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Effect of Renewable Bark Stimulant and PEG on Renewable Bark Growth and Rubber Production (*Hevea brasiliensis*) Clone PB 260

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ABSTRACT

The research was aimed to obtain alternative stimulant formulation from PEG to increase rubber production and accelerate renewable bark recovery of clone PB 260. This research was conducted at Nusantara III Plantation, Sungei Putih Estate and Sungei Putih Research Center, Rubber Research Center, Deli Serdang Regency, Province of North Sumatera. The research site is at 25 m above sea level with Ultisol soil type. The research is Randomized Block Design with 2 factors treatment and 3 replications. The factor treatment is ethephon stimulation concentration (S), consisted of 4 treatment levels namely S0 = no stimulant, S1 = N2O1 formulation, S2 = Etephon 1.5% + N2O1 formulation, S3 = 2.5% ethephon + N2O1 formulation and PEG concentration (P) consisted of 2 levels of treatment namely P0 = without PEG, P1 = PEG 3%. The results showed that the stimulant and PEG and treatment combination had significant effect on production, TSC, bark thickness, but no significant effect on the number of latex vessels and blockage index.

INTRODUCTION

Rubber tree (*Hevea brasiliensis* Muell, Arg) is one of the important plantation commodities in Indonesia, because natural rubber is one of the non oil and gas products that become large foreign exchange income source of the country. Foreign exchange income from this commodity in 2004 is reached US $ 2.25 billion, representing 5% of non oil and gas foreign exchange income (Anwar, 2012). The Ministry of Trade has released the value of foreign exchange generated by Indonesia in 2014 is US $ 4.7 billion (Joint Press Release, 2015).

Indonesia is a country with the world's largest rubber plantation, namely 3.4 million hectares, but the natural rubber production is still not optimal and left behind from Thailand as the world's first rubber producing country with only 2.4 million hectares (IRSG, 2007).

Generally, the economic cycle of rubber tree is 25 years which will produce latex around 36 tons per hectare, but currently, the economic cycle tends to be only up to 15 years. The main factors are the attack of white root fungus, wind, and dry tapping grooves. Tapping using a good and regular bark will realize a long period of economic life up to 25-35 years (Sumarmadji, 2000).

Today, tapping technique on large estates owned both by private and government tends to be wasteful of bark usage and the tapping quality is also decreasing and not qualified. Bark consumption in BO panel that should be for 5-6 years is often depleted in 3-3.5 years of tapping. Bark consumption is usually 25 cm per year and if done correctly for 5 years will be used about 125 cm. Besides, there is a perception that the thicker the bark is tapped the more latex would be yielded. Also, poor rubber sheeting causes waste and wood wounds. It can cause the occurrence of dry tapping grooves in the BO-2 panel (Andrianto and Tistama, 2014).

Existing of new superior clones from research institutes that have higher production potential will facilitate the planters to choose plant material to be planted. One of the superior clones that are widely used today by private and government planters are clones PB 260 (Sumarmadji, 2010).

Clone PB 260 is one of Quick starter clone. Quick starter clones have some specific properties such as high initial production, less responsive to stimulants, susceptible to dry tapping grooves and less potential of renewable bark so that the exploitation system for this clone does not use renewable bark, therefore, the renewable bark is often ignored (Sumarmadji, *et al*., 2012).

The problem of renewable bark of clone PB 260 is considered to be less potential, but it is actually very important and can affect the use of panels in BO-2. Ideally, the first until the fifth year, tapping is performed in panel BO-1 then some dry tapping grooves can be found. Therefore, tapping is done in panel HO-1 for four years to provide recovery for panel BO-1 and simultaneously to create good conditions for panel BO-2 (Sumarmadji *et al*., 2012).

The fact is tapping on the panel BO-1 is lasts for only 3-3.5 years. This is due to concerns about the emergence of dry tapping grooves in panel BO-2 due to slow recovery time in panel BO-1, so the panel BO-1 that should be used for 5 years forced to be conducted at panel HO-1 after 3-3.5 years. It certainly results not optimum yield from panel BO-1. In addition, the use of panel HO-1 bark is more wasteful due to tapping difficulty because the latex is on above. The result is the economic life of the tree reduced and the expected potential yield is less than the maximum.

The use of stimulant has long been known by the perpetrators of rubber agribusiness. Stimulants that are widely used in large treatments are Etephon. Etephon is a stimulant that applied to plants and prevents blockage of latex vessels so that latex drips longer. Yew (1988) found that stimulant with Etephon active ingredient has indirectly effect to increase production that is around 50%, whereas Karyudi *et al*. (2006) found that the use of ethylene gas stimulant can increase productivity around 75-100% on average, that is higher than conventional tapping system combined with Etephon stimulant.

From the information, it is known that conventional stimulant contains only ethylene which serves to increase rubber production. The next development of stimulants, besides ethylene active ingredients, is also added with vitamins to improve plant resistance. But until now there has been no stimulant that can increase latex production even speed recovery of renewable bark.

Latex is a secondary metabolite of rubber tree. External factors such as application of elicitors (PEGs) to induce stress conditions can be used to improve secondary metabolites. In various tissue culture experiments, PEGs added to the culture medium to create drought stress conditions by lowering water potential in the medium resulted in increased secondary metabolite content (Ehsanpour and Rezavizadeh, 2005 *cit* Zulhimi *et al*., 2012). Currently, stimulant formulation using PEG materials has been available.

PEGs is one of carrier which is often used as an additive in a formulation to improve solubility. This material is one type of polymer that can form polymer complexes in organic molecules when added to the formulation to increase the speed of dissolution (Martin, 1993). PEGs mechanism in increasing latex production and the added physiological effects are not known yet.

Due to the reason, research to get the right stimulant to increase rubber production and accelerate the recovery of renewable bark has been conducted.

MATERIALS AND METHODS

This research was conducted at Nusantara III Plantation, Sungei Putih Estate and Sungei Putih Research Center, Rubber Research Center, Deli Serdang Regency, Province of North Sumatera. The research site is at 25 m above sea level with Ultisol soil type.

The research is Randomized Block Design with 2 factors treatment and 3 replications. The factor treatment is etephon stimulant concentration (S), consisted of 4 treatment levels namely S0 = no stimulant, S1 = N_2O_1 formulation, S2 = Etephon 1.5% + N_2O_1 formulation, S3 = 2.5% etephon + N_2O_1 formulation and PEG concentration (P) consisted of 2 levels of treatment namely P0 = without PEG, P1 = PEG 3%.

Data Analysis

Data were analyzed using ANOVA; further testing used Duncan Multiple Range Test (DMRT) at 5% significant level. Data were analyzed using the Statistical Analysis System (SAS) Software 9.1.

RESULTS AND DISCUSSION

Latex Yield (g/p/s)

Latex yield data is a reflection of tree productivity when tapped which measured by the volume of latex per tree and then converted to dry production in grams per tree per tap (g/p/s) after multiplying by the Total Solid Content (TSC). The results showed that application of Stimulant and PEG, as well as the combination, had significant effect on latex production at 4 months after observation (Table 1).

Table 1 shows the highest latex yield is found at S_1 treatment namely N_2O_1 formulation at 34.77 g/p/s, while the lowest is on S_0 treatment (control) at 29.45 g/p/s. In this case, there is an increase in latex yield by 18.06 % with application of N_2O_1. PEG application had significant effect on latex yield, that is an increase of latex yield by 45.30%.

The combination of stimulants and PEG had significant effect on latex yield. The highest latex yield was found at S_1P_1 treatment (N_2O_1 and PEG formulations), while the lowest was S_0P_0 (control). Increased latex yield by application of N_2O_1 and PEG formulations is 90.48%.

Table 1. Mean of latex production (g/p/s) on treatment of stimulant and PEG at four Months after observation

Treatment	**PEG Concentration**		**Mean**
	P_0 (Control)	**P_1 (10%)**	
Stimulant Concentration			
S_0 (Control)	22.70 hH	36.21 cC	29.45 d
S_1 (N_2O_1 formulation)	26.31 fF	43.24 aA	34.77 a
S_2 (Etephon 1.5% + N_2O_1 formulation)	28.86 eE	31.96 dD	30.41 c
S_3 (Etephon 2.5% + N_2O_1 formulation)	25.47 gG	38.72 bB	32.10 b
Mean	25.83 bB	37.53 aA	

Note: The numbers followed by the same letter on the same row or column are not significantly different at the 5% Duncan test level and 1%

The most important response of rubber tree to the treatment is reflected by the production (yield). In this study, clone PB260 was used which characterized by high initial production, increased production continued and slow girder growth (Daslin *et al*., 2009). Interaction of stimulant S_1 treatment (N_2O_1 formulation) and PEG show significantly increased production. This is because the material formulation is containing palmitic acid (fatty acid). The palmitic acid would be converted into Acetyl Coenzyme A. Such Coenzyme will form Tricarboxylic Acid (TCAs) which will produce energy. The energy is used for vegetative growth (stem cell enlargement) that is the addition of bark thickness and the number of latex vessels. Some of

Acetyl Co A will produce terpenoid compounds such as politerpenes (latex). Therefore, application of a stimulant containing palmitic acid causes increasing the production of latex.

The presence of PEG will increases the osmotic pressure of tree and induces PEP carboxylase enzyme activity that plays in photosynthesis process (Singh *et al*., 1997). By increasing of photosynthesis activity there is an increase in sucrose as precursor of latex. In turn, the application of PEG will increase the production of latex.

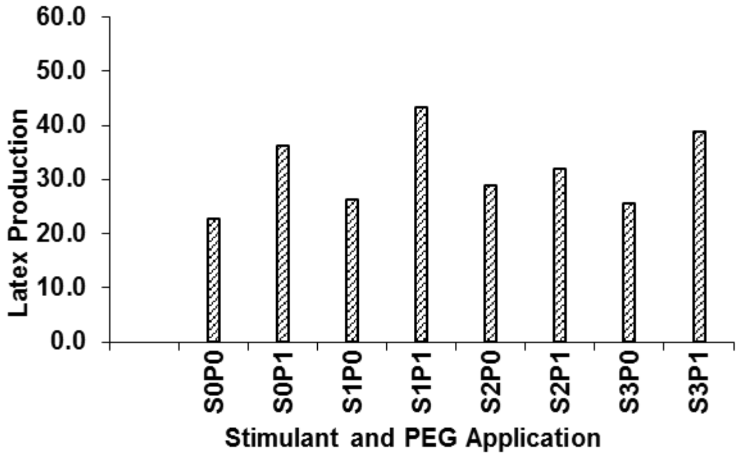

Figure 1. Histogram of latex production with stimulant and PEG treatment

Total Solid Content (TSC)

Total solid content is a reflection of the ability of *in situ* latex biosynthesis. The results showed that Stimulant and PEG treatment, as well as the combination, had significant effect on TSC at 4 months after observation (Table 2).

Table 2. Mean of Total Solid Content (TSC) of stimulant and PEG treatment at four Months after observation

Treatment	PEG Concentration		Mean
	P_0 (control)	P_1 (10%)	
Stimulant Concentration			
S_0 (Control)	38.76 fF	43.17 cC	40.96 bB
S_1 (N_2O_1 formulation)	41.72 dD	45.09 aA	43.41 aA
S_2 (Etephon 1,5% + N_2O_1 formulation)	41.33 dD	41.68 dD	41.50 bB
S_3 (Etephon 2,5% + N_2O_1 formulation)	40.57 eE	44.18 bB	42.38 abAB
Mean	40.59 bB	43.53 aA	

Note: The numbers followed by the same letter on the same row or column are not significantly different at the 5% Duncan test level and 1%.

The table showed that the highest TSC is found in S_1 treatment, that is the application of N_2O_1 formulation at 43.41%, while the lowest in S_0 treatment (control) at 40.96%. In this case, there is an increase of TSC by 7.97% by PEG application. PEG application is able to increase TSC by 7.24%.

The combination of stimulants and PEG had a significant effect on TSC. The highest TSC was found in S_1P_1 treatment (N_2O_1 formulation and PEG), whereas the lowest was found in S_0P_0 treatment (control). The increase of TSC by application of N_2O_1 formulation and PEG was 22.25%.

This is caused by the PEG application will increase photosynthesis activity. This is consistent with Singh *et al*., (1997) which states that the application of PEG in lemongrass induces the activation of PEG carboxylase enzymes that play in photosynthesis. By increasing of photosynthesis, carbohydrates will increase where these carbohydrates are part of TSC. While application of N_2O_1 can increase latex content by increasing the formation of Acetyl CoA which is a precursor in the formation of terpenoid compounds such as polyterpenoid. In this case, the rubber particles are the largest part of the TSC.

Due to the reason, increasing of TSC by application of N_2O_1 and PEG is through increasing of Acetyl Co A and photosynthesis activity. In addition, as indicated by Table 1, it is known that although production is increasing there is a decrease in TSC with the treatment, especially with S_1P_1 treatment. This is thought related to high latex production in S_1P_1 treatment where the decrease of TSC is due to the mass of latex that exists in large quantities so that fluid and serum contents in the latex also exist (Sumarmadji, 2010). But this decrease is still in reasonable latex because TSC is still above 25%.

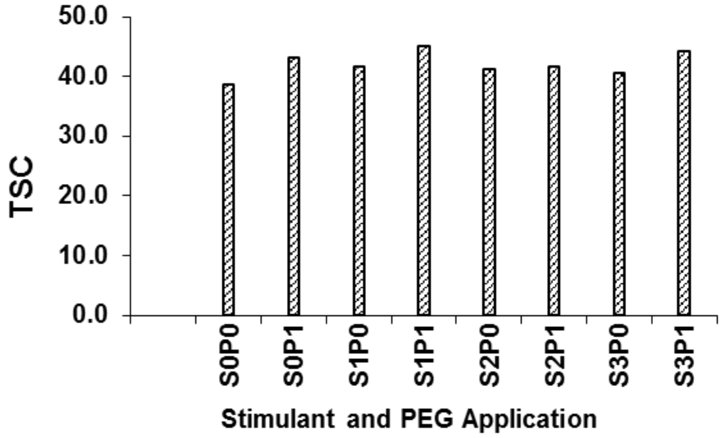

Figure 2. Histogram of TSC with stimulant and PEG application

Bark Thickness

Bark is the main capital of rubber cultivation. Therefore efforts should be conducted to make renewable bark can be formed well so that can be tapped again. The results showed that Stimulant and PEG treatment, as well as the combination, had significant effect on bark thickness at 4 months after observation (Table 3).

The table shows that the highest bark thickness found in S_1 treatment, namely application of N_2O_1 at 5.40 mm. While the lowest bark thickness found at S_0 treatment (control) namely 4.76 mm. In this case, there is an increase of bark thickness by 13.45% with application N_2O_1. Application of PEG is also significantly affected the thickness of renewable bark. PEG application is able to increase the renewable bark thickness by 9.33%.

Table 3. Mean of Bark Thickness on Stimulant and PEG Treatment at Four Month After Observation

Treatment	PEG Concentration		Mean
	P_0 (control)	P_1 (10%)	
Stimulant Concentration			
S_0 (Control)	4.42 cC	5.10 bB	4.76 bB
S_1 (N_2O_1 formulation)	4.92 cC	5.88 aA	5.40 aA
S_2 (Etephon 1,5% + N_2O_1 formulation)	5.09 bB	5.00 bB	5.04 abAB
S_3 (Etephon 2,5% + N_2O_1 formulation)	4.82 cC	5.09 bB	4.96 bB
Mean	4.82 bB	5.27 aA	

Note: The numbers followed by the same letter on the same row or column are not significantly different at the 5% Duncan test level and 1%

The combination of stimulant and PEG had a significant effect on the thickness of renewable bark. The highest thickness of renewable bark was found in S_1P_1 treatment (N_2O_1 formulations and PEG). The lowest thickness of renewable bark was found in the treatment of S_0P_0 (control). Increased thickness of renewable bark by application of N_2O_1 formulation and PEG is 33.83%.

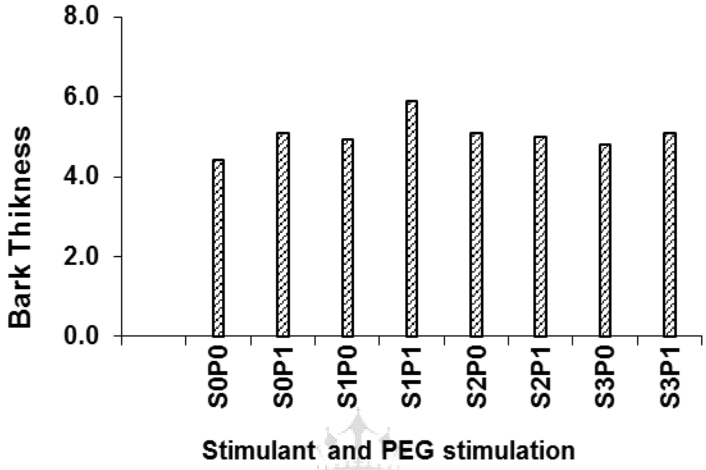

Figure 3. Histogram of renewable bark thickness with stimulant and PEG application

Number of Latex Vessel

Latex vessels have permanent and elastic walls. These vessels are close to the cambium, first forming a single cell and then forming vessel network. The results showed that stimulant and PEG treatment, as well as the combination, had no significant effect on the number of latex vessels at 4 months after observation (Table 4).

Table 4. Means of the number of latex vessels on stimulant and PEG treatment at four months after observation

Treatment	PEG Concentration		Means
	P_0 (control)	P_1 (10%)	
Stimulant Concentration			
S_0 (Control)	9.22	10.67	9.95
S_1 (N_2O_1 formulation)	9.45	9.78	9.61
S_2 (Etephon 1,5% + N_2O_1 formulation)	9.33	10.44	9.89
S_3 (Etephon 2,5% + N_2O_1 formulation)	9.44	10.45	9.95
Means	9.36	10.33	

Note: The numbers followed by the same letter on the same row or column are not significantly different at the 5% Duncan test level and 1%

The table above shows that the application of stimulant has no significant effect on the number of latex vessels. The highest number of latex vessels was found in S_0 treatment (control) namely 9.95. While lowest found at S_1 (N_2O_1 formulation) namely 9.61. PEG application has no significant effect on the number of latex vessels. However, PEG application is able to increase the renewable bark thickness by 10.36%.

The combination of stimulant and PEG did not significantly affect the number of latex vessels. However, there is a tendency for the largest number of latex vessels to be found in S_0P_1 treatment (control and PEG 10%), while the lowest number of latex vessels is found in the S_0P_0 treatment (control). Increasing number of latex vessels by stimulant (control) and PEG is 15.73%.

Table 3 and 4 showed that the application of N_2O_1 increases the bark thickness and the number of latex vessels. This is because N_2O_1 formulation is containing auxin (NAA) and cytokinin (kinetin) along with palmitic acid. The role of auxin in plants among others is to stimulate cell division and elongation while cytokinins play an important role in encouraging cell division and differentiation of plant tissues (Warner and Schmulling, 2009). Application of these two hormones will make cell division, enlargement and differentiation in the stem of the rubber tree so that there is increasing in bark thickness on the stem that exposed to the treatment.

While the palmitic acid in the formulation will be converted to Acetyl Co A which will form Tricarboxylic Acid (TCA) as one of the processes in respiration that will produce energy (ATP) where the energy (ATP) will be used by plants for vegetative growth such as increases the bark thickness and the number of latex vessels.

PEG application is also increased bark thickness and number of latex vessels (Tables 3 and 4). This is because PEG is capable of increasing the effectiveness of PEP carboxylase enzymes that play in photosynthetic activity (Singh *et al*., 1997). Increase in photosynthesis activity will be followed by increase in carbohydrate that acts as raw material for the formation of new cells, in this case, the cells are stem cells, so that increase in bark thickness followed by increasing number of latex vessels (Salisburry and Ross, 1995). In addition, auxin can also stimulate the cambium activity on the stem by increasing the synthesis of nucleic acids and proteins (Masuda, 1969).

Blockage Index

The blockage index is a value that describes the rate of latex coagulation in latex vessel tissue. The result showed that stimulant and PEG treatment, as well as the combination of, did not significantly affect the blockage index at 4 months after observation (Table 5).

Table 5. Mean of blockage index on stimulant and PEG treatment at four months after observation

Treatment	PEG Concentration		Means
	P_0 (control)	P_1 (10%)	
Stimulant Concentration			
S_0 (Control)	7.73	6.22	6.97
S_1 (N_2O_1 formulation)	6.32	5.56	5.94
S_2 (Etephon 1,5% + N_2O_1 formulation)	7.18	7.02	7.10
S_3 (Etephon 2,5% + N_2O_1 formulation)	6.89	6.87	6.88
Means	7.03	6.41	

Note: The numbers followed by the same letter on the same row or column are not significantly different at the 5% Duncan test level and 1%.

The table showed that stimulant application has no significant effect on the blockage index.

The lowest blockage index was found in S_1 treatment with application of N_2O_1 formulation, and the highest on S_2 treatment with ethephon 1.5% + N_2O_1 formulation. The decrease in the blockage index by application of N_2O_1 formulation compared with control (S_0) is 19.53%. PEG application has no significant effect on the blockage index but able to lowering the blockage index by 9.67%.

The combinations of stimulant and PEG have no significant effect on the blockage index. The lowest blockage index was found in S_1P_1 treatment (N_2O_1 formulation and PEG), while the highest found in S_0P_0 treatment. The decrease in the block index by application of N_2O_1 and PEG formulation compared with the controls is 39.03%.

The N_2O_1 formula can decrease the blockage index by increasing the energy derived from respiratory process (TCA) that generates ATP as energy source for the formation of Iso c (IPP) which will produce isoprene (latex).

This finding is confirming Sumarmadji (2000) which states that the blockage index is negatively correlated with production. Increasing production mean decreasing the blockage index. With the application of PEG, photosynthesis activity will also increase and in turn, NADN-oxidase also increases. Increased NADN-oxidase will also increase the enzyme Super Oxide Dismutase (SOD) which will stabilize the lutoid so that the blockage index becomes low. Thus production will increase.

CONCLUSION

1. Applied (proposed) stimulant is significantly lead to increased production, TSC and bark thickness, but have no significant effect on the number of latex vessels and the blockage index.
2. PEG application may encourage increased production, TSC and bark thickness, but not significant to the number of latex vessels and blockage index.
3. The combination of stimulant and PEG has significant effect on production, TSC and bark thickness, but not significant to the number of latex vessels and the index of blockage.

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