4 (212.14%), T5 (267.81%) and T7 (467.23%) were superior to un-inoculated control T1 (86%). It was noticed that, even half dose of SSP was enough to improve root fresh weight when combined with 50% dose of AM (T5- 267.81%). Full dose of AM inoculum (T7- 467.23%) augmented the effect on root fresh weight almost two times that of T5. Seedlings receiving only AM bio-fertilizer in full dose (T4- 212.14%) also produced good increase in root fresh weight which was comparable to that of chemical fertilizer alone. Treatment T8 (159.50%) was at par with the chemical control.

Similar pattern was observed when the root fresh weights of seedlings from each treatment were compared with that of the control seedlings (T1). The chemical fertilizer alone in full dose (T2) produced an increase of 26.42% over control T1. It is clear from the data that, even 50% dose of SSP was able to produce four times higher increase in root fresh weight when supplemented with half dose of bio-fertilizer (T5- 101.89%). When the same dose of SSP was supplemented with full dose of AM (T7- 155.4%) the increase was almost six times over T2.

Table No. 21: Comparison between effects of various treatments on increase in root fresh and dry weight of the seedlings from180DAS to 360 DAS (%).

Treatment	Root fresh weight (g) At 180 DAS	Increase in root fresh weight (180 DAS- 360 DAS)		Root dry weight (g) At 180 DAS	Increase in root dry weight (180 DAS- 360 DAS)	
		(In g)	(In %)		(In g)	(In %)
T1	6.00	5.16	86.00	1.93	1.66	86.01
T2	5.00	9.74	194.00	1.93	3.76	189.63
T3	4.50	0.47	10.44	1.61	2.48	10.55
T4	**4.20**	**8.89**	**212.14**	**1.25**	**2.65**	**212.00**
T5	**6.40**	**17.14**	**267.81**	**1.87**	**5.01**	**267.91**
T6	6.10	5.46	89.50	2.19	1.96	89.49
T7	**5.25**	**24.53**	**467.23**	**1.47**	**6.87**	**467.34**
T8	4.50	7.18	159.55	1.66	2.65	159.63
S.E.	0.976929	-	-	0.44931		
C.D. (0.5%)	2.864977	-	-	1.317663		

Figure No. 17: Comparison between effects of various treatments on root fresh weight of the seedlings from 180DAS to 360 DAS (%).

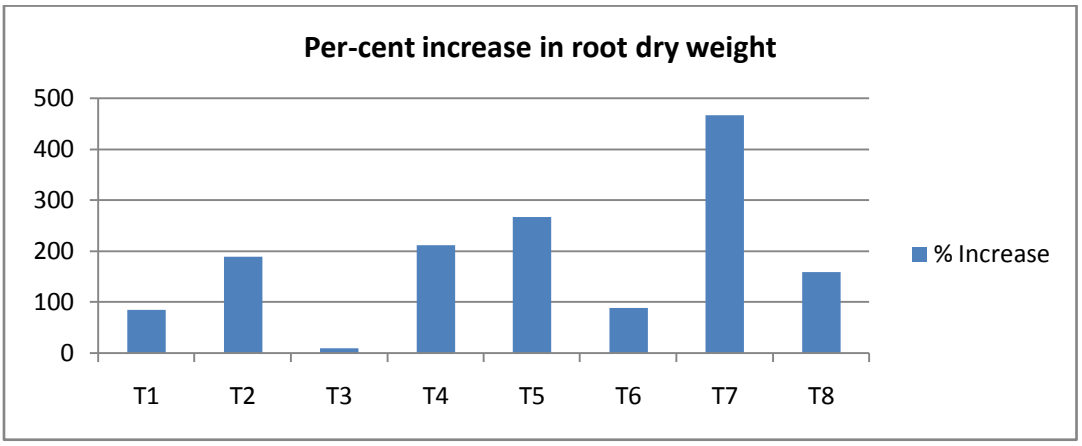


These results are well supported by those obtained by Sitaramaiah and Khanna (1997) in *Zea mays*, Katdare and Bagool (2006) in wheat and Jadhav (2011) on *Withania somnifera* and patchouli. While working on mango seedlings, Santosh

(2004) recorded significant enhancement of root parameters in the seedlings receiving AM inocula as compared to untreated ones.

After oven drying of the uprooted seedlings, per-cent increase in root dry weight over the initial dry weight was worked out. As given in the Table No. 22, the values obtained were very close to those obtained for fresh weights of the respective treatments.

Figure No. 18: Comparison between effects of various treatments on increase in root dry weight of the seedlings from 180DAS to 360 DAS (%).



Percent increase over control (T1) was calculated for all the experimental treatments. The most effective treatment was found to be T7 (132.31%) followed by T5 (91.64%). Both these treatments yielded greater effect over chemical control T2 (55.71%). Remaining experimental treatments *viz.* T3 (13.93%), T4 (8.64%), T6 (12.81%) and T8 (17.27%), were less significant, but better over un-inoculated control T1.

5B.7. Effect on shoot fresh weight and dry weight:-

Like all the above parameters, the increase in the fresh weight of shoot was noticed during the second half i.e. between 180 and 360 DAS. All the treatments were

statistically at par with the un-inoculated control (T1- 72.57%). The seedlings responded well to the chemical fertilizer (T2- 201.23%); however, its combination with AM inoculum produced better results (except T6 and T7). The shoot fresh weight of seedlings was 25% more when supplemented with both fertilizers in half dose (T5- 257.12%) and remained almost same even when both the fertilizers were applied in full doses (T8- 260.78%). Treatment T7 comprising full dose of AM and half dose of SSP produced almost the same result (191.31%) as that of SSP alone in full dose (T2). The data pertaining to per-cent increase in shoot fresh weight and dry weight is represented in Table No. 22 and Figure No. 21 & 22.

Per-cent increase in shoot dry weight during the period from 180th day to 360 days revealed that, the seedlings treated with chemical fertilizer and its combinations with AM inoculum produced significantly greater increase in dry weight than those inoculated with AM bio-fertilizer alone and un-inoculated control (T1- 76.92%). Seedlings treated with only chemical fertilizer (T2- 180.34%) showed increase of 189% in shoot dry weight. Maximum influence was recorded in T5 seedlings (257.24%) where chemical fertilizer dose was halved and added with 50% dose of AM inoculum. The increase in shoot dry weight caused by combination of 100% doses of both fertilizers (T8- 230.71%) was not less significant. Amongst other combination treatments, T7 (191.42%) was superior to both the controls, while treatments T4 (165.1%) and T6 (137.45%) were superior to un-inoculated control but less effective than chemical control T2.

Table No. 22: Comparison between effects of various treatments on increase in shoot fresh and dry weight of the seedlings from180DAS to 360 DAS (%).

Treatment	Shoot fresh weight (g) At 180 DAS	Increase in Shoot fresh weight (180 DAS- 360 DAS)		Shoot dry weight (g) At 180 DAS	Increase in Shoot dry weight (180 DAS- 360 DAS)	
		(In g)	(In %)		(In g)	(In %)
T1	7	5.16	72.57	2.34	1.70	76.92
T2	**8.1**	9.74	**201.23**	3.46	6.36	**180.34**
T3	7	0.47	24.85	2.81	0.70	24.91
T4	7.13	**8.89**	153.4	2.98	**4.92**	165.1
T5	**7.3**	**17.14**	**257.12**	2.83	**7.28**	**257.24**
T6	9.85	5.46	137.36	**5.34**	7.44	137.45
T7	7.83	**24.53**	191.31	3.15	**6.03**	191.42
T8	**10.2**	7.18	**260.78**	2.78	6.40	**230.71**
S.E.	1.20415	-	-	0.727962		
C.D. (0.5%)	3.53133	-	-	2.134849		

Figure No. 19: Comparison between effects of various treatments on increase in shoot fresh weight of the seedlings from 180DAS to 360 DAS (%).

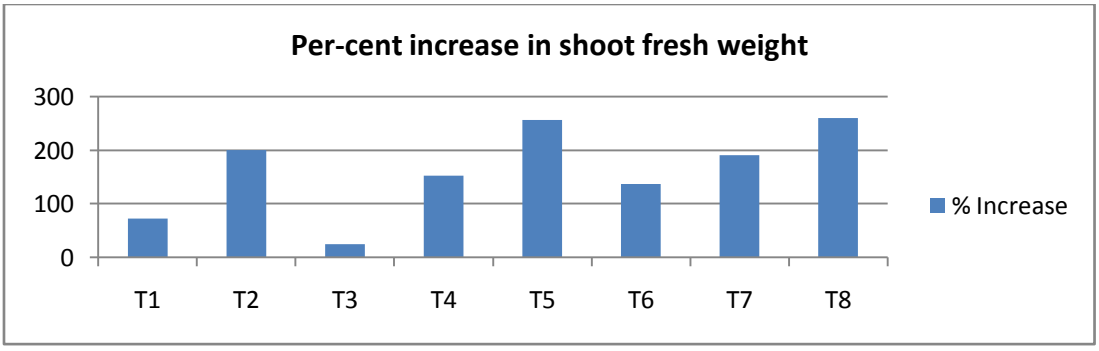
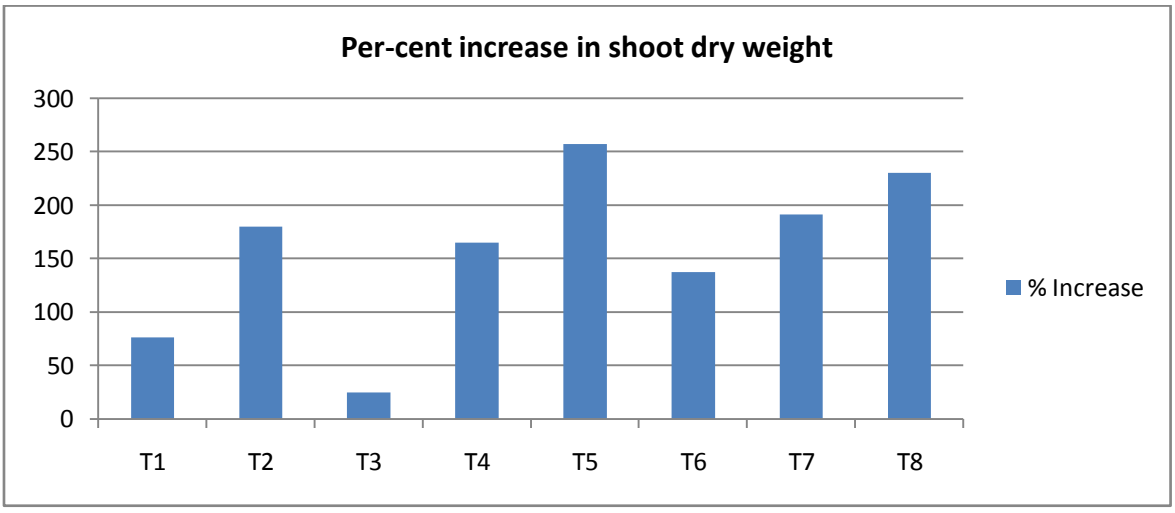


Figure No. 20: Comparison between effects of various treatments on increase in shoot dry weight of the seedlings from 180DAS to 360 DAS (%).



Percent increase over control (T1) was calculated for all the experimental treatments. The most effective treatment was found to be T7 (132.31%) followed by T5 (91.64%). Both these treatments yielded greater effect over chemical control T2 (55.71%). Remaining experimental treatments were less successful *viz.* T3 (13.93%), T4 (8.64%), T6 (12.81%) and T8 (17.27%) but better over un-inoculated control T1.

Previous work carried out by Santosh (2004) on mango seedlings, Katdare and Bagool (2006) on wheat and Jadhav (2011) on patchouli, have reports on positive effect of AM bio-fertilizer on fresh and dry bio-mass of the plants. Thus, the results obtained in present work are in agreement with the above reports.

5B. 8. Assessment of Mycorrhizal association

Mycorrhizal association in nursery seedlings was assessed by determining density of AM spores per 10g rhizosphere soil and percentage of root colonization for all the treatments. The data are given in table No. 14.

Colonization of roots by AM fungi was observed at 180 DAS and 360 DAS interval wherein, gradual increase was notable. In un-inoculated control (T1) as well as chemical control (T2) there was no addition of AM bio-fertilizer; thus the colonization represents the indigenous AM mycoflora. Seedlings in chemical control treatment T2 were poorly colonized by AM fungi which may be due to ready availability of phosphatic fertilizer where as un-inoculated control T1 showed significantly higher root colonization. As expected, amongst plants treated with AM alone, T3 plants (50% dose of AM inoculum) exhibited lower degree of colonization as compared to T4 treated plants (100% dose of AM inoculum- 80.48%). It was observed that the degree of colonization lowered wherever phosphatic fertilizer was

added (T5- 69.83%, T6- 63.28%, T7- 70.83% and T8- 66.47%); however, the differences amongst the treatments T5 to T8 were not very conspicuous. This is in concurrence with the general assumption that when there is readily available phosphorus in the soil, the AM root colonization is less. The same pattern of root colonization continued till the end of the experimental period i.e. at 360 DAS.

CHAPTER – VI

SUMMARY AND CONCLUSION

SUMMARY

Amongst various fruit crops, mango is the major horticultural crop of India that attracts international market. India is top ranked with 39% contribution to the total world production of mangoes (Singh, 2008). It is cultivated in many states of India as it is adaptable to wide range of soil and climatic conditions. However, the varieties grown in the *Konkan* region of Maharashtra state have more demand in national as well as international market because of the characteristic flavour and attractive colour. Therefore the sites were particularly selected from *Konkan* region so as to survey the status of AM fungi associated with this crop.

Sixteen sites were located from three districts (Raigad, Ratnagiri and Sindhudurga) in *Konkan* region and total of 58 samples comprising rhizosphere soil and the young roots were collected from selected mango plants.

The mango plants selected for sampling from various localities were of different ages ranging from 5 years up to over 100 years. The old plants (75-100 years) showed varied degrees of root colonization. Percent root colonization values determined for these plants ranged from as low as 4.16% and zero% (SdWP5- 100 years) to as high as 52.4% and 72.25% (RtKJ1-90 years) in season I and season II, respectively. Similarly, in younger plants (5-25 years) the lowest degree of colonization was found to be 2.73% and 2.91% (SdMM1) and the highest of 83.72% (RtPT3) & 88.23% (SdWJ4), respectively, in season I and II. The plants of moderate age (40-70 years) exhibited root colonization ranging between 3.47% and 68% in season I and zero to

71% in season II. This shows that, the extent of root colonization by AM fungi was not dependent on the age of the host plant.

The samples were collected twice *viz.* in pre-monsoon and post-monsoon season. The data revealed that, pH of the soil decreased slightly, invariably at all the collection sites during second season. A post-monsoon increase in moisture content was noticed in all the samples. The increased moisture content well supported soil microbial activity, as a result the soil pH showed shift towards acidic side. In about 70% of the samples, the spore density (per 10 g soil) and root colonization (%) decreased with increase in moisture content.

Total of eighteen diverse AM species were isolated from mango plants of Konkan belt and were identified. The collection comprised of ten identified and two unidentified *Glomus species,* two identified and three unidentified *Acaulospora species* and one species belonging to genus *Scutellospora.* Genus *Glomus* exhibited wide range in terms of types as well as distribution. Some AM species were widely distributed where as some were found to be restricted to particular collection sites.

In recent years, arbuscular mycorrhiza has been proved as a better form of bio-fertilizer and potential alternative to chemical fertilizer. Individual species or consortia of AM fungi have been used to test their effect on variety of crops. In many experiments, AM inocula have shown excellent results in terms of growth enhancement, increase in productivity and improving drought and disease resistance of the host plants.

In present work an attempt was made to study effect of AM bio-fertilizer on various growth parameters pertaining to mango seedlings of the variety *Villaicolumban*. It is

a homozygous variety and is always preferred as a root stock for grafting. Hence, it was selected as a test crop.

Effect of AM consortium in comparison with chemical fertilizer was assessed in the nursery experiment over a period of one year from germination. Various growth parameters *viz.* height of seedlings, number of leaves per seedling, stem girth at collar and nodal region, number of branches (all recorded at 30 DAS interval), root length, root and shoot fresh weight and dry weight, root colonization and spore density in the rhizosphere (all recorded at 180 DAS interval) were assessed.

Single super phosphate (SSP) is commonly used as a fertilizer for mango and the same was used as chemical fertilizer in the experiment. Its effect was clearly evident on the height of mango seedlings. On the other hand, treatments comprising AM inoculum alone were unsuccessful, in comparison with the chemical fertilizer. However, the combination treatments i.e. T5 (50% AM inoculum + 50% SSP), T6 (50% AM inoculum + 100% SSP), T7 (100% AM inoculum + 50% SSP) and T8 (100% AM inoculum + 100% SSP) showed greater potential in enhancing the growth parameters.

CONCLUSIONS

- Occurrence of AM association is found to be common even in mango plants of different varieties as well as ages.
- It is noticed in all the sites visited.
- The variations in AM spore density and root colonization is in response to changes in soil pH and moisture at most of the sites and to changes in phosphorus levels at some sites.
- The changes in AM spore densities and root colonization percentage are in inverse proportion during the two collection seasons i.e. spore count decreased from summer to winter season while root colonization increased during the same.
- *Glomus* is the prevalently occurring genus with great diversity of species followed by *Acaulospora* and scanty presence of *Scutellospora* genus, in the present study area.
- The combination treatments can form a better alternative to chemical fertilizer alone.
- The quantity of chemical fertilizer, required per plant, can be reduced if it is used in combination with AM inoculum. This, in turn, can reduce the cultivation cost and will also take care of soil health.
- The growth promoting effect of combination treatments takes place from the early stages of the seedlings (soon after transplantation). This property ensures healthy seedlings for their use as root stocks for grafting.
- AM being a biological agent, it is self multiplying and therefore it need not be applied as frequently as chemical fertilizer.
- Presence of AM bio-fertilizer enhances nutrient absorption, synthesizes PGRs and helps in acquiring disease and drought resistance.

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SYNOPSIS

OF

THE THESIS TO BE SUBMITTED TO

THE UNIVERSITY OF MUMBAI

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IN BOTANY

Title of the Thesis	**:** Survey of Arbuscular Mycorrhizal fungi and their application as bio-fertilizer in *Mangifera indica.*
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SYNOPSIS

INTRODUCTION:

Maharashtra is the third largest state of India. It has approximately 308,000 km^2 of area bordered by Madhya Pradesh to the north, Chhattisgarh to the east, Andhra Pradesh to the southeast, Karnataka to the south and Goa to the southwest. The state of Gujarat lies to the northwest with Arabian Sea lining the entire west coast of Maharashtra. The hilly range of Western Ghats, also known as 'Sahyadri' runs parallel to the west coast with Deccan plateau situated on the eastern side. In between the Arabian Sea and the Western Ghat is located the Konkan area as a narrow belt about 50 km wide. Though the tropical climate prevails in Maharashtra, the topographical variations result in climatic and soil type differences in various parts of the state. The state includes regions of heavy rain fall with cooler climate (Mahabaleshwar), hot and moist climate (Konkan), lower to scanty rainfall coupled with dry and hot climate, semi-arid and arid regions. The soil types and climate are the key factors that enable the farmers to cultivate variety of crops in this state of India.

Although, it is one of the most industrialized states of India, agriculture continues to be the only occupation of major part of the population. Total irrigated area under cultivation is about 33,500 km^2 (that counts to be 10% of the total area) of which remarkable part is used exclusively for cultivation of fruit crops. The major fruit crops that have become speciality of Maharashtra include oranges from Nagpur, grapes from Nasik and Sangli, strawberries from cold weather regions like Mahabaleshwar, chikoo from Thane region of Konkan and mangoes, cashews and coconut from rest of Konkan. The agro-climatic conditions in Konkan belt are more suitable for horticultural crops.

Mango:

Mango (*Mangifera indica* L., family *Anacardiaceae*), the native of India, is the most celebrated fruit worldwide. It is known to have been cultivated in India since ancient times. It is an evergreen plant occurring abundantly in forests as well as in cultivated lands. Almost all cultivars belong to the species *Mangifera indica* L. It is adaptable to a wide range of soil and climatic conditions and grows well right from Assam to the

southern limits of the country and from sea level up to about 1500 meters. It withstands both, fairly dry conditions and heavy rainfall, provided severe and recurring frosts in winter do not endanger the young trees (Randhawa, 1991).

The commercially grown varieties of mango have arisen through seedling selection made for different characters like colour, taste, flavour, size etc. Later, these varieties have been vegetatively propagated and cultivated in wide area (Mukherjee, 1972). Commonly, monoembryonic types are used for cultivation. Polyembryonic types are grown only in southern India, especially in coastal parts of Kerala, Karnataka and Goa. (Ravishankar *et al,* 2004).

Out of total world production of mangoes, 39 percent is contributed by India (Singh, 2008). Thus with respect to mango production and export, India is the major competitor in the international market. However, the yield is actually at its lowest level. Among many reasons leading to low yield of mangoes two important reasons are lack of education about correct agronomic practices and poor management of orchards (Venkatraman, 2002).

For cultivation of fruit crops, in general, majority of the farmers depend more on chemical phosphatic fertilizers than bio-fertilizers due to their faster effect. However, the amount of fertilizer applied is not completely available to the plants as it accumulates in the soil forming insoluble complexes. This harms the soil health slowly rendering it non-fertile. Nowadays, the research is directed towards developing better alternatives to the traditional chemical fertilizers.

Various classes of soil microorganisms have been screened for their efficiency in enhancing the plant growth. It includes nitrogen fixing bacteria like *Rhizobium, Azotobacter* etc.; phosphate solubilising bacteria, disease controlling microorganisms etc. In recent years, the Arbuscular mycorrhizal (AM) fungi have gained attention of the researchers as potential bio-fertilizer to boost production in agriculture and horticulture.

Mycorrhiza is a symbiotic relationship between a fungus (mycobiont) and the root system of the plant. This is a natural association of universal occurrence and is present in various plants from tiny herbs to tall trees. The mycorrhizae are categorized into

seven types on the basis of position of fungal partner in the root tissues and the type of associated host plant.

The AM fungi are obligate symbionts belonging to order *Glomales* (earlier known as *Endogonales)* forming symbiotic association with roots of plants. This association is a common phenomenon in the nature wherein, over 90% of the plants harbour AM fungi in their root tissues. Of all the types the arbuscular mycorrhizal (AM) fungi are more preferable as bio-fertilizer because of their inherent properties such as, their ubiquitous occurrence in wild as well as cultivated plants, remarkable ability of mobilizing insoluble phosphates and capacity to absorb various macro- and micro-nutrients. They also enhance the ability of the host plants to produce PGRs, to withstand water stress and provide protection against soil borne pathogens (Manoharachary *et al.,* 2009). This has been confirmed experimentally by various researchers working with different types of test crops (Hazarika *et al.,* 1999; Ratti *et al.,* 2002; Patil and Patil, 2005; Thakur *et al.,* 2005; Maiti *et al., 2008;*).

Over the years, diversity and distribution of AM fungal species have been surveyed in India as well as the other parts of the world (Rosendahl & Rosendahl, 1992; Rekha Rani & Mukerji, 1997; Khade & Rodrigues, 2003; Katdare & Bagool, 2004 and Hasan & Khan, 2005).

OBJECTIVES:

The main objectives of the present study were as follows:

1. To collect rhizosphere soil and root samples of various mango cultivars from different soil types and localities from three mango producing districts in Konkan region.

2. To isolate, identify and quantify the AM fungal spores from collected samples.

3. To perform physico-chemical analysis of soil samples and to find out possible correlation between some soil characters and AM distribution.

4. To carry out multiplication of AM spores and preparation of inoculum.

5. To study the effect of AM inoculum on germination of seeds and growth parameters of known variety of mango on nursery level (for one year).

The thesis is divided into six chapters.

Chapter I: Introduction

It includes the introduction to the subject, nature and types of mycorrhizal associations, taxonomic details of mycorrhizal fungi, ecological distribution and significance of AM as bio-fertilizer.

Chapter II: Review of Literature

A review of literature pertaining to the survey of AM fungi, their classification, physico-chemical analysis of soil and a brief account of beneficial aspects of AM fungi as bio-fertilizer in various test crops is covered in this chapter.

Chapter III to VI are divided in to two parts:

A. Survey
B. Nursery experiment.

Chapter III: Material and Methods

This chapter comprises the material used and the methodology followed to carry out the present research work as follows:

A. Survey :

   Collection of soil samples & young mango roots was done in orchards from sixteen localities of three districts *viz.* Raigad, Ratnagiri and Sindhudurg, in Konkan region of Maharashtra state. The mango plants selected for survey were of different age groups, varieties and were growing in different soil types. The material was collected twice i.e. in pre-monsoon and post-monsoon period.

1. The mycorrhizal status of the samples was recorded by assessing per-cent root colonization and measuring the spore density per unit soil from the rhizosphere.
2. Macro- and Micro-nutrient concentrations in the soil samples were determined by standard chemical estimation procedures.
3. AM spores were isolated from the soil samples, identified and were subjected to multiplication.

B. Nursery experiment:

1. A nursery experiment was set to check the effect of AM inoculum on growth parameters of mango seedlings of a polyembryonic variety- *Villaicolumban*, in comparison to chemical fertilizer and combinations of theirs.
2. Macro- and Micro-nutrient concentrations were estimated using standard methods.
3. The data obtained from nursery experiment were analysed statistically.

Chapter IV: Observations and Results

The observations and results pertaining to

A. survey and
B. nursery experiment

are presented in this chapter.

Chapter V: Discussion

The chapter comprises a discussion on the results obtained during

A. survey and
B. nursery experiment

with supportive evidences of the work carried out by other researchers in different parts of the world.

Chapter VI: Summery and Conclusion

This chapter includes summery of the results obtained and the conclusions drawn from the present work.

The major conclusions drawn from the research work are as follows:

- All the mango plants showed association with AM fungi irrespective of age and variety.
- The AM status of the sample plants was found to vary with respect to soil moisture, pH and soil phosphorus.
- *Glomus* was the dominant genus associated with the mango plants. Also few species of *Acaulospora* and *Scutellospora* were detected during screening.
- AM fungal inoculum in combination with chemical fertilizer (SSP) exhibited positive effect on growth of nursery seedlings of mango.

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