INFLUENCE OF HOST PLANT ON THE PHYSIOLOGICAL ATTRIBUTES OF FIELD GROWN SANDAL (*Santalum album* L.)

By

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(2009-17-104)

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Influence of host plant on the physiological attributes of field grown sandal (*Santalum album* L.)"** is a bonafide record of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society to me.

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Dedicated to my Family

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LIST OF ABBREVIATIONS

S	*Santalum album*
C	*Casuarina equisetifolia*
T	*Tectona grandis*
A	*Anacardium occidentale*
Co	*Cocos nucifera*
R	*Hevea brasiliensis*
Cc	*Theobroma cacao*
P	*Piper nigrum*

Introduction

INTRODUCTION

Sandalwood tree (*Santalum album* L.) is a precious tree well known for its fragrant heart wood (East Indian Sandalwood) and the scented oil derived from it (East Indian Sandalwood tree oil). It is commonly known as sandalwood tree or *chandan* and is a semi root parasite tree of the family *Santalaceae*. An individual growing tree can put an increment of 1.0 kg of heartwood per year and can attain a girth of over 1.5 metres (Rai, 1990). It has also been intimately associated with human civilization since time immemorial and is a part of Indian culture and heritage (Srinivasan et al., 1992). The heart wood of sandalwood tree is estimated to be fetching approximately 9 lakh per tonne in international market (Ananthapadmanabha, 2000). As per 2010 auction in Marayoor, Kerala, fifth class sandalwood tree was sold at rupees 7390 kg^{-1}. Over exploitation and illicit felling of sandalwood tree have resulted in decline of population and genetic erosion (Annapurna et al., 2007). In order to meet growing demand and sustainable utilization of *S. album*, regeneration and plantation techniques need to be standardized.

Distribution of genus *Santalum* is in the tropical region in between 30°N and 40°S, from India in the West to Juan Fernandez Islands in the East and from Hawaiian Archipelago in the North to New Zealand in the South (Brennan and Merlin, 1993). It comprises of 16 species (Hamilton and Conrad, 1990; Barret and Fox, 1997) and all of them are xylem tapping root hemi-parasites with highly valued aromatic heartwood (Shea et al., 1998). Four *Santalum* species namely *S. spicatum* (R. Br.) A. Dc., *S. acuminatum* (R. Br.) A. Dc., *S. morrayanum* (Mitchell) C. Gar., and *S. lanceolatum* (R. Br.) are native to Western Australia (Brand and Jones, 2000). Among the *Santalum* species, *Santalum album* has the highest oil content (6-7%)

while *S. spicatum* (2%) and *S. laneolatum* (3-5%) yield poorly scented wood and low quality oil (McKinnel, 1990).

S. album is found distributed in almost all the states of India covering a total area of 9040 sq. km and more than 90% lies in Karnataka and Tamil Nadu (Dutt and Verma, 2005). In Himachal Pradesh, it occurs at Bilaspur near main town and in the Kangra Valley at Jawala Mukhi (Venkatesan et al., 1995). Other important sandalwood tree bearing states include Andhra Pradesh, Orissa and Madhya Pradesh. A survey on the important sandalwood tree bearing areas of these states were done by Jain et al. (1998), which indicates that the sandalwood tree population has declined substantially due to biotic and abiotic factors. Sandalwood tree bearing forests of Kerala is mainly located on the drier parts of Eastern side of the Western Ghats in the Anjanad valley of Marayoor range in Munnar Forest Division (Hiremath, 2004). On a limited scale, sandalwood tree is also found in Ariankavu range of Thenmalai Forest Division. Isolated patches of sandalwood tree are also found in Walayar, Wadakancherry and Plamaram (Palghat district) forest areas. Sandalwood tree is also observed as a component of the homestead especially in Northern Kerala (Kumar et al., 1994).

The annual production of sandalwood tree has declined from 4000 tonnes in 1965-1975 to nearly 2000 tonnes during 1999-2000. The oil production has also decreased to 40-50 tonnes during 1999-2000 from 60 tonnes during 1981-1994 (Ananthapadmanabha, 2000). India exports around 2000 tonnes of wood and 100 tonnes of oil annually to various countries. This accounts for 99 per cent of sandalwood tree oil produced in the world (Lakshmisita and Bhattacharya, 1998). The sandalwood tree oil is present in the heartwood of stem and root and hence the tree is invariably harvested by uprooting (Hiremath, 2004). 30-60 year old trees having a girth of 40-60 cm generally have the best heartwood suitable for carving as well as for oil extraction (Shankaranarayana et al., 1998).

The depletion of sandalwood tree forest is attributed to factors like illicit felling, disease and smuggling, which are very rampant and is the major problem in the entire sandalwood tree growing states (Rao et al., 1999). Smuggling ultimately results in genetic erosion because smugglers remove genetically superior trees (Venkatesan, 1995). Umashankar et al. (2000) reported a decline in genetic diversity of natural population due to indiscriminate extraction of sandalwood.

The diminishing supplies of sandalwood tree from its natural habitat (forest) and its increasing demand, points to the need for expanding area not only in forest lands but also in farm lands. Its high economic value provides sufficient incentives to the farmers for growing sandalwood tree on a commercial scale. The area under sandalwood tree is decreasing fast, because of pilferage and the difficulty in establishing additional sandalwood tree forest. The gap between demand and supply are so wide that the price is sky rocketing. Considering the growing demand and the diminishing supply of sandalwood, there exists great potential for raising sandalwood tree in not only forest areas but also in private land like home gardens and other agroforestry systems.

Production of sandalwood tree wood can be increased by extensive plantation of this species after properly understanding the host-parasite relationship, proper production of planting materials and knowledge of silviculture of this species. The regeneration and establishment of sandalwood tree has been problematic because of the poor understanding of host-parasite relationships (Surendran et al., 1998). At the same time, only a few literatures are available indicating the relation of host in field grown sandalwood. Understanding of the haustorial anatomy is also important as sandalwood tree takes up food materials from the host plants through this specialized tissue.

Considering the above, investigations were carried out with the following objectives:

1. To understand the influence of host plants on carbon assimilation, water and nutrient absorption of sandalwood tree grown in the field.

2. To study the anatomy and functional status of haustoria in the field grown sandalwood tree.

Review of literature

REVIEW OF LITERATURE

The genus *Santalum* belongs to the family *Santalaceae*, which comprises herbs, shrubs and small trees. It has long been a source of sandalwood, a fragrant wood priced for its use in producing ornaments, cabinets and chests; incense for religious rites; and oil for perfume and medicines. *Santalum album* is the best known commercial species. It is found in southern India (but may have originally been introduced from Java, Indonesia and Northern Australia), especially in Karnataka, Tamil Nadu and Kerala. It is also found in Sri Lanka and other parts of South-Eastern Asia (Brandis, 1978). Various descriptions related with it are mentioned in Hindu mythology (Neil, 1990). Powdered wood in the form of a paste, with added pigments, is used in caste distinguishing marks (Drury, 1985).

Sandalwood tree (*Santalum album* Linn.) is a small to medium sized, evergreen hemi-parasitic tree with slender drooping branchlets, ordinarily attaining a height of 13.5 m to 16.5 m and a girth of 1.0 m to 1.5 m, though larger specimens are sometimes met with. In natural forests, the tree is observed in dry tropical forests. It is also seen in isolated farms and homesteads in Kerala (Kumar et al., 1994).

Due to hemi parasitic nature of sandalwood tree, there are various problems associated with its regeneration and silviculture. Sandalwood tree-host relationships, propagation methods, spike diseases, seed pre-treatment methods were the topic of interest for the sandalwood tree researchers. Tree improvement programmes, micropropagation of sandalwood tree and establishment of sandalwood tree plantations were getting attention in some parts of the world during the last few years.

2.1 Host Plants

The hemi parasitic nature of sandalwood tree was established for the first time by Scott (1871). Later on, the parasitic behaviour of *Santalum* had been described by Barber (1902 and 1907), Pilger (1935) and Rao (1942a). Barber (1902) found an abundance of root connections between sandalwood tree seedlings and other plants growing nearby. Rao (1903) and Lushington (1904) also could observe haustoria, which connect sandalwood tree roots to host plants and extract nutrients from the host.

The anatomy of the haustorial connections has been well studied. The haustoria of sandalwood tree, which rise laterally on roots, are exogenous. A young haustorium is formed by the epidermis and cortex of the root (Rao, 1942b). According to Pilger (1935), haustorium is derived from the root by the divisions of the cells of pericycle, endodermis and cortex. The young haustoria appear as small hemispherical outgrowths. The free end after coming in contact with the host gradually flattens.

Sandalwood tree is known to have sent out its roots up to a distance of 30 m for establishing the parasitic relationship (Rai and Sarma, 1986). Rao (1911) reported that the host, which is attacked by sandalwood tree, influences the extent and structure of haustoria. Taide (1991) in an anatomical study of sandalwood tree seedlings haustorium found that the sandalwood root and the host show direct vascular connections, which later undergoes secondary growth. The author also observed that vascular connection between the host and sandalwood becomes so intimate that host root and parasite root becomes almost a single physiological unit, catering to the nutritional requirements of the sandalwood. Ma Guo et al. (2005) found that sandalwood roots lack root hairs, but its vessels were well developed, which are suitable for absorption of water and nutrients from host roots.

The formation of haustoria is more or less confined to younger roots; the main roots probably take little part in the absorption of nutrients. If no host is met with, the haustoria remain small and underdeveloped, which ultimately wither away. But if a rootlet of a suitable host is met with, it grows rapidly assuming the shape of flattened bell. It is reported that sandalwood tree seedlings are incapable of growing beyond a year at the most unless nourished by attachment to the roots of other plants (Rao, 1903). Annapurna and Rathore (2006) observed the significant influence of host species on haustorial number, connection, size and chlorophyll content in sandalwood tree seedlings. Struthers et al. (1986) observed enhanced haustorial formation by the presence of *Acacia acuminata* roots. They also found out the competition for nutrients, especially calcium, from co-planted *Acacia acuminata* seedlings results in suppression of growth of young sandalwood tree compared with their growth in the absence of the host species.

The obligate parasitic nature of sandalwood tree is known since long, but there is no precise information about the nature and degree of its dependence on host. The presence of favoured host is considered to improve the establishment and growth of sandalwood tree.

Various researches have identified and classified several hosts of sandalwood tree. Iyengar (1965) has published a list of all known hosts till that time. The sandalwood tree hosts have been classified as good, medium and poor based on the complementary influence of the host species on sandalwood tree growth (Ananthapadmanabha et al., 1984). In Australia, the hosts are generally categorized into three groups namely pot host, intermediate host and long term hosts (Fox et al., 1990). All the three are critical for adequate survival and growth of sandalwood tree at various stages of its seedling growth and at various stages of the plantation growth. Characteristics of suitable pot host include fine root growth and even distribution of roots within the pot, ability to withstand top pruning, low level of competition, low allelopathic influences, low growth structure and persistence in the field after planting out (Fox and Doronila, 1993). Srinivasan et al. (1992) has recommended *Cajanus cajan* as a good primary host for sandalwood tree in the seedling stage whereas Surendran et al. (1998) reported *Albizia saman* as the best life time host for sandalwood tree based on growth attributes and amenability for pruning. Surata et al. (1995) found out that intercropping of sandalwood tree with *Arachis hypogaea* increased sandalwood tree seedling growth and survival. Taide et al. (1995) from the pot experiments on the influence of 15 host plants on the initial growth and development of sandalwood tree seedlings observed that some combinations had synergistic effect while others had allalopathic effect.

In India, earlier researchers have identified a range of pot hosts for the establishment of sandalwood tree plantations. Barber (1907) gave a list of 122 species and later Rao (1918) for 144 species of sandalwood tree hosts. Out of a large number of associates of sandalwood tree found in its natural habitat, it is difficult to classify the most favorable or suitable host species as sandalwood tree may show preference for different plants in different situations. The favoured hosts reported are *Desmanthus virgatus*, *Alternanthera* spp., and *Crotalaria juncea* in Timor (Surata, 1992), *Calotropis procera*, *Cassia siamea* , *Calliandra calothyrus* (Shinde et al., 1993), *Cajanus cajan* (Rai, 1990) and *Casuarina equisetifolia* (Taide, 1991 and Varghese, 1997).

Radomiljac (1998) reported that considerable variation existed between pot hosts in increasing the sandalwood tree survival and growth. Consequently, the utilization of appropriate pot hosts is critical to ensure successful sandalwood tree plantation establishment. Jin et al. (2010) found out that configuration time of pot host affected the height, ground diameter, and biomass as well as the number of haustoria of *Santalum album* seedlings after five month's growth.

Establishment of sandalwood tree plantations was mostly not successful due to several reasons. Being a semi parasite, the silvicultural requirements are unique and there is no adequate understanding of the same. Even though many investigations for identifying the best host for sandalwood tree in India and other countries are available, the growth stage at which the sandalwood tree needs the presence of a host and the complementary and competitive interactions between sandalwood tree and the host plants are not available in literature.

2.2 The Role of Host

The role of host plants in sandalwood tree, which is having independent root system and evergreen canopy capable of photosynthesizing, has aroused a lot of curiosity among the researchers. There are several reports indicating the necessity of host plants for acquiring some of the plant nutrients by sandalwood tree.

Srimathi et al. (1961) found that leaves of sandalwood tree did not have the basic amino acids in the absence of host, but when grown with leguminous plants, the sandalwood tree leaves showed high concentration of basic amino acids. Therefore, the authors concluded that for the supply of amino acids, sandalwood tree is dependent on its host. Iyengar (1965) reported that the dependence of sandalwood tree seedlings on the host is mainly confined to N and P, whereas it can directly absorb Ca and K.

Self-parasitism, a phenomenon in which a plant establishes haustorial connections with the same species was also observed in sandalwood tree by Iyengar (1965). Ananthapadmanabha et al. (1984) in a pot culture study observed that in many instances sandalwood tree seedlings have drawn the nutrients from hosts, but there are instances where some hosts derived benefit from sandalwood tree, by getting some amount of P, Ca, Mg. Rangaswamy et al. (1986) also suggested that sandalwood tree depends on its host for P, K and Mg and that in the absence of a host plant, it is incapable of growing normally. Brand (2002) observed significantly greater foliar concentrations of N and K and the K: Ca ratio in *S. spicatum* growing near *Acacia acuminata.*

Comparative analysis of leaves of sandalwood tree grown independent or with host shows appreciable differences in the mineral constituent of the leaves. The associations of host brought about higher accumulation of minerals and consequently better growth of sandalwood tree. In treatments without association of host plants, in spite of higher N content in the leaves, sandalwood tree showed poor growth. The experiments further indicated that the sandalwood tree depend on the host for P, K and Mg, although the plants not associated with hosts are capable of absorbing some minerals, but not enough to sustain growth (Rangaswamy et al., 1986). Subbarao et al. (1990) observed the number of nodules and nitrogen content of plants decreased in parasitized nodulating species with corresponding increase in the nitrogen content of sandalwood tree. Struthers et al. (1986) observed differences in K, Ca, N and Cu levels between parasitized and uninfected *Acacias* they confirmed the host plant contribution of nutrients to the sandalwood tree.

Hua et al. (2005) observed that sandalwood tree grow normally without host plant during its seed germination and early seedling stage. However, the subsequent growth needs roots of the host plant. They also observed that sandalwood tree root lack root hairs but its vessels are well developed, which are suitable for absorption of water and nutrients from hosts' roots. Kamalolbhavan (2002) reported the occurrence of Sandalwood tree-Arbuscular Mycorrhizal Fungi (AMF) associations in natural sandalwood tree growing forests and investigated the response of sandalwood tree seedlings to inoculation with commonly available cultures of AMF, shade levels and nature of host in a pot culture experiment. He reported that 50 per cent shade is the most favourable for the growth of sandalwood tree as well as for the better colonization of AMF.

Hence, it can be concluded that the interactions of sandalwood tree and host plants for the uptake and translocation of various mineral nutrients are very complex and need very precise and vivid researches. The manner of uptake of the mineral nutrients also needs to be understood very clearly in order to understand the physiological and anatomical formation of haustorium in sandalwood tree.

2.3 Haustorial Anatomy

The ecology, growth and host preference of the root hemiparasite *Santalum album* have been well documented (Ananthapadmanabha et al., 1984; Radomiljac, 1998; Tennakoon et al., 2001). While the functional attributes of *Santalum*-host interactions are relatively well understood, the structure and development of the *Santalum*-host interface and its implications for parasite nutrition have received adequate attention in the literature.

Parasitism in the angiosperms has evolved on at least seven separate occasions (Nickrent and Duff, 1996). Parasitic plants are a diverse polyphyletic group containing 3000 species and representing around one per cent of all plant species (Musselman and Press, 1995). They access their hosts' resources through a key organ called the haustorium, which provides a physical as well as a physiological bridge between the parasite and host, directing the hosts' resources to the parasite and functioning at the multiple stages in the parasitism (Kujit, 1969). A broad diversity is found in the internal structure of haustoria belonging to the different parasitic plant species (Hibberd and Jescheke, 2001). The morphology of the haustorium is directly related to the mechanism employed by the parasite to access host resources through either direct vascular continuity, interfacial parenchyma, or a combination of both (Pate et al., 1990). Furthermore, there is variability in the extent to which different nutrients and solutes are obtained by parasitic plants (Jiang, 2004). Riopel and Timko (1995) highlighted a structure in the haustoria of *Santalum album* called hyaline body, which is rich in nuclei, believed to be involved in resource translocation and processing. The other structures are the endophyte or the penetration peg (the projection of which enters the host root tissue), and the ellipsoidal disc (laterally flattened, relative to the host root, against the hosts' stele and the point of contact between the parasite and its hosts' vascular system) (Tennakoon and Cameron, 2006). Solute acquisition by *S. album* is not fully understood; increasing evidence points to the important role played by the unique structure and morphology of the juncture between *S. album* and its host. The type and magnitude of resource fluxes (nutrients, hormones and water) from the parasitized host via haustoria directly affect the growth and development of *S. album* (Tennakoon and Cameron, 2006). There are few studies investigating the anatomy and development of haustoria formed by *S. album* on any of its common hosts. Barber (1906, 1907) and Rao (1942b) undertook the first studies of the interaction between *S. album* and some of its hosts. In contrast to many other root parasitic genera in the families like *Orobanchaceae, Balanophoraceae, Rafflesiaceae* and *Lennoaceae* no involvement of chemical signals derived from the host roots of *S. album* in relation to the successful haustorial initiation and establishment was observed (Stewart and Press, 1990). However, close examination of the fully functional young haustoria revealed the presence of a darkly staining (purple) mucilaginous substance produced by the initial contact surface of the haustorium (Tennakoon and Cameron, 2006). The role and identity of this substance is unknown, although Baird and Riopel (1983) reported such exudation by the parasitic plant *Agalinis purpurea* and concluded that it was a hemicellulose compound.

Following attachment to compatible host roots, intrusive cells of haustoria penetrated the host epidermis and cortex between host cells. Concurrent with this endophytic development, the cortical fold of the haustorium partly encircled the host root (Tennakoon and Cameron, 2006). Similar observations have been reported for species of *Orobanche* (Lane et al., 1991), *Striga* (Losner-Goshen et al., 1998) and *Rhinanthus* (Cameron et al., 2005). The mature *S. album* haustorium consists of two regions, one external to the host root, the hyaline body, a structure with high metabolic activity and the penetration peg that makes the initial contact with the host root and penetrates the host tissue (Riopel and Timko, 1995).

According to Tennakoon and Cameron (2006), the finger-like projections of the developing endophyte extend up to the cambial tissue of the host root during the initial establishment of the haustorium. This tissue is mainly composed of characteristically elongated (tubular) thin walled parenchyma cells. As the projections elongated towards the host root xylem, they entwined with each other and gave a tubular appearance to the cells.

There was no biochemical evidence to support the involvement of either pressure or cell-wall-degrading enzymes in the development of *S. album* haustoria, although these factors are associated with the penetration process of haustoria formed by many other species of parasitic plants (Fineran and Hocking, 1983; Calladine and Pate, 2000; Rao, 1942a).

Darkly staining material at the host parasite interface of many parasitic plants has been described in literature (Dobbins and Kujit, 1974; Musselman and Dickison, 1975; Losner-Goshen et al., 1998; Kuo et al., 1989; Cameron, 2004). Tennakoon and Cameron (2006) illustrated the presence of darkly staining material at the host-parasite interface in the *S. album–Tithonia diversifolia* association. They also concluded that these may be the secreations (tip lysis) of tubular contact parenchyma emptied onto the surface of the host. This extruded material potentially aids in the firm adhesion of parasite tissue to host. However, some reports have suggested that these substances may aid penetration into host tissue (Heide-Jorgensen, 1989) or, in incompatible interactions, may represent induced defenses in the host (Gurney et al., 2003; Cameron, 2004).

Santalum album haustoria resembled the majority of other root hemiparasites' haustoria in lacking phloem connections with hosts (Pate, 2001; Shen et al., 2006; Tennakoon and Cameron, 2006). There are relatively few xylem elements in the haustorium that are typically short tracheary elements. Investigation by Tennakoon and Cameron (2006) revealed that direct lumen-lumen xylem connections between the xylem of the host and parasite are absent.

Hence, it can be concluded that haustorial formation is a very complex physical and physiological processes between host plants and sandalwood tree, which still need investigations before coming to a conclusion.

2.4 Nutrient Uptake

Many of the earlier workers were of the view that sandalwood tree probably is an obligate parasite entirely dependent upon the host for its nutrients (Barber, 1903; Lushington, 1904; Rangaswami and Griffith, 1939). But Brandis (1903) suggested that sandalwood tree may derive part of its nutrition from soil also. Later many workers have conducted isolation experiments by trenching to assert the extent of parasitism. But there was no consensus of opinion, to some it seemed like an obligate parasite while for others it was not so (Iyengar, 1965).

Rao (1933) after studying the parasite with and without host *Acacia farnesiana*, concluded that sandalwood tree depends on its hosts for N, P and K while Ca and Fe appear to be directly derived from soil. After studying soils under healthy and spiked sandalwood tree Iyengar (1965) concluded that sandalwood tree depends on the hosts for N and P while Ca and K are absorbed through roots from soil. He thus negated the view that sandalwood tree is an obligate parasite. He suggested that Ca:N ratio in the sandalwood tree may represent the balance of activity between root ends and haustoria. Rao (1938) reported that certain principles of the host such as the bitter principle in *Strychnos nuxvomica* and *Azadirachta indica* were translocated to the leaves of sandalwood tree. Iyengar (1965) in a study of physiology of root parasitism in sandalwood tree stressed the Barber's view that in healthy sandalwood tree both root ends and haustoria are very active, while in spike-diseased sandalwood tree both of them have ceased to function.

Subbarao et al. (1990) observed that sandalwood tree formed direct haustorial connections with root nodules of nodulating legumes in the field. In pot culture studies with sandalwood tree, *Cajanus cajan* and *Pongamia pinnata,* it was confirmed and the number of nodules and the N content of plants decreased in parasitized nodulating species with corresponding increase in N content of sandalwood tree.

Nayar and Ananthapadmanabha (1974) in a bioassay of tetracycline uptake in spike-diseased sandalwood tree observed that there is movement of tetracyclines from sandalwood tree to the host and host to sandalwood tree. The authors concluded that the haustorial connections may be permitting movement of substances in both the ways. Ananthapadmanabha et al. (1988) in a pot culture study observed that in most instances sandalwood tree have drawn nutrients from hosts, but some hosts derived benefit from sandalwood tree in getting some amount of P, Ca, Mg and N. This increase in the mineral elements in the hosts, when found associated with sandalwood tree might be possible by reverse transfer or by antagonistic processes, to the extent that the haustorial connections may serve as two way traffic.

Tracer technique studies have shown that calcium could be absorbed by the roots of sandalwood tree seedlings, while phosphate, organic substances, amino acids, sugar and mineral phosphates were drawn from the host plant (Kunda et al., 1974*a*, 1974*b*). Ashokan et al. (2008) with radiotracer studies observed that the translocation of the mineral nutrients and carbon were much efficient in sandalwood tree–casuarina haustorial association than in other host/crop plant. The translocation observed between sandalwood tree- cocoa association and sandalwood tree–teak associations were significantly more efficient than black pepper, cashew, rubber and coconut associations.

Varghese (1997) using radiotracer technique found that sandalwood tree could take up Ca directly from soil and its dependence on host for calcium was negligible.

The author also concluded that redgram parasitized by sandalwood tree had a higher uptake of Ca than the redgram grown alone, which may have been caused by the increased cation exchange capacity of roots of the parasitized plant. Kunda et al. (1974a) from radio tracer studies in sandalwood tree and a host (*Dolichos lablab*) and Iyengar (1965) after soil-plant analysis of spike-diseased and healthy sandalwood tree inferred that Ca is taken up directly from the soil by sandalwood tree. Parthasarathi et al. (1974) also concluded that increased uptake of ^{45}Ca by parasitized red gram may be due to the increased CEC of its roots.

Varghese (1997) with ^{35}S studies observed that sandalwood tree is taking up sulphur from soil or its dependence on host (redgram) for S is negligible. Haustoria acted as a two directional path way in the translocation of S, *i.e.* from sandalwood tree to host and host to sandalwood tree. He also observed more translocation of S from sandalwood tree to host (1.2 %) than Ca (0.44 %). He also found that sandalwood tree could take up P directly from soil and host may also provide a small fraction of the P requirement of sandalwood tree. If the soil source is not limiting, sandalwood tree may not have to depend on the hosts for P. It was also found that hosts differed in their ability to supply P to sandalwood tree. The translocation of ^{32}P from host to sandalwood tree was 0.7% in case of *Erythrina* and 8.7% in case of Casuarina to sandalwood tree which was considerably higher than that from *Erythrina*. Iyengar (1965) and Kunda et al. (1974b) also observed sandalwood trees' dependence on host for P. Varghese (1997) also concluded that there was translocation of carbon compounds between sandalwood tree and hosts and the extent of transfer varied depending upon the host plants.

2.6 Water potential

Sreenivasrao (1933) observed that the osmotic pressure in the tissue of sandalwood tree was higher, compared to that in the tissue of host plant and this may ensure unidirectional flow of nutrients from host to the parasite. Varghese (1997) observed that the predawn (at 600 IST) water potential of sandalwood tree is lower than that of the host species associated with in all the sandalwood tree-host associations examined. He also found the same trend of sandalwood tree plants maintaining consistently lower water potential throughout the day. Tennakoon et al. (2000) from Sri Lanka observed that the sandalwood tree seedlings always showed more negative water potential than associated host plants thus maintaining a water potential gradient favourable to sandalwood tree seedlings to derive water and nutrients from the host.

Hiremath (2004) opined that host plants may be helping the sandalwood tree plants to maintain higher water potential, as the water potential of sandalwood tree was higher in the presence of host. He also concluded that water stress decreases the water potential of both sandalwood tree and host.

Dhaniklal (2006) observed the predawn water potential of sandalwood tree seedlings after 270 days of planting. Sandalwood tree seedlings without host plant showed the highest value of predawn water potential and which was on par with sandalwood tree with *Casuarina equsetifolia* as host. This was followed by sandalwood tree seedlings with *Erythrina indica* as host. Ashokan et al. (2008) observed that in sole sandalwood tree and in sandalwood tree grown with agricultural crops, plant water potential was very low indicating high water stress for sandalwood tree in the absence of a preferred host. Water potential of sandalwood tree was high in sandalwood tree + Cocoa + Casuarina association (-2.05 MPa) followed by sandalwood tree + Cocoa (-1.84 MPa), probably due to the high transpiration demand of cocoa. Sandalwood tree grown without host plant showed lower water potential as compared to sandalwood tree + Casuarina (-0.68 MPa).

The review of the available literature reveals that though considerable investigations have been carried out on the parasitism of sandalwood tree, a clear cut understanding of the process has not yet been obtained, differences in opinion exists among scientists regarding which of the elements are absorbed by sandalwood tree directly from soil and which are absorbed from host. The influence of the host plant on the water relations of sandalwood tree is also not fully understood. The possibilities of raising sandalwood tree in the farmlands and the parasitic behaviour of field grown sandalwood tree on the host species in a farmland also not investigated.

Materials and Methods

MATERIALS AND METHODS

The present investigations were carried out at the sandalwood tree field plot (Plate 1) available in College of Forestry, Kerala Agricultural University, Vellanikkara, for studying the influence of host plant on carbon assimilation, water and nutrient absorption of field grown *Santalum album*.

3.1 Location of the study

The location comes under the Madakkathara panchayat of Thrissur district and lies between 10°32' N latitude and 76°16' E longitude. The climate is warm and humid with an average annual rainfall of 2668 mm. The mean maximum temperature varied from 28.4°C in July to 36.0°C in March. The mean minimum temperature varied from 21.6°C in November to 25.0°C in April. The diurnal variation in temperature is very narrow. The soil is of lateritic origin. The area has an altitude of about 40 m above MSL.

Experiment No: 1

Influence of host plants on carbon assimilation, water and nutrient status of sandalwood tree trees

This experiment aims at understanding the influence of the host plant on carbon assimilation, water and nutrient status in sandalwood tree grown in field. The existing field plot of sandal established as a part of an earlier research project during 2005 was utilized for this study. There were twenty trees without and thirty six trees with host (Casuarina) raised as part of the project. The available populations of sandalwood tree with host were grouped into two. In both the groups initial growth observations were taken at monthly intervals. Then in one group the host plants were

Plate: 1 Sandalwood tree plot of College of Forestry

cut and removed and observations on growth parameters were recorded for one month, at fifteen days intervals.

3.2 Experimental layout

The following were the treatments for the study.

T_1- Sandalwood tree without host (Casuarina) (Host plant dead naturally within 2 years after establishment of sandal)

T_2- Sandalwood tree with host (Casuarina)

T_3- Sandalwood tree with host (Casuarina) and the host plant cut and removed at six year stage of growth

Single plant experimental plot was used. The study was conducted in RBD with three treatments mentioned above and ten replications. Total number of trees used for the experiment was 30.

3.3 Observations

3.3.1 Height

The height of sandalwood tree was measured at monthly interval, using Vertex Hypsometer.

3.3.2 Diameter

The diameter at breast height (dbh) ie, 1.37 m was measured using a measuring tape at monthly interval.

3.3.3 Rate of photosynthesis

The rate of photosynthesis was measured using LI – 6400 Portable Photosynthesis System (LICOR, USA).

3.3.4 Plant water potential

The plant water potential of sandalwood tree were estimated during different seasons, before and after removing the host plants, using Scholander's pressure bomb type plant water status console (Soil Moisture Equipment Corporation, Ohio, USA).

3.3.5 Chlorophyll content

The estimation of chlorophyll content was done using Arnon's method (Arnon, 1948). Fresh leaf samples were collected at random and 100 mg of leaf sample were cut into smaller pieces, put in 7 ml dimethyl sulphoxide (DMSO) and kept in dark room, overnight. The supernatant was then decanted and the leaf tissues were discarded. The solution was then made upto 10 ml using DMSO. The absorbance value of the extract was taken at 645nm and 663 nm wavelength using DMSO as blank, in a spectrophotometer. The chlorophyll content was calculated using the following formula.

$$[\text{Chl } b] = 22.90 \cdot E^{645} - 4.68 \cdot E^{663}$$

$$[\text{Chl } a] = 12.70 \cdot E^{663} - 2.69 \cdot E^{645}$$

$$[\text{Chl } a + b] = 20.21 \cdot E^{645} + 8.02 \cdot E^{663}$$

Where [Chl *b*] is the chlorophyll *b* content in $mg.g^{-1}$ fresh weight of leaf

[Chl *a*] is the chlorophyll *a* content in $mg.g^{-1}$ fresh weight of leaf

[Chl $a + b$] is the total chlorophyll content in $mg.g^{-1}$ fresh weight of leaf

E is the absorbance values at respective wavelengths, observed using the spectrophotometer.

3.3.6 Leaf nutrient content of sandalwood tree

Fresh leaves of the sandalwood trees at the month of June and April and before and after removal of the host were analyzed for the nutrient content. Leaves were collected at random from different areas of the canopy, in paper bags and oven dried at 70 ± 5^0C. The dried samples were ground, mixed and analyzed for N, P, K, Fe, Cu, Zn and Mn.

Nitrogen

Nitrogen was determined by using Micro-Kjeldahl method. 0.2 g of the dried, powdered leaf samples were digested using 15ml of conc. H_2SO_4 and 3g of digestion mixture (K_2SO_4 and $CuSO_4$ at 10:1 ratio). The digestion was done at a temperature of $300\text{-}330^0C$ until the content turns into pale green colour. The digested sample was distilled with 40 per cent NaOH and the ammonia evolved was collected in 4 per cent boric acid. Distillation continued for 10 minutes so that the released steam is free of ammonia. Finally, the ammonium in the boric acid was titrated against 0.02 N H_2SO_4 taken in the burette. The end point was confirmed when the solution of boric acid regain its colour (Jackson, 1958).

Phosphorus

Phosphorus was determined by Vanado-molybdo-phosphoric yellow colour method in HNO_3 (Jackson, 1958). Dried, powdered leaf samples (0.2 g) were digested using diacid mixture of nitric acid and perchloric acid (2:1) and the digest made up to 50ml. 5 ml of diacid digest was added with 10 ml of Bartons reagent and made upto 50 ml using distilled water. After allowing the development of colour for 30 minutes, the intensity of the yellow colour developed was read in Spectrophotometer (Genesys 20) at a wavelength of 420 nm. Phosphorus content was calculated by referring the standard curve prepared with standard solutions of phosphorus (Koening and Johnson, 1942).

Potassium

An aliquot of the diacid digest of the leaf samples prepared for phosphorus estimation was used for estimating potassium content by using digital flame photometer (Jackson, 1958).

Micronutrients

An aliquot of the diacid digest of the leaf samples were used for the estimation of selected micronutrients Fe, Cu, Zn and Mn. It was estimated in atomic absorption spectrometer (Perkin Elmer Model: Analyst 400).

Experiment No. 2

Haustorial associations and anatomy

3.4.1 Anatomy of haustoria

Anatomical studies were conducted to understand the functional status of sandal-haustoria association. Thin (2-5μm) microscopic sections of haustoria were taken following standard procedures of fixing, tissue processing and staining.

Killing, fixing and aspiration

For killing and fixing of samples FAA (formalin, acetic acid and alcohol) was used. The samples were kept in FAA for 24 h and then aspirated in an aspirator. The remaining procedures of tissue processing was carried out in a Leica tissue processor.

Dehydration in alcohol series

30% ethyl alcohol

50% ethyl alcohol

60% ethyl alcohol

70% ethyl alcohol

90% ethyl alcohol

98% ethyl alcohol

Infiltration of paraffin (58-60^{0}C) in the paraffin solvent media tertiary butyl alcohol (TBA)

TBA

TBA: Wax (3:1)

TBA: Wax (1:1)

TBA: Wax (1:3)

Pure paraffin wax

Embedding

Embedding was carried out by manual embedding using the paper boat technique.

Microtomy

Sectioning of wax embedded and infiltered haustoria samples was carried out using a Leica Jung Multicut Rotary Microtome (Leica RM 2125) to obtain sections of 2-5 μm.

Dewaxing, staining and washing

Sections were dewaxed using alcohol, placed on slides pasted with Haupt's adhesive, stained using saffranin.

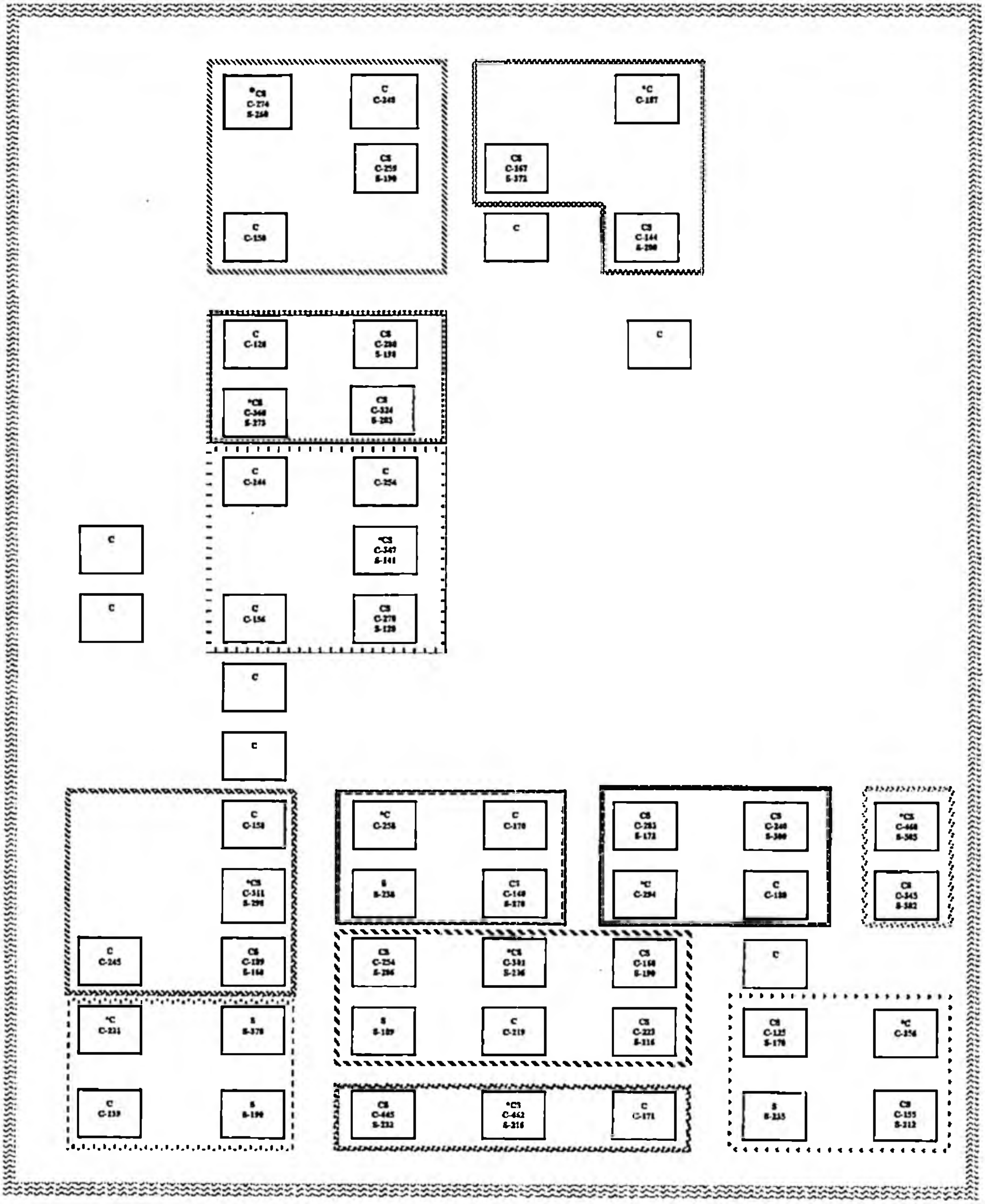
3.4.2 Haustorial association

Two sample trees, one with and the other without host (Casuarina) were excavated to investigate the haustorial association. Soil of one quarter of the area around sandalwood tree was carefully removed by loosening the soil with water spray. The length and diameter measurements of excavated sandalwood tree roots were recorded and the number of functional and nonfunctional haustoria on primary, secondary and tertiary roots of host at 15 cm length segments was also recorded.

Experiment No. 3

3.5 Radio-tracer studies on haustorial association

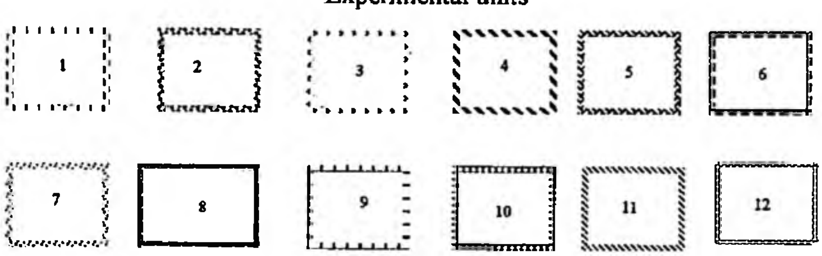
The layouts of the sandalwood tree plots used for the radiotracer studies are shown in the Fig. 1(a) and (b). Functional status of Sandal-haustoria was studied by observing the translocation of radio-labelled phosphorus (^{32}P) from host to sandalwood tree. The following were the treatments used for this study.



* ^{32}P labelled host

Fig.1 (a) Layout of sandalwood plot with casuarina-sandalwood associations and Jatropha border

Experimental units



CST	S	CS	CT
A	*AS	A	A
S	SC		S
S	S	S	
*C_oCSR	*RS	*C_oCSR	*C_oCSR
S	S	S	
CSR	CSR	*CSR	
RS	R	R	R
C_o		C_o	C_o
C_c	C_c		
*ACS	AC	AS	A
	TS	T	TS
CP	CS	CS	CS
T	S	TS	T
			S
CS	CS	CS	S
		S	S
A	*AS	A	A
CTS	TS	*TSC	S
CAS	*CAS	AC	CAS
		CS	CS
C_c	S	*C_cS	S
R	RC	*RSC	CR
TRS	*CST	TS	CST
CA	CA	CA	CAS
C	CPS	C	
*SCT	*SCT	T	T

* ^{32}P labelled tree

Fig. 1(b) Layout of the sandalwood plot with different host associations used for radiotracer studies

T_1- Labelling of host (Casuarina) with ^{32}P and tracing it in sandalwood tree

T_2- Labelling of host (wild grass) around sandal with ^{32}P and tracing it in sandalwood tree

The first treatment of labeling host plant with ^{32}P was done in the host plant (Casuarina) growing.between the rows and the host plant (Casuarina) growing with sandal in the same pit. Sandalwood trees as well as the casuarina growing around to the labelled plants were traced for ^{32}P.

Similarly, functional status of sandal-haustoria was also studied on sandalwood tree growing with agricultural crops like cashew, coconut, rubber, cocoa, black pepper and forest tree, teak.

Labelling of plants with ^{32}P

An aliquot (0.5 ml) of the ^{32}P, required for the study was obtained from Board of Radiation and Isotope Technology (BRIT), Mumbai, as orthophosphoric acid in hydrochloric acid solution was diluted with 1000 ppm KH_2PO_4 to 1 L. The diluted sample was transferred to the applicator and the output from the applicator was adjusted to required quantity, for root feeding the host plant (Plate 2). The feeder roots of the host plant were excavated and were inserted into a polyethene tube of size (2 x 15 cm^2). ^{32}P solution at the rate of 1.2 mCi in 20 ml, used for labelling one host (Casuarina) plant, was discharged to the polyethene tube with the root tip (After filling the bag it was sealed with cello tape). For labelling grass species growing around sandalwood tree, only 0.06 mCi, made up to 1ml was used.

Fresh leaf samples were collected from both host and sandal plant at 1h, 2h, 6h, 2 days, 8 days and 16 days after ^{32}P application and were assayed for ^{32}P activity.

Radioassay of plant samples

The plant leaf samples were collected in paper bags and were dried and powdered. One gram of this sample was digested using diacid mixture of nitric acid and perchloric acid (2:1). The digested sample was made upto 20 ml and introduced into the scintillation counting vials of 20 ml capacity. The radioactivity was determined in a computer controlled liquid scintillation system (Hidex-Triathler) using Cerenkove Counting mode and the activity was expressed as Counts per minute (cpm g^{-1}). All the radioisotope works were done by following the safety precautions prescribed by BARC, Mumbai.

3.6 Statistical analysis

Data obtained were subjected to statistical analysis using SPSS (v 17). The test included ANOVA with post hoc testing using Duncans' Multiple Range Tests (DMRT).

Plate: 2 Application of ^{32}P to casuarina by root feeding

Results

RESULTS

The results of the study on the influence of host plant on carbon assimilation, water and nutrient absorption in sandalwood tree grown in field are presented in this section.

Experiment No.1

4.1 Influence of host plants on carbon assimilation, water and nutrient status of sandalwood tree

4.1.1 Height

The height increment of six years old sandalwood tree with and without host plant is shown in Table 1. There were no significant differences in height (5%) due to the presence of the host, casuarina. However, sandalwood tree without host (host plant dead naturally within 2 years after the establishment of the sandalwood tree in the field) was found to be relatively shorter than the trees with host. There was only 8 cm increment of height in sandalwood tree without host. Sandalwood tree growing with host showed a height increment of 85 cm to 92 cm in twelve months.

4.1.2 Diameter (DBH)

The diameter (dbh) of sandalwood trees grown with and without host-trees is shown in Table 2. In the beginning of the investigation, sandalwood tree without host (host plant dead naturally within 1-2 years after the establishment of the sandalwood tree in the field) was found to have 9.69 cm diameter and at the end of the investigation (after one year) it was 13.27 cm. There was only 3.58 cm increment in diameter in sandalwood tree without host. In the second and third treatments of sandalwood tree growing with host showed an increment of 3.70 and 4.60 cm

Table 1: Effect of host on plant height of sandalwood tree (6 years age)

Months	Height (m)			SEm ±
	Sandal (H_0)	Sandal+Casuarina(H_1)	Sandal+Casuarina(*H_2)	
May 2010	3.79	4.16	4.25	0.18
June 2010	3.98	4.18	4.26	0.19
July 2010	4.13	4.26	4.32	0.22
Aug 2010	4.19	4.34	4.40	0.25
Sept 2010	4.24	4.41	4.49	0.25
Oct 2010	4.28	4.50	4.57	0.26
Nov 2010	4.36	4.58	4.64	0.27
Dec 2010	4.54	4.66	4.72	0.21
Jan 2011	4.57	4.76	4.78	0.26
Feb 2011	4.60	4.86	4.87	0.27
March 2011	4.63	4.95	4.93	0.28
April 2011	4.69	4.98	4.99	0.29
	NS	NS	NS	

H_0= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

*H_2= Sandalwood + Casuarina - Host removed after six years

Table 2: Effect of host on dbh of sandalwood tree (6 years age)

Months	DBH (cm)			SEm ±
	Sandal (H_0)	Sandal+Casuarina(H_1)	Sandal+Casuarina(*H_2)	
May 2010	9.69	9. 71	11.37	0.53
June 2010	9.71	10.19	11.66	0.54
July 2010	9.97	10.49	12.06	0.55
Aug 2010	10.43	10.77	12.59	0.57
Sept 2010	10.86	11.17	12.96	0.60
Oct 2010	11.23	11.60	13.41	0.61
Nov 2010	11.69	11.64	13.89	0.64
Dec 2010	12.10	12.86	14.40	0.65
Jan 2011	12.43	12.90	14.84	0.68
Feb 2011	12.89	12.94	15.23	0.74
March 2011	13.14	13.37	15.59	0.78
April 2011	13.27	13.41	15.97	0.83
	NS	NS	NS	

H_0= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

*H_2= Sandalwood + Casuarina - Host removed after six years

respectively in twelve months of growth. There was no significant difference between the diameter of sandalwood tree with and without host (5%). However, sandalwood tree growing with host plant showed relatively higher value compared to sandalwood tree without host.

4.1.3 Photosynthesis

The photosynthetic rates (carbon assimilation rate) of sandalwood tree during summer and rainy seasons are shown in the Table 3. Rate of photosynthesis of sandalwood tree without host, in the summer season (April) was 12.11μmol.cm^{-2}s^{-1}. In sandalwood tree with host it varied from 13.02 to 13.21μmol.cm^{-2}s^{-1}. There was significant difference in rate of photosynthesis between sandalwood tree with and without host (5%). Sandalwood tree growing without host showed a lower rate of photosynthesis compared to sandalwood tree with host.

Carbon assimilation rate of sandalwood tree with and without host, measured in the rainy season (June), showed that the rate has increased during the month. In this month also photosynthetic rate showed significant difference, the sandalwood tree growing without host showed a lower rate of photosynthesis.

4.1.4 Plant water potential

The plant water potential of sandalwood tree during summer and rainy seasons are shown in Table 4 (a) and (b). The peak reduction in water potential of sandalwood tree was observed from 12-1 pm. An improvement in water potential observed after sunset. The water potential ranged from -0.8 to -1.8. Sandalwood tree showed higher plant water potential during rainy season (June) compared to that in the summer (April). Significant differences were observed between the treatments of sandal growing with host and without host in different

Table 3: Seasonal variations in photosynthetic rate ($\mu mol.cm^{-2}s^{-1}$) of sandalwood tree

	Rainy season (July 2010)	Summer season (April 2011)
Sandal (H_0)	15.13[b]	12.11 [b]
Sandal+Casuarina(H_1)	17.34 [a]	13.02 [a]
Sandal+Casuarina(*H_2)	17.66 [a]	13.21 [a]
SEm ±	1.75	1.45

****Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

*H_2= Sandalwood + Casuarina - Host removed after six years

Table 4(a): Effect of host plant on plant water potential of sandalwood tree (MPa) in rainy season (June 2010)

	Time				
	6-7am	9-10 am	12-1 pm	3-4 pm	6-7 pm
Sandal (H_0)	-1.271 [a]	-2.443 [a]	-3.514 [a]	-3.014 [a]	-2.143 [a]
Sandal+Casuarina (H_1)	-0.857 [b]	-2.157 [b]	-3.134 [b]	-2.557 [b]	-1.874 [b]
Sandal+Casuarina (*H_2)	-0.900 [b]	-2.100 [b]	-3.314 [b]	-2.614 [b]	-1.964 [b]
SEm ±	-0.048	-0 .059	-0.21	-0.063	-0.094

** **Values with the same superscripts within a column are not significantly different**

H_0= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

Table 4(b): Effect of host on plant water potential of sandalwood tree (MPa) during summer season (April 2011)

	Time				
	6-7am	**9-10 am**	**12-1 pm**	**3-4 pm**	**6-7 pm**
Sandal (H_0)	-1.771 [a]	-2.943 [a]	-4.000 [a]	-3.514 [a]	-2.629 [a]
Sandal+Casuarina (H_1)	-1.386 [b]	-2.600 [b]	-3.814 [b]	-3.057 [b]	-2.386 [b]
Sandal+Casuarina (*H_2)	-1.471 [b]	-2.643 [b]	-3.800 [b]	-3.114 [b]	-2.371 [b]
SEm ±	-0.089	-0.046	-0.068	-0.061	-0.029

**** Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

seasons. In the rainy season, the sandalwood tree growing without host showed lower plant water potential compared to sandalwood tree growing with host. The plant water potential of sandalwood tree in summer season also showed significant difference due to the presence of the host (Casuarina) in the field. In this season also sandalwood tree without host showed lower plant water potential compared to sandal growing with host.

The plant water potential of sandalwood tree after the removal of host plant is showed in Table 4 (c and d). Significant difference was observed between the treatments of sandalwood tree growing with host and without host. The sandalwood tree, in which the host plant was removed, showed a significant reduction in water potential than sandalwood tree with host. The sandalwood tree growing without host showed lower plant water potential compared to sandalwood tree growing with host at all observations.

4.1.5 Chlorophyll content

The chlorophyll content of the leaves of the sandalwood trees was found to be influenced by the presence or absence of host plant. The mean values on chlorophyll content are shown in Table 5.

4.1.5.1 Chlorophyll *a*

Chlorophyll *a* content of sandalwood tree without host was lower when compared to sandal with host. Growing sandalwood tree without host showed a chlorophyll *a* content of 1.5 $mg.g^{-1}$ and sandalwood tree with host showed 1.7 and 1.8 $mg.g^{-1}$ respectively.

4.1.5.2 Chlorophyll *b*

Chlorophyll *b* content of sandalwood tree without host was lower than tree with host. Growing sandalwood tree without host showed a chlorophyll *b* content of 0.39 $mg.g^{-1}$ and sandalwood tree with host showed 0.53 and 0.85 $mg.g^{-1}$ respectively.

Table 4(c): Effect of host on plant water potential of sandalwood tree (MPa), 15 days after the removal of host (H_2)

	6-7am	9-10 am	12-1 pm	3-4 pm	6-7 pm
Sandal (H_0)	-1.814 ^{a}	-3.000 ^{a}	-4.043 ^{a}	-3.500 ^{a}	-2.671 ^{a}
Sandal+casuarinas (H_1)	-1.457 ^{b}	-2.514 ^{b}	-3.514 ^{c}	-3.100 ^{b}	-2.343 ^{c}
Sandal+casuarinas (*H_2)	-1.700 ^{a}	-2.813 ^{a}	-3.817 ^{b}	-3.543 ^{a}	-2.597 ^{b}
SEm ±	-0.034	-0.038	-0.062	-0.059	-0.027

**** Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

Table 4(d): Effect of host on plant water potential of sandalwood tree (MPa), 30 days after the removal of host (H_2)

	Time				
	6-7am	**9-10 am**	**12-1 pm**	**3-4 pm**	**6-7 pm**
Sandal (H_0)	-1.770^{b}	-2.957^{ab}	-4.000^{b}	-3.529^{b}	-2.729^{b}
Sandal+Casuarina (H_1)	-1.529^{c}	-2.629^{b}	-3.500^{c}	-3.043^{c}	-2.417^{c}
Sandal+Casuarina (*H_2)	-1.836^{a}	-2.986^{a}	-3.900^{a}	-3.776^{a}	-2.949^{a}
SEm- ±	-0.038	-0.029	-0.085	-0.063	-0.054

**** Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

Table 5: Effect of host on the chlorophyll content of sandalwood leaves

		Chl *a*	Chl *b*	Chl (*a*+*b*)
Chlorophyll ($mg.g^{-1}$)	Sandal (H_0)	1.5^{b}	0.4^{b}	1.9^{b}
	Sandal+casuarina (H_1)	1.7^{a}	0.8^{a}	2.2^{a}
	Sandal+casuarina (*H_2)	1.8^{a}	0.9^{a}	2.4^{a}
SEm ±		0.12	0.2	0.23

**** Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

4.1.5.3 Total chlorophyll

Total chlorophyll content of sandalwood tree without host was lower when compared to sandalwood tree with host. Growing sandalwood tree without host showed total chlorophyll content of 1.9 $mg.g^{-1}$ and sandalwood tree with host showed 2.2 and 2.6 $mg.g^{-1}$ respectively.

4.1.6 Leaf nutrient content of sandalwood tree

4.1.6.1 Nitrogen

The leaf nitrogen contents of the sandalwood tree in different seasons and after the removal of the host plant are shown in Table 6 (a, b and c). Nitrogen content observed in the rainy season (June) was marginally lower than the value in summer season (April). Significant difference in leaf nitrogen content was observed between the sandalwood tree with and without host. Sandalwood tree growing without host showed significantly lower leaf nitrogen content compared to sandalwood tree with host. The sandalwood tree, in which the host plant was removed, showed a significant reduction in leaf nitrogen content than the sandalwood tree with host.

4.1.6.2 Phosphorus

The leaf phosphorus content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). Phosphorus content observed in the rainy season (June) was slightly lower than in summer season (April). Significant difference in leaf phosphorus content was observed between the sandalwood tree with and without host. Sandalwood tree growing without host showed significantly lower value compared to sandalwood tree with host. The sandalwood tree, in which the host plant was removed, showed a significant reduction in leaf phosphorus content than the sandalwood tree with host.

4.1.6.3 Potassium

The leaf potassium content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). Potassium content observed in the rainy season (June) was slightly lower than in summer season (April). Significant difference in leaf potassium content was observed between the sandalwood tree with and without host. Sandalwood tree growing without host showed significantly lower leaf potassium content compared to sandalwood tree with host. The sandalwood tree, in which the host plant was removed, showed a significant reduction in leaf potassium content than the sandalwood tree with host plant.

4.1.6.4 Iron

The leaf iron content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). The Iron content observed in the rainy season (June) was slightly lower than in summer season (April). There was no significant difference in the leaf iron content in the sandalwood tree with and without host, during different seasons and before and after the removal of the host plant.

4.1.6.5 Zinc

The leaf zinc content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). There was no significant difference in leaf zinc content in the sandalwood tree with and without host, during different seasons and before and after the removal of the host plant.

4.1.6.6 Manganese

The leaf manganese content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). Manganese content observed in the rainy season (June) was slightly lower than in summer season (April). There was no significant difference in leaf manganese content between the sandalwood tree with and without host, during different seasons and before and after the removal of

Table 6 (a): Effect of the host plant on the leaf nutrient content of sandalwood tree during rainy season (June 2010)

	N	P	K	Fe	Zn	Mn	Cu
	Percentage			$mg.kg^{-1}$			
Sandal (H_0)	2.48^{b}	0.16^{b}	1.68^{b}	370^{a}	20.19^{a}	480^{a}	20.11^{a}
Sandal+Casuarina (H_1)	2.64^{a}	0.23^{a}	2.41^{a}	362^{a}	20.05^{a}	492^{a}	20.15^{a}
Sandal+Casuarina (*H_2)	2.65^{a}	0.24^{a}	2.31^{a}	381^{a}	20.13^{a}	474^{a}	20.09^{a}
SEm ±	0.11	0.20	0.13	20.00	0.18	19.00	0.41

**** Values with the same superscripts within a column are not significantly different**

H_0= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

Table 6 (b): Effect of the host plant on the leaf nutrient content of sandalwood tree during summer season (April 2011)

	N	P	K	Fe	Zn	Mn	Cu
	Percentage			mg.kg^{-1}			
Sandal (H_0)	2.54 [b]	0.15 [b]	1.775 [b]	436 [a]	21.31 [a]	495 [a]	20.29 [a]
Sandal+Casuarina (H_1)	2.63 [a]	0.25 [a]	2.49 [a]	445. [a]	20.48 [a]	502 [a]	21.47 [a]
Sandal+Casuarina (*H_2)	2.73 [a]	0.25 [a]	2.53 [a]	450 [a]	21.01 [a]	490 [a]	20.39 [a]
SEm ±	0.13	0.003	0.18	12.11	0.92	12.51	1.32

**** Values with the same superscripts within a column are not significantly different**

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

Table 6 (c): Effect of host on the leaf nutrient content of sandalwood tree, 30 days after the removal of host (H_2)

	N	P	K	Fe	Zn	Mn	Cu
	Percentage			mg.kg^{-1}			
Sandal (H_0)	2.45 ^{b}	0.18 ^{b}	1.79 ^{b}	431 ^{a}	27.14 ^{a}	438 ^{a}	24.16 ^{a}
Sandal+Casuarina (H_1)	2.53 ^{a}	0.25 ^{a}	2.54 ^{a}	440 ^{a}	26.27 ^{a}	444 ^{a}	21.10 ^{a}
Sandal+Casuarina (*H_2)	2.25 ^{c}	0.16 ^{c}	1.50 ^{c}	429 ^{a}	27.42 ^{a}	424 ^{a}	23.22 ^{a}
SEm ±	0.06	0.03	0.12	12.10	1.53	20.12	4.41

**** Values with the same superscripts within a column are not significantly different**

H_0= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

* H_2= Sandalwood + Casuarina - Host removed after six years

the host plant.

4.1.6.7 Copper

The leaf copper content of the sandalwood tree in different seasons and after the removal of the host plant is shown in Table 6 (a, b and c). There was no significant difference in leaf copper content in the sandalwood tree with and without host, during different seasons and before and after the removal of the host plant.

Experiment No. 2

4.2 Haustorial associations and anatomy

Root distribution pattern of two sample sandalwood trees; one with and the other without host (casuarina), after excavation are shown in the Plate 3. The connections between the host root and body of the haustorium were firm and not easily broken during excavations, probably because of the well co-ordinated tissue graft between the host and parasite. Number of functional and non-functional haustoria in each root at 15 cm length sections is shown in Table 7. Sandalwood without host showed longer roots (3.14 m) when compared to the root length of sandalwood with host (2 m).

Total number of hauatoria on the primary roots of the sandalwood tree grown along with host was 20, whereas in secondary roots they were 17, and in tertiary roots were 7. The total number of dead haustoria was 6 in the former. Sandalwood tree growing without host showed a total number of 11 haustoria on primary roots, 8 on secondary roots and 5 on tertiary roots. The total number of dead haustoria observed was 6.

4.2.1 Anatomical studies of haustoria

This study focused on the functional anatomy of haustoria formed by *Santalum album*, to understand the mechanism through which the parasite is able to develop such intimate connections with its host-root and how this fulfills its water and nutritional

Table 7: Number of sandal-haustoria on the excavated roots of host

	Functional haustoria			Non functional haustoria
	1^0 root	2^0 root	3^0 root	
Sandal + Casuarina	20	17	7	6
Sole sandal	5	2	5	6

requirements.

Newly initiated haustoria of *Santalum album* had a bell shaped configuration, tapering proximally by a narrow stalk joining onto its parent root. When it came into contact with host root (casuarina), they flattened against the surface and initiated the transition into young haustorium (Plate 4). Sandal-haustorium consists of hyaline body, the endophyte or penetration peg (the projection of which enters the host root tissue) and the ellipsoidal disc (laterally flattened, relatively to the host root, against the host's stele and the point of contact between the parasite and its host's vascular system). The intrusive cells of haustoria penetrate the host epidermis and cortex between host cells. Concurrent with this endophytic development, the cortical fold of the haustorium partly encircled the host root.

The finger like projections of the developing endophyte extended up to the cortical tissue of the host root. This tissue is mainly composed of characteristically elongated (tubular) thin walled parenchyma cells (Plate 5). As the projections elongated towards the host root xylem, they entwined with each other and gave a tubular appearance to the cells. Upon reaching the host root cambium, the penetration peg flattened out laterally to form a thin ellipsoidal disc closely pressed against the centrally located host root xylem. The haustorial parenchyma cells at the interface are distinguished from the rest of the parenchyma within the body of the haustorium by their irregular shape and a shift to a tubular structure. There are relatively few xylem elements in the haustorium that are typically short tracheary elements. Furthermore, our investigations revealed that direct lumen to lumen xylem connections between the xylem of the host and parasite are absent.

Haustorial sections of secondary and tertiary roots were taken and its dimensions are shown in the Plates 6 and 7. Since the primary host roots were thick, there sectioning was not feasible. Area of host root was higher in the case of

Plate: 3 Excavated sandalwood tree root

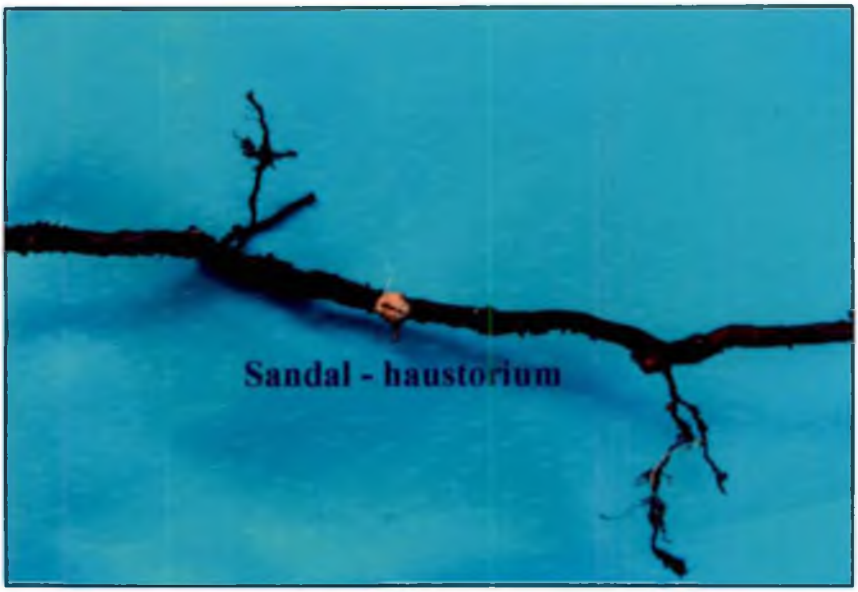


Plate: 4 Sandalwood-haustoria on casuarina root

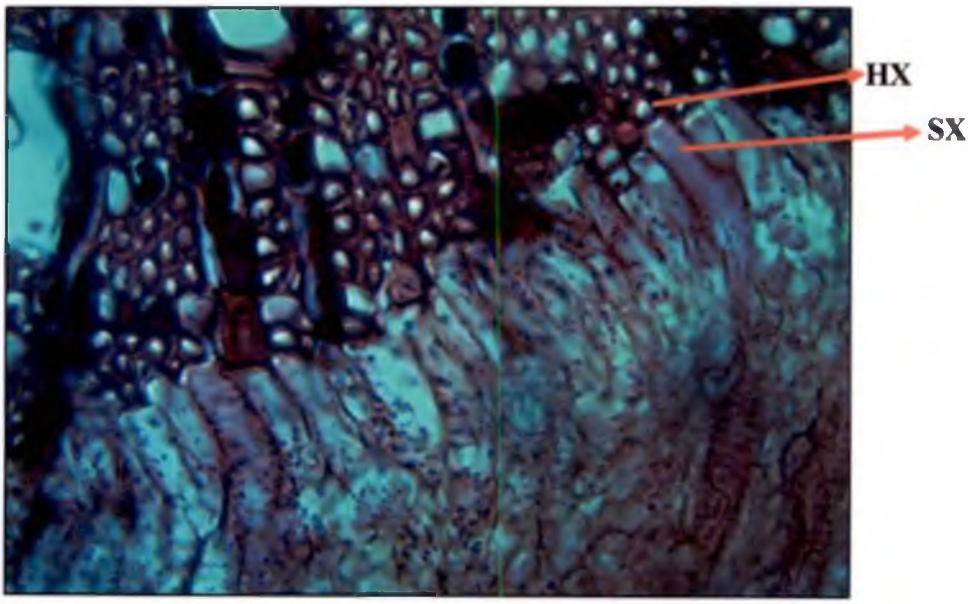


Plate: 5 LS of sandalwood haustoria showing xylem-xylem connection between sandalwood and host root (40 X)

HX: Host xylem
SX: Sandal-haustorial xylem

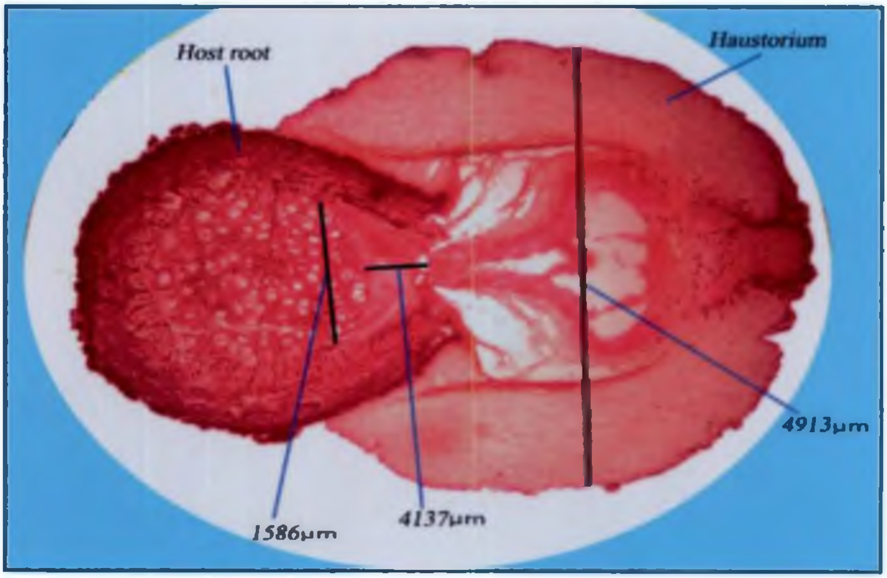


Plate: 6 LS of tertiary root haustorium of sandalwood with casuarina (10X)

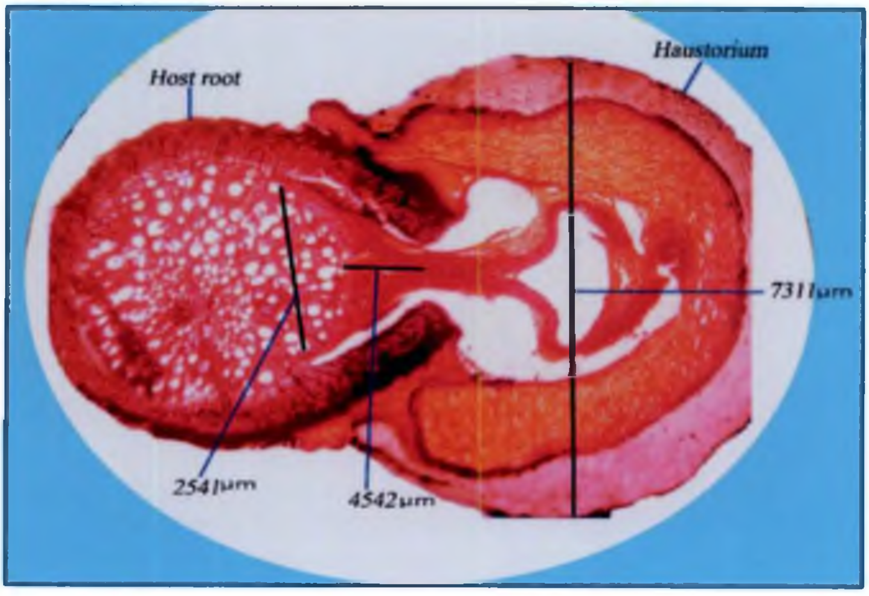


Plate: 7 LS of secondary root haustorium of sandalwood with casuarina (10X)

secondary root haustoria. Width of the ellipsoidal disc was higher (2541 μm) in the case of secondary root haustoria than in tertiary root haustoria (1586 μm).The thickness and area of ellipsoidal disc was also higher in secondary root haustoria. The length of penetration peg and area of haustoria was also higher in secondary root haustoria.

Experiment No. 3

4.2 Radio tracer studies on sandalwood-host association

The counts of ^{32}P in sandalwood tree, translocated from the host plant at different time intervals, after labeling the host plant with ^{32}P are indicated in Table 8. Counts observed after 2 h and 4 h after labelling were not significant. Significant count of translocated ^{32}P was observed in sandalwood tree after 6 h of labelling the host plant. There were marginal increase in ^{32}P count in sandalwood tree with time and this increase continued upto 194 h (8 days) to 384 h (16 days). Peak counts, in most cases were recorded on eighth day after labelling.

The ^{32}P in the labelled casuarina grown with sandalwood tree in same pit was more as compared to casuarina grown alone (Table 9). The ^{32}P from casuarina was translocated to sandalwood tree in both cases. Sandalwood tree in the same pit as casuarina showed a ^{32}P count of 283 $cpm.g^{-1}$and sandalwood tree which was 1.5 m away from casuarina showed a count of 216 $cpm.g^{-1}$ when sandalwood tree and casuarina was in the same pit and 260 $cpm.g^{-1}$when casuarina and sandalwood tree was in separate pits (1.5 m away). Sandalwood tree growing 1.5 m away from the host plant showed more or less same ^{32}P count as that of labelled casuarina (260 $cpm.g^{-1}$ and 263 $cpm.g^{-1}$). The ^{32}P count in sandalwood tree which was 2.5 m and 3 m away from labelled casuarina also showed appreciable count. However, the translocation of ^{32}P from casuarina grown in same pit as sandalwood tree to sandalwood tree in the adjacent pit 1.5 m and 2.5 m away was relatively less.

Table 8: Translocation of ^{32}P from host plant to sandalwood tree

Time interval	^{32}P counts (cpm g^{-1})			
	Sandal in same pit	Sandal at 1.5 m	Sandal at 2.5 m	Sandal at 3 m
6 h	260	216	180	168
48 h	298	270	240	200
192 h	390	310	270	268
384 h	300	263	210	201

Table 9: Translocation of ^{32}P from labelled host plant to sandalwood tree at different distances

Treatments	^{32}P count (cpm.g^{-1})				
	Labelled casuarina	Sandal in same pit	Sandal at 1.5 m from host	Sandal at 2.5 m from host	Sandal at 3 m from host
*C+S	360	283	216	180	-
*C	263	-	260	248	200

*** Indicate ^{32}P labelled plant**

The ^{32}P count in sandalwood tree translocated from wild grass growing around the sandalwood tree is shown in Table 10. There was significant transfer of ^{32}P from the labelled wild grasses to sandalwood tree.

Sandalwood tree and other hosts

Sandalwood tree grown with cashew, coconut, rubber, cocoa and teak appears to have developed hasustorial associations. ^{32}P labelled in these crop plants were detected in sandalwood tree in varying proportions (Table 11). The ^{32}P count in the labelled host plant and the sandalwood tree growing in the same pit showed considerable variation depending on the species of host plant. When cocoa was the host plant, the count in the llabelled plant was 102 $cpm.g^{-1}$, whereas the count in sandalwood tree was 251$cpm.g^{-1}$. More count in sandalwood tree than in the labelled host was observed when the host plant was cashew, teak and casuarina. When the host plant was rubber or coconut, ^{32}P count in the sandalwood tree was considerably less than the labelled host plant. In case of rubber as host, the labelled host showed a ^{32}P count of 436 $cpm.g^{-1}$ and that in sandalwood tree was 217 $cpm.g^{-1}$ in one case and in the another case, labelled rubber showed 372 $cpm.g^{-1}$ whereas sandalwood tree showed 142 $cpm.g^{-1}$. Similarly, labelled host plant, coconut showed a count of 527 $cpm.g^{-1}$ and sandalwood tree showed only 215 $cpm.g^{-1}$ in one case and labelled coconut showed a count of 289 $cpm.g^{-1}$ and the sandalwood tree showed only 120 $cpm.g^{-1}$ in another case.

^{32}P labelled to sandalwood tree (Plate 8) was traced in teak and casuarina growing along with sandalwood tree in the same pit (Table 12).

Table 10: ^{32}P count observed in sandalwood tree translocated from wild grass

Time interval	^{32}P counts (cpm g^{-1})
48 h	196
192 h	311

Table 12: Translocation of ^{32}P between sandalwood tree and host trees

Treatments	^{32}P counts (cpm g^{-1})		
	Sandal	**Casuarina	**Teak
*Sandal + Casuarina + Teak	513	183	275

* Indicate ^{32}P labelled plant

** All the host plants were planted in the same pit as that of sandalwood tree

Table 11: Translocation of ^{32}P from host tree to sandalwood trees

Treatments	^{32}P counts ($cpm.g^{-1}$)				Total count of sandal and treated host ($cpm.g^{-1}$)	Percentage count in sandal with treated host (%)
	Sandal	** Host 1	** Host 2	** Host3		
Sandal + Cocoa*	251	102			353	71.10
Sandal + Cashew*	320	275			595	53.78
Sandal + Cashew* + Casuarina +	198	224	170		422	46.91
Sandal + Teak*	542	376			918	59.04
Sandal + Teak* + Casuarina	321	479	119		800	40.12
Sandal + Coconut* + Casuarina	120	289	126		406	29.55
Sandal +Casuarina*	458	161			619	73.20
Sandal + Coconut* + Casuarina + Rubber	215	527	132	120	742	28.9
Sandal + Casuarina* + Rubber	483	132	124		615	78.53
Sandal + Casuarina* + Teak	196	155	316		351	55.84
Sandal + Rubber*	142	372			514	27.62
Sandal + Rubber* + Casuarina	217	436	99		653	33.23

*** Indicate ^{32}P labelled plant**
**** All the host plants were planted in the same pit as that of sandalwood tree**

Table 12: Translocation of ^{32}P between sandalwood tree and host trees

Treatments	^{32}P counts (cpm g^{-1})		
	Sandal	**Casuarina	**Teak
*Sandal + Casuarina + Teak	513	183	275

* Indicate ^{32}P labelled plant

** All the host plants were planted in the same pit as that of sandalwood tree

Plate: 8 Application of ^{32}P to sandalwood tree by stem injection

Discussion

DISCUSSION

Sandalwood tree is very precious, valued for its scented-heartwood and the oil. Considering the wide gap in demand and production of sandalwood and the high price of the crop, there is tremendous potential for growing sandalwood tree in forest lands as well as in farm lands/agroforestry systems. The hemiparasitic character of sandalwood tree and the requirement of a host plant for its satisfactory establishment and growth is reported as early as 1871 by Scott, and confirmed by various authors. However, there are wide variations in the reports on the roles of host plant in supporting sandal growth. Some of the authors reported that, host is necessary for the amino acid supply (Srimathi et al., 1961). Some others reported the necessity of the host for water and nutrient absorption (Barber, 1903; Rao, 1933; Sreenivasrao, 1933; Pilger, 1935; Iyengar, 1965; Ananthapadmanabha et al., 1984; Rangaswamy et al., 1986; Struthers et al., 1986; Varghese, 1997; Radomiljac, 1998; Tennakoon et al., 2000; Brand, 2002; Hiremath, 2004; Dhaniklal, 2006; Hua et al., 2005; Ashokan and Krishnambika, 2007; Ashokan et al., 2008). Lack of influence of host plant is also reported (Venkataraman, 1918; Fischer, 1922; Nagaveni and Srimathi, 1985). Most of these studies were on seedlings. The studies on field grown sandalwood tree are limited. Brand et al. (2003) reported the influence of host Acacia on the establishment and growth of sandalwood trees in the field. However, there are no reports on the influence of the host on field grown sandalwood tree especially physiological role of host. The present study throws some light on the influence of host plant (Casuarina) on sandalwood tree growth and their physiological interactions in field grown tree. The results observed are discussed below.

5.1 Height and DBH

The result of this field trial showed that, at 5-6 year stage of growth, the host plant (casuarina) did not influence the height and dbh of sandalwood tree significantly (Fig. 2 and 3). This observation is different from the result reported from earlier studies (Taide, 1991; Surata et al., 1992; Ananthapadmanabha et al., 1998; Ashokan et al., 2008). They observed that the height and dbh or collar diameters of sandalwood trees were increased due to the presence of host plant. However, most of those studies were conducted in seedlings. During the early stage of growth, sandalwood tree may require host support. From the field trial in Australia, Brand et al. (2003) reported that the host (*Acacia*) influenced the establishment and the growth of the sandalwood. They observed increased height and stem diameter in sandalwood grown with host at four years of establishment in the field. The lack of significant influence of host plant on sandalwood tree height and dbh indicates the possilbility that sandalwood tree may be producing haustoria on wild grasses and other trees present in the premises around the tree and tapping nutrients and water. This has been observed in the root excavation study conducted as a part of this project. Mal and Eric (2007) indicated the possibility of sandalwood tree partially depending on wild grasses for its nutrient supplies. However their physiological function was not established through translocation studies.

It was also observed that in certain pits the fast growth of the host plants inhibited the growth of sandalwood trees thus suppressing its growth. This may be due to the above ground and below ground competition for various resources between host and sandalwood tree (Plate 9).

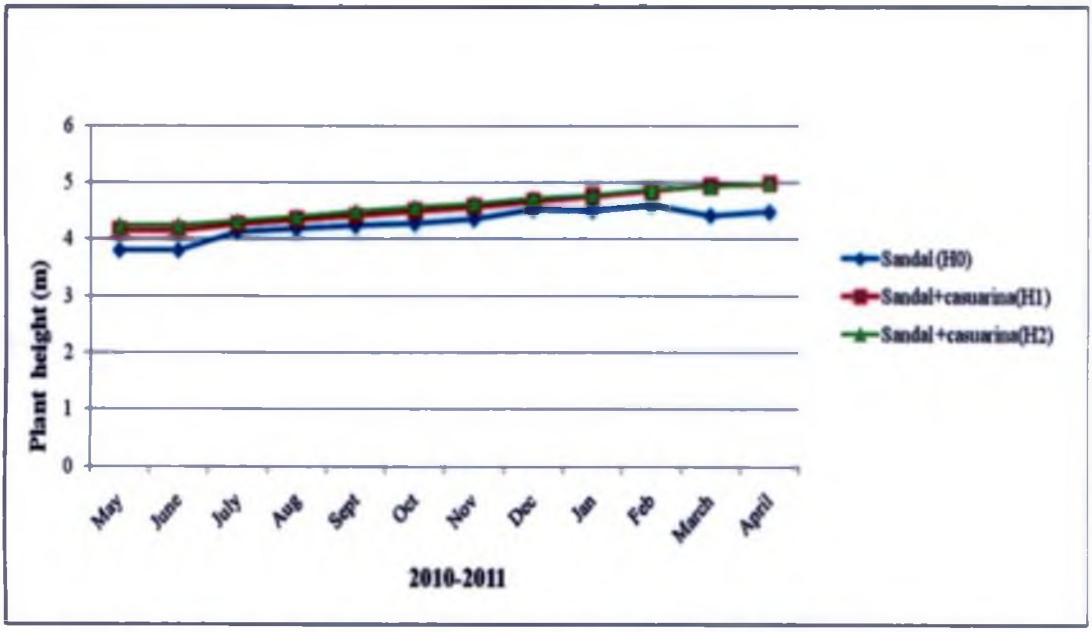


Fig. 2 Effect of host plant (casuarina) on plant height of sandalwood tree

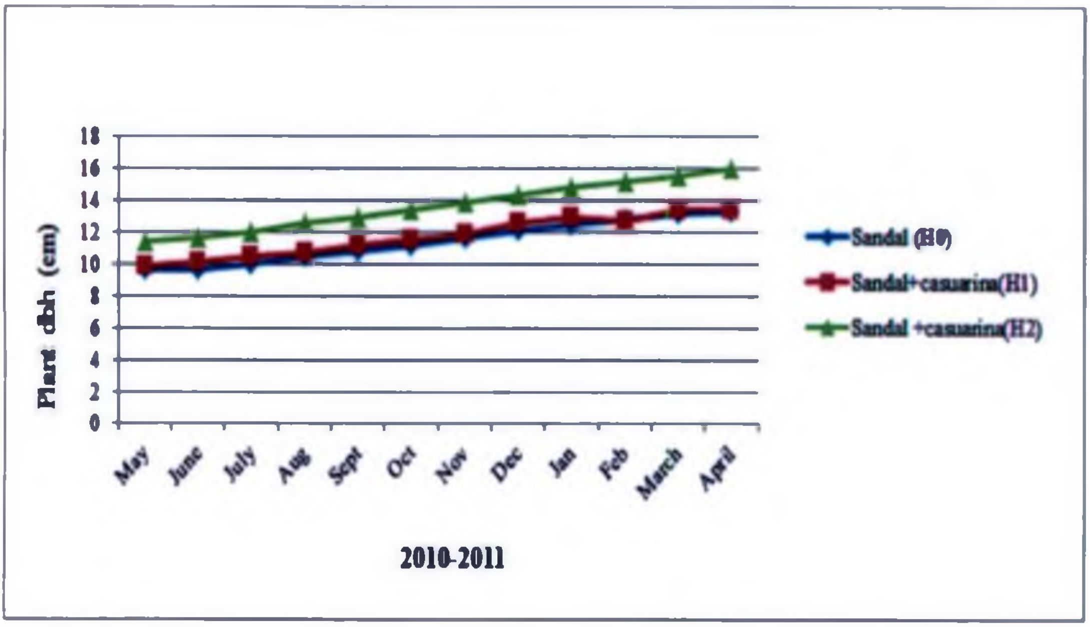


Fig. 3 Effect of host plant (casuarina) on dbh of sandalwood tree

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Throughout the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

Plate: 9 Suppressed sandalwood tree growing with host-Casuarina

Carbon assimilation

Sandalwood trees grown with host plants were found to have consistently higher rate of carbon assimilation (Fig. 4). This indicates that the host plant is influencing the physiology of sandalwood tree. Earlier studies of Varghese (1997), Tennakoon et al. (2000) and Hiremath (2004) showed that the host plant influences, mineral nutrient and water uptake by sandalwood seedlings. However, direct measurement of the assimilation rate of sandalwood tree with and without host is not reported earlier. The increased rate of carbon assimilation observed in sandalwood tree with host is an indication that in the long run the host plants may help sandalwood tree to grow faster and accumulate more biomass and sandalwood. However, the competition between sandalwood and host for solar radiation and soil resources cannot be ruled out, depending on the characters of the host. So selection of appropriate host and its field management is very critical for the success of sandalwood tree cultivation. The host selected shall offer minimum competition for aboveground and belowground resources. Considering the sparse canopy of casuarina with needles and nitrogen fixing nature, it could form an ideal host for sandalwood tree.

5.3 Plant water potential

The plant water potential measured in sandalwood tree with and without host indicates variation during different seasons, in the presence and absence of host [Fig. 5 (a), (b), (c) and (d)]. Sandalwood tree growing with host showed higher plant water potential during different seasons and the removal of the host reduced the plant water potential of sandalwood tree. Earlier studies of Varghese (1997), Tennakoon (2000), Hiremath (2004), Dhaniklal (2006) and Ashokan et al., (2008) showed that sandalwood tree growing with host had better plant water status.

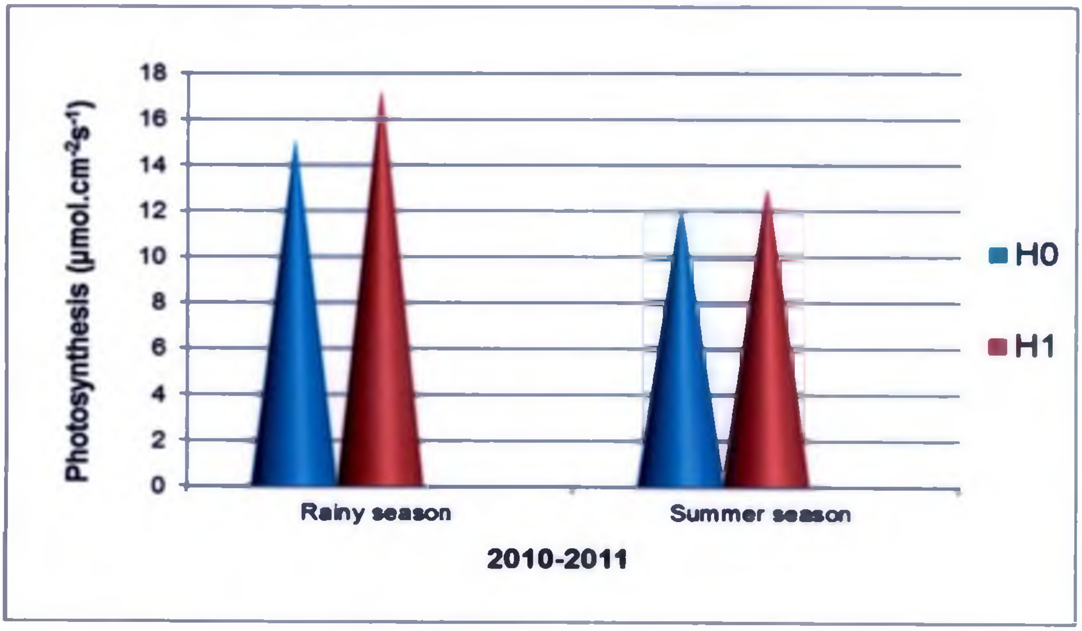


Fig. 4 Effect of host casuarina, on photosynthesis of sandalwood tree in different seasons

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

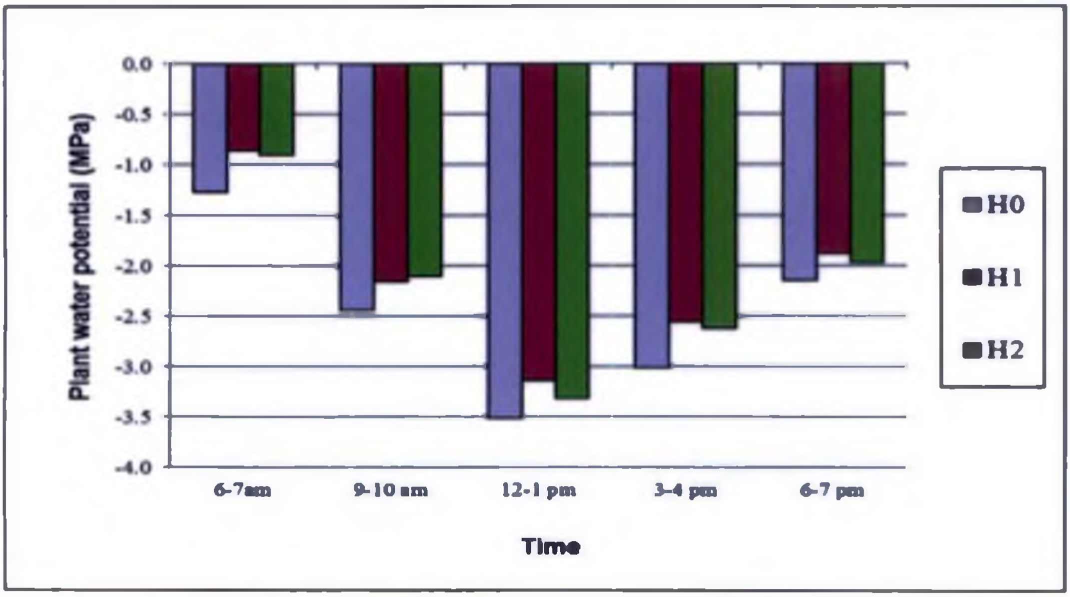


Fig. 5(a) Effect of host casuarina, on pre-dawn plant water potential of sandalwood tree in rainy season

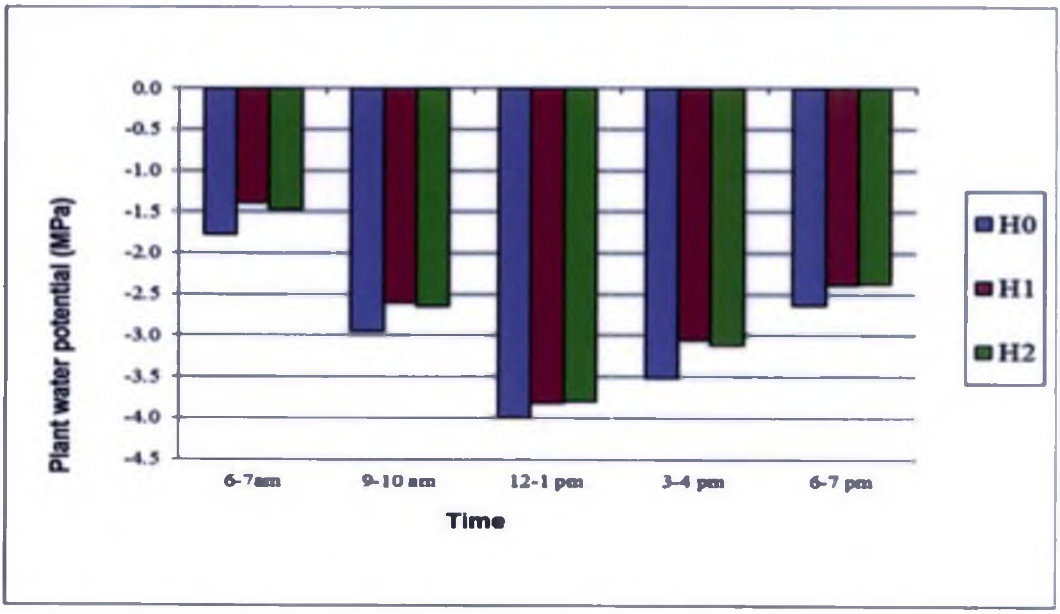


Fig. 5(b) Effect of host casuarina, on pre-dawn plant water potential of sandalwood tree in summer season

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

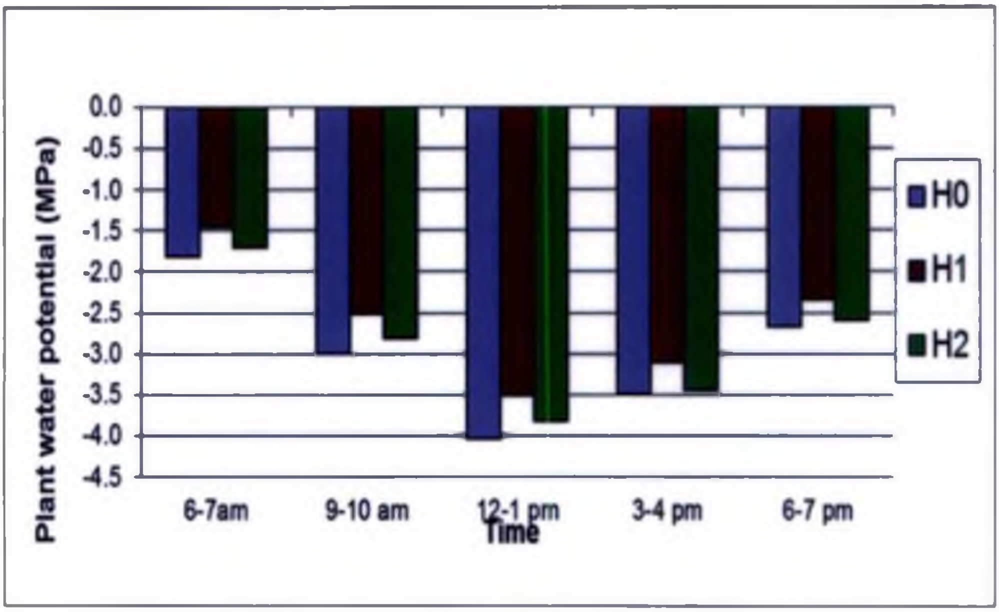


Fig. 5(c) Effect of host casuarina, on pre-dawn plant water potential of sandalwood tree, 15 days after the removal of host

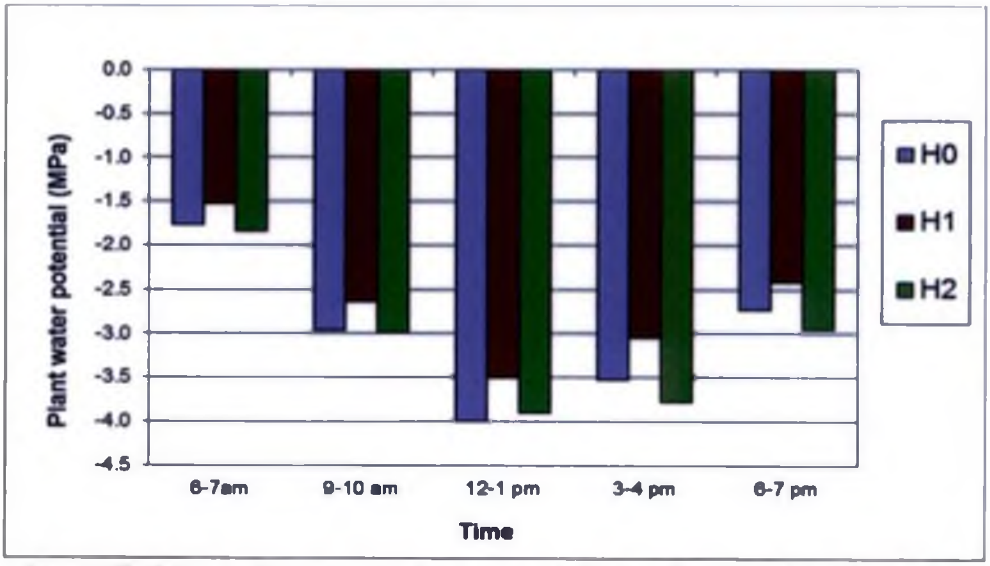


Fig. 5(d) Effect of host casuarina, on pre-dawn plant water potential of sandalwood tree, 30 days after the removal of host

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

The removal of host substantially lowers the water potential in sandalwood tree and thus leads to water stress in sandalwood tree. This finding is supported by the observation of consequent wilting and leaf shedding of the sandalwood tree after the removal of host plant and the grass growing around the sandalwood tree (Plate 10). Obviously the host plant is supporting sandalwood tree to maintain a plant water status. In the absence of the host sandalwood tree may be stressed for water.

5.4 Chlorophyll content of sandalwood leaves

The leaf chlorophyll content of sandalwood tree with and without host indicated significant variations (Fig. 6). Hiremath (2004) observed high a, b, and total chlorophyll content in the seedlings with host. Higher chlorophyll content observed may be due to the alleviation of solar radiation by host plants resulting in less photo destruction of chlorophyll in sandalwood tree. Higher light intensities are reported to destroy chlorophyll (Alberte et al., 1997). Hiremath (2004) also observed low chlorophyll content at low water status in sandalwood seedlings. Tennakoon et al. (2000) observed an increase in chlorophyll content and carbon fixation rates when sandalwood seedlings were planted with a pot host. Lower chlorophyll N content of sandalwood tree without host also supported by its low N content. This is also reported by Nazeem (1989) in nutmeg, Anoop (1993) in *Ailanthus* and Varghese (1997) in teak. Influence of N content on chlorophyll content of sandalwood tree was also observed by Barrett and Fox (1997).

5.5 Leaf nutrient content

The leaf nutrient content in sandalwood tree grown with and without host indicated variations in different nutrients in different seasons and in the presence and absence of host are shown in Fig. 7(a, b), 8(a, b) and 9(a, b). Interaction of sandalwood tree and host plant for the uptake and translocation of various mineral nutrient were reported by different authors (Iyengar, 1958; Anathapadmanabha et al.,

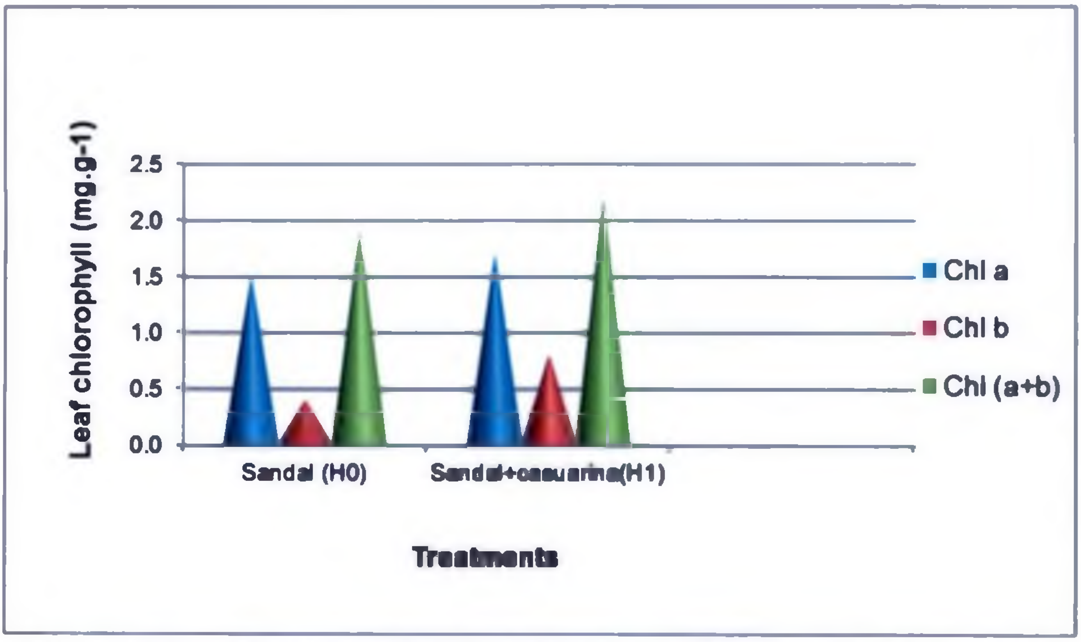


Fig. 6 Effect of host plant (casuarina) on the chlorophyll content of sandalwood leaves

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

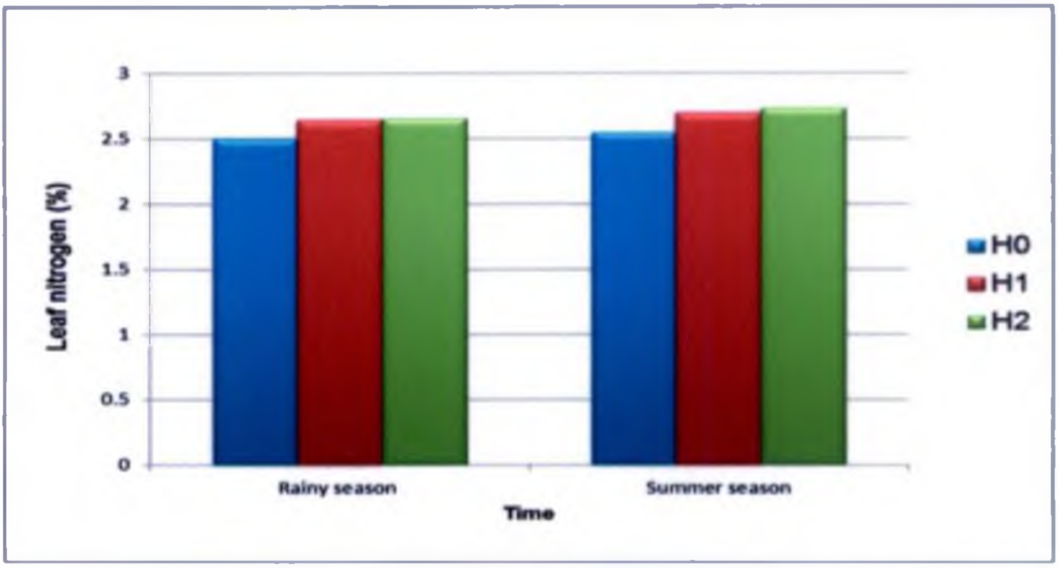


Fig. 7(a) Effect of host casuarina, on nitrogen content of sandalwood leaves in different seasons

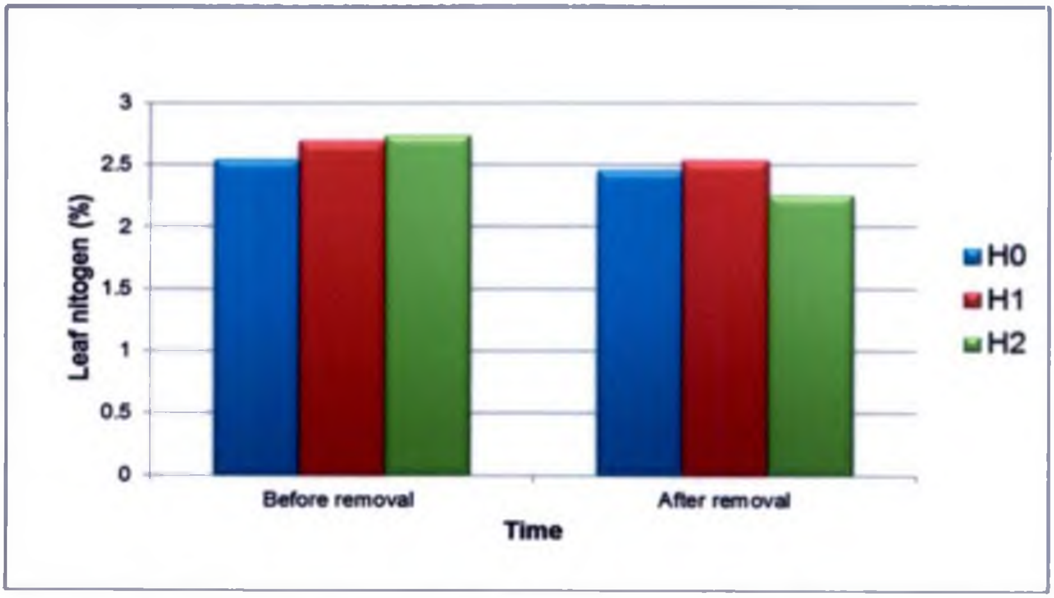


Fig. 7(b) Effect of host casuarina, on nitrogen content of sandalwood leaves before and after the removal of host plant

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

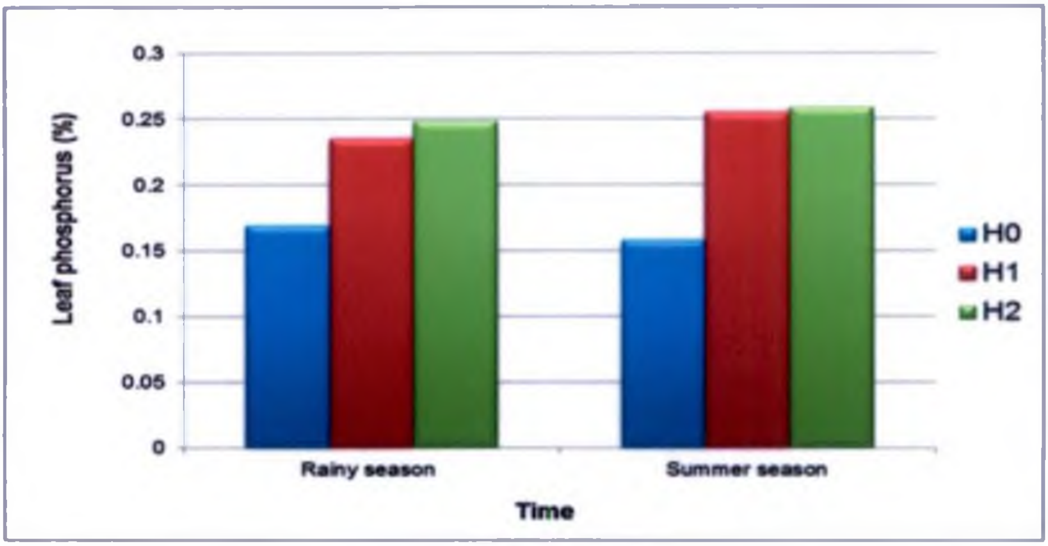


Fig. 8(a) Effect of host casuarina, on phosphorus content of sandalwood leaves during different seasons

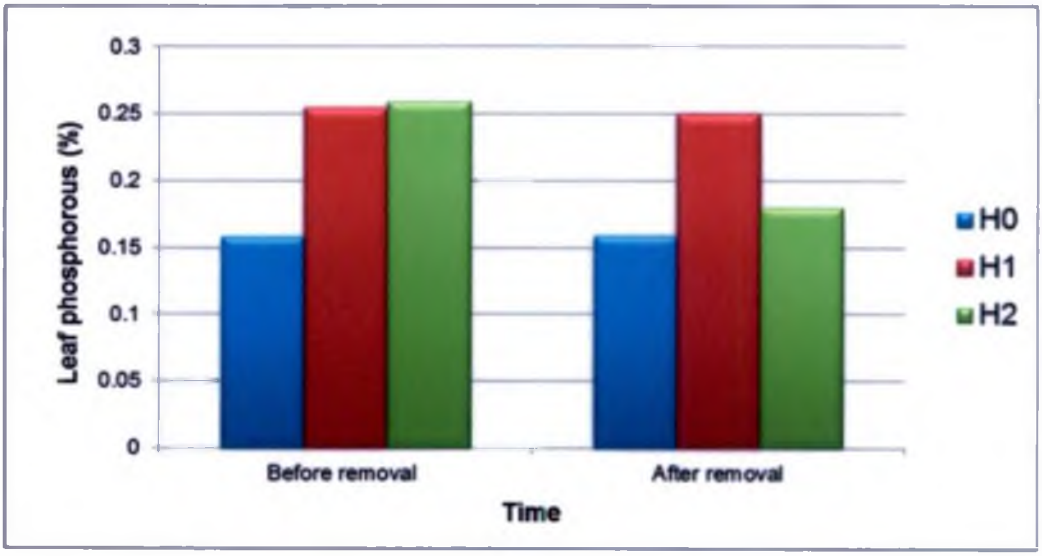


Fig. 8(b) Effect of host casuarina, on phosphorus content of sandalwood leaves before and after removal of host plant

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

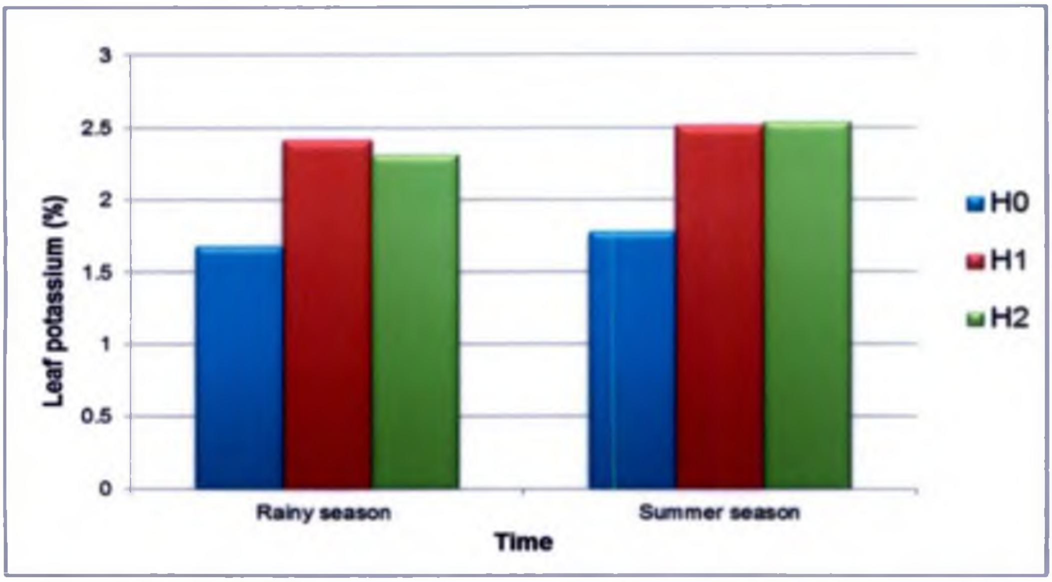


Fig.9(a) Effect of host casuarina, on potassium content of sandalwood leaves during different seasons

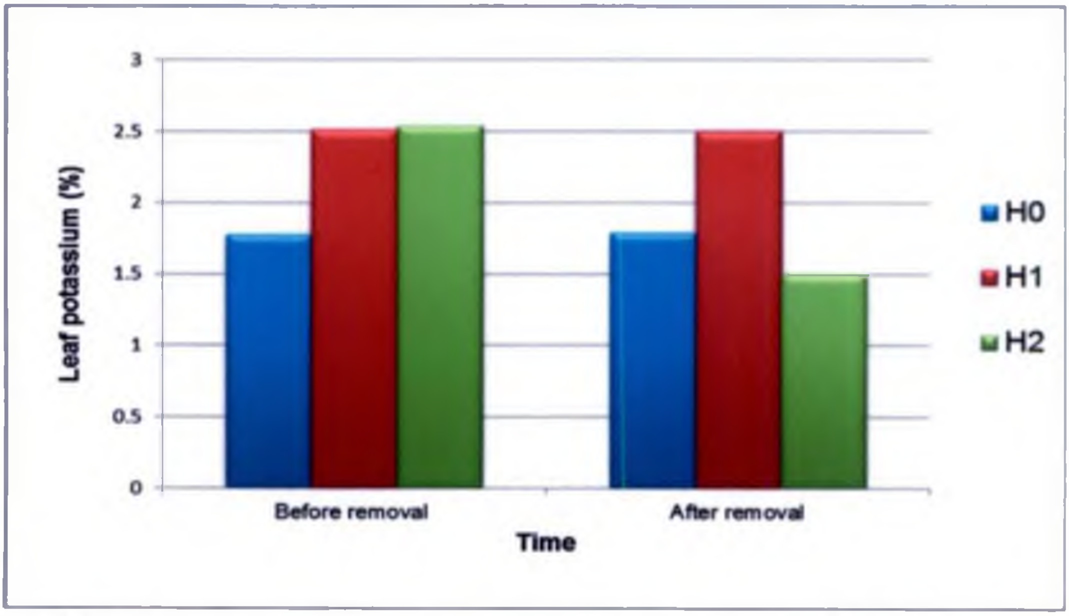


Fig. 9(b) Effect of host casuarina, on potassium content of sandalwood leaves before and after the removal of host plant

H_o= Sandalwood alone now - But provided with host upto two years

H_1= Sandalwood + Casuarina - Through out the experiment

H_2= Sandalwood + Casuarina - Host removed after six years

Plate: 10 Wilting and leaf-shedding of the sandalwood tree after the removal of host plant

1984; Rangaswamy et al., 1986; Brand, 2002). But their reports showed wide variations and no conclusions can be arrived at. But the current study reveals no considerable variation in the micronutrient content of sandalwood tree in the presence of host. However, significant variations were observed in the macronutrient content of sandalwood tree growing with host. Sandalwood tree grown with host showed higher content of N, P and K in the leaves.

The subsequent reduction in the leaf-nutrient status of sandalwood tree after the removal of the host plant also supports conclusion that a higher nutrient status is maintained in sandalwood tree by the host plant. It may be seen from the data that the potassium content of the sandal leaves decreased from 2.53 to 1.50 on removal of the host tree. This is reflected in the pre-dawn water potential. The role of K in regulating the water relation in plants is well established and its role in osmotic regulation and stomatal movements are known (De Costa and Liyanage, 1997).

5.6 Sandal-haustoria on host plant

Root excavation of sandalwood with and without host was conducted to investigate the haustrial associations. The formation of haustoria, its structure and mode of attachment to the roots of host plant was first reported by Barber (1906). The studies by Rao (1911), Venkatarao (1938), Srimathi and Sreenivasaya (1962) and Iyengar (1965) also highlighted the parasitic nature of sandal by the formation of haustoria in different host species. However most of these studies were conducted in seedlings. But the current study was on field grown sandalwood tree. The presence of functional haustoria indicates the translocation of water and nutrients between host and sandalwood. The haustorial connections were firm and not easily detected during the excavation is due to the tissue graft between the host root and the sandal-haustoria.

Sandalwood tree without host also formed haustoria with the roots of host growing in the adjacent pit. The sandalwood tree with and without host not showed much variation in the growth performances. This may be due to the presence of haustorial development, with host trees teak, cashew, coconut, pepper, cocoa and rubber present in the adjacent pits and reported that sandalwood roots can grow to far distances and form haustoria on other host trees.

5.7 Number of haustoria

Maximum number of haustoria was observed in the sandalwood tree with host. Within this, the peak number and the largest size of haustoria were noted on the primary root of the host followed by secondary and tertiary roots. Annapurna and Rathore (2006) observed maximum number of haustorial formation with good host and significantly enhancing the growth and nutrient status of sandal seedlings. Ashokan et al. (2008) also observed maximum number of haustorial connections with casuarina.

5.8 Anatomy of haustoria

From the anatomical studies of haustoria attached with the host, it can be concluded that they formed close connections between the sandalwood and the host. Taide (1991), Varghese (1997) and Singh (2008) also observed vascular connections between the host and the sandalwood tree through haustoria. The authors opined that the vascular connections between the host and the sandalwood tree became so intimate that the host root and the parasitic root became almost a single physiological unit catering to the nutritional requirement of sandalwood tree.

The area of clasping fold was noticed maximum in case of secondary root haustoria. This higher area of contact with host root may be influencing high translocation through secondary root haustoria than tertiary root.

Furthermore, our investigations revealed that direct lumen-lumen xylem connections between the xylem of the host and the parasite are absent. This implies that unimpeded mass treachery flow of water and nutrients from host through the haustorium to sandalwood is unlikely to occur. Therefore, movement of xylem sap from host could only occur principally via pits of host xylem elements.

5.9 Radio tracer studies on haustorial associations

The variations observed in the ^{32}P translocated from host plant to sandalwood tree depends on the species of the host, may be due to the difference in the number of the haustoria formed by sandalwood tree on the host, preference of host species by sandalwood tree, and the efficiency of translocation from host to sandalwood tree depending on the host species. From the data (Table 11) it can be deduced that the translocation from cocoa to sandalwood tree and casuarina to sandalwood tree are the most efficient followed by that from teak to sandalwood tree and cashew to sandalwood tree. The number of haustoria formed by sandalwood tree on these host species were reported earlier (Ashokan et al., 2008) and they observed maximum effective haustoria in teak, casuarina, cocoa and cashew. They observed no haustorial formation in rubber and coconut. They also observed the translocation of ^{32}P from these host plants to sandalwood tree during early seedling stage in the field.

The ^{32}P count observed in labelled cocoa is 102 $cpm.g^{-1}$and in sandalwood tree was 251 $cpm.g^{-1}$, whereas that in casuarina is 358 $cpm.g^{-1}$ and that in sandalwood tree was 291 $cpm.g^{-1}$. So it is evident that maximum translocation of ^{32}P from host to sandalwood tree is from casuarina. Cocoa being a broad leaved fast growing species, the chances of dilution effect is more. This may be the reason for the lower count in cocoa. On the other hand casuarina being a drought adapted species with needles in place of leaves, dilution effect is less and most of the ^{32}P absorbed is translocated to sandalwood tree. More accumulation of the absorbed ^{32}P in the host itself is seen in rubber and coconut. This may be due to the absence of sufficient number of haustoria to support efficient translocation from the host to sandalwood tree. Ashokan et al. (2008) observed that no sandal haustoria was observed on rubber roots at 3 year stage of growth.

The possible reverse translocation from sandalwood tree to host plant is evident from the data on ^{32}P count translocated from labelled sandalwood tree to host plants (Table 12). The labelled sandalwood tree showed a count of 513 $cpm.g^{-1}$ and the host in the same pit, teak and casuarina showed a count of 183 $cpm.g^{-1}$ and 219 $cpm.g^{-1}$ respectively. From the data, it is evident that translocation from sandalwood tree to host is also equally efficient. The anatomical studies showing the vascular connections with host, in the course of time, it developes and functions as single physiological unit (Taide, 1991). So the vascular connection between the host plant and sandalwood tree permits translocation in both directions.

The percentage of translocation from sandalwood tree to host casuarina was 26 and to teak was 34.89, whereas that from host to sandalwood varied from 27.6% to 78.5%. The percentage of the total ^{32}P count dectected in sandalwood tree and host plant also varied depending on the species of the host plant and the number of the host species present in the same pit as that of sandalwood tree. Percentage varied from 27.65, when rubber was host to 71%, when cocoa was the host. The second and third plant present in same pit as sandalwood tree also showed ^{32}P count translocated from the labelled host plant. As the host plants cannot have root connections, translocation from labelled host to other host plants in the pit or in the adjacent pit may be mediated through sandalwood tree which might have formed functional haustorial connections in all the host plants surrounding it. The same trend can be observed in the field observations made in other radiotracer study, where sandalwood and casuarina alone were involved. Here labelled ^{32}P was translocated from casuarina to sandalwood tree as well as from casuarina to casuarina, probably through sandalwood tree, which were separated by a distance of 1.5 m to 3 m.

The results from the radiotracer studies indicates that sandalwood tree forms a network of roots, connected through haustoria, between sandalwood-casuarina-sandalwood tree and even with the grasses growing around it (Fig.10) (Plate11). A possible pattern of root networking deduced from the translocated data of ^{32}P from sandalwood tree to host, host to host and host to sandalwood tree is shown in Fig. 11(a) and (b).

Fig. 12 shows the ^{32}P count observed in sandalwood tree on different days after labelling of the host plant. The ^{32}P count observed in sandalwood tree after six hours of labelling the plant casuarina, indicates that the rate of translocation of radio-labelled phosphorus from host to sandalwood tree is very rapid. The peak count of ^{32}P in sandalwood tree was observed on eighth day of the labelling, showing that translocation of ^{32}P progressed upto eighth day. The reduction trend after the eighth day may be due to the decay of ^{32}P.

The implication of the result from the radiotracer studies is that the host plants need not be present in the same pit as that of sandalwood tree. Sandalwood tree can extend its root to distance of 1.5 to 3 m (based on the data available from the present study) to form haustoria on host plant (Fig.13a and 13b). It may also be possible that the root extension may go further distances to meet the host plant, however data to confirm this is not available in the present study. This has to be investigated in future studies. Another fact which has to be considered in selecting host plant is the possible competition for above ground resources like solar radiation, CO_2 etc. So the best host would be that with more functional haustoria, but at the same time offers minimum competition for above ground resources. In this context casuarina is superior to cocoa, teak, cashew etc, because it may offer considerably less competition for light

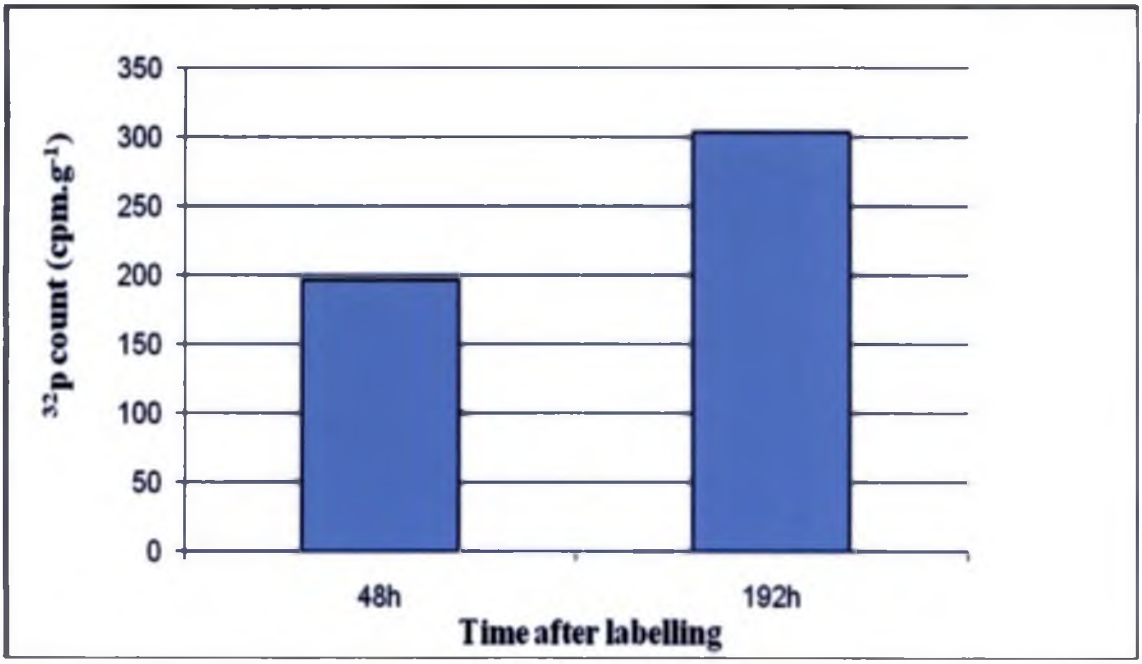


Fig. 10 Count of translocated ^{32}P from wild grass to sandalwood tree

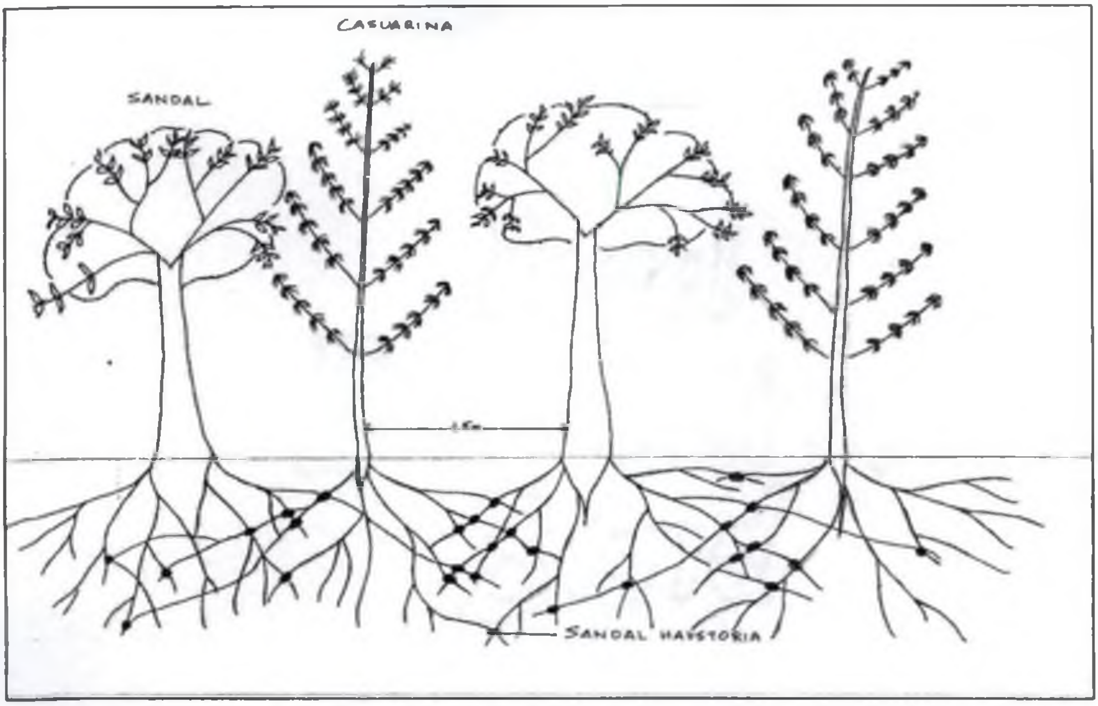


Fig. 11(a) Diagrammatic representation, showing interaction of sandalwood tree and host casuarinas through sandal haustoria, growing in different pits

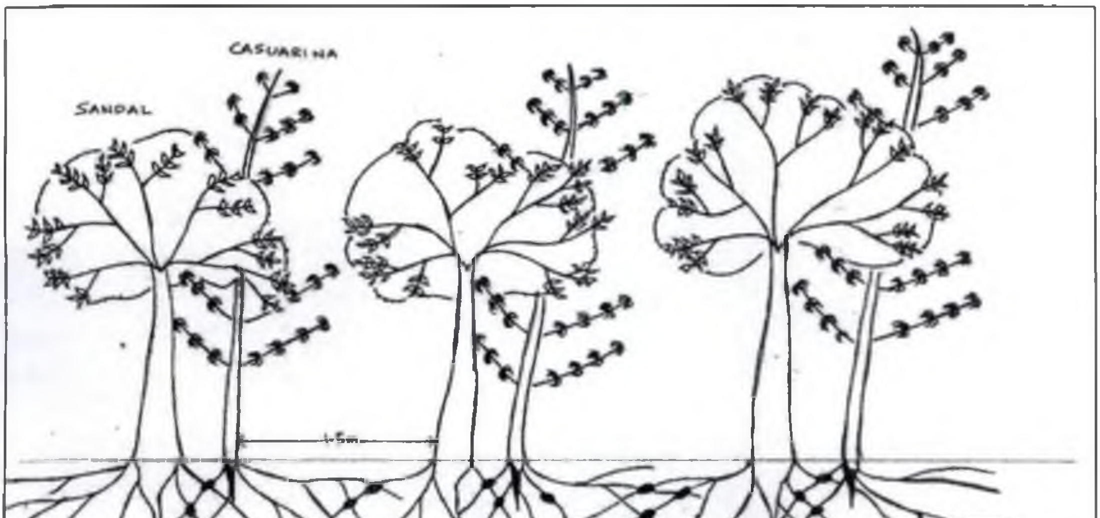


Fig. 11(b) Diagrammatic representation, showing interaction of sandalwood tree and host casuarinas through sandal haustoria, growing both in same pits

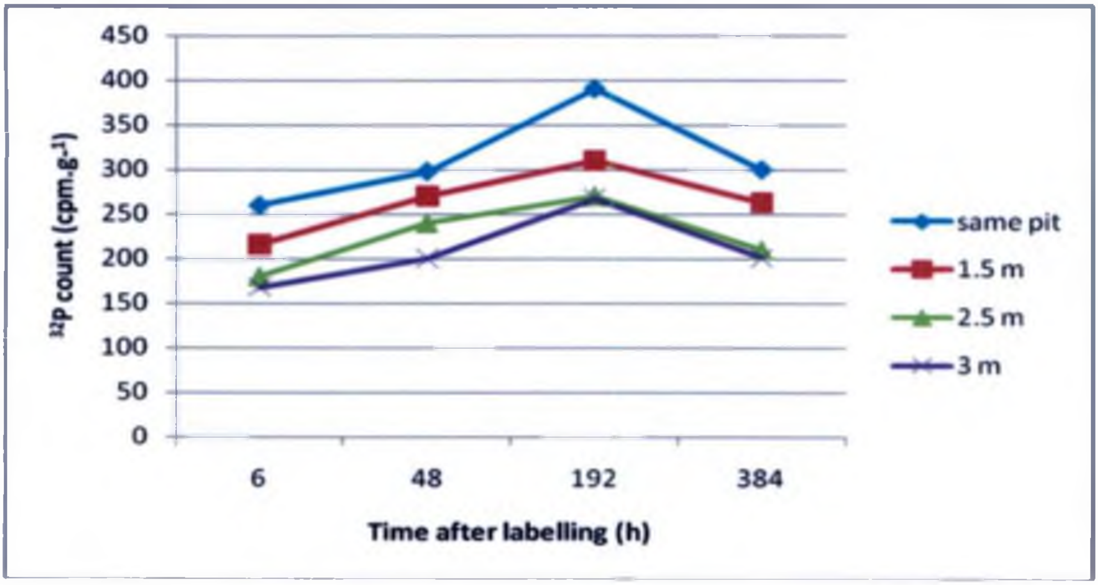


Fig 12. Count of ^{32}P translocated from host, casuraina obtained from sandalwood trees growing at different distances on different time intervals

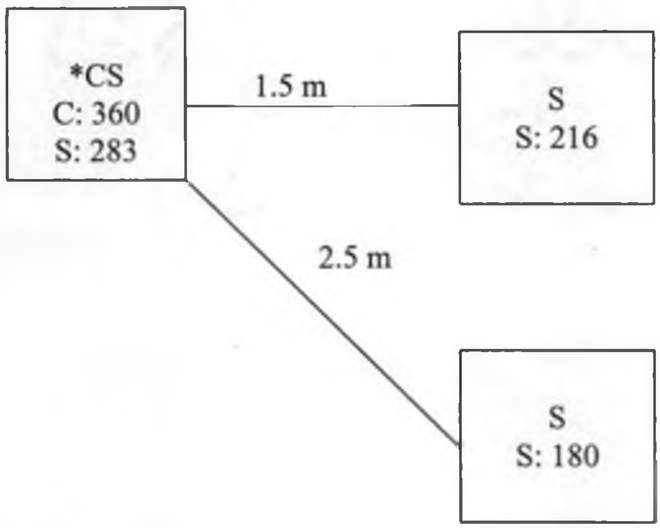


Fig: 13(a) Count of ^{32}P, translocated from labelled plant in sandalwood tree at different distances

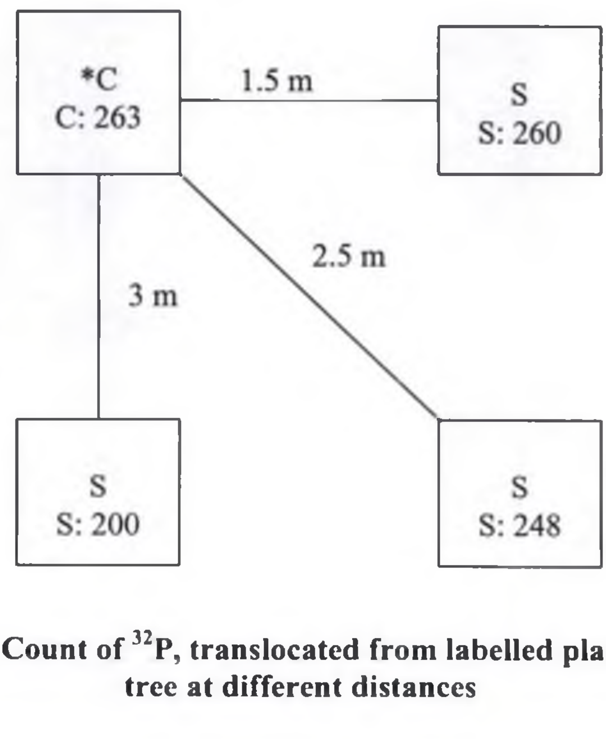


Fig: 13(b) Count of ^{32}P, translocated from labelled plant in sandalwood tree at different distances

Plate: 11 Presence of sandal haustoria on wild grass root

as the canopy is very sparse with needles in place of leaves. The cocoa, teak, cashew etc, which showed comparable trans-haustorial translocation of ^{32}P has broader leaves which may offer much higher competition for above ground resources. However, considering the value of these crops plants and timber value of teak, the possibility of planting sandalwood tree as an intercrop in suitable distances from the main crop can be considered. These crop plants will give periodical returns whereas sandalwood tree planted in field may yield significant income in the long term.

Summary

SUMMARY

The influences of host plant on the carbon assimilation, water and nutrient absorption of the field grown *Santalum album* L. were investigated in a field trial, at College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur during the year 2009-2011.

The salient results of the investigations are summarized below:

1. Height and diameter (dbh) of the sandalwood trees grown with and without host were on par. However, the sandalwood tree with host showed marginal superiority compared to the sandalwood trees growing without host.

2. Sandalwood trees grown with host showed higher rate of photosynthesis (carbon assimilation) than sandalwood trees grown without host.

3. Sandalwood grown without specific host can extend its root for finding a host in its vicinity, and form haustorial connections.

4. Sandalwood trees grown with host showed higher plant water potential than sandalwood trees grown without host. Plant water potential decreased significantly after the removal of host plant from the sandalwood pit. It also resulted in leaf wilting and leaf fall in sandalwood, indicating the contribution of host in maintaining higher water potential in sandalwood trees.

5. Sandalwood trees grown with host showed higher leaf nutrient content. The N, P and K content of the leaf decreased considerably after the

removal of host. The removal of host did not show any significant effect on leaf micronutrient content of sandalwood tree.

6. Sandalwood growing without host also formed haustoria with roots of host growing in adjacent pits. The sandalwood growing with host in the same pit showed more number of haustoria. Total number of haustoria was higher on the primary root of the host followed by secondary and tertiary roots.

7. Size of the haustorium, the depth of penetration, the length of the penetration peg and area of coverage of endophyte was higher in the case of secondary root haustoria than in tertiary.

8. Anatomical study of sandalwood haustoria revealed that elongated parenchyma cells of the haustorium projected towards the host root xylem making vascular connection between sandalwood root and host-root.

9. Radioactive phosphorus (^{32}P) application on host, casuarina and tracing in sandalwood revealed that there is inter translocation of nutrients between sandalwood and host through haustorial connections.

10. Radiotracer studies on different sandalwood tree-host associations showed that association of sandalwood with cocoa, as host, was most efficient in haustorial translocation of ^{32}P.

11. Labelling of wild grasses growing around sandalwood tree with ^{32}P revealed that there is translocation from these grasses to sandalwood tree and sandalwood also depend on wild grass for meeting its nutrient and water requirements.

It can be concluded from the above experiments that a host plant is not only essential for the initial establishment of sandalwood but also required in the main field, may be throughout its growth period. Sandalwood trees depend on host plant mainly for maintaining plant water status and reduce stress. The host plant is supplementing the N, P and K requirement of sandalwood trees through the haustorial connections. In the absence of a host plant in the immediate vicinity of sandalwood tree, it can extend its root for finding a suitable host. The results of this investigation indicate that one plant-one host system of planting is not required, may be a host in the middle of four sandalwood trees in enough, so that the suppression of sandalwood tree growth due to host competition for light can be minimized.

References

REFERENCES

Alberte, R.S., Thornbee, J.P., and Fiscus, E.L.1997. Water stress effects on the content and organization of chlorophyll in mesophyll and bundle sheath chloroplast of maize. Plant Physiol. 59: 351-353.

Ananthapadmanabha, H.S. 2000. Sandal wood and its marketing trend. My Forest. 36: 147-151.

Ananthapadmanabha, H.S., Nagaveni, H.C., and Rai, S.N. 1988. Influence of host plants on growth of sandal; My Forest. 24(2):154-160.

Ananthapadmanabha, H.S., Rangaswamy, C.R., Sarma, C.R., Nagaveni, H.C., Jain, S.H., Venkatesan, K.R., and Krishnappa, H.P. 1984. Host requirement of sandal (*Santalum album* L.). Indian Forester. 110: 264 – 268.

Annapurna, D and Rathore, T.S. 2006. Modern nursery practices in the production of quality seedlings of Indian Sandalwood (*Santalum album* L.) – Stages of host requirement and screening of primary host species. J. of Sustainable Forestry. 22(3): 33-55.

Annapurna, D., Rathore, T.S., and Joshi, J. 2007. Effect of various media on germination and seedling growth of *Santalum album* L. J. Non-Timber Forest Products. 14 (1): 33-36.

Anoop, E.V. 1993. Nutritional deficiency symptoms of Ailanthus (*Ailanthus triphysa* (Dennst.) Alston) seedlings. M. Sc. thesis. Kerala Agricultural University, Thrissur.

Arnon, D.I. 1948. Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. Plant Physiology. 24(1): 1-15.

Ashokan, P.K and Krishnambika, N.2007. Growing sandal in homegardens and other agroforestry systems – Potentials and problems. In: National Seminar on Conservation, Improvement, Cultivation and Management of Sandal (*Santalum album* L.). Dec12-13, 2007. Banglore, India. pp38-39.

Ashokan, P.K., Vijayakumar, N.K., and Santhoshkumar, A.V. 2008. Potentials of growing Sandal (*Santalum album*) on farmlands - Hemi-parasitic interactions of sandal with crop plants and selected host plants. KSCSTE – SRS project: Council order No.(T) 147/SRS/2005/CSTE. Final report. 33p.

Baird, W.M.V., and Riopel, J.L. 1983. Experimental studies of the attachment *Agalinis purpurea* to a host. Protoplasma. 118: 206-218.

Barber, C.A. 1902. The natural history of the sandal tree. Indian Forester. 29: 386-389.

Barber, C.A. 1903. Report on spike disease of sandal wood trees in Coorg. Indian Forester. 29: 21-31.

Barber, C.A. 1906. Studies in root parasitism. The haustorium of *Santalum album* L. early stages, up to penetration. Mem. Dept. Agric. India Bot. Ser. 1: 1-30.

Barber, C.A. 1907. Studies in root parasitism. The haustorium of *Santalum album*. L. The structure of the mature haustorium and the inter-relations between host and parasite. Mem. Dept. Agric. India Bot. Ser. 11: 1-58.

Barrett, D.R., and Fox, J.E.D. 1997. *Santalum album*: kernel composition, morphological and nutrient characteristics of the pre-parasitic seedlings under various nutrient regimes. Ann. Bot. 79: 59-66.

Brand, J.E. 2002. Review of influence of *Acacia* species on establishment of sandalwood (*Santalum spicatum*) in Western Australia. Conservation science Western Australia. 4(3): 125-129.

Brand, J.E., and Jones, P.J. 2000. The influence of landforms on sandalwood (*S. spicatum*) size structure and density in the Northern East gold fields, Western Australia. Rangeland J. 24(2). 219-226.

Brand, J.E., Robinson, N., and Archibald, R.D. 2003. Establishment and growth of Sandalwood (*Santalum spicatum*) in South-Western Australia: Accacia host trials. Australian Forestry. 66(4): 294-299.

Brandis, D. 1903. Treatments of sandal tree. Indian Forester. 29:3-6.

Brandis, D. 1978. *Indian trees*. International Book Distributors, Dehra Dun, India. 35p.

Brennen, B., and Merlin, M. 1993. Biogeography and traditional use of *Santalum* in the pacific region. In: McKinnel, F.H. (ed.). Sandalwood in the pacific region. Proceedings of the symposium at the XVII Pacific Science Congress; 2nd June, 1992; Honolulu, Hawaii, Australia. pp. 31-40.

Calladine, A., and Pate, J.S. 2000. Haustorial structure and functioning of the root hemiparasite tree *Nuytsia floribunda* (Labill.) R. Br., and water relations with its hosts. Ann. Bot. (Lond.). 85: 723-731.

Cameron, D.D. 2004. A role for differential host resistance to the hemiparasitic angiosperm *Rhinanthus minor* in determining the structure of host plant communities. Ph.D. thesis, University of Aberdeen, School of Biological Sciences (Plant and Soil Science), UK.

Cameron, D.D., Hwangbo, J.K., Keith, A.M., Geniez, J.M., Kraushaar, D., Rowntree, J., and Seel, W.E. 2005. Interactions between the hemiparasitic angiosperm *Rhinanthus minor* and its hosts: From the ceel to the ecosystem. Folia Geobot. 40: 217-230.

De Costa, W.A.J.M ., and Liyanage, L.P. 1997. Effects of potassium and water availability on water use efficiency of common beans (*Phaseolus vulgaris* L.). J.Natn.Sci.Coun.SriLanka.25(4): 241-254.

Dhaniklal, G. 2006. Influence of host plants on soil moisture stress on the water relations in sandal. M. Sc Thesis. Kerala Agricultural University, Thrissur. 64p.

Dobbins, D.R., and Kujit, J. 1974. Anatomy and fine structure of the mistletoe haustorium (*Phthirusa pyrifolia*). I. Development of the young haustorium. Am. J. Bot. 61: 535-543.

Drury, C.H. 1985. *The useful plants of India* (2nd ed.). International Book Distributors, Dehra Dun. 131p.

Dutt, S., and Verma, K.S. 2005. Effect of collection of time, pre-sowing treatments and sowing time on the germinability of sandal (*Santalum album* L.) seeds under nursery conditions. J. Non-Timber Forest Products. 12 (4): 205-208.

Fineran, B.A., and Hocking, P.J. 1983. Features or parasitism, morphology and haustorial anatomy in Loranthaceous root parasites. In: The Biology Of Mistletoes. M. Calder and P. Bernhardt (eds.). Academic Press, Sydney. pp. 205-227.

Fischer, C.E.C. 1922. Host plants of *Santalum album* L. Indian Forester. 48:113.

Fox, J.E.D., and Doronila, A.I. 1993. Selection of primary host (pot stage) for *Santalum album*. Report to the Sandalwood Research Institute. Perth. Mulga Research Center, School of Environmental Biology. Cutin University of technology. pp. 38-44.

Fox, J.E.D., Surata, I.K., and Sutarjo, S. 1990. Nursery potting mixture for sandal in Timor. Mulga Res. Cent. J. 10: 38-44.

Gurney, A.L., Grimanelli, D., Kanampiu, F., Hoisington, D., Scholes, J.D., and Press, M.C. 2003. Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides,* a wild relative of maize. New Phytol. 160: 556-568.

Hamilton, L., and C.E. Conrad (eds.). 1990. Proceedings of the Symposium on Sandalwood in the Pacific, April 9–11, 1990, Honolulu, Hawaii. General Technical Report PSW–122. Pacific Southwest Research Station, USDA Forest Service, Berkeley, California.

Heide-Jorgensen, H.S. 1989. Development and ultrastructure of the haustorium of *Viscum minimum* Harvey. I. The adhesive disk. Can. J. Bot. 67: 1161-1173

Hibberd, J.M., and Jescheke, W.D. 2001. Solute flux into parasitic plants. J. Exp. Bot. 52: 2043-2049.

Hiremath, V.J. 2004. Influences of soil moisture regimes and stage of host introduction on seedling growth of sandal provenances. M. Sc. thesis. Kerala Agricultural University, Thrissur, Kerala. 149p.

Hua, M. G., Min, H. Y., Feg, Z. J., and Lian, C. F. 2005. Study on semi-parasitism of sandalwood seedlings. J. of Tropical and Subtropical Botany. 13(3): 233-238.

Iyengar, A.V.V. 1958. Spike disease of Sandal. A retrospect. Indian Forester. 8: 603-612.

Iyengar, A.V.V. 1965. The physiology of the root parasitism in sandal (*Santalum album* Linn.). Indian Forester. 91: 246-256.

Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice Hall Inc. USA. 363p.

Jain, S.H., Angadi, V.G., Ajeevalochan, A.N., Shankernarayana, K.H., Theagarajan, K.S., and Rangaswamy, C.R. 1998. Identification of provenances of sandal in India for genetic conservation. ACIAR proceedings of the conference on sandal at Institute of Wood Science and Technology; 18-19 Dec.; Bangalore, India. No. 84. pp. 117-120.

Jiang, F. 2004. Water, mineral nutrient and hormone flows and exchanges in the hemiparasitic association between root hemiparasite *Rhinanthus* *minor* and the host *Hordeum vulgare*. Ph. D. thesis, Julien-von-Sachs Institut fur Biowissenschaften, University of Wurzburg, Germany.

Jin, X., Ping, D.X., Nan, N.Z ., Sheng, Z., and Fu, H.C. 2010. Effects of pot host configuration on the growth of Indian sandalwood (*Santalum album*) seedlings in South China. Forest Research, Beijing. 23(6): 924-927.

Kamalolbhavan, B.N. 2002. Response of sandal (*Santalum album* Linn.) seedlings to shade and mycorrhizal association. M. Sc. thesis. Kerala Agricultural University, Kerala. 64p.

Koening, H.A., and Johnson, G.R. 1942. Colorimetric determination of phosphorus in biological materials. Ind. Eng. Chem. (Anal). 14: 155-156.

Kujit, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, Calif.

Kumar, B. M., George, S.J., and Chinnamani, S. 1994.Diversity, structure and standing stock of wood in the home gardens of Kerala in Peninsular India. Agroforestry system. 25: 243-262.

Kunda, S.D., Vasantharajan, V.N., and Bhatt, J.V. 1974*a*. Uptake and translocation of ^{45}Ca and ^{32}P by angiospermic root parasite *Santalum album* and by one of its host plants *Dolichos lablab*. Proc. symp. Use of Radiations and Radioisotopes in studies on plant productivity. G.B. Pant. Univ. of Agric., and Tech., Pantnagar, April, 1974. pp. 11-27.

Kunda, S.D., Vasantharajan, V.N., and Bhatt, J.V. 1974*b*. Movement of organic substances from the host to the hemiroot parasite *Santalum* *album* L. Proc. symp. on use of Radiations and Radioisotopes in studies on plant productivity. G.B. Pant. Univ. of Agric., and Tech., Pantnagar, April, 1974. pp. 696-706.

Kuo, J., Pate, J.S., and Davidson, N.J. 1989. Ultrastructure of the haustorial interface and apoplastic continuum between host and the root hemiparasite *Olax phyllanthi* (Labill.) R. Br. (Olacaceae). Protoplasma. 150: 27 – 39.

Lakshmisita, G., and Bhattacharya, A. 1998. cDNA cloning and characterisation of proline (or hydroxyproline) rich protein from *Santalum album* L. In: Radomiljac, A.M., Ananthapadmanabha, H.S., Welbourn, R.M., and Satyanarayana Rao, K. (eds.). *Sandal and its products*. ACIAR proceedings. ACIAR, Canberra, Australia. No. 84. pp. 29-35.

Lane, J.A., Bailey, J.A., and Terry, P.J. 1991. An *in vitro* growth system for studying parasitism of cowpea (*Vigna unguiculata*) by *Striga gesneriodes*. Weed Res. 31: 211-217.

Losner-Goshen, D., Portnoy, V.H., Mayer, A.M., and Joel, D.M. 1998. Pectolytic activity by the haustorium of the parasitic plant *Orobanche* L. (Orobanchaceae) in host roots. Ann. Bot. (Lond.). 81: 319-326.

Lushington, P.M. 1904. Notes on sandal. Indian Forester. 30: 13-1.5.

Lushington, P.M. 1918. Spike disease in Sandal.an interesting isolated area and its treatment. Indian Forester. 44(2): 114-117.

Mal,G.H., and Eric, B. 2007. Embryology and pollination trials support dichogamy in *Santalum album* L. J. of Integrative Plant Science. 48: 300-306.

Ma Guo, H., He Yue, M., Zhang, J.F., and Chen F.L. 2005. Study on semi-parasitism of sandalwood seedlings. J. of Tropical and Subtropical Botany.13(3): 233-238.

McKinnel, F.H. 1990. Status of management and silviculture – Research on sandalwood in Western Australia and Indonesia. In: Hamilton, L., and Conrad, C.E. (ed.). Proceedings of the symposium on sandalwood in Pacific; 9-11 April 1990; Honolulu, Hawaii. U.S. Forest Service General Technical Paper. PSW.-122. pp. 19-25.

Musselman, L.J., and Dickison, W.C. 1975. The structure and development of the haustorium in parasitic Scrophulariaceae. Bot. J. Linn. Soc. 70: 183-212.

Musselman, L.J., and Press, M.C. 1995. Introduction to parasitic plants. *In* Parasitic plants. *Edited by* M.C. Press and J.D. Graves. Chapman and Hall, London. pp. 1 – 11.

Nagaveni. H.C., and Srimathi, R.A. 1985. A note on haustoria-less sandalplants. Indian Forester. 64: 656-669.

Nayar, R., and Ananthapadmanabha, H.S. 1974. Bioassay of tetracycline uptake in spiked sandal (*Santalum album* L.). J. Indian Acad. Wood Sci. 5 (2): 108-112.

Nazeem, P.A. 1989. Deficiency symptoms of mineral nutrients in clove *(Syzigium aromaticum* (L) Merr. and Perry*)*. Ph.D. Thesis, Kerala

Agricultural University, Vellanikkara, Kerala. pp. 185.

Neil, P.E. 1990. Growing Sandalwood in Nepal-Potential Silvicultural Methods and Research Priorities. USDA Forest Service Gen. Tech. Rep. PSW-122. 72p.

Nickrent, D.L., and Duff, R.J. 1996. Molecular studies of parasitic plants using ribosomal RNA. In: Advances in parasitic plant research. Moreno, M.T., Cubero, J.I. , Berner, D., Joel, D., Musselman, L.J., and Parker, C (eds.). Junta de Andalucia, Direccion General de Investigacion Agraria, Cordoba, Spain. pp. 28-52.

Parthasarathi, K., Gupta, S. K., and Roa, P. S. 1974. Differential response in the cation exchange capacity of the host plants on parasitisation by sandal (*Santalum album* Linn.). Current. Sci. 43: 209p.

Pate, J.S. 2001. Haustoria in action: case studies of nitrogen acquisition by woody xylem- tapping hemiparasites from their hosts. Protoplasma. 215: 204-217.

Pate, J.S., Kuo, J., and Davidson, N.J. 1990. Morphology and anatomy of the haustorium of the root hemiparasite *Olax phyllanthi* (Olacaceae), with special reference to the haustorial interface. Ann. Bot. (Lond.). 65: 435-436.

Pilger, R. 1935. *Santalaceae* (In: Engler, A., and Prantl, K.). Die naturalichen pflanzen familien, Leipzig. 206p.

Radomiljac, A.M. 1998. The influence of pot host species, seedling age and supplementary nursery nutrition on *Santalum album* Linn. (Indian sandalwood). Plantation establishment, Ord river irrigation area, Western Australia. Forest Ecol. Manage. 102: 193-202.

Rai, S.N. 1990. Status and cultivation of sandalwood in India. In: Hamilton, L., and Gornad, C.E. (eds.). Proceedings of the symposium of sandalwood in the pacific; 9-11 April 1990; Honolulu, Hawaii. PSW-122. pp. 66-71.

Rai, S.N., and Sarma, C.R. 1986. Relationship between height and diameter increment of sandal (*Santalum album* Linn.). Van Vigyan. 24: 105-138.

Rangaswami, S., and Griffith, A.L. 1939. Host plants and spike disease of sandal. Indian Forester. 65: 335-345.

Rangaswamy, C.R., Jain, S.H., and Parthasarathi, K. 1986. Soil properties of some sandal bearing areas. Van Vigyan. 24: 61-68.

Rao, N.M., Padmini, S., Ganeshaiah, K.N., and Umashankar, R. 1999. Sandal genetic resources of South India: threats and conservation approaches. In: National symposium on role of plant tissue culture in biodiversity conservation and economic development. Kosi-Katarmal, Alomora, UP. 63p.

Rao, M. 1918. Host plants of *Santalum album*. Indian Forester. 44:58-65.

Rao, L.N. 1942*a*. Parasitism in Santalaceae. Ann. Bot. 6: 131-150.

Rao, L.N. 1942b. Studies in the Santalaceae. Ann. Bot. (Lond.). 6: 151-175.

Rao, M.R. 1903. Root parasitism of sandal trees. Indian Forester. 29: 386-389.

Rao, M.R. 1911. Host plants of the sandal tree. Indian Forester Rec. 2: 159-207.

Rao, S.Y.V. 1933. Contributions to the physiology of sandal (*Santalum album* L.) Part I. Nature and extent of parasitism. J. Indian Inst. Sciences. 16A: 167-184.

Riopel, J.L., and Timko, M.P. 1995. Haustorial initiation and differentiation. In: M.C. Press and D. Graves (eds.): *Parasitic plants*. Chapman and Hall, London. pp. 39-79.

Scott, J. 1871. Notes of Horticulture in Bengal. No. 2. Loranthaceae the mistletoe order of their germination and mode of attachment. J. Agric. Hort. Soc. Indian. 2: 287.

Shankaranarayana, K.H., Ravikumar, G., and Rangaswamy, C.R. 1998. Content and composition of oil from the central and transition zone of sandal wood disc. In: Radomiljac, A.M., Ananthapadmanabha, H.S., Welbourn, R.M., and Rao, S. K. (Eds.). Sandal and its products. ACIAR proceedings Canberra, Australia. No. 84. pp. 86-88.

Shea, S.R., Radomiljac, A.M., Brand, J., Jones, P. 1998. An overview of sandalwood and the development of sandal in farm forestry in Western Australia. In: Radomiljac, A.M., Ananthapadmanabha, H.S., Welbourn, R.M., Satyanarayana Rao, K. (eds). Sandal and Its Products. ACIAR: Canberra. pp. 9-15.

Shen, H., Ye, W., Hong, L., Huang, H., Wang, Z., Deng, X., Yang, Q., and Xu, Z. 2006. Progress in parasitic plant biology: host selection and nutrient transfer. Plant Biol. 8: 175-185.

Shinde, S.R., Ghagte, R.D., and Mehetre, S.S. 1993. Growth and development of sandalwood and its host (*Casuarina*) with teak in mixed plantation. Indian J. For. 22: 253-257.

Singh, S.H. 2008. Influence of host, light and mineral nutrition on the growth of sandal seedlings (*Santalum album* L.). M.Sc. Thesis. Kerala Agricultural University, Kerala. 234p.

Srimathi, R.A., Babu, D.R.C., and Sreenivasaya, M. 1961. Influence of host plants on the amino acid make up of *Santalum album* Linn. Curr. Sci. 30: 417.

Srimathi, R.A., and Sreenivasaya. 1962. Occurance of auxins in the haustoria of *Santalum album*. J. Sci. Res. 21: 131.

Srinivasan, V.V., Shivaramakrishan, V.R, Rangaswamy, C.R., Ananthapadmanabha, H.S., and Shankaranarayan, K. 1992. SANDAL (*Santalum album* Linn.). Monograph by the institute of wood science and technology (ICFRE) Malleshwaram, Bangalore, India. 233p.

Sreenivasarao, Y.V. 1933. Contributions to the physiology of sandal (*Santalum album* L.). J. Indian Inst. Sci. 16: 167-184.

Stewart, G.R., and Press, M.C. 1990. The physiology and biochemistry of parasitic angiosperms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 127-151.

Struthers, R., Lamont, B. B., Fox, J. E. D., Wijesuriya, S., and Crossland, T. 1986. Mineral nutrition of sandalwood (*Santalum spicatum*). J. of Exptl. Bot. 37(9): 1274-1284.

Subbarao, N.S., Yadav, D., Padmanabha, H.S., Nagaveni, H.C., Singh and Kavimandan, S.K. 1990. Nodule haustoria and microbial features of *Cajanus* and *Pongamia* parasitized by Sandal (Sandal wood). Plant and soil. 128: 249-256. Kluwer Academic Publishers, the Netherlands.

Surata, K. 1992. Effect of host plants on the growth of sandalwood (*Santalum album* Linn.) seedlings. Santalum. 6: 1-13.

Surata, K., Harisetijono., and Sinaga, M. 1995. Effect of intercropping on growth of sandalwood (*Santalum album* L.). Santalum. 20:17-24.

Surendran, C., Parthiban, K.L., Bhuvaneshwaram, C., and Murugesh, M. 1998. Silvicultural strategies for augmentation of sandal regeneration. ACIAR proceedings of the conference on sandal at Institute of Wood Science and Technology; 18-19 Dec., 1997, Bangalore, India. No. 84. pp. 69-73

Taide, Y.B. 1991. Biology of the seedling of sandalwood (*Santalum album* Linn.). M. Sc. thesis, Kerala Agricultural University, Thrissur. 69p.

Taide, Y.B., Babu, L. C., and Abraham, C. C. 1995. Influence of host species in the initial growth and development of sandal (*Santalum album* Linn.). Indian Journal of Forestry. 17 (14): 288-292.

Tennakoon, K.U., and Cameron, D.D. 2006. The anatomy of *Santalum album* (Sandalwood haustoria). Can. J. Bot. 84: 1608-1616.

Tennakoon, K.U., Etampawala, E.R.L.B., and Gunatilleke, C.V.S. 2001. Silviculture of the economically important woody root hemiparasitic tree *Santalum album* L. (Sandalwood). In Tropical ecosystems: structure, diversity and human welfare. *Edited by* K.N. Ganeshaiah, . Uma-Shaanker, and K.S. Bawa. Oxford and IBH, New Delhi, India. pp. 41-45.

Tennakoon, K.U., Ekanayake, S.P., and Etampawala, E.R.L.B. 2000. An overiew of *Santalum album* research in Sri Lankha. Sandalwood Research Newsletter. 11:1-4.

Umashankar, R., Ganeshaiah, K.N., and Nageshwara Rao, M. 2000. Conservation of sandal regeneration research in India: problems and prospects. In: International conference of science and technology for managing Plant Genetic Diversity in 21st Century. Kuala Lumpur, Malaysia. 73p.

Varghese, S. 1997. Parasitic interference of sandal (*Santalum album* Linn.) on common agricultural crops from the homesteads. M. Sc. thesis. Kerala Agricultural University, Thrissur. 66p.

Venkata Rao, M.G. 1938. The influence of host plants on sandal spike disease. Indian Forester. 64: 656-669.

Venkatesan, A.R., Srimathi, R.A., and Kulkarni, H.D. 1995. Survey of Sandal populations. Recent Advances in Research and Management of Sandal (*Santalum album* L.) in India. pp.3-52.

Appendix

APPENDIX - I

Weather parameters during the study period (May 2010 to April 2011)

Source: Department of Agricultural Meteorology, KAU, Vellanikkara.

Months	Max. Temperature (^{0}C)	Min. Temperature (^{0}C)	RH (%)	Rainy days	Rainfall (mm)	Sunshine (hr)
May (2010)	33.1	24.8	87	7	123.8	166.5
June (2010)	30.4	23.7	83	24	700.4	89.7
July (2010)	29.2	22.8	85	25	552.0	56.8
Aug (2010)	29.3	23.2	81	16	224.1	78.6
Sept (2010)	30.5	23.2	70	17	326.7	125.6
Oct (2010)	29.7	23.2	58	18	667.6	129.5
Nov (2010)	30.4	23.7	55	11	282.8	122.5
Dec (2010)	30.9	23.9	64	2	24.5	206.7
Jan (2011)	32.7	32.7	73	0	00.0	263.0
Feb (2011)	33.7	33.7	77	3	77.5	239.1
Mar (2011)	34.8	34.8	89	2	10.0	268.9
April (2011)	34.3	34.3	88	5	207.1	199.2

INFLUENCE OF HOST PLANT ON THE PHYSIOLOGICAL ATTRIBUTES OF FIELD GROWN SANDAL (*Santalum album* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Effects of host plant, casuarina on the carbon assimilation, water and nutrient absorption in field grown sandalwood was studied at College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur during the year 2009-2011. Radioisotopic study to understand the trans-haustorial translocation from the host to field grown sandalwood tree and anatomical studies of sandalwood haustoria were also taken up during the investigation. The investigations were carried out in a six year old sandal-field, where sandalwood tree with and without host were established as a part of an earlier research project. The experiment was laid out with single tree plants, in RBD with three treatments viz, T_1 - Sandalwood tree without host (Casuarina) (Host plant dead naturally within 2 years after establishment of sandal), T_2 - Sandalwood tree with host (Casuarina), T_3 - Sandalwood tree with host (Casuarina) and the host plant cut and removed at six year stage of growth.

The host plant casuarina did not influence the height and diameter (dbh) of the sandalwood tree significantly during its six years growth. Sandalwood trees growing with host showed higher rate of photosynthesis (carbon assimilation) than sandalwood trees growing without host. Sandalwood tree growing without specific host can extend its root for finding a host in its vicinity, and forming haustorial connections, for meeting its water and nutrient requirements. Sandalwood trees grown with host showed higher plant water potential than sandalwood trees grown without host. Plant water potential has decreased significantly after the removal of host plant from the sandalwood pit. It also resulted in leaf wilting and leaf fall in sandalwood tree, indicating the contribution of the host in maintaining higher water potential in sandalwood trees. Sandalwood trees growing with host showed higher leaf N, P and K content. The N, P and K content of the sandalwood tree leaf decreased considerably after the removal of the host plant. The removal of host did not show any significant effect on leaf micronutrient content of sandalwood tree. Sandalwood growing without host also formed haustoria with roots of

host plants growing in the adjacent pit. The sandalwood growing with host in the same pit showed higher number of haustoria. Total number of haustoria was higher on the primary root of the host followed by secondary and tertiary roots. Size of the haustorium, the depth of the penetration, the length of the penetration peg and area of coverage of endophyte was higher in the case of secondary root haustoria than in tertiary. Anatomical study of sandalwood haustoria showed that elongated parenchyma cells of the haustorium projected towards the host root xylem, making vascular connection between sandalwood tree root and the host plant root. Radioactive phosphorus (^{32}P) labelling on host plant, casuarina and tracing in sandalwood tree and other hosts revealed that there is inter-translocation of nutrients between sandalwood and host plants, sandalwood and sandalwood, host and host. The data indicated that a network of roots of sandalwood tree and the hosts are formed through haustorial connection resulting in xylem-translocation. Peak count of ^{32}P was obtained on the eighth day of its application. Radiotracer studies on different sandalwood tree-host associations showed that association of sandalwood and cocoa as host was efficient in haustorial translocation of ^{32}P. Labelling of wild grasses growing around sandalwood tree with ^{32}P revealed that, sandalwood also depends on wild grass for meeting its nutrient requirements.