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RESEARCH ARTICLE



Combination of exploitation systems to optimize yield of GT1 clone in smallholder rubber (Hevea brasiliensis) plantation in Sumatera Utara, Indonesia

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Abstract

Clone GT1 is one of the low metabolism clones and is recommended for smallholder and large plantations. These clones are responsive to stimulants and reach production peak at the mid-economic cycle period. Its production can reach 2200-3000 kg⁻¹ha⁻¹year⁻¹ and is relatively more resistant to high exploitation pressures if the agroecosystem conditions are suitable. This study aimed to determine the effect of the combined application of liquid stimulant concentration and tapping interval on the physiology and productivity of latex in rubber clone GT1. This research was conducted at Naga Rejo Village, Galang District, Regency of Deli Serdang, Province of Sumatera Utara, located at an altitude of 49 masl with Ultisol soil type. The research design is factorial randomized completed block design (RCBD) consisting of 9 levels, namely S2d4 without a stimulant, S2d4 ET 3.5%, S2d4 ET 4.5%, S2d5 without a stimulant, S2d5 ET 3.5%, S2d5 ET 4.5%, S2d6 without a stimulant, S2d6 ET 3.5%, S2d6 ET 4.5 % with three replicas of each treatment. The results showed that the tapping system on clone GT1 obtained the highest annual production with tapping system S2d4 ET 4.5% (DS_3) of 214.51 g/t/y. The lowest production is obtained in January-March when the leaves fall. High sucrose levels in the non-stimulant treatment (8.54 -12.98 mM) indicated that at least sucrose was converted into latex. The pH of latex is directly proportional to the stimulant, so an increase in the concentration of the stimulant raises the pH and metabolism of latex formation.

Keywords

Hevea brasiliensis; slow starter; tapping interval; stimulant concentration; latex physiology; latex yield

Introduction

Indonesia is one of the main natural rubber producers in the world, with the largest land area of 3.6 million hectares, consisting of smallholding plantations of 3.1 million hectares (85%), private companies at 8%, and state -owned companies at 7%. The value of rubber exports for the last five years reached USD 3.24 billion. The productivity of smallholding plantations in Indonesia was an average of 1.025 kg/ha/year; state-owned companies yielded around 1,379 kg/ha/year and private companies yielded 1,542 kg/ha/ year (1). Compared to smallholding plantations in other producing countries such as Malaysia with 1,100 kg/ha/year, Thailand with 1,600 kg/ha/year, India with 1,334 kg/ha/year, and Vietnam with 1,358 kg/ha/year, the productivity

of rubber in smallholder plantation in Indonesia is far lower (2). The main issues of Indonesian smallholder rubber include the limited use of clonal planting materials, minimum upkeep, and rubber plants that are mainly old or damaged, leading to low productivity.

The problem of smallholder rubber was also affected by high production costs, especially tapping costs, amidst low rubber prices (3). Tapping is the largest cost component, accounting for 40-60% of total costs. The farmers usually apply high tapping intensity to increase revenue; however, due to their limited knowledge of clonal typology and harvesting system, the high tapping intensity induces Tapping Panel Dryness (TPD) leading to a significant yield drop.

Tapping costs can be reduced by lowering tapping frequency and the use of stimulants (4). A study (5, 6) suggested that decreasing the tapping frequency from d3 to d4 can increase the yield. Other works (7, 8) showed that d6 tapping frequency reduced costs by 58-59% compared to control (d2); while d3 reduced costs by 25-28%, it produced the highest yield compared to other tapping frequencies. A low Tapping Frequency (LTF) system is applied by reducing tapping frequency with high ethephon stimulation intensity as the compensation. This tapping system has several advantages, including low labour cost and a longer rubber lifespan (9, 10).

Tapping power is the largest component in production costs, which accounts for 30-40%. Efficiency requires companies to reduce production costs, especially in tappers, and tappers must be able to increase yields. The use of wholesale labour on young plants is feared to be less controlled for taps' quality and the bark's consumption. In addition, competition with other commodities in terms of manpower also causes the commitment of tappers to decrease, resulting in high numbers of empty hancas. The conditions in the field are such that there are still many rubber plantations that rely on the d3 frequency to explore production. The frequency of tapping every three days (d3) requires more manpower than what is available. Therefore, the low-frequency tapping approach with a certain treatment is expected to solve the problem of high cost and a limited tapping power. The results of previous research (11), with a low tapping frequency (up to d7/weekly tapping) showed that the cumulative production gain was not significantly different from the tapping frequency of d3, even d2. The research results of another study showed that the frequency of tapping d4 in clone PB 260 resulted in higher g/p/s than the d3 system (5). Still, cumulative production (kg/ha/year) decreased by about 10% due to fewer tapping days in the d4 system. So further testing is needed with various clones and tapping frequencies as well as stimulant applications. In addition to reducing energy requirements, low frequency tapping (LFT) system can reduce production costs. Still, production achievement is relatively the same compared to the d3 frequency tapping system. Tapping costs can be reduced by 60% using LFT d6 compared to d2 tapping (7).

Ethephon stimulation is widely used in rubber

plantations as part of the harvesting system. The concentration and the intensity of ethephon stimulation play important roles in obtaining high yield; the stimulation regime should be adjusted according to the rubber clonal typology (12). Ethephon is a compound that contains an active ingredient called 2-chloroethyl phosphonic acid, which plays a role in the process of releasing ethylene. In its physiological process, it is rapidly hydrolysed into ethylene, hydrochloric acid, and phosphoric acid (13). The effect of ethephon stimulation includes an increase in osmotic and turgor pressures and a decrease in the plugging index leading to a prolonged latex flow (14). According to a study (15), several factors influence the effects of ethephon stimulation on yield, including the type of clone, plant age, method, dose, and nutrient status of the plant. Over-stimulation might induce disturbances in metabolic processes leading to bark thickening, necrosis, and bark cracking (16).

Clone GT1 is one of the common clones planted by rubber smallholders and is very responsive to stimulants. To identify the best combination of Low Tapping Frequency (LTF) and ethephon stimulation, it is necessary to carry out a study on the application of various ethephon concentrations and low tapping frequencies in this clone. The result of this study will help smallholding farmers optimize their rubber plants.

Materials and Methods

Experimental Sites and Planting materials

The study was conducted at a smallholder rubber plantation in Naga Rejo Village, Deli Serdang Regency, Sumatera Utara Province, Indonesia (3°29'20" N, 98°52'04" E). The location is one of the centers of smallholder rubber plantations in North Sumatra. The experimental site is flat, 49 m above sea level of altitude, and belongs to Ultisol soil type. During the experiment, the average rainfall was 167.64 mm/month with an average of around 14 rainy days per month. The observation was performed from January to July 2021 on the 22-year-old GT1 rubber clone planted in 2.5 m x 5 m planting space (800 trees/ha).

Experimental Design

The experiment was arranged based on Randomized Completed Block Design (RCBD). Two factors were tested in this study i.e., tapping frequency, consisting of d4, d5, and d6 (tapped every four, five, and six days, respectively) and ethephon concentration, consisting of 3.5% and 4.5%, with no stimulation as the control. The description of the treatment notation used in this experiment is presented in Table 1. Each treatment used 30 sample trees with three replications. The sample trees were selected with a girth of 65 - 70 cm, measured at 100 cm from the soil surface.

Tapping and Ethephon Stimulation

The downward half spiral tapping (S2) was applied on renewed bark (BI-2). The ethephon stimulation was carried out every 15 days using the groove application method (Ga) with water as a carrier (Fig. 1). During the defoliation period (February-March), the ethephon stimulation was stopped and continued in March. The parameters assessed

	- Tapping notation	Description					
Treatment		Cut length	Tapping frequency	Ethephon concentration	Stimulation frequency		
DS_1	S2 d4	Half spiral	Tapped every 4 days	without stimulation	-		
DS_2	S2 d4 ET3.5%	Half spiral	Tapped every 4 days	Ethephon 3.5%	15 days		
DS ₃	S2 d4 ET4.5 %	Half spiral	Tapped every 4 days	Ethephon 4.5%	15 days		
DS ₄	S2 d5	Half spiral	Tapped every 5 days	without stimulation	-		
DS_5	S2 d5 ET3.5%	Half spiral	Tapped every 5 days	Ethephon 3.5%	15 days		
DS_6	S2 d5 ET4.5 %	Half spiral	Tapped every 5 days	Ethephon 4.5%	15 days		
DS ₇	S2 d6	Half spiral	Tapped every 6 days	without stimulation	-		
DS_8	S2 d6 ET3.5%	Half spiral	Tapped every 6 days	Ethephon 3.5%	15 days		
DS ₉	S2 d6 ET4.5%	Half spiral	Tapped every 6 days	Ethephon 4.5%	15 days		



Fig. 1. Ethephon stimulation on BI-2 panel

in this experiment included yield, plugging index (PI), sucrose content, inorganic phosphorus content (Pi), and thiols content.

Yield and Plugging Index Measurement

The yield was collected in the form of a cup lump one day after the tapping. The yield data presented in this paper is dry rubber yield per tree per tapping (g/t/t). It was calculated by multiplying the fresh cup lump weight with the dry rubber content (DRC) (equation 1). For DRC measurement, a 150 g fresh cup lump was pressed to remove water and obtain \pm 2 mm uniform sheet followed by a 60°C oven-dried for 8 hours. The DRC is the ratio between the sheet dry weight and the fresh cup lump sample (Equation 2).

 $GTT = \frac{\frac{dry \text{ weight (g)}}{total \text{ yield sample tree (t)}}}{\text{total tapping day (t)}} \dots \dots (Equation 1)$

 $DRC = \frac{\text{wetweight}}{\text{dry weight}} \times 100\%$ (Equation 2)

The plugging index (PI) was observed every month. The PI data presented in this paper is the average of a seven months observation. The PI was calculated by dividing the initial flow rate (IF) by the total fresh latex volume of the associated tree (17). The IF is defined as the average of latex volume per minute in the first 10 minutes after tapping. Latex pH was observed using litmus paper following the protocol of (18).

Latex Physiological Assessment

The latex physiological assessment was performed at the end of January and June. Latex serum for sucrose, Pi, and thiols content determination was obtained by adding 1 mL fresh latex with 9 ml of 2.5% trichloroacetic acid (TCA); clear serum was obtained by removing the coagulated rubber. Sucrose content determination was performed according to (19) protocol. Samples serum, 150 μ L in volume, was diluted with 2.5% TCA to reach a total volume of 500 μ L. The solution was then reacted with a 3 mL anthrone reagent. Following 15 minutes of submerging in boiling water and rapid cooling treatment, the absorbance was measured at λ 627 nm using Beckman DU 650 spectrophotometers (Beckman Coulter, Brea, California, USA).

The Pi was measured based on the method of (20). The diluted serum, 100 μ L in volume, was added to Ferrosulphate (FeSO₄) solution and incubated for 5 minutes at ambient temperature. The absorbance measurements were carried out at λ 750 nm. The thiol content measurement was performed following (21) protocol. Latex serum (1.5 mL) was added by 2.5% TCA to reach a total volume of 1.5 mL. The solution was then reacted with 75 μ L of 10 mM 5.5'-dithiobis 2-nitrobenzoic acid (DTNB) and 1.5 mL of Tris buffer and incubated at ambient temperature for 30 minutes. The absorbance was assessed at λ 412 nm.

Data analysis

Data was analyzed using the Statistical Analysis System (SAS) Software version 9.1 (SAS Institute, Cary, North Carolina, USA). The Analysis of Variance (ANOVA) was performed for all parameters, followed by Duncan's Multiple Range Tests (DMRT) at $\alpha = 0.05$.

Results and Discussion

Six Days Tapping with High Ethephon Stimulation Gave High Yield but Might not be Suitable for Smallholder Farmer

The monthly yield of each treatment is presented in Table 2. The combination of tapping frequency and ethephon concentration significantly affected latex production in January, April, May, June, and July, while in February and March, no significant difference among treatments was encountered. In January, the highest yield (2.42 g/t/t) was found in DS₈ (S2d6 ET3.5%), not significantly different from DS₇ (S2d6 without stimulation), which reached 2.17 g/t/t of yield. This month, defoliation had not occurred, and the trees were relatively in full-canopy condition.

Table 2. Monthly yield of each treatment from January to July.

more adaptability to various agroecosystems (23). A study showed that clone GT1 has a higher leaf area index and yield than RRIC 100, BPM 24, and PB 260 clones in the leaf fall period (22). Therefore, this clone is likely suitable for a smallholder farmer to avoid significant yield drops during leaf fall.

The yield increased from April to July as the ethephon stimulation resumed. The highest yield in April was found in treatment DS_8 and DS_9 (d6 tapping with ET3.5% and 4.5%, respectively) with a similar yield of 4.62 g/t/t. The DS₉ showed the highest yield in May, June, and July compared to others treatments, with 4.77 g/t/t, 4.77 g/t/t, and 4.80 g/t/t, respectively. The lowest yield was DS₄ with 1.30 g/t/t, 1.33 g/t/t, and 1.32 g/t/t, respectively, not significantly different from DS₁. The increase in yield compared to which leaf fall period was supported by active photosynthetic activity as new leaves have fully developed. Carbohydrate substrate is required as an energy source and raw material for latex biosynthesis. These results indicate that the stimulation significantly impacts the yield by taking advantage of carbohydrate conservation in a low tapping frequency system.

Tuestantent	Yield (g/t/t)						Annual yield		
Treatment	January	February	March	April	Мау	June	July	Average	(g/t/y) (%)
DS_1	1.52 cd	1.63 a	1.10 a	1.30 e	1.47 de	1.52 d	1.32 e	1.41	121.14 (100)
DS_2	1.38 cd	1.42 a	1.17 a	3.15 c	2.92 c	3.57 c	3.12 c	2.39	205.54 (170)
DS₃	1.89 bc	1.83 a	1.23 a	3.08 c	2.85 c	3.53 c	3.05 c	2.49	214.51 (177)
DS_4	1.65 cd	1.63 a	1.20 a	1.78 de	1.30 e	1.33 d	1.32 e	1.46	99.18 (82)
DS₅	0.74 e	1.25 a	1.18 a	3.93 b	3.70 b	3.63 bc	3.62 bc	2.58	175.34 (145)
DS_6	1.33 d	1.40 a	1.25 a	4.20 ab	4.32 ab	4.32 ab	4.28 ab	3.01	204.97 (169)
DS ₇	2.17 ab	1.80 a	0.97 a	2.01 d	2.02 d	2.00 d	1.98 d	1.85	103.60 (86)
DS_8	2.42 a	2.21 a	1.30 a	4.62 a	4.03 b	4.05 bc	4.07 b	3.24	181.60 (150)
DS ₉	1.17 de	1.53 a	1.30 a	4.62 a	4.77 a	4.77 a	4.80 a	3.28	183.68 (152)

Note: Numbers in the same column followed by different letters indicate significantly different according to Duncan test ($\alpha = 0.05$). The estimated annual yield was calculated using 86, 68, and 56 tapping day per year for d4, d5, and d6 respectively.

In February and March, the yield was not significantly different among treatments, ranging from 1.25 g/t/t to 2.21 g/t/t in February and from 0.97 g/t/t to 1.30 g/t/t in March. In this period, trees were encountering the defoliation period, so the ethephon stimulation was not applied. The yield indicated that the low tapping frequency (d4, d5, and d6) there was no difference in yield. Rubber trees generally require a two-day recovery for rubber regeneration; thus, the tapping intervals d4, d5, or d6 are sufficient (7). The lowest yield in all treatments occurred in March; this was the period of new leaf formation. According to a previous study, the yield in the leaf fall period, including new leaf formation, tends to decrease as carbohydrate reserves are mostly used for the growth and development of new leaves so that the allocation for latex biosynthesis is reduced leading to a yield decline. In addition, during that period, photosynthetic activity in rubber plants is limited (22). According to the previous study, some rubber clones have

High ethephon stimulation increased yield per tapping regardless of the tapping frequency. However, due to the discrepancy in annual tapping days, stimulation in d4 gave a higher yield than d5 and d6. Using DS₁ (S2d4 without stimulation) as the control, and estimated annual tapping days of 86 days, 68 days, and 56 days per year for d4, d5, and d6, respectively, the highest annual yield was found in SD₃ (S2D4 ET4.5%) with an annual yield of 214.51 g/t/y (177% compared to SD₁). The lowest was SD4, which only reached 82% of SD1's yield. SD9, which consistently had the highest g/t/t, only had a 152% yield compared to SD1. This should be considered, as it might determine the annual income for the farmers. The low tapping frequency aims to reduce labour costs. However, in the case of rubber smallholders, who generally have limited land tenure, this factor may not be a priority. Therefore, tapping d5 or d6 may not be suitable for rubber smallholders.

Ethephon Stimulation Increase Latex Salinity and Plugging Index

The intensity of tapping with a dose of liquid stimulant has a significant effect on latex acidity (pH) and plugging index (Table 3). The highest latex pH was found in DS₉ (6.90), which were significant from other treatments, while the lowest pH was in DS₁ and DS₄, which shared a similar pH of 6.20. The increase in ethephon concentration resulted in increased the latex pH of clone GT1 observed. According to the previous study, alteration in the pH occurs due to changes in enzyme (24). Ethylene activates the H⁺ATPase in the lutoid membrane and increases the invertase activity involved in the rubber biosynthesis mechanism by providing energy from the glycolysis process. Ethephon stimulation can cause the cytosol to become alkaline; when combined with an adequate water supply, it may affect the lutoid stability leading to a longer latex flow.

Treatment	n H	Plugging	
meatment	рп	index	
DS1	6.20 f	19.47 h	
DS_2	6.30 ef	24.60 g	
DS₃	6.40 de	29.20 d	
DS ₄	6.20 f	25.73 f	
DS₅	6.50 cd	25.73 f	
DS ₆	6.60 bc	30.80 c	
DS ₇	6.40 de	27.80 e	
DS ₈	6.70 b	36.42 b	
DS ₉	6.90 a	37.80 a	

Table 3. Latex acidity (pH) and plugging index of each treatment.

Note: Numbers in the same column followed by different letters is significantly different according to Duncan test (P = 0.05).

The tapping interval with the administration of a liquid stimulant concentration affects the PI plugging index; the lower the tapping frequency and the higher the concentration of the stimulant, the higher the latex PI. The highest plugging index was found in the DS₆ treatment (37.80%), significantly higher than other treatments, while the lowest was found in the DS₁ (19.47%). Our result contrasts the previous studies that showed a significant decrease of PI in ethephon stimulation (25, 26).

The cessation of latex flow occurs due to the coagulation of rubber particles in the latex vessels, induced by the lutoid bursting and releasing coagulation factors into the cytoplasm. The PI negatively correlates with yield; rubber clones with low PI tend to have high latex yields (27, 28). The increase in IP by stimulants application and tapping time is due to disrupted response of physiological characters, such as increasing pH to activate sucrose-breaking enzymes. However, minimal sucrose is produced, and energy / Pi is used for low metabolic processes and low thiol levels that function to maintain lutoid stability. While applying a 5% stimulant concentration did not significantly reduce the IP value (14), the plant might have experienced physiological disturbances, leading to a subsequent increase in the blockage index. Lutoid membrane damage is suggested as the cause of rubber particle coagulation and subsequent latex flow stoppage (29). A high blockage index, as mentioned in (4), can result in a quicker cessation of latex flow. The blockage of latex vessels occurs due to the coagulation of rubber particles, ultimately affecting latex flow (30, 31). In general, the physiological characteristics of latex can be divided into two categories: those related to latex flow and those related to latex regeneration.

The blockage index exhibits a negative correlation with latex yield; higher blockage index values indicate a higher level of latex coagulation in the latex vessel (32). This coagulation process within the latex vessel network accelerates the cessation of latex flow (33). A study by Sumarmadjiet al. (34) also emphasized that the latex flow will stop more rapidly with a high blockage index.

The blockage of latex vessels occurs as a result of rubber particle coagulation within the latex vessels, directly affecting latex flow. However, it is important to note that after treatment, the pH of the latex increases. The higher concentration of stimulants leading to an increase in pH prolongs the coagulation process. According to Chern & Chen (35), the coagulation process occurs due to a decrease in pH, making it necessary to increase the pH of latex. Additionally, latex coagulation can be induced by lowering the electric charge (dehydration), which can be achieved through the addition of acid (36, 37).

Effect of tapping intervals combination with the application of liquid stimulant concentration on the physiology of the latex clone GT 1

The result showed that sucrose decreased with high ethephon concentration, except in d4 tapping (Table 4). The highest sucrose content (12.98 mM) was found in DS₇ (d6 tapping without stimulation). Interestingly, the lowest sucrose was found in DS₉ (d6 ET4.5%), suggesting that the decrease in sucrose was more influenced by the ethephon concentration than tapping in the low tapping frequency system. It should be noted that the 3.5% and 4.5% ethephon concentrations applied in this study are higher than the general recommendation of 2.5%. Several factors can affect the response to ethephon stimulation, including the clone type, plant age, application technique, dose, and environmental conditions (38). Ethephon stimulation can alter the latex's physiological status, including sucrose content (39). Increasing the frequency of stimulation would lead to increased consumption of latex sucrose, resulting in a hyperbolic pattern of the relationship between the frequency of stimulants and the levels of latex sucrose (40).

The Pi content indicates cellular metabolic activity. The result showed that the d5 tapping frequency without stimulation resulted in a higher Pi content (27.06 mM) than other treatments, while the lowest Pi level was in the SD₉ (19.34 mM). Our result contrasts with previous studies which show that ethephon stimulation increased Pi (14). Pi levels were also higher in the treatment without stimulant, 12.80 mM, compared to stimulant application, 10.88 mM. Thiol levels are also higher without stimulant application, namely 0.32 mM compared to treatment with a stimulant, namely 0.33 mM. Pi is low after stimulants application because sucrose is broken down into rubber particles. Such a process requires energy, thus decreasing energy (Pi) required for sucrose breakdown into latex particles,

Table 4. Physiological parameter of each treatment.

Treaturent	Sucrose	Pi	Thiols
Treatment	(mM)	(mM)	(mM)
DS1	9.55 b	12.80 f	0.32 a
DS ₂	8.04 c	11.20 g	0.33 a
DS₃	8.78 bc	10.88 g	0.30 b
DS ₄	8.54 c	27.06 a	0.25 d
DS ₅	8.78 bc	25.22 b	0.27 c
DS ₆	7.85 cd	23.60 c	0.24 d
DS ₇	12.98 a	22.98 c	0.22 e
DS ₈	7.05 de	21.16 d	0.22 e
DS ₉	6.74 e	19.34 e	0.24 d

Note: Numbers in the same column followed by different letters is significantly different according to Duncan test (P = 0.05).

namely 10,88 mM due to stimulant application. Likewise, low thiol levels after stimulant application, which is below the minimum limit of 0.22 mM, make the lutoids membrane easily broken (22). Refer to (6), the Pi content generally tends to decrease if the plant is intensively tapped and by stimulants application.

The higher the tapping intensity, the lower latex's thiol content (R-SH) in the latex. The function of the stimulant is to start ethepon hydrolysis into ethylene gas which then activates $\mathsf{H}^{\scriptscriptstyle +}$ and transports sucrose from cytosol to lutoid (41). This process makes the pH in the cytosol become alkaline and the lutoid becomes acidic and activates cytosol activity to supply waterin the tapping panel so that the latex does not coagulate. This process also activates turgor pressure that triggers increase in elasticity and makes latex flow easily. Inorganic Pi (plant energy) is also activated to convert the basic material sucrose into latex. According to the study (42), the optimal content of inorganic phosphate in rubber plants ranges from 10 - 20 mM. Thiol's content indicates lutoid protection from oxygen radicals. The thiol content shows a negative correlation with tapping stress; as the exploitation intensity increases, the thiol contents decrease. According to (43), thiols content ranged from 0.4 mM to 0.9 mM. The present study found that the highest thiols content (0.33 mM) in the DS2 treatment (S2d4 ET3.5%) was not significantly different from DS1 (0.32 mM). Conversely, the DS7 and DS8 treatments had the lowest thiols content, both measuring at 0.22 mM. Several factors, including the exploitation system, season, and plant age, determine the thiols content (44). Further, the tapping frequency d4 showed higher thiols content compared to d5 and d6 in the present study.

Conclusion

Based on the present study, it can be conclude that the most suitable tapping system to optimize rubber production in the GT1 clone is the S2D4 ET 4.5% tapping system, which resulted in the highest annual production. However, it should be noted that the use of stimulants in this system can lead to an increase in pH, potentially reducing sucrose stock, Pi, and thiol blockage index. Moreover, excessive levels of stimulants may negatively impact the physiological performance of rubber plants.

Therefore, careful consideration should be given to the application of stimulants in the tapping system to ensure optimal yield and plant health.

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Authors' contributions

For the Authors' contributions, we suggest the following format (please use initials to refer to each author's contribution): YP conducted the research design, participated in data collection and drafted the manuscript. YA perform statistical analysis of data. HS and NN participated in the preparation and alignment of the manuscript, as well as general coordination. MSR participated in data collection. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest : Authors do not have any conflict of interests to declare..

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