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Effect of Fertilizer rates and Soil Series on Root Morphological Traits and Root: Shoot Ratio of Immature Natural Rubber (*Hevea brasiliensis*)

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Abstract— Report of this research emphasized the importance of roots morphological traits and root: shoot ratio (RSR) of selected clones of *Hevea brasiliensis* in nutrients utilization for the production of high vigor planting materials. Quantitative traits of root were examined with WinRHIZO image analysis; different from many previous similar studies on natural rubber. The study was conducted to determine the influence of fertilizer rates and soil series on three selected clones of natural rubber. It was observed that the fertilizer rates influenced root morphological traits such as root length, average diameter, surface area and root volume and root: shoot ratio (RSR). The root increases as fertilizer rates increase. Optimum level recommended for growth was 150% (234gm^{-2}). The control had poor performance followed by 50% (78g m^{-2}), and 100% (156g m^{-2}). Holyrood (Ultisols) soil series promotes roots morphological traits when compared to Munchong (Oxisols) soil series. A high significant difference ($p<0.5$) was observed among the clones and RRIM 3001 had the best performance followed by RRIM 2001 while RRIM 2025 recorded least performance. Holyrood soil series (Ultisol) noticeably support root morphological traits of *Hevea* seedlings with fertilizer rate at 150%. Munchong (Oxisol) significayly support root: shoot ratio (RSR) at the same fertilizer rate. Both soils are suggested when considering the interaction between roots and shoot and the overral root morphology of the plant. The fertilizer rate is considered optimum for the rubber seedlings especially where RRIM 3001 is selected as planting material to be planted on any of the soils, Oxisols and Ultisols.

Keywords: soil series, roots morphological traits, root:shoot ratio, *Hevea brasiliensis*, fertilizer rates

1 INTRODUCTION

There are only a few plants that have positively contributed to the modern civilization than this tree *Hevea brasiliensis*. This tree has made an impact in the modern industrial development through transportation and much of its modern products. The tree could be found in the tropics and represents one of the industrial plants that have been continuously domesticated [1]. In the short period of 100 years, no single species of the plant made a remarkable impact globally in the lifestyles of a human being than *Hevea brasiliensis* [2]. Natural rubber originated from a single cultivated species, the para rubber tree (*Hevea brasiliensis*) and widely used as industrial raw materials including tires and non-tyre industries [3]. The increasing demand of natural rubber with the evolution of modern civilization and industrial development is posing a great concern on the rubber producing countries.

Although a motivating scenario for natural rubber was revealed, this will continue until the year 2020 and likely beyond. It was speculated that global consumption will be from 9.9 million tons in 2010 to 12.6 million tons in 2015 and 13.4 million tons in 2018. In addition, world output was forecasted to increase from 9.3 million tons in 2010 to 11.5 million tons in 2015 and 13.2 million tons in 2019 as expected to catch up with the demand [4]. Emanating challenges which may threaten the production are also envisaged such as unpredictable climate, inadequate and cost of skilled manpower, non-availability of required land for expanding rubber cultivation. In addition, [5] stated that Ultisols and Oxisols soils are other factors which have to be considered in rubber cultivation. These soils lack organic matter that could help to supply required essential plant nutrients for plant growth and development.In their work, they noted that these soils are widely used in Malaysia and other topics for rubber cultivation.

In view of this, [6] suggested that fertilizer application should be given priority during vegetative development stage (first six years) because of nursery and young rubber respond well to N.P.K fertilization which contributes to their rapid growth. Meanwhile, the plants need less Mg and Ca as this may result in instability of latex vessel. As those nutrients may cause hazard or positively impact crops based on management practices adopted [9] Furthermore, nutrients uptake in the different agricultural system can be improved with attention on roots characteristics [10]. However, the role of root in water and nutrients uptake cannot be overemphasized because it serves as nutrient uptake. However, the roots surface must be in contact with the soil solution because the nutrients mobility in the soil is a minimal and morphological appropriation of the root properties has to be distributed in such a way that will allow adequate soil nutrients to reach the root surface by their own movement [11]. In addition, it helps in raising quality seedlings and aids biological control of plant diseases through the acquisition of nutrients and water for adequate growth and development [12].

Knowledge of rooting patterns is an important aspect of crop production because it gives background information such as crop combination for a given land use system, efficient use of fertilizer. Several methods could be adopted in determining the impact of roots depending on the objective of the study. Physical methods known as excavation had proved to be reliable because it gives substantial information on root surface area, root length, root volume, the thickness of root e.t.c although it is labor intensive and time-consuming [13]. Despite this, the method has been widely adopted because it helps to

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evaluate the impact of the whole system of root compared to previous methods [14]. On the other hands, fertilizer requirement of crops could be based on the relationship between root and shoot of a particular crop. In addition, [15] observed a relationship between root and shoot which could be best described using root: shoot ratio which is the most acceptable method for estimating root biomass from the more easily measured shoot biomass. Also, noted that root: shoot ratio is preferred and needed because it gives information about effective use of land. The knowledge of root: shoot ratio of Hevea, either budding or seedlings is essential and considered for selecting planting materials to be planted in a particular environment [7]. In addition, Leong et al., (1985) established that success could be achieved in Hevea cultivation with root: shoot ratio RSR and other cultural practices. Therefore, the study was conducted optimum fertilizer rate for the growth of rubber seedlings and point out a well-performed clone in relation to their root morphological traits and root-shoot ratio in different soil series.

2 Materials and Methods

2.1 Trial Site, Treatments and Experimental design

The study was conducted in the winter and summer between at the experimental research field 2 of the Universiti Putra Malaysia, Malaysia under rain shelter of 3^0 02'N latitude, 101^0 42'E longitude, and 31m above sea level altitude. The area has a tropics climate with temperatures between 25.01 °C and 33 °C and frequent rainfall throughout the year with an average monthly relative humidity of 93.4 %. The soils used were Ultisols and Oxisols named as Holyrood and Munchong soil series in Malaysia and widely used for rubber cultivation. Physical and chemical properties characterization of the soils used showed that Munchong soil series comprises of 62.79% clay, 10.89% silt, and 26.21% sand with Cation Exchange Capacity (CEC) 8.1 $cmol^{+}kg^{-1}$ and acidity (pH) of 4.20.

While Holyrood soil series has 67.57% sand, clay 22.39%, and salt 9.93% with Cation Exchange Capacity 4.7 cmol + kg^{-1} (CEC) and an acidity (pH) of 4.40. The experiment was conducted in complete randomized design (CRD) in factorial with three replications arranged side by side. The polyethylene bags used as planting material for the immature rubber were large size 15 x 33cm filled (15kg/bag) with the soil series and arranged side by side according to the treatments. The respective treatments had the equivalent of control no fertilizer F1, 50% F2 (78g m^{-2}), F3 100% (156g m^{-2}), F4 150% (234gm^{-2}), F5 200% (312gm^{-2}). This was in agreement with the recommendations of Industry Smallholder Development Authority (RISDA) with formulation ratio of 10.7%N: 16.6%P205:9.5% K20:2.4%; Mg. RISDA is a company responsible for the supply of fertilizer to the smallholders in Malaysia. The seedlings used were RRIM 2001, RRIM 2025 and RRIM 3001 rubber clones among latex timber clones (LTC) collected from Malaysia Rubber Board and widely planted in Malaysia. Fertilizer application was carried out at four weeks interval. Manual irrigation was adopted at every other day and weeding was carried as at when due.

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2.2 Root Scanning

At the end of the experiment, after the last harvest, root morphological traits were examined. Roots were gently separated from the soil and polybag, washed thoroughly with water to remove excess soil. Root samples were scanned using Win-RHIZO pro software (Regent Instrument Inc.). Parameters measured in all the treatments were total length, average diameter, surface area and total root volume.

2.3 Measurment of root: shoot ratio (RSR)

Samples of roots, stems and leaves were collected, kept in brown paper and oven dried at 70°C for 48 hours. In order to calculate the ratio between roots and shoots of the plants, weight of each of the dried samples were determined with digital weighing balance and weighed (g) to constant weight. Root/shoot ratio was also determined using the following equation [16] Root to shoot ratio = (Total Root Dry Weight/Total Shoot Dry Weight).

3 Statistical analysis

The study was conducted in complete randomized design (CRD) in factorial with three replications. Data were analyzed with Analysis of variance (ANOVA) using Statistical Analysis System SAS (Version 9.1) Duncan's multiple range tests at $p<0.05$ was employed for mean comparison.

4 Results

4.1 Root morphological traits

Roots morphological traits of different clones using image analysis machine showed the growth and yield response in relation to soil series and fertilizer rates (Table 1). The growth performance of RRIM 2001, 22025 and RRIM 3001 regarding the root length, surface area, average diameter and root volume were significantly different. The values recorded in the respective clones RRIM 2001, RRIM2025 and RRIM 3001 in terms root length were 124.8, 109.2 and 154.6 and surface area 58.4, 52.6, and 74.2 respectively. However, RRIM 3001 performed better when compared to other clones. This may be attributed to its traits as a latest clone developed by Rubber Research Institute of Malaysia followed by RRIM 2001 while RRIM 2025 recorded worst performance.

In addition, performance of RRIM 2025 is yet to be fully established and still under evaluation different micro – climate, soils and environment by Malaysia Rubber Board. Similarly, the results of average diameter and root volume showed

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Table 1: Root morphological traits of different clones

Clones	Root Length (cm^2)	Average Diameter (mm)	Surface area (m^{-2})	Roots volume (cm^3)
RRIM 2001	124.8b	2.191b	58.4c	22.6b
RRIM 2025	109.2c	1.861c	52.6b	20.5c
RRIM 3001	154.6a	2.543a	74.2a	28.3a

Means with the same letter are not significantly different at ($p < 0.05$, ANOVA)

Table 2: Effect of soil series on the roots morphological traits

Soil series	Root length (cm^2)	Ave diameter mm)	Surface area (m^{-2})	Roots volume (cm^3)
Munchong	125.7b	1.976b	59.8b	22.6b
Holyrood	133.4a	2.421a	63.6a	25.0a

that RRIM 2025 had a poor performance compared to other clones with values of 1.861mm which may be as a result of the established factors relating to soil and environment as mentioned earlier that the clone is still under evaluation. However, RRIM 2001 performed better with 2.191mm while RRIM 3001 recorded 2.543mm and had the best growth and significantly different in terms of root volume when compared to other clones with 28.3mm followed by RRIM 2001 22.6mm while RRIM 2025 recorded the least performance 20.5mm. Roots morphological properties in soil series (Table 2) showed the performance of soil type in relation to roots growth of *Hevea*. In this regards, Holyrood soil series showed a significant deferent in all the morphological traits measured when compared to Munchong soil series which may be due to a high clay content in Munchong series which causes compaction and inhibition of roots movement. Roots morphological traits were significantly increased and catch up with fertilizer rates on both soils (Table 3 and 4). However, fertilizer rates on Holyrood series made a more positive impact and aids plant growth when compared plants in Munchong series in terms of total roots length and other parameters taken with image roots analysis. This was also being observed in the increment in the number of leaves.

Generally, roots parameters measured were significantly low on treatments without fertilizer on both soils. Furthermore, indications from total root length which ranges in the following treatments F1 73.647cm², F2 76.543cm² and F3 88.027cm² in Munchong soil series showed that there were no significant differences among the fertilizer rates. In terms of an average diameter, it also appeared no significant differences between fertilizer rates of F2 1.49833mm and F3 1.62667mm in the same soil. This may be as a result of environmental factors and other cultural practices. In the same vein, the roots length on Holyrood soil series appeared no significant differences among fertilizer rates F1 89.93cm² and F2 117.7cm² also between F4 234.43cm² and F5 207.61cm² which may still be attributed to the above mentioned factors. Similarly, there were no significant differences among F3 61.387cm² and F4 70.377cm² in total roots surface area. In this study, 150% (234gm^{-2}) fertilizer rate is recommended as optimum required for healthy root growth for the crop maximum yield because 200% (312gm^{-2}) appeared to be too high for the plant due to undesirable visual symptoms such as yellowish of the leaves and small quantities of roots obtained after application of this rate which may be as result of high nitrogen content and other growing factors. There was a significant different in the treatments with 150% and 200% and the former rate performed better than the later throughout the period of the study. This shows that 150% could be best adopted as fertilizer level required for rubber cultivation in the nursery and by extension in the estate. Furthermore, all the treatments without fertilizer (control) recorded the worst performance. This shows that the plant cannot survive without adequate fertilization. However, most of the soils in the tropics like Malaysia require fertilizer for crop maximum growth and development.

Table 3: Effect of fertilizer rates on roots morphological traits of different clones in Holyrood (Ultisols) soil series.

Treatments	Root Length (cm^2)	Ave diameter (mm)	Surface Area (m^{-2})	Root Volume (cm^3)
F_1 0%	89.93c	1.70833e	45.818d	25.362bc
F_2 50%	117.77c	2.03833d	61.387c	22.362c
F_3 100%	155.38b	2.52500c	70.377c	27.108b
F_4 150%	234.43a	3.90667a	105.293a	37.462a
F_5 200%	207.61a	3.15333b	87.900b	32.827ab

Means with the same letter are not significantly different at ($p < 0.05$, ANOVA)

Table 4: Effect of fertilizer rates on roots morphological traits of different clones' rubberplanted in Munchong (Oxisols) soil series**.**

Treatments	Root length (cm^2)	Ave Diameter (mm)	Surface Area (m^{-2})	Root volume (cm^3)
F_1 0%	73.647c	1.26833d	34.782b	11.322b
F_2 50%	76.543c	1.49833c	37.973b	15.922ab
F_3 100%	88.027c	1.62667c	47.302ab	21.448ab
F_4 150%	144.580a	2.39500a	57.457a	28.550a
F_5 200%	107.587b	1.86333b	57.412a	28.362a

5 Influence of Fertilizer Rates on Root: Shoot Ratio of Rubber Clones

Root: shoot ratio (RSR) of plants are quantitative measurement of plant tissues, it involves monitoring overall health of plant which reflect in both growth and yield of present plant. This includes (stem and leaves) as above ground and (dry roots) as bellow ground. It was observed from the study that Munchong soil series adequately support root: shoot ratio and plant performed better when compared to Holyrood soil series. Also, Fertilizer rates improve the plant health and support growth of plant tissues especially at F4 150%. This showed the toxicity level and optimum required because there was a declined in growth of plant tissues when F5 200% was applied. For instance, on Munchong soil series (Fig. 1), RRIM 3001 which was found to perform best recorded the highest values 6.4 at F4 (150%) followed by (F5 200%) 5.07, F2 50% 4.5 and F3 100% 4.9 while (F1 0%) had the lowest value 3.4. Similarly, RRIM2001 recorded highest values 5.1 at F4 (150%) optimum level followed by (F5 200%) 4.5. However, (F2 50%) and F3 100% had low values and found to be insufficient while F1 (0%) without fertilizer recorded the lowest values and tissues obtained were relatively small. Furthermore, RRIM 2025 poorly performed and had the lowest values when compared with different fertilizer rates to other clones.

The highest values were recorded at F4 with 3.1 followed by F5 (200%) 3.09, (F2 50%) 2.04 and (F3 100%) 3.06 also had lowest values and proves to be insufficient for plant growth. However, without fertilizer control recorded the lowest value (1.02). On the other hands, response of plant on Holyrood soil series (Fig. 2) varies and the values obtained were generally low when compared to those on Munchong soil series. Though, RRIM 3001 performed better. For instance at (F4 150%) which was found to be optimum is 5.1 followed by (F5 200%) 4.4 and F2 and F3 had 3.2 and 4.02 respectively. In the same vein, (F1 0%) had the lowest value 3.07. Also, on RRIM 2001 highest value were recorded at (F4 150%) 4.1 followed by (F5 200%) 3.01 while low values were recorded in F2 2.3 and F3 2.7. Similarly, poor performance and lowest value 2.01 was recorded in plants without fertilizer. Furthermore, RRIM 2025 at (F4 150%) 2.3 followed by (F5 200%) 1.7 low values were recorded with (F2 50%) 1.4 and (F 3 100%) 1.5 while, treatment with (F1 0%) poorly performed and had the lowest value 1.0.

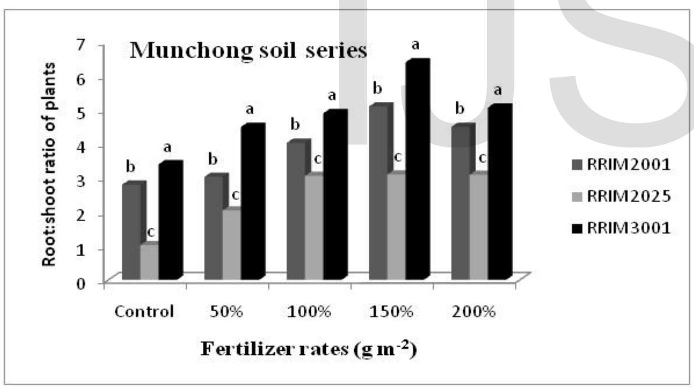

Fig. 1: Effect of fertilizer rates on different clones of *Hevea* in Munchong (Oxisols) soil series

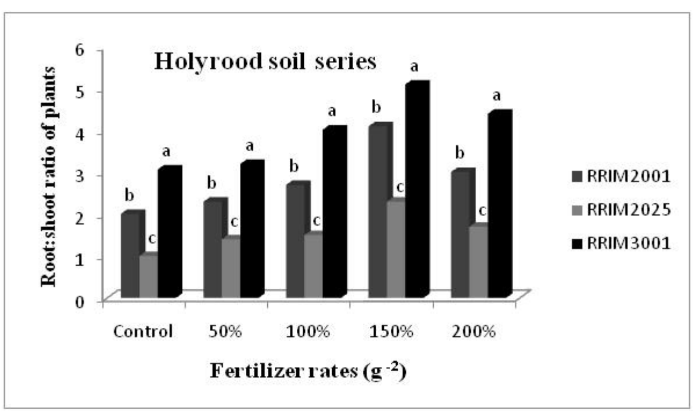

Fig.2: Effect of fertilizer rates on different clones of *Hevea* in Holyrood (Ultisols) soil series.

6 Discussion

Root growth could be used as one of the parameters to measure the rate at which plant absorbed nutrients and by extension quantify the plants growth which has been recognised as part of the factors that may affect crop yield [17].

However, [18] noted that compaction due to the nature of the soil such as Munchong (Oxisols) could alter root growth and reduce the free movement of water and nutrient into other parts of the plant and in turn reduce growth and development of the plants. On the other hands, the performance of Holyrood soil series (Ultisols) could be attributed to the lower moisture retention and better aeration [19]. This however, made the soil performed better than Munchong soil series (Table 2).

Furthermore, image analysis machine has been recognised as one of the most reliable machines which give precise measurement of root morphological parameters [20]. The primary determinants of root's ability to acquire water and nutrients are root length and surface area [21]. However, noted in their work that small roots efficiently utilize carbon to collect underground resources than large roots diameter (Table 1 and 2). Efficient land use requires adequate attention on roots systems for nutrients utilization of most field crops [22]

Furthermore, observed that root length and surface area can be relied upon as an important indicator for potential uptake of nutrients from the soil. Total root length and surface area can influence the nutrient uptake and contribute to the growth performance of crops planted in an area of a given land [23] In addition, decrease in the root:shoot ratio shows nutrients deficiency symptoms in the plants. Apparently when the nutrients supply is localized in the soil; it will have positive effect on the root branch and vehemently change the fineness of a root system ranging from the increase in root length and other root patterns [24]. Different studies showed that fertilizer with high P tends to increase plant biomass especially root length of different plants species [25]. Other factors which may affect activities of root and shoot are controlled osmotic and specific nitrate. This may negatively affect rooting system and favour shoot growth system [26]. Generally, the soils used for rubber in south East Asia and other tropics negatively affect root and shoot because it is highly weathered with low organic carbon [27].

As a result, root:shoot ratio would be automatically decreased in any affected plants. This shows that a wide range of factors may affect root and shoots in a particular crop. For instance inadequate water may hinder plant nutrients acquisition in root zones and this may result in poor transportation to the shoot [28]. In addition, capability of the soil in absorbing water could be ultimately achieved through a higher root length and volume [29]. Moreover, function of the root systems of any crop could be best assessed using average diameter [30]. Although, the mass flow and diffusion which helps in the transportation of nutrients to roots largely depend on so many factors such as nutrients concentration and soil moisture in the planting area [31].

Furthermore, assimilation of inorganic fertilizer by root and shoot may be affected by elevated CO_2 concentrations and frequent rainfall. This can only be adjusted by global change such as adequate supply of nutrients and C to the root ecosystem [32]. Adequate fertilizer for any crop planted in the tropics is required because the area is dominated by Ultisols and Oxisols soils which lack organic matter that could complement essential plant nutrients for plant growth. However, [34] in their previous work using RISDA fertilizer observed that 200% ($312gm^{-2}$) of the rates appeared to be high and detrimental to the plant due to high nitrogen content in the formulation which causes scorching and other undesirable visual symptoms of the plant.

Munchong (Oxisols) and Holyrood (Ultisols) soils occupies about 24 million ha or 72% of the total land area in Malaysia, and possess low Cation Exchange Capacity C.E.C, high aluminium and devoid of most essential plant nutrients. Effective fertilizer management must be given priority [35]. Nonetheless, these two soil types has been adopted and widely used in Malaysia for rubber cultivation [36]. Thus, a balanced fertilizer recommendation with any suitable soil series adopted in the tropics will help in the production of high vigour planting materials of *Hevea brasiliensis*.

7 CONCLUSIONS

The root morphological traits and root:shoot ratio of *Hevea* are essential in order to ensure adequate absorption of plant nutrients for plant growth and yield especially in tropical countries like Malaysia where most of the planting areas are predominantly Ultisols and Oxisols. Increase in the length of roots and other morphological traits except average diameter were achieved on both soil series with optimum fertilizer levels at 150% ($156g\ m^{-2}$) of fertilizer rates of Rubber Industry Smallholders Development Authority (RISDA1) recommendation. However, 200% appeared to be high for the plants growth which resulted in slow growth and detrimental to the plants. In the same vein, 50% and 100% are not suitable for the plants while the plants experienced retarded growth without fertilizer. The plants also perform well while considering root:shoot. Munchong soil series support the root:shoot of the treatments with the same observed fertilizer rate. In addition, Holyrood soil series promotes root growth compared to Munchong soil series which suggest that planting materials could be successfully raised in polythene bags with Holyrood soil series because it is well drained and allowed free movement of roots. Furthermore, there was a significant difference among the clones while RRIM 3001 performed better compared to RRIM 2001 and RRIM 2025 on both soils and fertilizer levels. Moreso, economic loss could be avoided in natural rubber cultivation with the adoption of this clone RRIM 3001 where these soils are found in Malaysia and other tropical countries in the world with balanced fertilizer recommendation.

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