# THE EFFECT OF DIFFERENT CONCENTRATIONS OF MYCORRHIZA ON THE PERFORMANCE OF GINGER (*Zingiber Officinale*)

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## BORANG PENGESAHAN TESIS

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## ABSTRACT

An experiment to evaluate the effect of AM (Abuscular Mycorrhiza) on ginger was undertaken at Universiti Malaysia Sabah, Sandakan, Sabah from February to December 2017. The topsoil used was autoclaved to remove microorganisms could affect the mycorrhiza performance on the inoculation with ginger roots. The experiment was conducted with 5 treatments (T1) with no application of mycorrhiza and (T2) with 25 g of mycorrhiza application (T3) with 50 g of mycorrhiza application, (T4) with 75 g of mycorrhiza application and (T5) with 100 g of mycorrhiza application with 5 replicates per treatment. Each concentration was applied at a depth of 10 cm from the surface of the topsoil. Analysis of data was done following one-way ANOVA. The experimental design was arranged following a Completely Randomised Design (CRD) and mean separation was be done using Tukey's Honest Significant Difference (HSD). The parameters that were taken for data analysing are plant height, number of leaves per plant, number of tillers, number of flower, rhizome fresh weight, rhizome dry weight and mycorrhiza root colonisation. Root colonisation showed to have the only parameter with significance. The overall results showed that The T3 and T4 had shown to be the overall best treatment and T2 and T5 were the worst performing treatments as compared to T1. However T3 was the overall best performing treatment as out of the seven parameters taken which are plant height, number of leaves per plant, number of tillers, fresh weight, and colonisation of mycorrhiza parameters except rhizome fresh weight. Mycorrhiza root colonisation showed significant difference as compared to other parameters. External condition played a role in the inoculation of AM on the roots of mycorrhiza as disturbances of soil could disrupt the colonisation of the mycorrhiza with ginger roots. In conclusion, high amounts of mycorrhiza application could improve the inoculation of ginger roots if external disturbances were lessened.

# KESAN KONSENTRASI BERBEZA MIKRORIZA TERHADAP PRESTASI HALIA (*Zingiber officinale*)

## ABSTRAK

Eksperimen untuk menilai kesan AM (Abuscular Mikroriza) pada halia telah dijalankan di Universiti Malaysia Sabah, Sandakan, Sabah pada Februari untuk Disember 2017. The tanah atas yang digunakan ialah tanah yang suah autoklaf untuk menghapuskan mikroorganisma boleh menjejaskan prestasi mikroriza pada inokulasi dengan akar halia. Eksperimen ini dijalankan dengan 5 rawatan (T1) tanpa permohonan mikroriza dan (T2) dengan 25 g permohonan mikroriza (T3) dengan 50 g permohonan mikroriza, (T4) dengan 75 g permohonan mikroriza dan (T5) dengan 100 g permohonan mikroriza dengan 5 replikasi setiap rawatan. Setiap rawatan diaplicasi pada kedalaman 10 cm dari permukaan tanah atas. Analisis data yang digunakan ialah sehala ANOVA. Rekabentuk analisis akan disusun mengikut Rekabentuk Rawak Penuh (CRD) dan pemisahan mean telah dilakukan dengan menggunakan Tukey "Honest Significant Difference" (HSD). Parameter yang telah diambil untuk menganalisis data adalah ketinggian tumbuhan, bilangan daun setiap tumbuhan, bilangan anak pucuk, bilangan bunga, berat basah rhizom, berat kering rhizom dan akar kolonisasi mikroriza. Akar kolonisasi mikroriza menunjukkan kepada mempunyai satu-satunya parameter dengan perbezaan yang ketara. Keputusan keseluruhan menunjukkan bahawa T3 dan T4 telah ditunjukkan untuk menjadi rawatan terbaik keseluruhan dan T2 dan T5 adalah rawatan persembahan paling teruk berbanding T1. Walau bagaimanapun T3 adalah keseluruhan rawatan yang berprestasi terbaik daripada tujuh parameter yang diambil, ketinggian tumbuhan, bilangan daun setiap tumbuhan, bilangan anak pucuk, berat segar rhizom, dan innokulasi mikroriza dengan akar kecuali berat kering rhizom. Innokulasi mikroriza dengan akar mempunyai perbeezaan ketara berbanding parameter lain. Faktor luaran dapat memainkan peranan dalam menginokulasi AM pada akar mikroriza kerana gangguan tanah boleh mengganggu penjajahan Mikroriza dengan akar halia. Kesimpulannya, innokulasi mikrorhiza pada akar akan menjadi lebih ketara jika pengangguan fak tor luaran dikurangkan.

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| | |
|---|---|
| AMF | Arbuscular Mycorrhizal Fungi |
| ANOVA | Analysis of Variance |
| cm | Centimeter |
| g | Gram |
| kg | Kilogram |
| nm | Nanometer |
| ppm | Parts per million |
| SAS | Statistical Analysis System |
| USD | US Dollar |

## LIST OF FORMULA

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# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

There are many ginger producing countries in the world. As time progresses, ginger producers will need to plan to increase yield as so to increase production. World trade in ginger is estimated at USD190 million per year. India's share in ginger trade is only 6 per cent, while China's share is 57 per cent. In ginger oil and oleoresin trade India dominates with 50 per cent share. Garbled or ungarbled and bleached or unbleached variants occupy predominant position in ginger export. Cochin ginger is popular and it fetches a premium price because of its superior quality. The estimated world import of ginger is 300,000 tonnes per year. In Malaysia, from the year of 1961 to the year of 2013, there was an increase in the production of yield of ginger, to yields of 9378 tons in the year of 2013 from 1300 in the year of 1961 (FAOSTAT, 2015).

Ginger has starring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer. It also has anti-inflammatory and anti-oxidative properties for controlling the process of aging. Furthermore, it has antimicrobial potential as well which can help in treating infectious diseases. Generation of free radicals or reactive oxygen species (ROS) during metabolism beyond the antioxidant capacity of a biological system results in oxidative stress, which plays an essential role in heart diseases, neurodegenerative diseases, cancer, and in the aging process. The bioactive molecules of ginger like gingerols have shown antioxidant activity in various modules. Inflammatory disorders such as gastritis, esophagitis, and hepatitis, which are cause not only by infectious agents such as viruses, bacteria, and parasites but also by physical and chemical agents like heat, acid, cigarette smoke, and foreign bodies, are recognized as risk factors for human cancer ( Mashhadi *et al.*, 2013).

The symbiosis, mycorrhiza is named after the Greek "mycos" and "rhiza" meaning "fungus-root," and it is probably the oldest and most widespread plant symbiosis on Earth. Such symbioses between plants and beneficial soil microorganisms like arbuscular-mycorrhizal fungi (AMF) are known to promote plant growth and help plants to cope with biotic and abiotic stresses (Jung *et al.*, 2012). Profound physiological changes take place in the host plant upon root colonization by AMF affecting the interactions with a wide range of organisms below- and above-ground. Protective effects of the symbiosis against pathogens, pests, and parasitic plants have been described for many plant species, including agriculturally important crop varieties. Besides mechanisms such as improved plant nutrition and competition, experimental evidence supports a major role of plant defenses in the observed protection. Arbuscular mycorrhizas are mutualistic associations formed between the roots of 80 % of terrestrial plant species and fungi from the small phylum Glomeromycota (Jung *et al.*, 2012).

Indeed, fossil records and phylogenetic evidence date their existence back more than 450 million years. In recent years, the induced disease resistance of plant by Arbuscular Mycorrhizal Fungi (AMF) has become a hot spot in chemo-ecological study and in biocontrol of plant disease. There were many reports indicating that AMF had antagonistic function to soilborne disease pathogen, or could suppress the growth of pathogen, and increase the resistance or tolerance of mycorrhizal plants to soilborne disease. In mycorrhizosphere, there are interactions among microbial community, in which, AMF could suppress the growth of pathogen and promote the growth of beneficial microbe. Thus, AMF maybe used as biocontrol fungi with other antagonism microbe. There were several hypotheses about the mechanisms of the increased resistance in mycorrhizal plants which initially involves improvement of plant nutrient status, competition, changed roots morphology and structure; changed microbial flora in rhizosphere and lastly induced resistance or systematic resistance in plant. Hence, inoculation on ginger with mychorhizal on the roots of ginger plant will be studied on the possiblility of developing resistance on the growth of ginger with the assistance of mychorhizal inoculation (Huang *et al.*, 2003).

## 1.2 Objectives

1) To evaluate the effects of innoculating mycorrhiza on ginger roots and to improve the performance of ginger using top soil.

2) To compare the effect of different concentrations of Mycogold on the performance of ginger.

## 1.3 Justification of Study

Possible harmful chemicals used currently which is harmful for human health and also the environment could be replaced with the inoculation of mycorrhiza on ginger. Besides that, this research could potentially provide farmers with alternative to encourage cost saving methods such as mycorrhiza application as microorganism are able to reproduce, making it a one-time application. This research would potentially bring about a natural way to handle biotic and abiotic stress from the environment. Countries that uses ginger as an important plant source can also be exported to as to overcome lack of supply for the country that require it and as well be given continual supply, as climate from the country may not be conducive for such crop to be grown for their country usage. Inadequate amounts of research as to provide future research in the relationship between researchers and farmers can be conducted as to ensure that in depth research of mycorrhiza and ginger plant benefit.

## 1.4 Significance of Study

This study could potentially be a breakthrough for understanding of the role of Abascular mycorrhiza in its effect on ginger plants in terms of how such relationship could work. Besides that, this study can determine if such method can help in the increase in the yield of ginger during time of harvest. This study can help farmers to save cost, as mycorrhiza can colonise the root of plant, hence there it is cost saving in the application of additional nitrogen the soil. From this study what can be achieved from the research can help in the understanding of possible roles of mycorrhiza in as well benefiting similar family species of ginger, Zingiberceae.

## 1.5 Hypothesis

$H_0$: There is no significant difference between different concentrations of mycorrhiza in the performance of ginger.

$H_A$: There is significant difference between different concentrations of mycorrhiza in the performance of ginger.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 *Zingiber officinale*

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is widely used around the world in foods as a spice. Ginger originated in the Indo-Malayan region and is now widely distributed across the tropics of Asia, Africa, West Indies (Ghosh *et al.*, 2011), America and Australia. It was domesticated in India and China, which represent the centre of origin of the species (Kizhakkayil and Sasikumar, 2011) . In China, ginger is a leading high value crop in south-eastern China and a primary source of income for the region's farmers (Li *et al.*, 2010).

Ginger is a perennial herb, cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States. The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs from parts of East Asia and part of the tropics of Australia. (Mishra *et al.*, 2012)


Figure 2.1 Sketch of the ginger plant showing the origin of shoots, inflorescence, and flower.
AS: Aerial shoot, R: Rhizome, Fl: Flower, P: Peduncle (scape), S: Spike.

Source: Wimmer, 2016

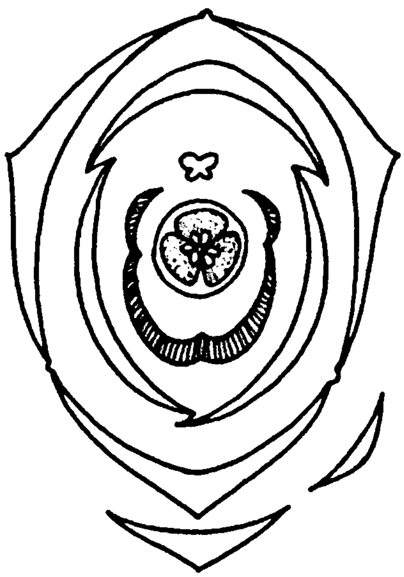

Figure 2.2 Floral diagram of ginger flower

Source: Wimmer, 2016

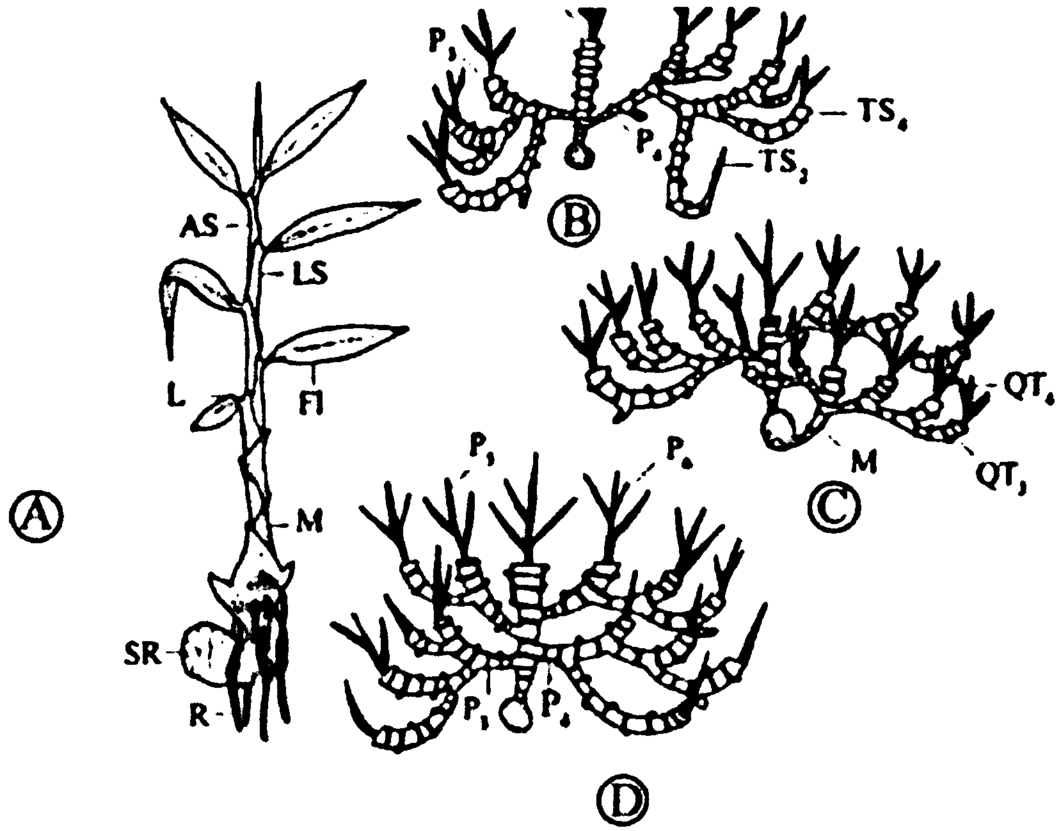


Figure 2.3 Growth pattern of the ginger rhizome.
A. Habit. B. Typical mode of growth pattern (nodes are represented by dark horizontal lines and dormant buds by a black spot). C. Two main axes developing from the seed rhizome, and their subsequent branches developing in the same plane. D. A main axis with four primary branches and their subsequent branches developing in the same plane.

Source: Wimmer, 2016

### 2.1.1 Morphology

The ginger plant is a plant with an erect, perennial herb with thick (Salmon *et al.*, 2012), underground creeping (Sharma *et al.*, 2010) hard laterally compressed, reed-like plant with annual leafy stems which is often palmately branched type of rhizomes, covered with small scale of leaves and fine fibrous roots while the rhizome is of pale yellow colour in cross section reaching up to 90 cm in height under cultivation. The stems are both erect and vertical, and is generally sterile, with leaf sheaths covering, and reaches to height of up to 1.5 m height. Leaves are of structure of linear, lanceolate, acuminate, alternate, sessile, distichous, lamina 15-30 cm long (Shakya, 2015) and 2-3 cm wide, which continues into a transparent sheath on the stem.

Flowers bloom at the axil of a bract of the plant. It has greening calyx, and three sepals joined at the base. The corolla yellowish-orange in colour. One out of the three stamens is fertile while the two sterile stamens are joined together to form a blade, which is of 2 cm long and 1.5 cm wide, divided into three lobes with the blade (lip) being reddish purple in colour with lighter patches. The shape of the Stigma is in a fringed disc. Ginger produces clusters of white and pink flower buds blooming into yellow flowers (Egbuchua and Enujeke, 2013). Fruit capsule are three loculi but fruits are very rarely found. The seeds are however shaped in angular, small, black, arillated form and they very rarely develop. Ginger is often used as landscaping around subtropical homes because of its aesthetic appeal and the adaptation of the plant to warm climates.

### 2.1.2 Area of Use

Ginger use is fairly high in USA, England and Scandinavian countries apart from the countries it is cultivated today. It has been used as a prominent spice and medicinal plant across the world since ancient times. The ginger consume in fresh or dried from is the *Rhizoma zingiberis* drug obtained from *Z. officinale* rhizomes. Traditionally, ginger is carminative, pungent, stimulant, used widely for indigestion, stomach ache, malaria and fevers (Moghaddasi and Kashani, 2012). It is chiefly used to cure diseases due to morbidity of Kapha and Vata. Ginger with lime juice and rock salt increases appetite and stimulates the secretion of gastric juices. It is said to be used for abdominal pain, anorexia, arthritis, atonic dyspepsia, bleeding, cancer, chest congestion, chicken pox, cholera, chronic bronchitis, cold extremities, colic, colitis, common cold, cough, cystic fibrosis, diarrhoea, difficulty in breathing, dropsy, fever, flatulent, indigestion, disorders of gallbladder, hyperacidity, hypercholesterolemia, hyper glycemia, indigestion, morning sickness, nausea, rheumatism, sore throat, throat ache, stomach ache and vomiting. Ginger forms an important constituent of many pharmacopoeial Ayurvedic formulations.

### 2.1.3 Antimicrobial Agent

Ginger as antimicrobial agent Ginger has some antifungal properties as well inhibits *Aspergillus* sp, a fungus known for the production of aflatoxin, a carcinogen (Nielsen and

Rios, 2000). Fresh ginger juice showed inhibitory action against *Aspergillus niger, Saccharomyces cerevisiae, Mycoderma* sp. and *Lactobacillus acidophilus.* It provides protection against invading microorganisms, including bacteria such as *E. coli* and *Staphylococcus aureus* (a common cause of skin infections) and fungi, including *Candida albicans.* The benzene extract of *Zingiber officinale* rhizome showed highest antibacterial activity against drug resistant *P. aeruginosa* isolated from wound and pus samples while the ginger extract exhibited maximum antimicrobial activity against *P. aeruginosa.*

### 2.1.4 Other Usage

Ginger was also found to be protective against DNA damage induced by $H_2O_2$ and enhanced health (Khaki *et al.*, 2009).The rhizome of ginger (*Zingiber officinale*) has been reported to possess a broad spectrum of prophylactic and therapeutic activities (Ernst and Pittler, 2000). Ginger is effective in the control of a range of bacterial, viral, fungal and parasitic. Cultured fish suffer from a wide variety of bacterial, viral, parasitic and fungal diseases. The extract of ginger inhibits the growth of *Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Streptococcus pyogenes* and Salmonella (White, 2007)

## 2.2 World Ginger Production

Area, production and yield of ginger in India are shown in (Abubacker, 2009). Area and production of ginger in India have continuously increased over the years. What was observed under ginger cultivation in the world was 429,481 hectares areas that were cultivated in 2007. The largest area under ginger cultivation is in Nigeria, which is about 55 per cent of the total area under ginger cultivation in the world. World production of ginger was 1,387,445 metric tons in 2007. India has a predominant position in ginger production contributing 36 per cent of the total world production, but productivity is very poor compared to USA.

World trade in ginger is estimated at USD190 million per year. India's share in ginger trade is only 6 per cent, while China's share is 57 per cent. In ginger oil and oleoresin trade India dominates with 50 per cent share. Garbled/ungarbled and bleached/unbleached variants occupy predominant position in ginger export. Cochin ginger is popular and it fetches a premium price because of its superior quality. The estimated world import of ginger is 300,000 tonnes per year. India's share in ginger export is estimated at 13,000 tonnes per year, which is only two per cent of its production. The main competitors in ginger export are China, Nigeria and Thailand.

### 2.2.1 Ginger Production in Malaysia

In Malaysia, gingers are commercially grown in Bentong (Pahang), Keningau and Tambunan (Sabah), and Bakun (Sarawak). The main varieties preferred by the entrepreneurs are Bentong, Bara, Chinese, and Indonesian gingers. Malaysian ginger demands for domestic and international markets are very high especially for Bentong ginger. Bentong ginger has a better quality compared to gingers from Indonesia, Thailand, China (Hong Kong), Taiwan, and the United States of America. Therefore, Bentong gingers have high demands in Hong Kong and Britain's markets. Potential and Viability Analysis for Most of high quality gingers are conventionally grown in mountain slopes such as in Janda Baik and Bukit Tinggi, Bentong, Pahang. Nomadic cultivation is still practised (Suhaimi *et al.*, 2014).

Second phase planting on the same land can only be done after the land has been left (without any commercial crop) for at least 6 years. The shrinking of the cultivation area is causing low production thereby limiting the amount of export capacity. The low production is due to the lack of suitable land for cultivating high quality ginger. The soil-borne diseases that easily attack ginger crops are also causing the lack of interest among farmers to cultivate gingers. The fertigation method using coco peat in black plastic bags is an alternative technique in ginger cultivation to overcome the problems in ginger cultivation. The role of agriculture sector in the economic growth of developing countries is important (Nya *et al.*, 2010; Piya *et al.*, 2012). The use of technology is crucial in increasing the yield and income of farmers. Thus, the adoption and utilization of new technology is critical in agricultural growth. Ginger cultivation in lowland areas using fertigation method is also capable of increasing the average yield of ginger rhizomes (5.4 kg per clump) up to two or threefold compared to the conventional method (900 g/clump). The use of fertigation system could also enable gingers to be cultivated in the same area repeatedly for years (Kay *et al.*, 2011)

## 2.3 Mycorrhiza

The word mycorrhiza is derived from the Classical Greek words for 'mushroom' and 'root'. Certain fungi are capable of infecting roots and forming a symbiotic relationship with them. The resulting structure is called a mycorrhiza, or literally fungus-root. In a mycorrhizal association the fungal hyphae of an underground mycelium are in contact with plant roots, but without the fungus parasitizing the plant. In fact the association is commonly (but by no means always) mutually beneficial. Through photosynthesis a chlorophyll-containing plant makes simple carbohydrates (using carbon dioxide, water and sunlight). While it is clear that the majority of plants form mycorrhizas, the exact percentage is uncertain, but it is likely to lie somewhere between 80% to 90%. In many of these associations between 10% to 30% of the food produced by the plant moves through to the fungi.

The benefits of mycorrhizas to plants are well documented and include efficient nutrient uptake, especially phosphorus; enhanced resistance to drought stress; and direct and indirect protection against some pathogens. From an ecosystem standpoint, Mycorrhizal benefits extend much further in space and time. In addition to participation in nutrient cycling and food web processes, Mycorrhizal fungi provide linkages among plants that affect plant community development and resiliency. Indeed, many fungi can form mycorrhizas with diverse plant species, including across plant families (Rajak, 2004). Under field conditions a new flow of carbon can move from a donor plant to shaded recipient plant via Mycorrhizal fungus linkages necessary for their survival and propagation (Bonfante and Genre , 2010). Thus, understorey plants many receive partial nourishment from overstorey plants via a shared Mycorrhizal fungus network.

Mycorrhizas are mutualistic associations between fungi and plant roots. They are described as symbiotic because the fungus receives photosynthetically derived carbon compounds from green plant and the plant increase access to mineral nutrients and sometimes water. The two most common associations are the arbuscular endomycorrhizas (AM) formed by Zygomycete fungi, and the ectomycorrhizas (ECM) formed by Basidiomycetes, Ascomycetes and a few Zygomycetes. AMF could have played a major role in the colonization of land by plants (Redecker *et al.*, 2000). This hypothesis is also consistent with recent reports describing the capacity of some AMF species to infect the most ancient plant lineages (e.g., liverworts) and improve their overall fitness (Humphreys *et al.*, 2010). Other mycorrhizal associations include the

Orchid, Ericoid, Arbutoid, Monotropoid and ectendo- mycorrhizas. Mycorrhizal associations predominate in most natural terrestrial ecosystems. Whereas the endomycorrhizas fungi are widespread geographically and have a very extensive host range, the ectomycorrhizal fungi are more restricted, forming associations predominantly with genera of important woody plants.

Nevertheless endomycorrhizas fungi are dominant components of the ground-dwelling macro-fungi in ecosystems of the following plant families: Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, Pinaceae, Ulmaceae, Salicaceae. Ectomycorrhizas fungi are common in tropical and sub tropical forests of Asia but are uncommon in many forests like China, India and Nepal. The number of host species tends to increase with altitude and at higher latitudes.

### 2.3.1 Benefits to Plants

The experiments have shown that mycorrhizal fungi can overcome nutrient limitation to plant growth by enhancing nutrient acquisition, especially phosphorus (Smith *et al.*, 2011) The most common of these strategies worldwide is AM symbiosis. Scattered through the plant kingdom are other strategies that enhance Pi availability or uptake, such as the formation of dense "cluster roots" that produce organic anions that release Pi from poorly available inorganic forms, but these are much less common (Cheng *et al.*, 2011; Lambers *et al.*, 2011). A notable exception is the element boron which is often the main micronutrient limiting growth in Asia. According to the plant species and to the growing practices and conditions, mycorrhizae provide different benefits to the plants and to the environment: Increase yields and crop quality, reduce disease occurrence , enhance flowering and fruiting, increase plant establishment and survival at seedling or transplanting, produce more vigorous and healthy plants, improve drought tolerance, allowing watering reduction, optimize fertilizers use, especially phosphorus, increase tolerance to soil salinity, contribute to maintain soil quality and nutrient cycling, contribute to control soil erosion.

Although many mycorrhizal fungi such as AM can access inorganic forms of N and P through their pathway (Smith and Smith, 2011), some litter-inhabiting Ectomycorrhiza fungi produce proteases and distribute soluble amino compounds through hyphal networks into the root.

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