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Assessment of the early growth of agarwood (*Aquilaria crassna*) seedlings under different sources of nutrients

Kkiu Arunakumara[1], Bc Walpola[1], Chathura Karunatunga[2]*

[1]*Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Matara, Sri Lanka*
[2]*Sadaharitha Plantations Ltd., Colombo, Sri Lanka*

**Corresponding author: kkiuaruna@crop.ruh.ac.lk*

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Abstract: The effect of different sources of nutrients on the growth of *Aquilaria crassna* seedlings was assessed by raising seedlings in polybags treated with inorganic fertilizer (IF), organic fertilizer (OF) and biofertilizer (BF) either alone or in combinations. The pots were established following a completely randomized block design with eight treatments: (T1) soil without IF, OF or BF − control; (T2) soil + IF; (T3) soil + OF; (T4) soil + BF; (T5) soil + IF + OF; (T6) soil + IF + BF; (T7) soil + OF + BF; (T8) soil + IF + OF + BF each replicated five times. Plant height, stem diameter, dry weight of shoots and roots, leaf area and chlorophyll index were measured six months after planting. Soil pH (H_2O) and available soil P content were measured as soil parameters. The best growth performances (54.30 cm, 6.48 mm, 7.10 and 5.92 g·plant^{-1}, 435.33 cm^2 and 58 for plant height, stem diameter, shoot and root dry weight, leaf area and chlorophyll index, respectively) were recorded in T8, which also resulted in the highest available P content (18.96 mg·kg^{-1} soil). The lowest soil pH (H_2O) value (6.02) was recorded in T7 followed by T6 (6.17). The application of IF, OF and BF as a combination could be recommended to promote the growth of *Aquilaria* seedlings.

Keywords: biofertilizer; organic fertilizer; inorganic fertilizer; phosphate solubilizing bacteria

Agarwood, a well-known resinous non-timber forest product, is extracted from the plants of *Aquilaria* spp. and *Gyrinops* spp. of the family *Thymelaeaceae*. On account of the economic value of agarwood, it has become an export commodity as well as a good source of income for the community who are involved in their cultivation (Azren et al. 2019). The high demands of agarwood are primarily due to its wide range of applications in perfume and cosmetics industry, medicinal preparations as well as for religious ritual purposes (Liu et al. 2013). The Middle East region and East Asian countries are reported to be the major consumers of agarwood (Tan et al. 2019).

The production of agarwood is known to be associated with physical damage to the plant stem heartwood, subsequent stress and fungal infections (López-Sampson, Page 2018). As a defensive action, the plant secretes a resin which deposits around the wounds and forms agarwood with some volatile compounds incorporated into it (Subasinghe, Hettiarachchi 2015). Although the infected area becomes dark brown or black (Mohamed et al. 2014) and infected trees apparently exhibit certain symptoms, it is hard to identify such agarwood formation by visual observations.

The species Walla patta of the genus *Gyrinops* is endemic to Sri Lanka (Sri Lankan agarwood) and it is naturally found in some parts of the country. However, the species of the genus *Aquilaria* have not been naturally found in Sri Lanka and there is increasing interest in establishing *Aquilaria* plantations especially those of the species *Aquilaria crassna*. This is largely in response to the high rate

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of decline of the wild population of *Gyrinops* spp. due to excessive harvesting. Therefore the cultivation of *Aquilaria* spp. as a source of agarwood has become popular among local communities and private organizations. However, reliable recommendations on seed propagation, nursery management, field planting, nutrient application, and other management practices are not available yet. Therefore, a scientific approach to the management of *Aquilaria* is of utmost importance.

Adopting optimum nutrient management practices for *Aquilaria* plants in the seedling stage is an essential prerequisite for raising quality seedlings needed for large-scale plantations. Generally, seedlings are maintained at the nursery for about twelve months before field planting. Most of the nursery seedlings are raised in potting mixtures without assessing the nutrient requirements of the plants. A planting medium with sufficient nutrients induces resistance in the seedlings and enables them to withstand the adverse conditions in the field (Srivastava, Behl 2002). Therefore the present study was undertaken to assess the effect of integrated nutrient management practices (organic, inorganic and biofertilizers) on the early growth performance of *Aquilaria crassna* seedlings.

MATERIAL AND METHODS

Study area. The location of the experimental study was the Faculty of Agriculture, University of Ruhuna, Sri Lanka. The area receives an annual rainfall of around 2 500 mm with an annual mean air temperature of 22–30 °C. The distribution of rain is bimodal. These climatic conditions are suitable for agarwood cultivation as stated by Quang and Huu (2011).

Seeds. Healthy seeds of agarwood were collected from well-matured trees grown in Matara district, Sri Lanka. The seeds were soaked in water for about 2 h and then surface-sterilized by shaking in 5% NaClO for 5 minutes. They were thoroughly rinsed twice in sterile distilled water and sown in plastic trays containing sand media sterilized by autoclaving. Trays were kept under the shade net (55% shade net) until the germination was completed.

Soil preparation and planting. The soil used as a potting medium belongs to the Red-Yellow Podzolic great soil group and is classified as Hapludults according to the USDA soil taxonomy (Mapa et al. 1999). The soil was collected from 0–15 cm soil depth at the faculty research farm. The air-dried, crushed and sieved (2-mm sieve) soil was used in analyzing physicochemical characteristics (Table 1). The soil was autoclaved at 121 °C for 20 min to eliminate native microorganisms and exactly 3 kg of the soil were weighed and placed in each sterilized plastic pot (25 cm in diameter, 35 cm in height).

Three-week-old seedlings were transplanted to plastic pots (two plants per pot). They were kept in a polytunnel and with a spacing of 1 m among pots. After 4 weeks, the weak seedling of each pot was removed allowing the remaining plant to grow. Plants were protected from rain and grown under natural light (mean temperature 30 °C day and 25 °C night, relative humidity 16–89%, day length approximately 12 h, maximum light intensity 196 $W \cdot m^{-2}$). They were watered with tap water to a level near the field capacity twice a week. Weed and pest control was carried out manually.

Treatments. The inorganic fertilizer treatment (IF) comprised an application of 5 g of commercially available Yara Mila Unik 16 fertilizer (N : P : K; 16 : 16 : 16) to the base of the seedling immediately after planting and then in 8 and 16 weeks after planting. The organic fertilizer treatment (OF) comprised an application of 13 g of compost (wet basis) to the base of the seedling immediately after planting and then in 8 and 16 weeks after planting. The selected basic properties of OF used in this study

Table 1. Some important physicochemical properties (means ± standard deviation) of soil used in the study

Soil properties	Units	Value
Sand		84 ± 2.46
Silt	(%)	12 ± 1.01
Clay		4 ± 0.73
Soil texture	–	loamy sand
Bulk density	($g \cdot cm^{-3}$)	1.52 ± 0.06
pH	–	6.69 ± 0.41
Organic carbon	(%)	0.85 ± 0.07
Total N		0.15 ± 0.01
NH_4^+– N		82 ± 3.24
NO_3^- – N	($mg \cdot kg^{-1}$ soil)	31 ± 2.25
Available P		4.8 ± 0.84
Available K		118 ± 4.78
CEC	($cmol^{(+)} \cdot kg^{-1}$ soil)	12.1 ± 0.85

CEC – cation exchange capacity

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are shown in Table 2. The biofertilizer treatment (BF) comprised an application of previously isolated phosphate solubilizing bacterial (PSB) suspension (10 mL) with the concentration of 10^5 CFU·mL^{-1} to the base of the seedling immediately after planting and then in 8 and 16 weeks after planting. According to the analysis of the 16S rRNA sequence, the bacterial strain used to prepare BF was identified as *Enterobacter cancerogenus* and the sequences of the strain were deposited in the GenBank nucleotide sequence data library under accession number KX815170 (Walpola, Hettiarachchi 2020).

Compost used as the OF in this study was collected from the faculty farm. Composite samples were taken, crushed into smaller particles by hand pressing, homogenized and passed through a 1-mm sieve before use. The addition of compost was done on the basis of the recommended mulch application rate of 10 tons dry matter per hectare, assuming that top 15 cm of 1 ha of land contains 2.31×10^6 kg of soil (bulk density of the soil 1.522 g·cm^{-3}).

The pots were established as a completely randomized block design with 5 replications per treatment. The experimental plan was based on eight treatments as follows; (T1) soil without IF, OF or BF – control; (T2) soil + IF; (T3) soil + OF; (T4) soil + BF; (T5) soil + IF + OF; (T6) soil + IF + BF; (T7) soil + OF + BF; (T8) soil + IF + OF + BF.

Growth promoting effects of different sources of plant nutrients were assessed by measuring plant height, stem diameter, dry weight of shoots and roots, and P uptake of agarwood plants after six months from planting. Soil pH and available soil P content were measured as soil parameters.

Plant height and stem diameter. Seedling height was measured from the base of the stem to the tip of the main stem (to the base of the terminal bud) using a scale in centimetres (cm). Stem diameter was measured at 3.8 cm above the root collar using a Vernier calliper in millimetres (mm).

Dry matter content of plants. At the end of the experiment, all plants were uprooted, root and shoot portions were separated. The root portion was washed gently over a 2-mm sieve under running tap water to separate them from soil particles. Then both root and shoot portions were air dried for two days. They were then oven dried at 70 °C to a constant weight. The shoot and root dry weight was recorded separately and the average dry weight of plants was expressed in g·plant^{-1}.

Leaf area and chlorophyll index. The total leaf area was measured using a leaf area meter (Delta-T Devices Ltd, China) in square centimetres (cm^2). The chlorophyll index which reflects the relative chlorophyll content was measured using a handheld chlorophyll meter (Konica Minolta SPAD 502 Plus, Japan) with a randomly selected leaf from each seedling.

Soil analysis. The samples of rhizosphere soil were aseptically separated from roots to measure soil pH and phosphorus content. Soil pH was measured in a 1 : 2.5 soil/water suspension with a pH meter. Available phosphorus extracted by the bicarbonate method was determined following the molybdenum blue method (Murphy, Riley 1962).

Statistical analyses. The data generated from the study were subjected to a test of the homogeneity of variance. The Kolmogorov-Smirnov and Shapiro-Wilk tests were employed to test the normality. As the *P*-values were greater than 0.05, the data were assumed to be normally distributed. The data were then used for the analysis of variance (ANOVA) using SAS package (Version 9.1, 2003). Duncan's multiple range test (DMRT) was applied to separate the treatment means (α = 0.05).

Table 2. Selected basic properties (mean ± standard deviation) of OF (compost) used in the study

Properties	Units	Values
pH	–	7.4 ± 0.22
EC	(mS·cm^{-1})	3.3 ± 0.05
Organic carbon	(%)	11.25 ± 0.87
NH_4^+ - N	(mg·kg^{-1} soil)	173 ± 15.4
NO_3^- - N		118 ± 12.3
Available P		38.3 ± 1.52
Available K		453 ± 16.7

OF – organic fertilizer; EC – electrical conductivity

RESULTS AND DISCUSSION

Significantly ($P \leq 0.05$) increased plant height, stem diameter, shoot and root dry weights, leaf area and chlorophyll index of *Aquilaria* plants were recorded in the seedlings which received nutrients through external treatments compared to the control (Tables 3 and 4). Seedlings treated with a combination of IF, OF and BF (T8) showed the significantly best growth performances (54.30 cm, 6.48 mm, 7.10, and 5.92 g·plant^{-1}, 435.33 cm^2, 58

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for plant height, stem diameter, shoot and root dry weight, leaf area and chlorophyll index, respectively) compared to all the other treatments. Seedlings treated with a combination of IF and OF (T5) and combination of IF and BF (T6) recorded the second best growth performances though no significant differences ($P > 0.05$) were found between them. A significant ($P \leq 0.05$) decrease in soil pH (H_2O) was recorded in all the rhizosphere soil samples taken from the pots treated with different sources of nutrients compared to the control. The lowest soil pH (H_2O) value (6.02) was measured in the pots treated with the combination of OF and BF (T7) followed by seedlings treated with the combination of IF and BF (T6: 6.17). No significant ($P > 0.05$) difference in soil pH (H_2O) was found between OF (compost) and BF either alone or combined with IF. A significant ($P \leq 0.05$) increase in available P content was observed in rhizosphere soil samples collected from seedlings treated with different sources of nutrients compared to the control. The highest available P content (18.96 mg·kg^{-1} soil) was recorded in soils of seedlings treated with the combination of IF, OF and BF (T8) followed by treatment T6 with the application of IF and BF (17.86 mg·kg^{-1} soil).

Table 3. Effect of different sources of nutrients on plant height, stem diameter, shoot and root dry weight of *Aquilaria crassna* seedlings

Treatments	Plant height (cm)	Stem diameter (mm)	Shoot dry weight (g·plant^{-1})	Root dry weight (g.plant^{-1})
Soil without IF, OF, BF (T1)	28.07 ± 0.35^{e}	3.03 ± 0.06^{f}	4.22 ± 0.11^{d}	2.01 ± 0.16^{d}
Soil + IF (T2)	38.93 ± 0.56^{c}	5.55 ± 0.08^{c}	5.41 ± 0.25^{c}	3.98 ± 0.12^{b}
Soil + OF (T3)	35.28 ± 0.56^{d}	4.51 ± 0.22^{e}	4.98 ± 0.12^{c}	3.31 ± 0.16^{c}
Soil + BF (T4)	36.47 ± 0.91^{cd}	4.50 ± 0.13^{e}	5.06 ± 0.14^{c}	3.29 ± 0.16^{c}
Soil + IF + OF (T5)	45.18 ± 1.29^{b}	6.06 ± 0.19^{b}	6.14 ± 0.17^{b}	4.49 ± 0.28^{b}
Soil + IF + BF (T6)	44.62 ± 1.26^{b}	6.18 ± 0.20^{ab}	6.22 ± 0.12^{b}	4.48 ± 0.26^{b}
Soil + OF + BF (T7)	43.21 ± 0.66^{b}	4.92 ± 0.08^{d}	5.87 ± 0.09^{b}	4.14 ± 0.28^{b}
Soil + IF + OF + BF (T8)	54.30 ± 1.57^{a}	6.48 ± 0.09^{a}	7.10 ± 0.21^{a}	5.92 ± 0.23^{a}
CV	18.63	21.38	15.42	27.92

$^{a-f}$means with the same letters are not significantly different from each other within each column at α = 0.05 (N = 5); CV – coefficient of variation; IF – inorganic fertilizer; OF – organic fertilizer; BF –biofertilizer

Table 4. Effect of different combinations of nutrients on soil pH, soil available P content, leaf area and chlorophyll index of *Aquilaria crassna* seedlings

Treatments	Soil pH	Soil available P (mg·kg^{-1} soil)	Leaf area (cm^{2})	Chlorophyll index
Soil without IF, OF, BF (T1)	6.69 ± 0.06^{a}	4.78 ± 0.84^{f}	322.67 ± 8.02^{e}	36.67 ± 1.53^{e}
Soil + IF (T2)	6.56 ± 0.02^{a}	15.97 ± 0.57^{de}	375.67 ± 9.02^{bc}	43.33 ± 1.53^{cd}
Soil + OF (T3)	6.20 ± 0.08^{bc}	15.09 ± 0.37^{ef}	350.67 ± 3.51^{d}	39.33 ± 1.53^{de}
Soil + BF (T4)	6.18 ± 0.05^{bc}	16.91 ± 0.28^{bcd}	347.33 ± 5.03^{d}	39.67 ± 2.08^{de}
Soil + IF + OF (T5)	6.21 ± 0.07^{bc}	16.79 ± 0.18^{cd}	389.00 ± 6.56^{b}	48.67 ± 2.08^{bc}
Soil + IF + BF (T6)	6.17 ± 0.06^{bc}	17.86 ± 0.43^{b}	391.33 ± 5.51^{b}	49.33 ± 3.21^{b}
Soil + OF + BF (T7)	6.02 ± 0.18^{c}	17.15 ± 0.41^{bc}	361.00 ± 6.56^{cd}	42.00 ± 1.00^{de}
Soil + IF + OF + BF (T8)	6.30 ± 0.05^{b}	18.96 ± 0.28^{a}	435.33 ± 11.68^{a}	58.00 ± 2.00^{a}
CV	3.56	8.18	9.01	15.41

$^{a-f}$means with the same letters are not significantly different from each other within each column at α = 0.05 (N = 5); CV – coefficient of variation; IF – inorganic fertilizer; OF – organic fertilizer; BF – biofertilizer

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According to the present results, the combined application of IF, OF and BF (T8) favoured the growth of *Aquilaria* seedlings with a significant increase in plant height, stem diameter, dry weight of shoots and roots, leaf area and chlorophyll index. This may be due to the continuous supply of nutrients by the fast-release IF at the initial stages and the supply of nutrients at the later stages ensured by the slow-release OF and BF.

OF (cattle manure, chicken manure, poultry manure, farmyard manure, compost etc.) not only supply essential nutrients to growing plants, but also they increase soil organic matter content and improve soil physical and chemical properties through the decomposition of their substances (Huang et al. 2021). OF are also capable of producing growth regulators and humic acid which facilitate the growth of plants (Han et al. 2016). BF mainly comprise nitrogen fixer, phosphate and potassium solubilizers/mobilizers and plant growth promoters (Elsayed et al. 2020). Phosphate solubilizing microorganisms not only provide phosphorus to plants, but also they could enhance plant growth through several different mechanisms, such as symbiotic nitrogen fixation, production of growth-promoting substance or organic acids, enhancing nutrient uptake and controlling phytopathogenic microorganisms (Rawat et al. 2020). Therefore, the application of OF and/or BF has proved to be an economically viable alternative to the more expensive IF and thus they possess a greater economic utility. The phosphate solubilizing bacterial strain used in this study as BF exhibited the capacity to produce a phytohormone (indoleacetic acid/IAA – personal communications). IAA stimulates and supports the cell elongation and multiplication so that the seedlings have a greater absorption capacity of nutrients, particularly of phosphorus. Therefore, IAA might also have contributed to enhance plant height, stem diameter, shoot and root dry weight, leaf area and chlorophyll index in *Aquilaria* seedlings.

Though the concentrations of nitrogen, phosphorus, and potassium (macroelements) measured in the OF were relatively small, during the decomposition process, it acidifies the soil medium facilitating the dissolution of fixed sources of nutrients such as phosphates which are already available in the soil. Therefore increased plant height, stem diameter, shoot dry weight, chlorophyll index and leaf area recorded in the seedlings treated with OF are possibly due to increased nutrient availability and the improvements in the quality of the soil (Han et al. 2016). Furthermore, it is well known that OF also contain other essential nutrients which were not measured in the present study. Similar growth patterns were reported by Suryanto and Hariyono (2018) after the application of OF (poultry manure) treatment for *Aquilaria malaccensis* L. newly planted seedlings.

Plant roots play an important role in seedling growth through essential functions such as water uptake, nutrient acquisition, and anchoring into the soil system. The changes in the soil microenvironments (e.g., soil bulk density, soil moisture, temperature, fertility, mechanical strength, and soil porosity, etc.) affect the growth of plant roots (Freschet et al. 2017). Greater root dry weight observed in this study with the application of OF (compost) either alone or combined with IF might reflect the effect of organic matter on soil structure improvement by increasing the soil pore spaces and decreasing the soil compaction/bulk density.

Application of IF, OF along with BF (T8) increased the leaf area and chlorophyll index. This might be due to the fact that the crop had better balanced nutrition supply especially of N and K from IF source and P from both IF and BF sources, which enhanced leaf area and chlorophyll index. The present findings are in accordance with Jayachandran (2013), who reported that the application of N, P_2O_5 and K_2O (200, 300 and 200 mg) along with Vermicompost (5 g), Azospirillum (5 g) and phosphobacteria (5 g) increased the leaf area and chlorophyll content of *Aquilaria malaccensis* seedlings.

The increase in soil available P content in BF treatments compared to other treatments might be due to the phosphate solubilization by phosphate solubilizing bacteria through the production of organic acids such as gluconic, succinic, oxalic, citric acid etc. (Walpola et al. 2014). Furthermore, the application of OF and BF to soil could increase density and diversity of microorganisms in the soil and it indirectly assists in increasing phosphorus availability to plants by solubilizing insoluble phosphates (Fankem et al. 2006; Hu et al. 2006). Therefore, combined application of OF and BF containing phosphate solubilizing bacteria could enhance phosphate solubilization which in turn would increase the availability of phosphorus to plants. Therefore, the activation of phosphate solubilizing microorganisms and solubilization of insoluble phosphates through the production

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of organic acids could be expected to occur in soils that are amended with organic fertilizer, which may explain the highest soil available P recorded in the combined application of IF, OF and BF (T8).

With the application of treatments, the pH (H_2O) of the potting media decreases and the reduction is found to be slightly varying (from 6.69 to 6.02), which might be due to the high buffering capacity of soil used in this study. The addition of OF substantially increases the soil organic carbon content (Xu et al. 2006), which ultimately enhances the buffering capacity of soil. The decrease in soil pH (H_2O) (from 6.69 to 6.56) in the potting media which received IF may be attributed to the acid producing nature of nitrogen containing a fertilizer which can release a considerable amount of H^+ ions to the soil through nitrification (Liu et al. 2010; Schroder et al. 2011). The addition of OF and BF also resulted in a decrease in soil pH (H_2O). This reduction was higher with the combined application of OF and BF (T7). The decrease in soil pH (H_2O) by adding OF might be attributed to the production of organic acids and CO_2 during the organic matter decomposition. The organic acid production by phosphate solubilizing microorganisms contributes significantly to the process of acidification and pH reduction of the BF applied rhizosphere soil. These findings are in agreement with Bhat et al. (2020), who observed a similar decrease in soil pH when *Aquilaria agallocha* L. seedlings were treated with IF, OF and BF. Agarwood plants prefer well-drained deep sandy loam soils rich in organic matter. The soil of traditional agarwood growing areas is slightly acidic in many countries which is proved to be preferred by *Aquilaria* plants. The optimal soil pH for *Aquilaria* plants is about 5.6 to 8 (Prastyaningsih, Azwin 2017). This might be due to the fact that mycorrhiza and other beneficial fungi require acid soil for their population build-up (Adhikari et al. 2021).

CONCLUSION

The combined application of IF, OF and BF to promote the growth performance of seedlings was proved to be the best among all the tested treatments. This can be documented in terms of increased plant height, stem diameter, dry weight of shoots and roots, leaf area and chlorophyll index. Therefore, integrated nutrient management could be recommended to enhance the growth of *Aquilaria* seedlings during their early stages. This information could be used for further development of nutrient management practices of *Aquilaria crassna* plants.

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