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SHORT COMMUNICATION

GROWTH PERFORMANCE OF SANDALWOOD DURING NURSERY STAGE AS AFFECTED BY DIFFERENT HOST PLANTS

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ABSTRACT

***Santalum album* L. is a hemiparasitic tree that requires parasitism of host plants in three stages, where the primary host at the nursery stage is critical. The effects of six legume species (*Desmodium triflorum*, *Mimosa pudica*, *Alysicarpus vaginalis*, *Crotalaria retusa*, *Vigna radiata* and *Crotalaria juncea*) and two non-legume species (*Alternanthera sessilis* and *Tagetes erecta*) were investigated to select the most promising pot host for sandalwood in the nursery stage. Each host plant was established in 10 poly bags with four plants/bag, in a completely randomized design. When host plants are one month old, 45-day-old S. album seedling per bag was introduced. After six months the number of leaves, seedling height (cm), dry weight (g), root collar diameter (mm), root length (cm), the number of root interactions were recorded and sturdiness quotient (SQ) and quality index (QI) were calculated. S. album seedlings grown with *C. retusa* had a significantly ($P \leq 0.05$) higher number of leaves (26), taller (32.4 cm) and higher root collar diameter (7.5 mm), followed by seedlings grown with *M. pudica*. The highest SQ (5.18) of *S. album* seedlings grown with *M. pudica* implies lower growth and survival in the field. Non-legumes produced taller seedlings than legume hosts except for *M. pudica* and *C. retusa*. Sandalwood seedlings with *C. retusa* had the significantly highest number of ($P \leq 0.05$) root interactions (38), tallest shoot (3.3 g), greatest root dry weight (1.1 g), highest QI (0.58) and shortest root length (13 cm). *C. retusa* was identified as the preferred host for *S. album* at the nursery stage.**

Keywords: Growth parameters, Indian Sandal, Nursery stage, Parasitism, Pot host

INTRODUCTION

Sandalwood (*Santalum album*) is a semi root parasitic tree of the family Santalaceae. Numerous species of genus Santalum yield a profoundly fragrant oil that can be separated from the heartwood and used in the production of perfume, and the oil also has therapeutic properties (Subasinghe *et al*. 2019). S. album can bring promising monetary benefits through well-managed plantations (Sundararaj and Muthukrishnan 2011). Sandalwood is a protected species in Sri Lanka under the Flora and Fauna Act (1964) as amended in Fauna and Flora Protection Amendment Act No. 22, 2009. Commercial-scale cultivation of sandalwood has become popular in Sri Lanka since 2010 with silvicultural studies in different climatic zones (Subasinghe *et al*. 2012). The tree gets certain supplements from host trees by means of root associations called haustoria, which are vascular connections between the roots of sandalwood and its host plant (Rocha *et al*. 2015). In natural populations of sandalwood, 2% of the seedlings do not produce haustoria and fail to survive beyond three years (Rai 1990). The establishment of sandalwood plantations requires parasitism during nursery propagation with intermediate hosts that bridge the nursery and the field, and long-term secondary hosts (Ehrhart and Fox 1995). Raising sandalwood seedlings along with a post host plant is critical in improving their quality prior to out planting. Pot host is planted into a S. album seedling pot during the nursery propagation. The pot host relationship should be maintained even after the field planting until the secondary host is introduced. These primary hosts create

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varying influences on the sandalwood seedlings (Surata 1995) and host-parasite relationships are poorly understood (Rocha *et al*. 2015). The selection of suitable host plants is consequently the single most important silvicultural parameter for sandalwood nurseries since there is a great potential to grow sandalwood trees in forests, home gardens and in other agroforestry systems. Numerous studies have been carried out on the relationship between sandalwood and different host species. And there are numerous plants which have not been accounted for to be haustorised by sandalwood. In view of these facts, this experiment was designed to select the most promising of six leguminous and two non-leguminous primary host species for the growth and development of sandalwood seedlings at the nursery stage.

MATERIALS AND METHODS

A pot experiment was conducted at the Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya in Matara district, Sri Lanka. The average annual temperature of the area is about 27 – 30°C. The mean annual rainfall of the area exceeds 2500 mm. The experiment was conducted in poly bags with 20 cm height and 10 cm wide. The medium was a mixture of compost, sand, top soil 1:2:1 by volume. There were nine treatments, i.e. eight host plant species and one control without a host (Table 1), with ten replicate pots per treatment. Six nitrogen-fixing herbaceous annuals and 2 non-nitrogen-fixing herbaceous annuals were included in the treatments (table 1). The pots were arranged in a completely randomized design. Host plants were watered daily and pruned at two-week intervals for four weeks before *S. album* seedlings were introduced to the pots.

Mature *S. album* seeds were soaked in water for two hours to facilitate removal of the seed coat, then air-dried for two days under 27 °C in the laboratory. Thereafter, seeds were stored in a refrigerator under 7 °C for three weeks and pretreated with 0.05% gibberellic acid (GA3) for 16 hours (Annapurna *et al*. 2006). Treated seeds were germinated in sand trays under 60% shade house and transplanted one seedling per pot at the two-leaf stage (45 days old) into the pots containing the host plants.

Pots containing sandalwood seedlings and host plants were watered each morning and hand weeded as necessary. Host plants were pruned 30 days after the introduction of S. album seedlings, and subsequently at 15-day intervals (Annapurna *et al*. 2006). After six months, sandalwood seedlings were uprooted and washed with running tap water to remove the adhered soil particles without disturbing root interactions. The number of leaves , plant height and root length and root collar diameter were measured. Number of root interactions was also counted, Morphological features were described using two criteria: the sturdiness quotient (SQ) (Roller 1977) and the quality index (QI) (Dickson *et al*. 1960). SQ was calculated by dividing seedling height (cm) by collar diameter of root. Seedlings were oven-dried at 80°C to constant weight, then the QI (Dickson *et al*. 1960) was calculated according to the following equation.

Table 1: Different treatment codes and relevant pot host species.

Treatment code	Host species	Family
T_1	Green gram (*Vigna radiata*)	Fabaceae
T_2	Mukunuwenna (*Alternanthera sessilis*)	Amaranthaceae
T_3	Marigold (*Tagetes erecta*)	Asteraceae
T_4	Nidikumba (*Mimosa pudica*)	Fabaceae
T_5	Adanahiriya (*Crotalaria retusa*)	Fabaceae
T_6	Sunhemp (*Crotalaria juncea*)	Fabaceae
T_7	Aswanna (*Alysicarpus vaginalis*)	Fabaceae
T_8	Udupiyaliya (*Desmodium triflorum*)	Fabaceae
T_9	Control treatment	-

$$QI = \frac{sdw}{\left(\frac{h}{d}\right)+\left(\frac{t}{r}\right)}$$

Where,
sdw = seedling dry weight (g), h = height (cm), d = collar diameter (mm), t = shoot dry weight (g) and r = root dry weight (g).

Data obtained for all parameters were subjected to analysis of variance (SAS User Guide 2007). Duncan multiple range test was employed for mean separation at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The growth of the sandalwood seedlings varied among pot hosts (Fig. 1), with Crotalaria retusa, having the significantly highest number of leaves (26/plant), seedling height (32.4 cm) and root collar diameter (7.52 mm) ($P \leq 0.05$), followed by *M. pudica*. According to Surata (1995), growth variations of sandalwood seedlings occur due to the varying capabilities of primary host plants to absorb nutrients. The high performance of parasitic plants with legume hosts is thought to be related to the lowered competition for soil nitrogen between the hosts and the parasites (Radomiljac *et al*. 1999). Among the pot host species we tested, the legume *C. retusa* may have the highest capability to supply plant nutrients to the hemiparasitic sandalwood seedlings (Brand *et al*. 2000). Similar to our findings, Radomiljac (1998) reports that sandalwood seedlings parasitized with *C. retusa* performed well at the nursery and promoted good early growth of sandalwood seedlings over host plants such as Alternanthera nana, *Sesbania formosa, Atalaya hemiglauca, Acacia hemignosta, Crotalaria retusa.*

Seedlings grown with *M. pudica* (T4) had the greatest SQ of 5.18 ($P \leq 0.05$), and those grown with *D. triflorum* (T8) had the lowest SQ of 2.99 ($P \leq 0.05$) (Fig.1). The lower the SQ value, the less spindly the seedlings and the better their chance of survival and growth in the field: SQ value > 6 is undesirable as it is a parallel diameter in predicting survival and growth in the field (Roller 1977). In our experiment, SQ was < 6 for all the treatments, including the control treatment (SQ = 4.37). This shows that even without a host plant, S. album seedlings survived in the nursery and promised to survive in the field, because seedlings that do not interact with a host can survive <3 years (Rai 1990). Deepa and Yusuf (2016) reported that sandalwood plants associated with legume hosts have better growth parameters than non-legume hosts. However, Annapurna *et al*. (2006) has identified *M. pudica*, as the best primary pot host for *S. album* seedling growth in terms of height and root collar diameter. However, in the present experiment, sandalwood seedlings with *M. pudica* (T4) had significantly fewer leaves (19), a smaller root collar diameter (4.8 mm), and their height was less (24.4 cm) than those with *C. retusa*. Annapurna *et al*. (2006) used a potting medium containing: sand, soil, compost, rice husk and charcoal in the ratio of 25:15:50:5:5 (v/v) along with supplementary nutrients, whereas in the present experiment the potting mixture was compost: sand: topsoil in the ratio of 1:2:1 (v/v). According to Annapurna *et al*. (2006), the best growth performance of sandalwood seedlings was with *A. sessilis* as a non-legume host. In this experiment, sandalwood seedlings grown with A. sessilis (T2) had significantly fewer leaves (16), shorter height (20.7 cm), and a smaller root collar diameter (4.5 mm) than those grown with *T. erecta* (T3), which is the other non-legume host. This shows that *T. erecta* has a greater capability to supply nutrients to the sandalwood seedlings than *A. Sessilis* at our conditions. According to Radomiljac (1998) sandalwood seedlings (54 days old) introduced to another *Alternanthera* species (*A. nana*) and fostered for 4.5 months in the nursery reached 37.4 cm tall with a collar diameter of 3.9 mm. In the present experiment, SQ values of sandal seedlings grown with the two non-legume host plants were not significantly different ($P \leq 0.05$). Also, sandalwood seedlings grown with the two non-legume pot hosts had more leaves ($P \leq 0.05$) than those on other legume pot hosts except *C. retusa*. Also they had higher seedling height than other legume pot hosts except *M. pudica* and *C. retusa* and significantly smaller collar diameter to *C. retusa* same to *M. pudica* and higher than all

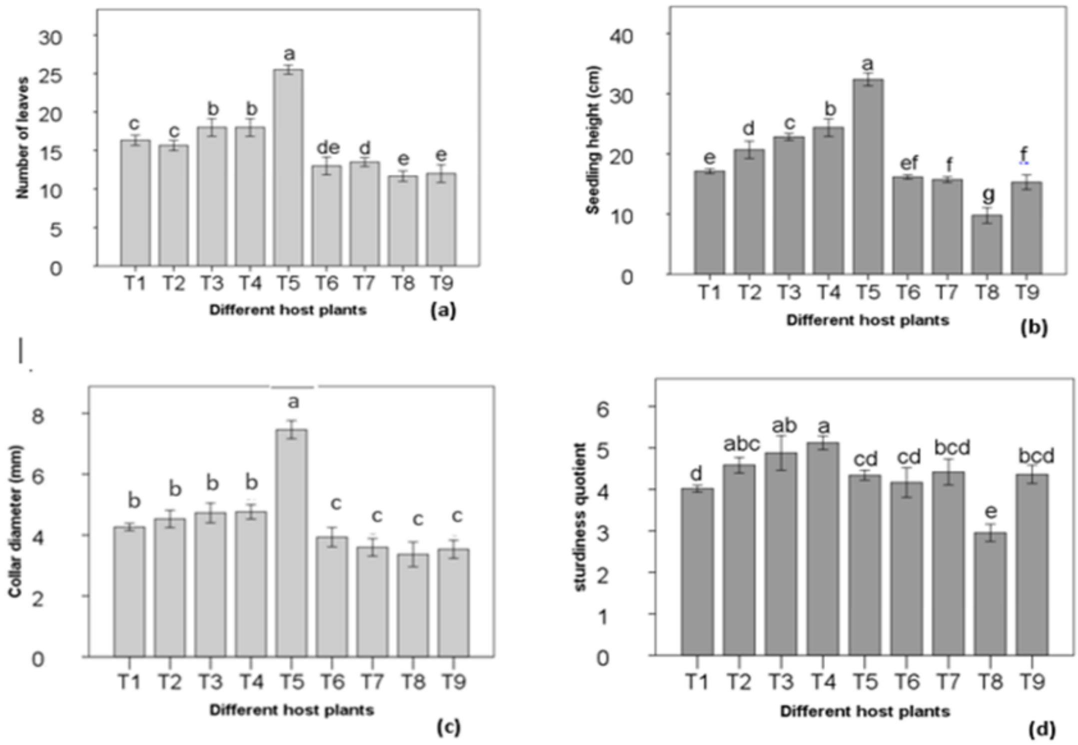

Figure 1: **Effect of different pot host plants on the growth of *S. album* seedlings: the number of leaves (a); seedling height (b); root collar diameter (c); and, calculated sturdiness quotient values for each treatment (d). (T1: *Vigna radiata*, T2: *Alternanthera sessilis*, T3: *Tagetes erecta*, T4: *Mimosa pudica*, T5: *Crotalaria retusa*, T6: *Crotalaria juncea*, T7: *Alysicarpus vaginalis*, T8; *Desmodium triflorum*, T9: Control). *Means followed by the same letter within a figure are not significantly different from each other (P ≤ 0.05).***

other legume pot hosts. Though it is stated that legumes confer more benefits to the parasite, our results suggest that the suitability of pot hosts depends on their efficacy in supplying nutrients and moisture and that legumes are not necessarily superior to non-legumes. This supports the findings of Radomiljac (1998) as he states that *A. nana* which is a non-legume host significantly increased S. album survival, height and diameter over some other legume species. Height differences of *S. album* seedlings and root interactions with some host species are given in plate 1.

Effect of different host plants on root length and number of interactions of *S. album* are displayed in the figure 02.

Successful haustorial development is a key importance for the survival of individual sandalwood trees. Haustorium provides a physical and physiological bridge between the host and the parasite directing resources from the host to the parasite (Tennakoon and Cameron 2006). However, no haustorial interaction was possible with the control treatment. The root length of the sandalwood seedlings in the control treatment (T9) was significantly greater (28 cm) than the root length of seedlings in other treatments. This may be due to the negative effect on the vertical distribution of roots from different host plants. Significantly lowest root length (13 cm) and the most interactions (38) were observed in the seedlings grown with *C. retusa* (T5). Among the non-legume host species used, *A. sessilis* had a significantly lower root length (16 cm)

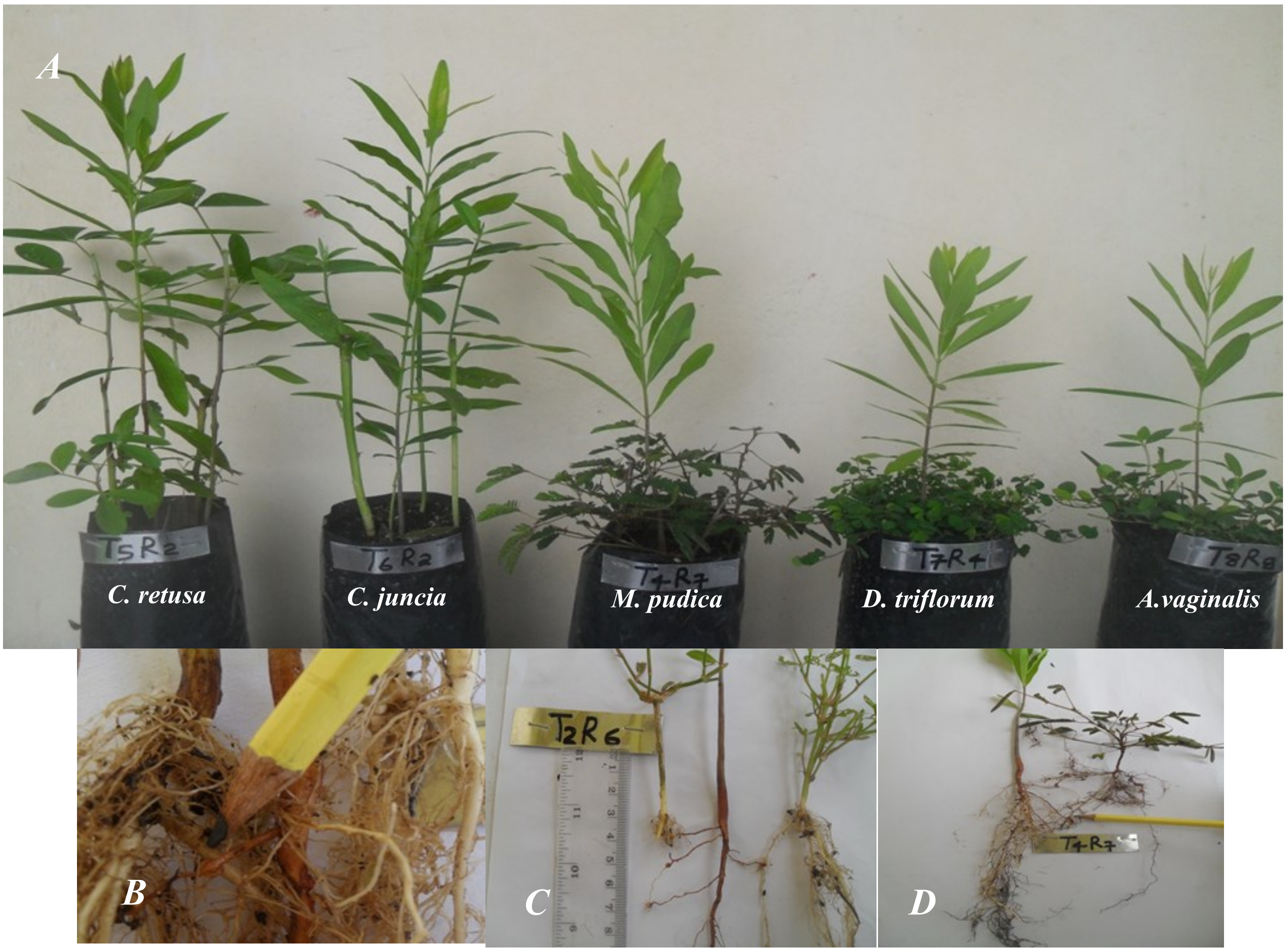

Plate 1: **Height differences of *S. album* seedlings with different pot hosts (a), root interactions of; *S. album* x *C. retusa* (b), S. *album* x *A. sessilis* (c) and *S. album* x *M. pudica***

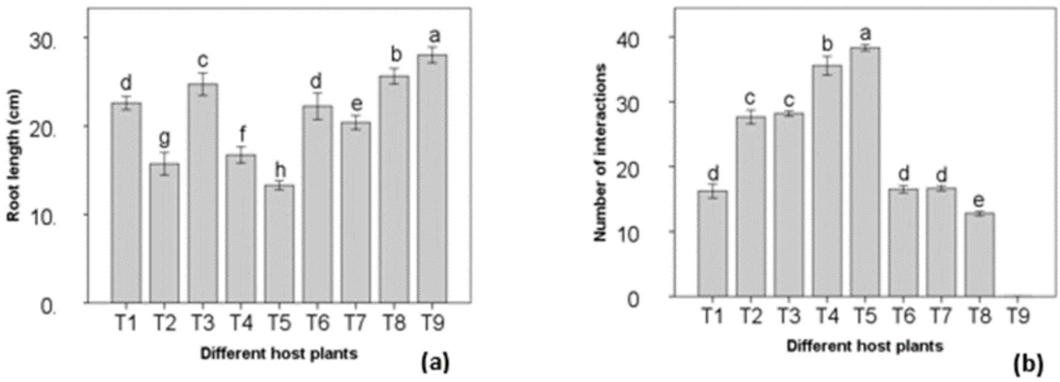

Figure 2 : Effect of different host plants on root length (a) and the number of interactions (b) of *S. album* seedlings (T1: *Vigna radiata*, T2: *Alternanthera sessilis*, T3: *Tagetes erecta*, T4: *Mimosa pudica*, T5: *Crotalaria retusa*, T6: *Crotalaria juncea*, T7: *Alysicarpus vaginalis*, T8; *Desmodium triflorum*, T9: Control). *Means followed by the same letter within a figure are not significantly different from each other (P ≤ 0.05).*

when compared to *T. erecta* (25 cm). The number of interactions was not significantly different for the two host species ($P \leq 0.05$). According to Annapurna *et al*. (2006) maximum number of small size haustoria and effective haustorial connections could be observed with *A. sessilis* while a larger size and the small number of haustoria could be observed with *M. pudica*. However, in contrast to that finding, in this experiment seedlings with host *M. pudica* had significantly ($P \leq 0.05$) more interactions (36) than to *A. sessilis* (28). This variation may be attributed to the effect of the growing media utilized in these two experiments. Further, the number and the size of the haustoria also could be varied with the nature of the host plant root system (Annapurna *et al*. 2006).

S. album seedlings grown with *C. retusa* as the pot host had a significantly ($P \leq 0.05$) greater dry weight of shoots (3.3 g) and whole seedlings (4.4 g). In contrast, Surata (1995) reported that *Alternanthera spp* were the best hosts in terms of the dry weight of sandalwood seedlings among the legume and non-legume species they tested. No significant difference ($P \geq 0.05$) was observed in the root dry weight of sandalwood seedlings grown with all pot host plants. The highest-quality index was observed in the seedlings grown with *C. retusa* (0.58) and

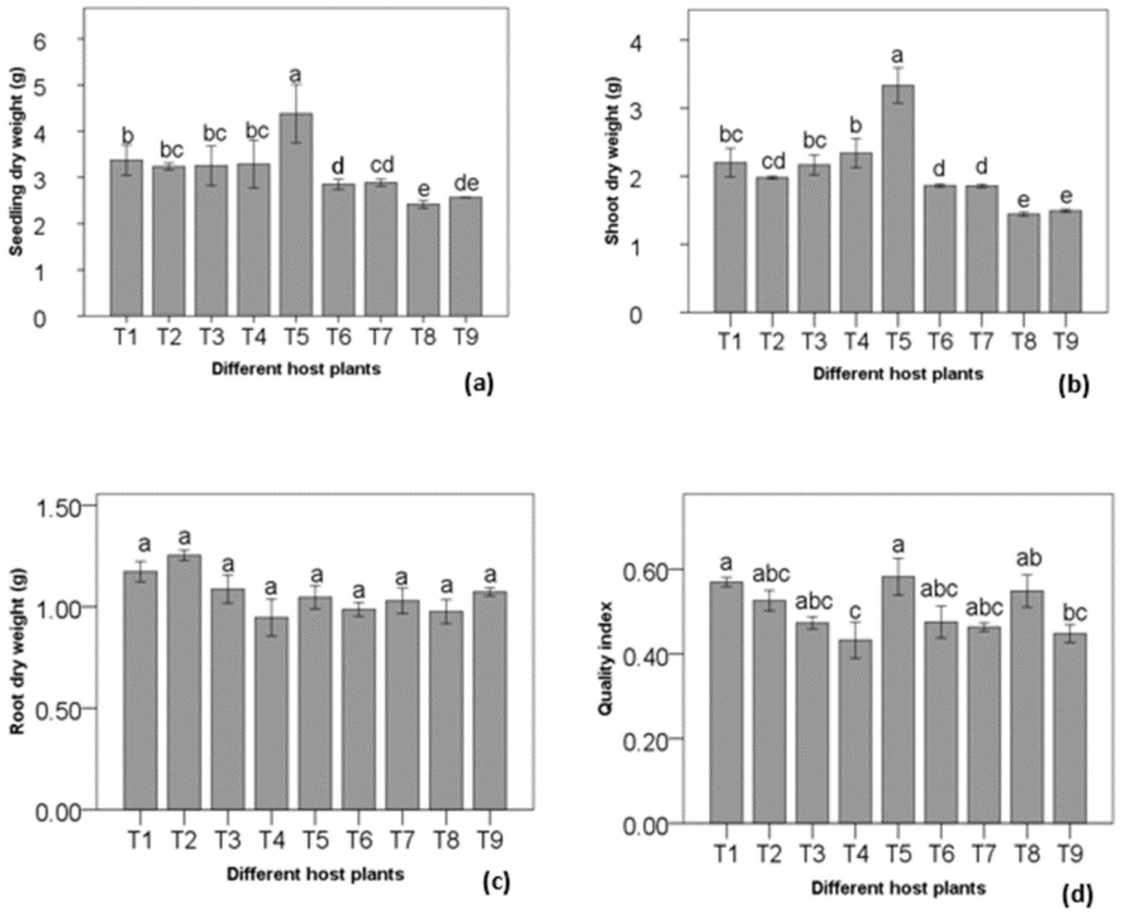

Figure 3: Effect of different host plants on seedling dry weight (a), shoot dry weight (b), root dry weight (c) of *Santalum album* seedlings and calculated quality index (d). (T1: *Vigna radiata*, T2: *Alternanthera sessilis*, T3: *Tagetes erecta*, T4: *Mimosa pudica*, T5: *Crotalaria retusa*, T6: *Crotalaria juncea*, T7: *Alysicarpus vaginalis*, T8; *Desmodium triflorum*, T9: Control). *Means followed by the same letter within a figure are not significantly different from each oth-*

was not significantly ($P \geq 0.05$) different from the seedlings grown with *V. radiata* as the pot host (0.56). A higher value for QI indicates the quality of the seedling (Deepa and Yusuf 2016). According to Ramya *et al* (2010), *Vigna species* can function as superior host species for sandalwood plants. In their experiment, the QI of sandal seedlings grown with *Vigna unguiculata* was reported as 2 ± 0.11. Sandalwood plants grown with legume pot hosts such as *Pongamia pinnata* and *Vigna unguiculata* reported increased quality index and shoot-root ratio (Ramya *et al*. 2010). In addition, *M. pudica* is a legume pot host which increased the QI, total dry weight and shoot: root ratio of sandal seedlings at the nursery stage (Annapurna *et al*. 2006). In the same study, seedling dry weight (3.3 g), shoot dry weight (2.3 g) and quality index (0.43) of the seedlings grown with *M. pudica* (T4) were significantly lower ($P \leq 0.05$) than the seedlings with *C. retusa*.

CONCLUSION

Sandalwood plants require nutritional support from their hosts in order to grow and thrive. Of the nine potential host species for S. album tested, *C. retusa* is the preferred nursery pot host based on all performance measured in this study. Understanding the suitable type of hosts at different stages of the plant growth will sustain the sandalwood cultivations.

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AUTHOR CONTRIBUTION

NN designed the experiment, interpreted data and drafted the manuscript. AMU performed the experiment. HIGK contributed in drafting the manuscript. SS critically revised the manuscript.

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