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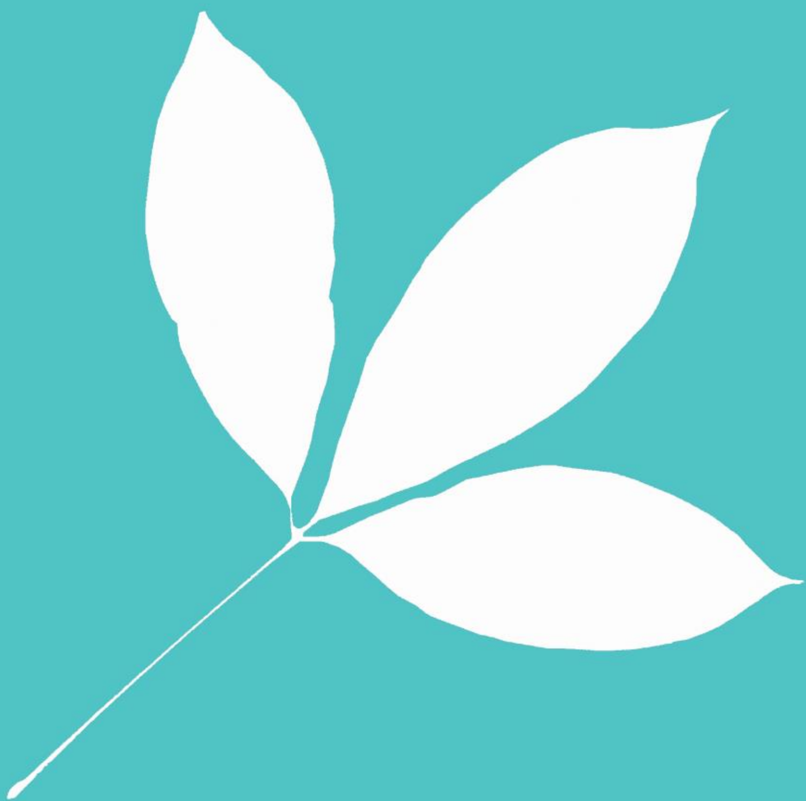

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Coir pith and elephant dung: sowing substrates alternative to river sand for rubber (*Hevea brasiliensis*) nurseries

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Abstract

The sowing medium plays an important role in the production of good quality budded plants of rubber. Nowadays, the search for alternative high-quality and low-cost materials as sowing media in rubber nurseries is a necessity due to the increasing demand, environmental constraints and rising costs for river sand, the most widely used sowing medium during the last decades. To study the effects of different media on seed germination and seedling growth of rubber, experiments were conducted using river sand as the control and several other media viz., leached coir pith, dried elephant dung, reclaimed sea sand, fine quarry dust, course quarry dust, rubber wood saw dust and rubber wood chips. Germination attributes were significantly affected by the sowing medium. In the first experiment, seeds sown in coir pith recorded significantly higher values for germination percentage, germination value, germination index, rate of germination (lowest E_{50}*), as compared to those in river sand (control) and other media. In the second and third experiment, seeds sown in coir pith and elephant dung recorded significantly higher values for germination as compared to those in other media and the values were on par with those in river sand. Seedlings raised from seeds sown in all media in the first experiment achieved buddable size (>6mm stem diameter) after three months. Considering the cost, availability and germination attributes, leached coir pith and dried elephant dung are identified as ideal sowing substrates alternative to river sand for rubber nurseries.*

Key words: alternative sowing media, coir pith, elephant dung, river sand, rubber seeds

Introduction

Rubber (*Hevea brasiliensis*) seeds are used for raising root stock for bud grafting of clones. The successful production of healthy and vigorous root stock in rubber depends on the use of good quality seeds and management practices followed in the nursery. Rubber seeds are classified as recalcitrant that are susceptible to deterioration and lose their germination capacity in a short time (Dias *et al.*, 2010). Fresh rubber seeds take 7-10 days to start germination in a sand bed and vigorous seedlings (about 50% of the early germinators) should be harvested every other day (only for three rounds up to 14 days), to raise stock plants even if the entire population germinates (Anon, 2016).

Seeds for commercial rubber nurseries are purchased from different suppliers who collect and store seeds for few days to weeks to supply them in bulk quantities. In certain nurseries, such seeds take more than 14 days to start germination in sand beds. Due to delays in germination, low germination percentage and asynchronous germination, large quantities of seeds are required to establish in germination beds. Moreover, the production of seeds in some rubber growing areas in Sri Lanka has been very poor in the recent past and therefore, finding sufficient quantities of good quality seeds during seed fall season is difficult. Hence, it is essential to search for alternative sowing media which can speed up germination, improve germination synchronicity and increase the percent germination of rubber seeds.

Seed germination can be influenced by many factors such as the type of substrate used and environmental factors. River sand has been used as the sowing medium for rubber seeds in commercial nurseries during the last decades. Due to the recent growth in the construction industry in Sri Lanka, the demand for river sand has increased tremendously (Ratnayake *et al.,* 2014). Over exploitation of river sand, the rapid expansion of the mining of river bed and river bank sand, has caused widespread environmental, social and economic problems linked to the livelihoods of people, agriculture, health and land (Piyadasa, 2011). Because of these environmental constraints, the government of Sri Lanka has imposed various restrictions on the extraction and transportation of river sand. Consequently, high cost and scarcity of river sand have become the major problems faced by industrial workers in Sri Lanka. Therefore, the search for alternative high-quality and low-cost materials as sowing media in rubber nurseries is a necessity and the use of river sand must be progressively reduced. In the selection of new materials, environmental considerations have become as important as performance and economic cost. In this context, there has been a justifiable emphasis on materials derived from agricultural, industrial and municipal waste streams (Chong, 2005). The disposal of such materials already presents an environmental problem, and their re-use as sowing media might provide a convenient solution.

The use of materials like reclaimed sea sand and quarry dust in place of river sand is on the rise in building construction (Ratnayake *et al.,* 2014; Rajapaksha and Sooriyarachchi, 2009).

An extensive research has been carried out regarding the use of different farm, industrial and consumer waste by-products as components of nursery substrates (Chong, 2005). Different residual biomasses, such as coir (also known as coir pith, coir meal, coir dust, coco peat), a waste product of the coconut (*Cocos nucifera*) industry (Joseph and Jessy, 2015; Kumarasinghe *et al.,* 2015), saw dust and wood chips (Joseph and Jessy, 2015) have been studied as partial or total substrate components. Tons of elephant dung is accumulated daily at Elephant Orphanage, Pinnawala (Kegalle District) and dried elephant dung can be collected free of cost for usage (personal communications, Assistant Curator, Elephant Orphanage). Therefore, this study was undertaken to ascertain the effectiveness of some alternative materials as sowing substrates for river sand in rubber nurseries.

Materials and Methods

Experiment 1

This experiment was conducted in a plant house at the Dartonfield Estate of the Rubber Research Institute of Sri Lanka (RRISL) from August - December, 2017. Fresh rubber seeds of the clone RRISL 217 were collected and were sown in germination beds with different media *viz.*, river sand (control), leached coir pith, reclaimed sea sand, fine quarry dust and course quarry dust at a thickness of 5cm in randomized complete block design (RCBD) in 4 replicates. Forty seed were used in each treatment per replicate with a total of 160 and 1120 seeds in each treatment and all treatments, respectively. Watering was done manually using a watering can and providing the same amount of water for each treatment once daily. The management practices of germination beds were adopted as per recommendations of the Rubber Research Institute of Sri Lanka (Anon, 2016).

The effects of different sowing media were assessed by counting of the number of germinated seeds at 5, 7, 9, 11, 14 and 17 days after sowing. A seed was considered germinated when the tip of the radicle emerged free of the seed coat. Germination related parameters were calculated as follows;

a) The imbibition period (number of days from sowing to commencement of germination) was recorded.

b) Germination percentage: calculated as the percent of germinated seeds in relation to the total number of seeds per bed recorded on each day.

c) Rate of germination: the number of days taken to attain (emerge) 50% germination (E_{50}) was calculated according to Du *et al.* (2019).

$$E_{50} = t_i + [(N/2 - n_i)(t_j - t_i)]/(n_j - n_i) \quad (1)$$

where N is the final number of emerged seeds; n_i and n_j are the cumulative numbers of emerged seeds counted at time t_i and t_j, respectively, where $n_i < N/2 < n_j$.

d) Mean germination time: calculated according to Du *et al.* (2019).

$$MGT = \frac{\sum Dn}{\sum n} \quad (2)$$

where n is the number of emerged seeds on day D, and D is the number of days counted from the beginning of the emergence

e) The germination value (GV): which is a composite value that combines both germination speed and total germination was calculated according to Jessy and Joseph (2015) by using the following formula

GV = (EDGs/N) X GP/10: (3)

where, GP: germination percentage at the end of the experiment. DG: daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing, EDGs: total germination obtained by adding every DGs value obtained from the daily counts, N: total number of daily counts starting from the first germination, 10 is constant.

f) The emergence index (EI) was calculated as described by Du *et al.* (2019).

$$EI= \frac{\text{No.of emerged seeds}}{\text{Days of first count}} + \frac{\text{No.of emerged seeds}}{\text{Days of final count}} \quad (4)$$

A preliminary experiment was conducted in the government rubber nursery, Gurugoda (Kaluthara District of the Wet Zone of Sri Lanka), from January-February, 2018, with a few seeds provided by the nursery using the aforementioned media, in order to evaluate the effects of sowing media under general nursery conditions. However, seeds provide by the nursery were not that much fresh when compared to that used in the experiment 1 in Dartonfield. Except for the fine quarry dust and course quarry dust, a satisfactory germination was recorded in all sowing media. Meantime, another preliminary experiment was conducted in the same nursery and during the same period using some other materials *viz.*, dried elephant dung, rubber wood saw dust and rubber wood chips, along with river sand, to evaluate the effects of sowing media on seed germination. Seeds provided by the nursery were used for this experiment too. Satisfactory results were obtained from all media in this preliminary experiment too (results not shown). Therefore, to evaluate the effects of above sowing media, except for the fine and course quarry dust, on seed germination under general management conditions in government rubber nurseries, two large-scale experimental trials were conducted in Gurugoda and Middeniya nurseries belong to wet zone (WL1a) and intermediate zone (IL1b) from August-September, 2018.

Experiment 2

In Gurugoda nursery, polyclonal seeds provided by the nursery were sown in germination beds with different media *viz.*, river sand (control), leached coir pith, dried elephant dung, saw dust, wood chips and reclaimed sea sand at a thickness of 5cm on top of the existing sand beds in the nursery in a randomized complete block design (RCBD) in three replicates. There were six (6) treatments and a total of 23 400, 3900 and 1300 seeds were used for all treatments, each treatment and in each replicate respectively. The germinated seeds were counted after 7, 10, 12, 16 and 20 days of sowing, instead of alternative days, due to practical difficulties.

Experiment 3

In Middeniya nursery, polyclonal seeds provided by the nursery were sown in germination beds with different media *viz.*, river sand (control), leached coir pith, dried elephant dung, saw dust, wood chips and reclaimed sea sand at a thickness of 5cm on top of the existing sand beds in the nursery in a randomized complete block design (RCBD) in three replicates. There were six (6) treatments and a total of 23 400, 3900 and 1300 seeds were used for all treatments, each treatment and in each replicate respectively. The germinated seeds were counted after 7, 10, 12, 16 and 20 days of sowing, instead of alternative days, due to practical difficulties.

Seedling attributes

Germinated seeds resulted in the first experiment were transplanted in black polythene bags filled with soil. Polybags were arranged in a nursery according to an RCBD with five blocks, 30 plants per treatment. All other management practices were the same as recommended by the RRISL (Anon, 2016). Growth attributes of rubber seedlings raised from the experiment 1 were assessed after three months from transplanting. Ten plants from each treatment were removed and growth parameters *viz.*, stem diameter, plant height, number of leaves, chlorophyll content and dry weights of shoots and roots were recorded.

Data analysis

Germination data were subjected to arc-sin transformation where appropriate. Significance of the observed treatment differences was tested by analysis of variance using proc ANOVA procedure of the SAS software package (version 9.1) and significant means were separated using the least significant difference (LSD). An economic analysis was done for river sand and coir pith for an area of $0.05m^3$.

Results

Seed germination

Germination percentage

Seed germination started 7 days after sowing in all sowing media in the first experiment (Fig. 1). However, in the second and third experiments, no germinated seeds were recorded within 7-10 days, but, germination started after 12 days of sowing (Fig. 2 & 3). Significant variations were observed in the germination of seeds in different media after different sowing days in all experiments (Fig. 1, 2 & 3).

In the first experiment, a significantly ($p \leq 0.05$) higher germination percentage was recorded in coir pith as compared to river sand (control) and other media after 9 (54.37%) days of sowing. Also, after 11 days, coir pith recorded a significantly higher germination percentage (66.25%) as compared to other media and the value was on par with that in river sand (control). Nevertheless, after 14 days, no significant differences were recorded in seeds germinated in different media (Fig. 1). In the second experiment in Gurugoda nursery, the highest germination percentage was recorded in elephant dung after 12 (26%), 16 (58.66%) and 20 (81.3%) days of sowing (Fig. 2). Nevertheless, after 16 days of sowing, germination value in elephant dung was on par with those in river sand (control) and coir pith (Fig. 2). In the third experiment in Middeniya nursery, the highest germination percentage (39%) was recorded in river sand (control) after 12 days of sowing and the value was on par with those in coir pith, elephant dung and wood chips. After 16 days, higher germination values (58.66%) were recorded in both river sand and elephant dung and the values were on par with those in wood chips and coir pith. Nevertheless, after 20 days, the highest value (65.66%) was recorded in coir pith which is on par with those in river sand, elephant dung and wood chips (Fig. 3).

Rate of germination (time taken to attain (emerge) 50 percent germination, E_{50})

A significant difference in the rate of germination was observed among treatments. In the first experiment, seeds sown in coir pith recorded significantly the highest rate of germination or the least time taken to attain 50 percent germination (E_{50}=8.63) when compared to other media and the value was on par with that in river sand (control) (Table 1). The highest E_{50} value (12.17), the highest time taken to attain $E_{50,}$ was recorded in fine quarry dust and the value was on par with that in course quarry dust. In the second experiment in Gurugoda nursery, seeds sown in coir pith, elephant dung and river sand reached 50% germination after 16 days when compared to those in saw dust, wood chips and reclaimed sea sand (Fig. 2). In the third experiment in Middeniya nursery, seeds sown in coir pith, elephant dung, wood chips and river sand reached 50% germination after 16 days when compared to those in saw dust and reclaimed sea sand (Fig. 3). Moreover, seeds sown in saw dust and reclaimed sea sand in Middeniya nursery and those in saw dust and wood chips in Gurugoda nursery did not reach 50% germination even after 20 days of sowing (Fig. 2 & 3).

Table 1. *Effect of sowing media on germination attributes of rubber seeds*

Treatments	**Germination value**	**Mean germi-nation time**	**Germination index**	**Rate of germination (E_{50})**
River sand (control)	10.72±0.64[b]	11.69±2.18 [ns]	3.25±0.10[b]	8.95±0.26[c]
Coir pith	13.55±0.78[a]	9.26±0.90	3.68±0.10[a]	8.63±0.12[c]
Reclaimed sea sand	9.45±0.48[bc]	10.67±0.74	3.07±0.07[bc]	11.15±0.20[b]
Fine quarry dust	7.25±0.99[c]	13.24±1.26	2.69±0.19[c]	12.17±0.06[a]
Course quarry dust	9.30±0.81[bc]	10.91±0.78	3.04±0.14[bc]	11.62±0.06[ab]
P value	0.0089	0.3767	0.0179	0.0001
LSD ($p \leq 0.05$)	2.514	-	0.4309	0.5689

Means followed by the same letter (s) are not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT). ns: non-significant, ± indicates the standard error of the mean.

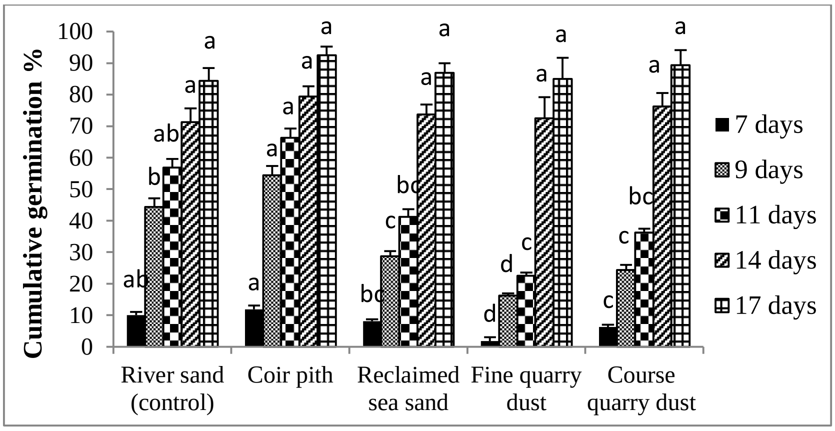

Fig. 1. Effect of different sowing media on germination percentage of rubber seeds after 7, 9, 11, 14 and 17 days of sowing in the plant house, Dartonfield (Experiment 1). Vertical bars above each mean denote the standard error. Letters indicate a significant difference at p≤0.05 according to LSD

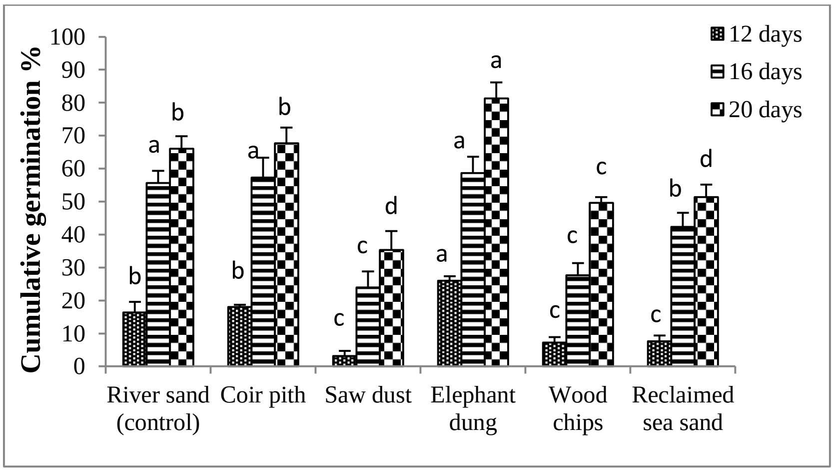

Fig. 2. Effect of different sowing media on germination percentage of rubber seeds after 12, 16 and 20 days of sowing in Gurugoda nursery (Experiment 2). Vertical bars above each mean denote the standard error. Letters indicate a significant difference at p≤0.05 according to LSD

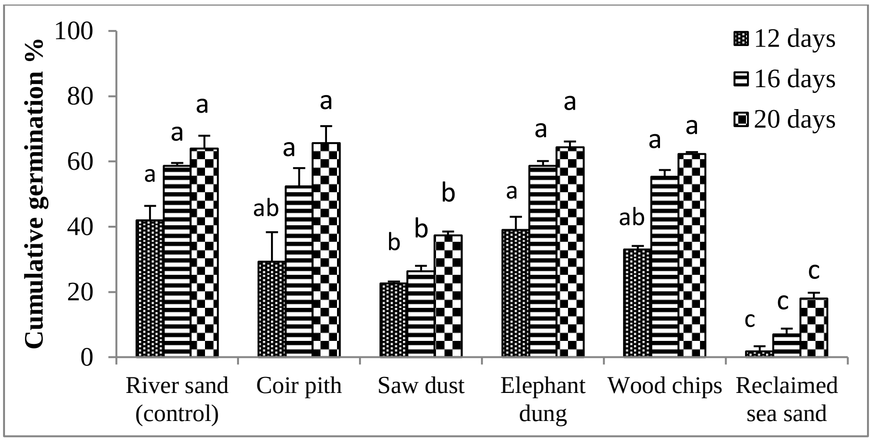

Fig. 3. Effect of different sowing media on germination percentage of rubber seeds after 12, 16 and 20 days of sowing in Middeniya nursery (Experiment 3). Vertical bars above each mean denote the standard error. Letters indicate a significant difference at p≤0.05 according to LSD

Mean germination time, germination value and germination index

The mean germination time did not differ significantly in different sowing media in the first experiment (Table 1). Coir pith recorded significantly the highest germination value as compared to other media and the control (river sand) (Table 1). The lowest germination value was recorded in fine quarry dust and the value was on par with that in course quarry dust and reclaimed sea sand (Table 1). The germination index was the highest (3.68) in coir pith as compared to that in river sand (control) and other media. The lowest value for germination index (2.69) was recorded in fine quarry dust and the value was in par with that in course quarry dust and reclaimed sea sand (Table 1).

Seedling attributes

In the first experiment, no significant differences were recorded for seedling attributes *viz.*, stem height, no. of leaves, leaf chlorophyll content, dry weight of shoots and roots after three months from transplanting in polybags (Tables 2 & 3). However, seedlings raised from seeds sown in reclaimed sea sand recorded a significantly higher stem diameter as compared to those in river sand and the value was on par with that in coir pith and course quarry dust (Table 2). Nevertheless, seedlings raised from seeds sown in all media achieved buddable size (> 6mm stem diameter) after three months. Also, the seedlings raised from seeds sown in river sand, coir pith and elephant dung in the second and third experiment (Gurugoda and Middeniya nurseries) reached buddable size after 4-5 months respectively under general nursery management conditions (results not shown).

Table 2. *Effect of sowing media on seedling attributes of rubber after three months of transplanting in polybags*

Treatments	Stem height (cm)	Stem diameter (mm)	No. of leaves	Leaf chlorophyll content (SPAD value)
River sand (control)	58.9±3.07[ns]	6.89±0.17[b]	8.20±0.73[ns]	8.95±0.86[ns]
Coir pith	55.4±2.52	7.46±0.26[ab]	8.28±0.58	8.63±3.05
Reclaimed sea sand	71.0±4.05	7.60±0.27[a]	3.07±0.48	9.20±1.26
Fine quarry dust	55.8±2.31	6.97±0.21[b]	2.69±0.58	7.20±1.29
Course quarry dust	56.7±2.39	7.36±0.18[ab]	3.04±0.80	8.20±1.17
P value	0.0516	0.0373	0.0783	0.4918
LSD ($p \leq 0.05$)		0.5737	-	-

Means followed by the same letter (s) are not significantly different at $p \leq 0.05$, according to Duncan's Multiple Range Test (DMRT). ns: non-significant, ± indicates the standard error of the mean

Table 3. *Effect of sowing media on dry weights of shoots and roots of rubber after three months of transplanting in polybags*

Treatments	Dry weight (g) of shoot	Dry weight (g) of root
River sand (control)	10.15±0.35[ns]	4.10±0.26[ns]
Coir pith	10.41±1.21	5.33±0.82
Reclaimed sea sand	14.58±1.10	4.61±0.46
Fine quarry dust	9.83±0.66	3.89±0.18
Course quarry dust	11.28±1.00	3.76±0.30
P value	0.089	0.2256

ns: non-significant, ± indicates the standard error of the mean

Economic analysis

A cost analysis of the coir pith which recorded a significantly higher germination percentage as compared to river sand is shown in the Table 4. The market prices of one tipper load (100 cubic feet) of river sand and coir pith are around Rs.14,000.00 and Rs.6,000.00 respectively. The cost of river sand and coir pith were around Rs.3.00 and Rs.26.00 per kg respectively, considering the weight of a tipper load/100 cubic feet of sand and coir pith are 4618 kg and 230 kg respectively. The quantities of river sand and coir pith required for an area of 0.05m^3 were 80 kg and 3.6 kg and the cost were Rs.242.00 and Rs.93.60 respectively. Around 50% of sand can be reused as germination medium during the following year and therefore, the cost of sand for an area of 0.05m^3 would be around Rs.121.00. Approximately, one tipper load/100 cubic feet of river sand can cover 57m^2 of an area in the germination bed whilst one tipper load of coir pith or elephant dung can cover an area of 76m^2 in the germination bed with a height of 5cm. The dried elephant dung can be collected from Elephant Orphanage, Pinnawala free of cost. Transportation cost should be added to Elephant dung to be transported from Pinnawala to the nursery concerned. Similarly transportation cost for river sand and coir pith should be added considering the distance from the supplying place and area to the nursery concerned.

Table 4. *Comparative cost analysis for an area of 0.05m^3 in a germination bed*

Treatment	Required amount (kg) for an area of 1m X 1m X 0.05m (0.05m^3) in the nursery	Weight (kg) of 1 tipper load (100 cubic feet)	Price (Rs.) of 1 tipper load (100 cubic feet)	Price (Rs.) of material for an area of 0.05m^3
Sand	80	4618	14,000.00	240.00
Coir pith	3.6	230	6000.00	93.60

Discussion

Seed germination

Seed germination is a complex process, comprising events from seed imbibition to radicle emergence. In the present study, different germination responses were observed for the various sowing media. Rubber seeds used in the first experiment germinated within seven days, *i.e.* low imbibition time, in all sowing media. Nevertheless, rubber seeds used in the second and third experiment in Gurugoda and Middeniya nurseries started germination after 12 days (*i.e.* high imbibition time). This could be attributed to the differences in the quality of seeds used in different experiments.

Being recalcitrant, *Hevea* seeds lose their viability within a few days after falling from the tree. Therefore, it is essential to collect fresh seeds from early seed fall and transport to nurseries at the earliest possible. Fresh (high quality) rubber seeds generally take 7-10 days to start germination in a sand bed (Anon, 2016). Fresh seeds of the clone RRISL 217 were used in the first experiment. However, in the second and third experiment, polyclonal seeds that had been brought to each government nursery were used. Seeds for government rubber nurseries are purchased from different suppliers who collect and store seeds for a few days to weeks to supply them in bulk quantities. When the seeds were stored even for a few days, they lose their viability. Moreover, large quantities of seeds are required to be transported to each nursery on long distances, which can result in the deterioration of seeds to a certain extent. Therefore, these low-quality seeds require more time to start germination in sand beds. According to Wongvarodom *et al.* (2014), high-quality rubber seeds had a greater emergence percentage and took less time to emerge compared to the low-quality seeds.

The current recommendation for harvesting germinated seeds from germination beds is to use only those germinated within the second week (until 14 days) of sowing, to ensure production of the vigorous rootstock. About 50% of the early germinators should be harvested every other day, only for three rounds, although the entire population germinates (Anon, 2016). Germination percentage/capacity in all sowing media in the first experiment after 14 days was above 70%. Moreover, no significant differences were recorded for mean germination time among media. Mean germination time is a measure of the rate and time-spread of germination (Soltani *et al.*, 2015). Therefore, all sowing media (river sand, coir pith, reclaimed sea sand, fine quarry dust and course quarry dust) appear to be suitable when fresh (high quality) rubber seeds are used. However, the highest germination index and germination value were recorded in coir pith as compared to river sand (control) and other media. The germination index represents the speed and uniformity of germination (Du *et al.*, 2019) and the high germination value is indicative of high vigour of the seeds (ISTA, 1995). The time taken to reach E_{50} was lower in coir pith followed by river sand. E_{50} is the time taken for cumulative germination to reach 50% of its maximum and interpolate from the germination progress curve versus time (Soltani *et al*. 2001). The results show that coir pith is superior to river sand as a sowing medium. Supporting the present study, Joseph and Jessy (2015) reported that leached coir pith was ideal to river sand as a germination medium as far as availability, cost, and germination capacity are concerned.

In the second and third experiment with low quality seeds, the sowing media elephant dung, coir pith and river sand recorded a satisfactory germination percentage (>50%) after 16 days of sowing in both Gurugoda and Middeniya nurseries. A growing medium should have various favorable characters such as light-weight, good porosity, well-drained but with optimum water holding capacity, slightly acidic with optimum cation-exchange-capacity, constant volume when wet or dry (Miller and Jones, 1995). Ndor *et al.* (2012) reported that good seed material contact and firmness of the medium contributed better moisture availability to trigger a better germination process. According to Jessy and Joseph (2015), the water holding capacity (WHC) of different media was in the order of coir pith (937.33%) > sawdust (271.62%) > wood chips (227.688) > river sand (30.48% > rock powder (11.91%). Accordingly, coir pith recorded the highest WHC whilst rock powder (quarry dust) recorded the lowest. Coir pith provides a favorable balance of air and water to plant roots and it has a high re-wetting capacity (Blok and Wever, 2008). The physical appearance and properties of dried and crushed elephant dung are similar to that of coir pith and therefore, we can expect a high WHC in elephant dung too. In government nurseries, sand germination beds are irrigated daily throughout the germination period. It shows that irrigation intervals to germination beds filled with coir pith or elephant dung can be reduced to alternative days or once in two days and thereby, minimize labor cost while saving water. Very low germination percentage recorded (results not shown) in low-quality seeds sown in fine and coarse quarry dust in the preliminary experiment in Gurugoda nursery might be due to its poor water holding capacity and some other unfavorable properties.

Even though the WHC of river sand is less, the germination attributes in river sand are on par with those in coir pith. River sand was sufficiently firm and dense to hold the seeds in place during germination and it retained enough moisture for germination (Ekwu and Mbah, 2001). Although reclaimed sea sand recorded satisfactory seed germination attributes in the first experiment, it didn't perform well in the second and third experiment. This can be attributed to the differences in sand and seed qualities. The properties, especially the salt and chloride content in sea sand, may vary depending on the leaching duration. Sea sand stock piles should be kept inland for 1-2 years to remove chloride ions through natural washing before being used to construction purposes (Ratnayake *et al.,* 2014). Reclaimed sea sand was purchased from one supplier in 2017 but from a different supplier in August 2018. Perhaps, germination had been negatively affected by salinity stress (the presence of high chloride (Cl^-) (Ratnayake *et al.*, 2014) and other ions in the latter sea sand). The process of germination starts with the uptake of water by the dry seed (imbibition) and is completed by when a part of the embryo, usually the radicle, penetrates the seed coat (Bewley, 1997). Salinity stress affects seed germination either through osmotic effects, by preventing or delaying germination (Welbaum *et al.*, 1990), or through ion toxicity, which can render the seeds unviable (Huang and Reddman, 1995).

In the second and third experiment, seeds sown in sawdust had a low germination percentage as compared to those in coir pith, elephant dung and river sand even though the WHC in sawdust was very high (Joseph and Jessy (2015). The sawdust used in the preliminary trial in Gurugoda was old and well dried. However, the sawdust used in the second and third experiment was somewhat fresh. Moreover, germination percentage in wood chips in Middeniya nursery was satisfactory but a low germination percentage was recorded in wood chips in Gurugoda nursery. Here, wood chips used in Gurugoda nursery were somewhat fresh as compared to those used in Middeniya nursery. The release of soluble tannin related phenolic compounds from fresh sawdust and wood chips (Thampan, 2000) might have contributed to the low germination percentage in seeds.

Several coir pith types (leached, partially leached and fresh) are available in the market. Although we used leached coir pith for the major experiments, other coir pith types were also tested in different sub experimental trials in Gurugoda nursery using both fresh and old seeds. In all the trials, satisfactory germination (>50%) was recorded in both seed types sown in all three types of coir pith after 14 days. Interestingly, fungal infections/growth were not recorded in/ on seeds or in sowing media in all experiments throughout. Moreover, no seeds were rotten due to high moisture and bacterial infections. In general, nursery managers apply a fungicide solution, preferably of Captan, in sand beds before sowing seeds due to fear of fungal infection. Therefore, experimental trials were also conducted in Gurugoda nursery with or without a fungicide treatment in the sowing media such as river sand and elephant dung. None of the trials recorded fungal infections in any of the sowing medium even after one month irrespective of fungicide treatments.

Seedling growth

Seedlings raised from seeds sown in different media in the first experiment showed no significant variations in growth parameters except for the stem diameter. Seedlings derived from seeds sown in reclaimed sea sand recorded a significantly higher stem diameter and the value was on par with that in coir pith and course quarry dust. This suggests that some substances in sea sand, probably mineral ions (Na^+ and K^+), might have induced some growth attributes in germinated seeds before transplanting in polybags. Nevertheless, seedlings raised from seeds sown in all media in the first experiment achieved buddable size after 3 months and those in the second and the third experiment achieved after 4-5 months and it showed that there was no effect of sowing media on growth of rubber seedlings.

Economic analysis

The performance of an alternative sowing medium must be balanced against its cost. This includes the market value of the material per unit volume, transport cost and the cost of any secondary processing required for its effective use (Lu *et al.*, 2006). Coir pith can be purchased at a low price as compared to river sand. In order to minimize the long distance transportation cost, usage of coir pith can be encouraged in nurseries located in areas where coir pith is readily available. Dried and crushed elephant dung can be collected free of cost at Elephant Orphanage, Pinnawala, Kegalle and transported in bulk quantities similar to that of coir pith. Therefore, elephant dung may be an ideal alternative sowing medium for nurseries located especially in Kegalle district.

Conclusion

Seed germination in coir pith and elephant dung is superior to/on par with that in river sand. Environmental and economic benefits can also be enjoyed with elephant dung and coir pith where possible. Therefore, coir pith and elephant dung may be used as alternative substrates for river sand in rubber nurseries.

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Effect of biofilmed biofertilizer on plant growth and nutrient uptake of *Hevea brasiliensis* nursery plants at field condition

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Abstract

Beneficial microbes through their direct and indirect mechanisms support plant growth in a sustainable manner. This study was conducted to understand the effect of biofilmed biofertilizer (BFBF) on dry matter production and nutrient uptake of Hevea brasiliensis nursery plants. The BFBF was formulated by phosphorus solubilizing bacteria Bacillus spp. and commonly found fungi Aspergillus spp. associated with H. brasiliensis rhizosphere. Rubber Research Institute of Sri Lanka (RRISL) recommended inorganic fertilizers were applied at the rates of 0%, 50% and 100% of the recommended level with and without the application of BFBF at a rate of 100 ml per rubber nursery plant monthly. Plant dry matter production, nutrient contents of plant parts and their nutrient uptake were determined before and after bud grafting stages. Results showed that many observed growth parameters; diameter, height, shoot dry matter, root dry matter and total dry matter were significantly higher in BFBF applied treatments compared to its respective non BFBF treatments. There was no consistent trend on the effect of BFBF on leaf nutrient contents at both growth stages. There were no significant differences with 100%F, 50%F+BFBF and 100%F+BFBF treatments in relation to total N, P and K uptake at before bud grafting stage and total N and K uptake at after bud grafting stage. At after bud grafting stage, significantly higher total P uptake was observed in T5 (50%F+BFBF) and T6 (100%F+BFBF) treatments (81 mg P/plant and 87 mg P/plant respectively) than in T3 (100%F) (64 mg P/plant) treatment. This study suggest that reduced amount of inorganic fertilizer with BFBF gave no significant difference or significantly higher values in relation to plant growth and total nutrient uptake of rubber nursery plants compared to recommended fertilizer application.

Key words: biofilmed biofertilizer, *Hevea brasiliensis*, nursery plants, nutrient uptake, plant growth

Introduction

In general, soils in rubber growing areas of Sri Lanka are deficient in plant nutrients and consequently, considered as less productive (Dissanayake *et al.*, 1999). The same agricultural cropping system adopted with the same management practices throughout the last several decades have resulted in decreasing soil fertility (Samarappuli, 1995 and Samarappuli and Yogaratnam, 1996). Therefore, fertility improvement has been identified as an accepted practice (Samarappuli and Yogaratnam, 1997) and requires a number of nutrients for normal, healthy growth and development of rubber. At present, mineral fertilizers are used to compensate the shortages of nutrients and to manage the soil fertility (Bockman *et al*., 1990; Thennakoon, 1990). There is a potential loss of nitrogen, phosphorus, potassium and magnesium that could be occur through leaching and runoff while nitrogen could get lost through volatilization and denitrification. Hence, loss of applied fertilizers from the soils leads to marked economic losses and negative environmental impacts (Bockman *et al*., 1990). Therefore, minimizing leaching of nutrients and volatilization of nitrogen losses associated with chemical fertilizers are required for prevention of unbalanced nutrition promoted through indiscriminative fertilization practices (Ayoub, 1999).

Among the sources available for fertilizing, the use of biofertilizers in agriculture is considered to be an eco-friendly practice which is more cost effective than chemical fertilizers. They are highly advantageous in enrichment of soil fertility and fulfilling the plant nutrient availability. The conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes as biofertilizers may not furnish the highest microbial effect, which may be achieved by biofilm formation (Bandara *et al*., 2006). Biofilm is a community of microbes and considerable attention has been focused recently on BFBF and their potential to increase the nutrient availability in soils (Jayasinghearachchi and Seneviratne, 2004a; Seneviratne and Jayasinghearachchi, 2005) and enhance plant growth (Bandara *et al*., 2006; Seneviratne *et al*., 2009). The bradyrhizobial-fungal biofilm with nitrogenase activity has showed N_2 fixing symbiosis with soybean, but the bradyrhizobial strain alone did not N_2 fixing symbiosis (Jasinghearachchi and Seneviratne, 2004b). Moreover, fungal-rhizobial biofilms could be used more effectively in biosolubilizing of poorly soluble Eppawala Rock Phosphate (ERP) (Seneviratne and Jasinghe-arachchi, 2005, Hettiarachchi *et al*., 2015). The application of BFBF developed from effective microbes have shown to stimulate restoring degraded tropical agricultural lands with improved ecosystem functioning and sustainability (Seneviratne *et al*., 2011; Seneviratne, 2012). Several studies conducted so far with the BFBF under laboratory, nursery and field conditions for soybean, mung bean, wheat, rice, anthurium and tea in Sri Lanka have shown positive results in relation to soil fertility and crop growth (Seneviratne and Jayasinghearachchi, 2005; Seneviratne *et al*., 2009 and Seneviratne *et al*., 2011; Seneviratne and Kulasooriya, 2013).

Therefore, this study was conducted with the aim of understanding the effect of BFBF on plant growth and nutrient uptake of *Hevea* seedling nursery plants.

Materials and Methods

The top soil belonged to Red Yellow Podzolic (RYP) great soil group and classified as Hapludults according to the USDA soil taxonomy (Mapa *et al*., 1999) was used without stones, pebbles and roots particles. This top soil was the recommended medium for filling poly bags (Tillekeratne and Nugawela, 2001) with a size of 6” wide, 15” long (lay flat dimension) and contain about 2 kg of soil. Prior to filling the poly bags the soil was air dried and crushed gently to pass through 2 mm sieve. Fifty grams of Higher Grade Eppawala Rock Phosphate (HERP) and 50 g of compost per bag were mixed with soil prior to filling according to the Rubber Research Institute Sri Lanka (RRISL) recommendation for rubber nursery plants. Bags in a single row were arranged close to each other and kept 5 cm distance between two rows. Two weeks old one germinated seed per bag was placed and Rubber Research Institute recommended management practices were conducted throughout the experimental period (Tillekeratne and Nugawela, 2001). Germinated seeds were grown in poly bags to raise young buddings were bud-grafted by the green budding technique at the age of 3-4 months. Considering that bud-grafting activity two periods were identified as before the bud-grafting and after the bud-grafting. Two weeks after planting of seedlings, fertilizer application was started and it was continued throughout the before and after bud-grafting stages until two weeks before planting at field conditions following the recommendation of fertilizer application for rubber nursery plants by RRISL (Tillekeratne and Nugawela, 2001). Recommended N, P, K and Mg fertilizers were used at 0% (0%F), 50% (50%F) and 100% (100%F) of the currently recommended level with and without the application of developed BFBF. There were 6 treatment combinations. The required quantities of N, P, K and Mg fertilizers were dissolved in water and 100 ml of the solution per bag was applied at monthly intervals. Isolated phosphorus solubilizing bacteria *Bacillus* spp. and fungi *Aspegillus* spp. from rubber root rhizosphere had an ability to form complex, multicellular biofilm community called BFBF. It was evaluated under *in-vitro* conditions and proved its ability of phosphorus solubilizing, N_2 fixing and secreting plant growth promoting hormones. The BFBF medium was examined under the microscope using a hemocytometer and cell density was adjusted to 10^8 – 10^9 cells ml^{-1}. The freshly prepared BFBF was applied as a liquid at equal rate for inorganic fertilizers. Each treatment had 5 replicates and each replicate consisted of 10 poly baged plants. Each replicate was maintained in different steps of the land and there was a variation in sunlight at the site. Hence, treatments were arranged in a randomized complete block design.

When the seedling plants reached their grafting stage, 6 mm in diameter above the soil level (Tillekeratne and Nugawela, 2001) was grafted with the clone RRISL 203. One month after, successfully grafted seedling plants were pollarded and facilitated for sprouting the desired bud patch. Therefore, both stages (before and after bud grafting) important for the final outcome of the preparation of quality planting material for rubber plantation. End of four months of before and after bud grafting stages, randomly selected five plants from each treatment were collected for data collection.

Growth assessments

Plant height was measured as the total height above the soil level and diameter was measured at 1 cm above the base of the plant and bud union at before bud grafting stage and after bud grafting stage respectively throughout the experimental period at one month interval.

Plant dry matter

At the end of four months at before and after bud grafting stages each plant was separated into leaves, stems, roots and their dry weights only were recorded by drying the components at 60ºC for constant weight. At the time of separating plants into their parts special care was taken to collect almost entire root system. Soil and dust particles adhered to the root system were removed carefully.

Plant analysis

Dried samples were ground in a Willy mill. 0.2g of finely ground plant materials were weighed into a pyrex tube and 5 ml Se/H_2SO_4mixture and 1 ml of '100 vol' H_2O_2were added to each. The tubes were then heated to 350-400°C for around two hours in a digestion unit. When the heated samples became colorless, the solution was kept to cool for another 2 hours. Ten ml of distilled water were added to the digest and the digest was thoroughly washed with distilled water and transferred to 50 ml volumetric flasks and made up to volume. This solution was used for the determination of nutrient contents (RRIM 1971b).

Nitrogen (N) and phosphorus (P) contents were determined using a SKALAR San^{++} Auto analyzer and potassium (K) using a GBC 203 Atomic Absorption Spectrophotometer. Nutrient uptake was calculated as the nutrient content of plant part; leaf, stem and root multiply by their total dry weight of each plant part.

Statistical analysis

Statistical analysis of the experimental data *viz*; plant height, plant diameter, total plant dry matter, nutrient contents; N, P and K of leaf, stem and root, N, P and K nutrient uptake of shoot, root and total plant were done by Analysis of variance (ANOVA) is for the RCBD. Subsequently, mean separation was done employing Duncan's Multiple Range Test (DMRT).

Results

Growth parameters were measured at before and after bud grafting stages are given in Table 1 and 2 respectively.

Plant growth parameters; diameter, height and shoot dry weight gave significantly higher values with 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments compared to other treatments; 0%F (T1), 50%F (T2) and BFBF (T4) treatments at before bud grafting stage. Same pattern could be observed with diameter, shoot dry weight, root dry weight and total dry weight at after bud grafting stage. There is no significant difference in many growth parameters with 100% F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments. Other growth parameters were higher in BFBF included treatments; 50%F+BFBF (T5) and 100%F+BFBF (T6) compared to 100%F (T3) treatment.

Table 1. *Plant growth parameters were measured at before bud grafting stage of nursery plants as affected by different combinations of fertilizer applications*

Treatment	**Diameter (mm)**	**Height (cm)**	**Shoot dry matter (g/plant)**	**Root dry matter (g/plant)**	**Total dry matter (g/plant)**
0%F	6.79^{c}	69.4^{c}	12.7^{c}	6.6^{c}	19.3^{c}
50%F	7.8^{b}	68.4^{c}	21.4^{b}	8.6^{b}	30^{b}
100%F	8.5^{a}	78.0^{ab}	25.3^{a}	9.6^{b}	34.9^{ab}
BFBF	7.3^{bc}	69.75^{c}	19.3^{b}	8.7^{b}	28^{b}
50%F+BFBF	9.05^{a}	82.16^{a}	25.9^{a}	11.1^{a}	37^{a}
100%F+BFBF	8.7^{a}	85.08^{a}	28.6^{a}	12.6^{a}	41.2^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Table 2. *Plant growth parameters were measured at after bud grafting stage of nursery plants as affected by different combinations of fertilizer applications*

Treatment	**Diameter (mm)**	**Height (cm)**	**Shoot dry matter (g/plant)**	**Root dry matter (g/plant)**	**Total dry matter (g/plant)**
0%F	6.3^{c}	69.7^{c}	15.9^{c}	7.0^{c}	22.9^{d}
50%F	7.0^{b}	71.1^{c}	18.4^{b}	8.7^{b}	27.1^{c}
100%F	8.1^{a}	78.6^{b}	22.6^{a}	9.9^{a}	32.5^{b}
BFBF	7.5^{b}	68.7^{c}	17.75^{bc}	7.6^{c}	25.3^{c}
50%F+BFBF	8.5^{a}	78.9^{b}	29.3^{a}	10.3^{a}	39.6^{a}
100%F+BFBF	8.6^{a}	85.7^{a}	29.6^{a}	10.2^{a}	39.7^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Nutrient concentrations were measured in the leaves collected at before and after bud grafting stages are given in Table 3. In both stages P concentrations of the leaf samples were not significantly affected by the treatments. 0% fertilizer (T1) treatment and BFBF treatment (T4) gave significantly lower values of leaf N concentrations compared to other treatments; 50%F (T2), 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) at both growth stages. At before bud grafting stage 100%F (T3) treatment increased leaf K level significantly compared to other treatments. Furthermore, significantly lower value of leaf K contents could be observed with 0%F (T1) treatment compared to other treatments.

At after bud grafting stage, BFBF applied treatments; BFBF (T4) and 50%F+BFBF (T5) gave significantly higher values of leaf K contents compared to their respective non BFBF treatments; 0%F (T1) and 50%F (T2).

Table 3. *Effect of different combinations of fertilizer applications on leaf nutrient contents of nursery plants at before and after bud grafting stages*

Treatment	Leaf nutrient contents (%) at before bud grafting stage			Leaf nutrient contents (%) at after bud grafting stage		
	N	P	K	N	P	K
0%F	1.83^{c}	0.23^{a}	0.597^{c}	1.77^{c}	0.23^{a}	0.63^{b}
50%F	2.27^{ab}	0.215^{a}	0.720^{b}	2.42^{ab}	0.22^{a}	0.69^{b}
100%F	2.59^{a}	0.233^{a}	0.807^{a}	2.64^{a}	0.24^{a}	0.8^{a}
BFBF	1.78^{c}	0.245^{a}	0.740^{b}	1.76^{c}	0.25^{a}	0.8^{a}
50%F+BFBF	2.05^{b}	0.233^{a}	0.700^{b}	2.08^{b}	0.25^{a}	0.76^{a}
100%F+BFBF	2.2^{ab}	0.24^{a}	0.765^{b}	2.56^{ab}	0.26^{a}	0.76^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Table 4. *Effect of different combinations of fertilizer applications on total nutrient uptake of nursery plants at before and after bud grafting stages*

Treatment	Total nutrient uptake (mg/plant) at before bud grafting stage			Total nutrient uptake (mg/plant) at after bud grafting stage		
	N	P	K	N	P	K
0%F	116^{c}	19^{c}	123^{c}	205^{c}	38^{c}	176^{c}
50%F	319^{b}	41^{ab}	262^{b}	283^{b}	50^{c}	270^{b}
100%F	377^{a}	46^{ab}	334^{ab}	421^{a}	64^{b}	342^{ab}
BFBF	159^{c}	37^{b}	193^{c}	173^{c}	48^{c}	209^{c}
50%F+BFBF	340^{ab}	53^{a}	410^{a}	403^{a}	81^{a}	407^{a}
100%F+BFBF	347^{ab}	52^{a}	390^{a}	450^{a}	87^{a}	398^{a}

(Means with same letters along the column are not significantly different at 5% probability level)

Total nutrient uptakes of the plant at both growth stages were calculated by using the data of nutrient concentrations in different plant parts and their dry weights are given in Table 4.

Calculation of nutrient uptake

Nitrogen uptake of shoot = leaf dry wt. x leaf nitrogen content + shoot dry wt. x shoot nitrogen content

Phosphorus uptake of shoot = leaf dry wt. x leaf phosphorus content + shoot dry wt. x shoot phosphorus content

In both stages, total N uptake gave significantly higher value with 50%F (T2), 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments compared to 0%F (T1) and BFBF (T4) treatments. Same pattern could be observed with total K uptake at both growth stages. Moreover, there were no significant differences with 100%F (T3), 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments in relation to total N and K uptake at both stages. At after bud grafting stage significantly higher total P uptake with 50%F+BFBF (T5) and 100%F+BFBF (T6) treatments (81 mg P/plant) and 87 mg P/plant) than those in 100%F (T3) (64 mg P/plant) treatment.

Discussion

Fertilizer application according to RRISL recommendations is an accepted practice for rubber in their different stages of rubber nursery, immature rubber and mature rubber to achieve their maximum growth and yield. This was further confirmed by an observation of significantly higher growth parameters of nursery plants with recommended fertilizer application treatment (T3) compared to no fertilizer application treatment (T1) in both growth stages on this study.

Due to low value of cation exchange capacity (CEC) in rubber growing soils added fertilizers can be frequently lost through leaching as well. Phosphorus availability is very low in rubber growing soils and found that P fixation is very high in respect to their high availability of Fe and Al contents in the soil (Dharmakeerthi *et al*., 2010; Silva *et al*., 1977).

Biofilm showed high nitrogenase activity (Jayasinhearachchci and Seneviratne, 2004b; Seneviratne and Jayasinghearachchi, 2005) and biosolubilization of rock phosphate enhanced availability of nitrogen and phosphorus in the soil (Jayasinghearachchi and Seneviratne, 2006a; Seneviratne and Indrasena, 2006). Bandara *et al*. (2006) observed that the conventional practice of plant inoculation with monocultures or mixed cultures of microbes may not facilitate the highest microbial effect in biological N_2 fixation, mineral nutrient release organic acids and plant growth hormone production etc. and may only be better achieved by biofilm formation. Hettiarachchi *et al*., 2012 observed that BFBF treated soils showed significantly higher microbial biomass content (MBC) which reflected the buildup of soil microbial communities. Soil microorganisms are of critical importance in nutrient cycling processes and also source and sink of plant nutrients.

Results showed that 60% of the observed plant growth parameters were significantly higher in BFBF treatments compared to its respective non BFBF treatments at both growth stages. No significant differences between 100%F (T3) treatment and 50%F+BFBF (T5) treatment for more growth parameters and rest of them were significantly higher with 50%F+BFBF treatment compared to 100%F treatment (Table 1&2).

Several studies conducted so far with biofertilizers and BFBF have shown positive results. Hettiarachchi *et al*., 2012 observed that the combined use of 50% recommended fertilizer with BFBF recorded the highest values for most growth parameters in rubber nursery plants under greenhouse conditions. Seneviratne *et al*., (2008a) observed that biofilm attached to the plant root of some crops help in cycling of nutrients and biocontrol of pests and diseases, resulting in enhancing of plant growth accompanied with improved agricultural productivity. Improved growth parameters with reduced rates of chemical fertilizer and BFBF were also recorded with tea plantations in Sri Lanka (Seneviratne *et al.*, 2009 and 2011) and China (Hvistentendahl, 2010). Khan, 2018 observed the performances of rice plant were better when 25% less inorganic N was applied with *Trichoderma* and combined application of *Trichoderma* and *Azospirillum.* Nafady *et al*., 2018 observed that the total dry mass of *Vicia faba* was increased significantly by the application of arbuscular mycorrhizal fungi and biofertilizer compared with non-inoculated plants. Mahanta *et al*., 2014 observed that the inoculation of phosphorus solubilizing bacteria (BSB) and vesicular arbusculatar (VAM) could substitute 50% P of soybean – wheat cropping system with better root property and higher grain yield.

According to the enhancement of growth parameters, improvement of leaf nutrient status could not be observed under present study. Further leaf nutrient contents and total nutrient uptake showed that 25% of the observed nutrient levels were significantly higher in BFBF treatments compared to its respective non BFBF treatments (Table 3 & 4). Rest of the observation gave no significant differences between BFBF treatments compared to its respective non BFBF treatments except one occasion. Out of 12 combinations of 100%F (T3) and 50%F+BFBF (T5) for different leaf nutrients and their total nutrient uptake showed no significant differences except three occasions. Dawwam *et al*., 2013 reported that inoculated potato plants showed significant differences in vegetative growth parameters as well as photosynthetic pigments and N, P and K concentrations compared with control. Similar to that Silva *et al*., 2016 observed high rate application of bio protector and biofertilizer gave increased chemical characteristics and nutrient uptake of melon plant compared to soluble fertilizer applied in the recommended rate.

Moreover, some other studies have reported similar to above mentioned observations of their studies by the application of microbial inoculants as biofertilizers (Mukhtar *et al*., 2017; Dutta *et al.*, 2017; Andrade *et al*., 2013; Gupta *et al*., 2012; Tejada *et al*., 2016). Biofertilizer or BFBF is a term used for the products including living or dormant micro-organisms (Rai, 2006). They are an alternative to mineral fertilizers for increasing soil productivity and plant growth in sustainable agriculture. Recently, there is an increasing interest in this type of environmental friendly, sustainable agricultural practices to alleviate deterioration of nature and environmental pollution (Gauda *et al.*, 2018). They may support the plant growth by several mechanisms such as decomposing organic materials and release inorganic nutrients, increasing the availability of nutrients in the soil by solubilization, chelation, oxidation and reduction processes, increasing root surface area by inducing root growth promotion, enhancing other beneficial symbiosis associated with plant and by combination of mode of actions *etc.* (Vessely, 2003; Anderson *et al.*, 1993; Whiting *et al.*, 2001; Jing *et al*., 2007).

Conclusion

RRISL recommended fertile top soil is not available in most of the rubber nurseries in Sri Lanka. Soil Analysis of the poly bag filling indicated mean values for the: pH 4.6; CEC 2.3 (c mol (+) kg^{-1}; total nitrogen 0.097%; available phosphorus 10 – 12 ppm; potassium 112 ppm and magnesium 223 ppm. Due to low values of CEC in the soils added fertilizers can be frequently lost through leaching as well. Therefore, fertility management is an important aspect for the production of quality planting material of rubber.

This study showed that the enhancement of many growth parameters (around 60%) could be observed with BFBF treatments compared to its respective non BFBF treatments. Further, 50% F + BFBF (T5) and 100%F (T3) gave comparable growth parameters for more assessments and rest of them were significantly higher with 50%F+BFBF (T5) treatment compared to 100%F (T3) treatment. Moreover, their total nutrient uptake showed no significant differences frequently.

It can therefore be concluded that there is a possibility of using BFBF to improve growth and nutrient uptake of rubber seedling plants with modified levels of chemical fertilizers under field condition.

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Surface treated natural rubber latex sludge as a potential filler for natural rubber compounds

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Abstract

Natural rubber (NR) latex sludge, a byproduct generated from centrifuged latex manufacturing industry, contains a large portion of inorganic materials and presently treated as a waste material. In this study, the sludge was converted to a powdery material through drying and grinding processes followed by a surface modification with a long chain fatty acid with a view improving its compatibility with rubber composites.

Composition and structure analysis of the latex sludge powder showed that it is mainly a crystalline material of phosphate mineral with approximately 8% of trapped rubber particles within the sludge material. Processed NR latex sludge powder was surface treated with a long chain fatty acid to offer organophilic character to the sludge. FTIR analysis confirmed that latex sludge was successfully modified with the long chain fatty acid.

A series of NR compounds were prepared with different loading levels of the surface treated sludge using a laboratory scale internal mixer to study the effect of the surface modified sludge on curing and mechanical properties in comparison to unmodified latex sludge filled NR compounds. Curing characteristics measured under low shear strain rate have shown that surface modification improves the processing safety without a significant impact on other curing characteristics.

Mechanical properties of surface treated sludge filled NR vulcnizates have shown an improvement in tensile properties, especially strength and elongation at break, in comparison to untreated counterparts. However, on the other hand, stiffness as measured with hardness was reduced at higher loadings of surface treated sludge filled NR vulcanizates. Both surface treatment and the increase of filler loading increased the compression strength and abrasion weight loss of the compounds.

***Key words**:* fatty acids, mechanical properties, Natural rubber, NR latex sludge, surface treated

Introduction

Fillers are a group of materials frequently incorporated in to rubber composites as one of the major additives among all the other rubber compounding ingredients such as activators, accelerators, vulcanizing agents, antioxidants, softeners, thickeners, *etc*.

to meet various product performance requirements (Okiemen *et al.*, 2003). It has been well established for decades that incorporation of filler has two main advantages; improvement of the reinforcement of the rubber composites and/or reduction of the cost of the final product (Hepburn & Blow, 1971). Incorporation of reinforcing filler into NR matrix improves physico-mechanical properties such as oil resistance, abrasion resistance and tensile strength of rubber vulcanizate by increasing the number of cross-links in rubber chains, which share load applied to the matrix. The reinforcement efficiency of the particulate filler depends on particle size, particle size distribution, particle shape, surface activity, and the interaction between the filler and the rubber matrix. (Ski, 1970; Parkinson, 1957; Hepbium, 1984). Non reinforcing fillers, known as diluents increase the bulk volume of the composite while reducing the cost of the materials.

A large number of studies on effective usage of various industrial and agricultural by-products such as wood flour, rice husk, palm kernel husks, fly ash as low cost filler materials, in rubber composites has been reported by many research groups (Okiemen & Imanah, 2003; Sae- Oui, P. *et al*., 2002; Muniandy *et al*., 2012; Pangamol *et al*., 2018). These studies have revealed that the use of such materials in NR composites has multiple benefits such as low cost, certain property enhancements and in addition, the positive environmental impacts offered by restricting their disposal to the environment. However, preparation of rubber composites filled with these types of fillers derived from industrial waste materials to yield useful technical properties, overcoming unnecessary agglomeration within the rubber matrix, is a challenging task.

Surface modifications of filler using various modifiers such as titanates, silanes, phosphates, fatty acids *etc.* have been widely reported as one of the main techniques to overcome the above challenge (NikNurAzzaNikAdik *et al*., 2016). Stearic acid, a low molecular weight organic compound, has been used successfully to modify the surface of the different filler types like calcium carbonate and magnesium hydroxide. Coating with stearic acid on filler surface can improve the compatibility between rubber matrix and the coated filler, resulting in improved interfacial interaction and improved uniform dispersion within the rubber matrix due to the bi functional character (polar head and non-polar tail) of the stearic acid structure. Consequently, rubber composites with improved properties could be generated (Mihajlovc *et al*., 2009).

Latex sludge containing magnesium ammonium phosphate which is a by-product generated in the magnesium removal process during centrifuged latex manufacturing process is one of the potential source for deriving a such low cost filler. Manufacture of 100 kg (dry rubber) of concentrated NR latex generates about 4-5kg of latex sludge. This is a considerable quantity when consider the total concentrated latex production of approximately 30,600 MT

(Rubber Development Department, 2016). In general practice, this sludge is disposed as a waste material, creating environmental issues such as emanating bad smell, contamination of water bodies and increasing the soil basicity. Even though agronomic benefits of the latex sludge have been investigated (Okiemen *et al.,* 2002), there is no reported literature available on its wide usage in the agriculture sector. This may be probably due to the presence of rubber particles trapped in the sludge which could decrease the rate of biological degradation. Effect of natural rubber processing sludge on the degradation of crude oil hydrocarbons in soil has also been investigated and found out that the extent of crude oil degradation was markedly increased by the sludge (Okieimen & Okieimen, 2002). A study on potential use of sludge ash as a filler in NR has revealed that there is a potential of using sludge ash as filler in the rubber industry (Intiya *et al*., 2016). Use of untreated sludge as a semi reinforcing filler along with Carbon black for NR compounds has been reported by (Priyanka & Rathnayake, 2012). This study has revealed the use of sludge as a partial filler in carbon black filled rubber composites suitable for selected industrial applications.

Modification of sludge surface is a potential route to achieve the improved compatibility between latex sludge filler and the rubber matrix and subsequent enhance the dispersibility, widening its applications in the rubber industry.

Therefore, this study was carried out to investigate the effect of surface modification of latex sludge on the performance of NR compounds filled/treated sludge. Sludge material was first characterized for its chemical composition and structure. Filler surface was treated with stearic acid and curing and mechanical performances of latex sludge filled NR compounds were investigated with a view to studying the effect of surface modification of latex sludge.

Materials and Methods

Materials

RSS No 1 grade of natural rubber obtained from Dartonfield Factory, Rubber Research Institute of Sri Lanka was used as the rubber material. NR latex sludge, a by-product of centrifuged latex manufacturing process, used in this study was obtained from Lalan Rubber (Pvt.) Ltd., Bulathsinghala, Sri Lanka. Other compounding ingredients used were of commercial grade chemicals supplied by local rubber chemical suppliers.

The latex sludge collected from Lalan Rubber (Pvt.) Ltd., was first sun dried for 4 days and further dried at 100^{o}C in an oven for 24hrs and ground in to a powder. This powder was further dried at120^{o}Cin an oven for 2hrs to assure a moisture free material.

Preparation of surface treated sludge

A volume of water (172ml) was heated to 75 ^{o} C and stearic acid (20.3 g) was added in to the heated water. The mixture was further heated for another 15min. Ammonia solution (25 w/w %) was then added while stirring and the mixture was homogenized for 30min. Sludge suspension was prepared by mixing 640 g dried sludge powder into 3.2 L of water. The temperature of the suspension was adjusted to 75 ^{o} C. Two mixtures were then mixed together and the mixture was stirred for one hour at 75 ^{o} C. Coated sludge was separated off and dried in an oven at 55^{o} C for, 24hrs, and again ground to a fine powder.

Characterization of latex sludge

Chemical composition**,** especially metal iron concentration, of dried latex sludge powder was characterized with Atomic Absorption Spectrophotometer (AAS), model GBC Avanta l, whilst phosphate content was determined using ASTM 4500-P (Vandomolybdophosphoric acid colorimetric method).

Structural analysis of sludge carried out using an X-ray diffractometer (Bruker D8 diffractometer and the analysis was performed at a wave length of 1.54Å of Cu Kά radiations to determine the crystalline structure of the sludge, scanning over a two theta (2θ) range from 1 to 60 degrees, at a rate of 0.01^{0}/seconds.

Rubber hydrocarbon content, acetone extract, and volatile material content were determined using ISO test methods, ISO 5945-19 2E, ISO 1404 and ISO 1976-E, respectively.

Fourier Transmission Infra-red Spectroscopy (FTIR) analysis was carried out in order to find out the presence of stearic acid coating on the latex sludge using Nicolet 380 FTIR spectrometer. The analysis was done within the wavelength range of 400 to 4000 cm^{-1}.

The morphology of the sludge particles were studied with the HITACHI SU 6600 scanning electron microscope after the samples were sputter coated with a thin gold layer.

Compounding

Table 1 shows the formulation for each compound containing different loading levels of coated and uncoated sludge as filler.

For the compounding and mixing, a Brabender plasticorder (model: PL-2000) was used. Latex sludge filled NR composites were prepared by mixing them in the Brabender for 15 minutes at temperature of 60 ^{0}C and rotor speed of 60rpm. Various rubber additives were added to the masticated natural rubber prior to the addition of NR latex sludge and carbon black. Finally, accelerator and sulfur was added. Six natural rubber composites were produced incorporating different sludge loading levels from 0-100 phr at 20 phr intervals.

Table 1. *Formulations of sludge filled NR composites*

Ingredients	Amount (phr)	
	Uncoated Sludge filled Composites (USC)	**Coated Sludge filled Composites (CSC)**
Natural Rubber (RSS)	100	
Zinc oxide	5.0	
Stearic acid	2.0	
N-isopropyl n-phenyl1,4-phenylendiamine	1.0	
Carbon black	30	
N-cyclohexyl-2-Benzothiazole sulphenamide (CBS)	1.2	
Tetramethyl thiuram monosulfide (TMTM)		
Sulfur	0.4	
Processing oil	2.0	
Sludge uncoated	5.0	
Sludge coated with stearic acid	Variable (0-100)	-
	-	Variable (0 -100)

Cure characteristics

Cure characteristics of the composites were determined using a moving die rheometer, model (MDR:Ekron EKT-2000s) according to ASTM 5289 at 130 ^{0}C. Cure characteristics of the latex sludge filled NR compounds were derived from the respective rheographs.

Mechanical properties

NR vulcanisates were prepared by compression molding of rubber compounds according to respective optimum cure times (t_{90}) using a Hot press (KAO-Tech compression Moulding Machine) at 130^{o}C. Tensile properties and tear strengths of the rubber vulcanisates containing coated and un-coated sludge were analyzed using an Instron, model 3365 Universal Tensometer following ASTM D 412-68 and ASTM 624-54 standards methods respectively. Five specimens were analyzed from each composite and the average results were recorded.

Results and Discussion

Composition and structure characterization

Composition of the latex sludge dried at 120^{0}C is presented in Table 2. The results show that magnesium and phosphorus are the main inorganic constituents in the sludge. There is also a considerable amount of polyisoprene which indicates the natural rubber (measured as polyisoprene) trapped in the sludge material. Volatile matter percentage is about 5.54 (w/w) which consists of both ammonia and moisture of the sample.

Table 2. *Chemical composition of NR latex sludge dried at 120^{0}C*

Constituent	**(w/w %)**
Oxygen (based on phosphate content)	36.0
Magnesium	27.73
Phosphorus	18.26
Poly isoprene content	8.31
Volatile matters	5.54
Ammonical Nitrogen content	0.16
Zinc	0.001
Other materials	add to 100

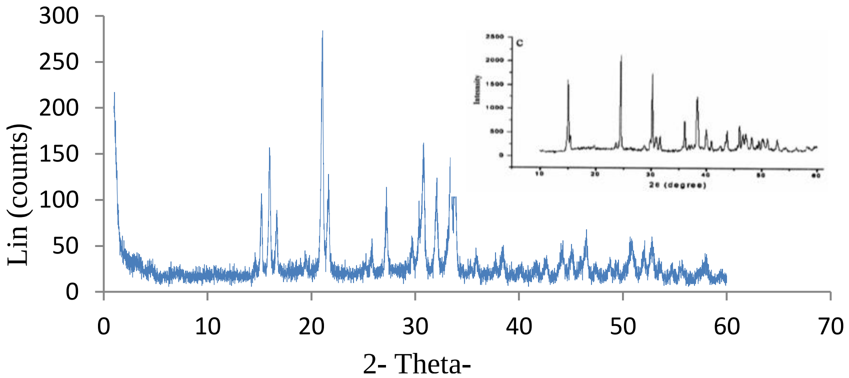
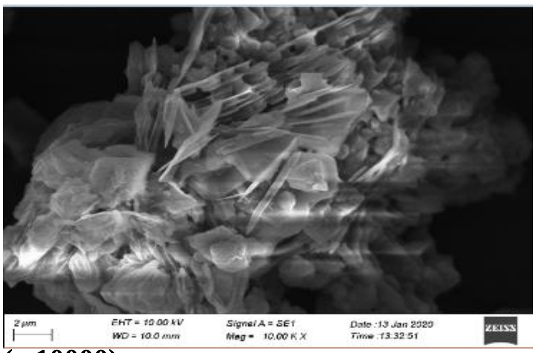


Fig. 1. The XRD spectrum of uncoated NR latex sludge

The diffraction peaks are appeared at 15 ^{0},45^0, 46^0, and 47^0 in the -XRD spectrum of the sludge are analogous with the characteristic peaks for magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4.6H2O$), a phosphate mineral known as struvite. Therefore, XRD analysis suggests that sludge is a phosphate rich mineral with a structure similar to struvite (Fig.1).

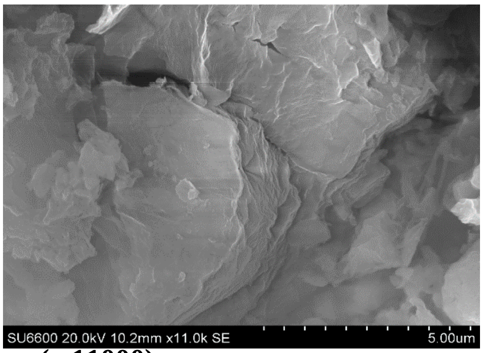
Morphological studies

Figure 2 (a) illustrates the SEM micrographs of the latex sludge. It could be seen that uncoated sludge particles exist as agglomerates with irregular sizes and shapes. SEM micrographs of the sludge observed at higher magnification are shown in Figure 2 (b). It clearly shows platelet like laminated sheets structure of the sludge particles. Due to these laminated structure, sludge particles have a higher aspect ratio, which has a potential reinforcing effect in a polymeric composites matrix. When the micrographs of untreated and surface treated filler samples at same magnification (Figures 2(a) and 2 (c) are compared, agglomerates of relatively smaller sizes could be seen in surface treated sludge. It could be suggest that the surface coating has restricted the sludge particle agglomeration to a certain extent.



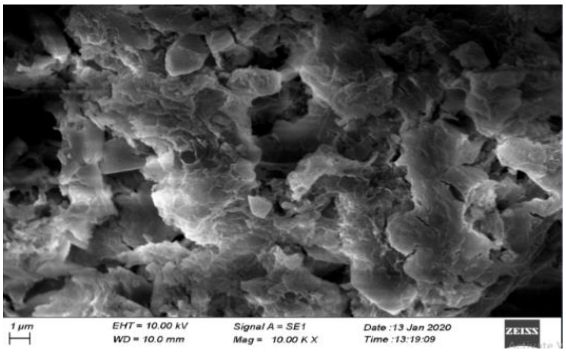
(x 10000)

a. Uncoated sludge particles



(x 11000)

b. Uncoated sludge particles higher magnification



(x 10000)

c. Coated sludge particles

Fig. 2. Illustrates the SEM micrographs of the sludge

FTIR analysis

Figure 3 shows the infrared spectra of coated sludge and uncoated sludge samples. Peaks at 2326 cm^{-1}due to stretching vibration of NH4 group, 1480 cm^{-1} due to bending and vibration of crystalline water, 1070, 990 and 913 cm^{-1} peaks due to bending and vibration of $PO4^{-3}$ (Stefov *et al.,* 2005) they are common to both coated and uncoated spectrums and further confirmed the sludge as crystalline magnesium ammonium phosphate hexahydate. IR band appear at 1241 cm^{-1}due to CO-O stretching in esters and two additional peaks at 2854 $cm^{-1}$and 2919 cm^{-1} seen in the FTIR spectrum of coated sludge. These two additional peaks correspond to symmetric and asymmetric stretching and vibrations of aliphatic groups $–CH_2$-groups. Presence of these peaks in the spectrum of stearic acid coated sludge suggests that, there is CO-O groups and C-H bonding typical to CH_2 groups of stearic acid (CH_3 $(CH_2)_{16}$ COOH). Therefore, it is evidenced that modification of the sludge surface with stearic acid is successful.

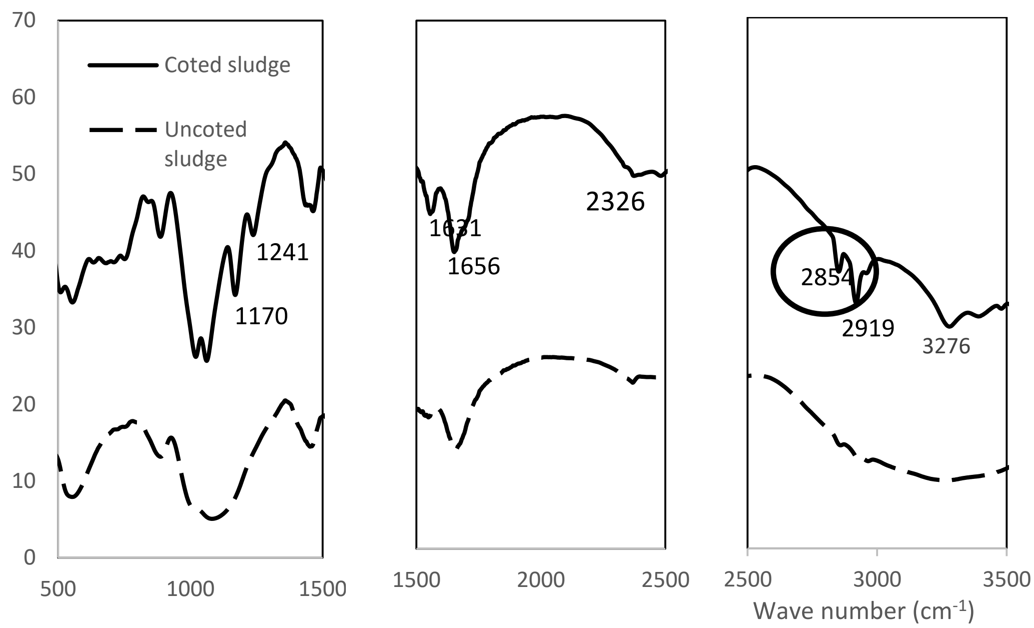


Fig. 3. FTIR of Stearic acid coated and uncoated sludge

Cure characteristic and processability of NR/sludge compounds

Figures 4 to 8 show the effect of stearic acid coated sludge and uncoated sludge on the curing characteristics of the rubber compounds.

Figure 4 shows that incorporation of sludge has initially resulted an increase in the scorch time in both uncoated and coated composites. Maximum increase was registered at 20phr sludge loading for both types of compounds. For unmodified filler incorporated composites, the scorch time gradually decreases after passing through the maximum values while treated composites remains its scorch time almost unchanged. Incorporation of filler may retard the formation of activator-accelerator pre-cursors due to the adsorption of them by the filler agglomerates. The composition analysis of the sludge confirmed that it mainly consist of magnesium ammonium phosphate hexahydrate. Ammonium salt which could increase the basic nature of the rubber compounds and hence supports the curing reactions. Therefore, increase in ammonium salt content with increasing filler loading speeds up the formation of the activator-accelerator complexes loading at higher filler loading as the surface area of filler agglomerates dose not increase proportionally to its volume. Therefore, decrease in scorch time of un-coated sludge rich compounds could be elucidated. It has been reported in literature that addition of various types of fatty acids at high concentrations could retard the curing reactions due to the various complex reactions associated with the soluble Zn ions formed in the compound (Coran, 1965, Poh & Tang 1995, Hanafi Ismail & Tajur Arus Ruhaizat,1997). In surface treated sludge, it has two major chemical substances namely Magnesium ammonium phosphates and stearic acid, both influence the curing reactions in two opposite directions. This may results in the almost unchanged higher scorch time of treated sludge incorporated compounds due to the compensation of opposite effects of each other. Therefore, it could be seen in Figure 4, that stearic acid coating has diminished the effect of filler loading on the scorch time of the treated sludge filled composites after a certain filler loading (20 phr).

In contrary to the effect on scorch time of the composites, there is a reduction in T_{90} of the both types of filled compounds even at 20 phr sludge loading (Fig. 5). T_{90} of Uncoated Sludge Compounds (USC) exhibited a gradual reduction may be probably due to the presence of ammonium slats in the sludge. CSCs show an almost unaffected T_{90} values with sludge loading. The acceleration effect of ammonium salts and retardation effect of excessive fatty acid concentration in curing reactions of NR compounds incorporated with treated sludge explain this trend. Higher scorch time and lower T_{90} values of 20 phr sludge filled compounds than that of NR gum compounds suggests that adsorption of curing ingredients by filler particles has become significant only at the low loading levels.

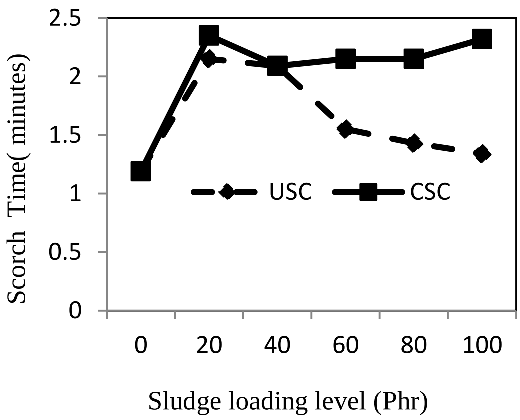


Fig. 4. Scorch time vs sludge loading

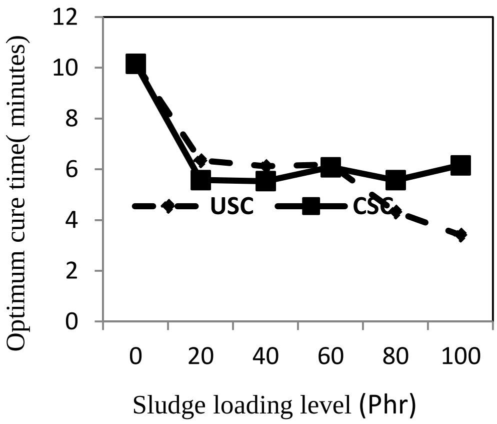


Fig. 5. Curve time vs sludge loading

As it could be seen in Figure 6, for uncoated sludge filled composites, cure rate index gradually increases with the sludge loading. In other words, a faster cure rate is obtained for the composites with increasing sludge loading. This may be due to the ammonia complexes formed between ammonia and compounding ingredients yielding new substances which accelerate the curing process. Coating of sludge may delay the curing reactions as it could be acted as a barrier in emitting moisture and ammonia which cause to increase the cure rate (Egwaikhide *et al.*, 2013) which is in par with the earlier observations made by other researchers (Poh & Tang 1995, Hanafi Ismail & TajurArus Ruhaizat 1997). From the results, it is evidenced that coating diminishes the effect of sludge loading on the cure rate of coated sludge filled NR compounds. Modification of sludge surface with stearic acid seems to allow incorporation of higher sludge loading without much adverse effect on the total cure time and processing safety.

As expected, incorporation of sludge has increased the minimum torque which indicates the processability of the rubber compound (Fig. 7). Higher the minimum torque, lower the material's processability. This is because of the restriction exerted by the filler particles against the mobility of rubber macromolecules. Maximum torque also increases for both types of composites, until it reached certain loading, beyond which maximum torque has again decreased with the sludge loading. When the composites are vulcanized, rubber molecules are cross linked and restrict their mobility in addition to the restriction applied by filler agglomerates. As the filler loading increases, larger agglomerates are formed in cross-linked rubber matrix and it may become unstable due to formation of larger agglomerates under experienced strain yielding lower torque values. It could be further seen in Figure 8 that the maximum torque of rubber compounds modified with stearic acid is lower than the corresponding uncoated sludge containing compounds. This may be due to the plasticizing effect of excess stearic acid in the modified sludge.

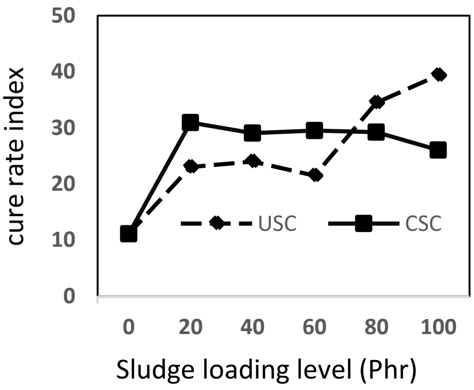


Fig. 6. Cure rate index vs sludge loading

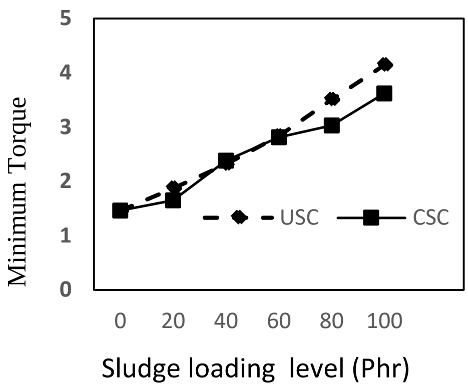


Fig. 7. Minimum torque vs sludge loading

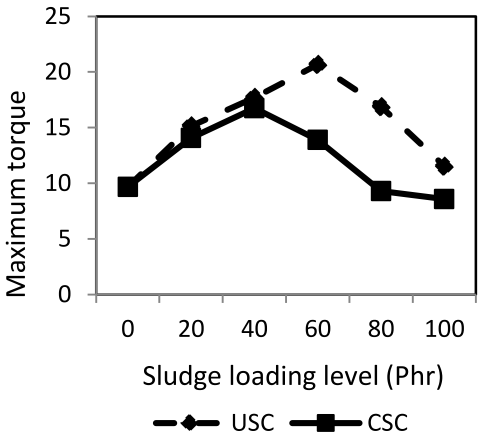


Fig. 8. Maximum torque *vs*. sludge loading

Mechanical properties

Figure 9 and 10 show the stress-strain responses of both unmodified (uncoated) and surface modified (stearic acid coated) latex sludge filled NR vulcanisates as a function of filler loading.

As expected, tensile strength (Fig. 9) of both uncoated and coated latex sludge filled vulcanisates was shown a reduction with the increase of sludge loading. In general, natural rubber gum compounds have shown strain-induced crystallization and the material crystallizes under increasing stress (Ismail *et al*. 2010). However, incorporation of sludge (*i.e.* both modified and unmodified latex sludge) may disrupt the strain-induced crystallization process of NR. In addition, as the filler loading increases, there is a trend to form larger sludge agglomerates resulting in inhomogeneous distribution of sludge agglomerates and poor interactions between sludge particles and rubber matrix and as a result of incompatibility of the filler and rubber. These agglomerates could serve as stress concentration points and flaws yielding poor tensile properties. Similar observations have been reported in literature for NR composites filled with other incompatible filler/natural rubber compounds (ShuhairiahDaud *et al.*, 2016). However, it could also be inferred from the results that surface modification with stearic acid has improved the filler dispersion within the rubber matrix and hinder the sludge agglomeration to a certain extent as evidenced by the higher tensile strength values of coated sludge filled NR vulcanisates in comparison to that of uncoated sludge filled NR vulcanisates (Fig. 9), especially, at lower sludge loading up to 60 phr. Beyond 60 phr, coated and uncoated samples showed same values in tensile strength. This may be due to agglomeration of even coated latex sludge particulates at a higher loading.

According to Figure 10, % elongation at break is gradually decreased with increase of uncoated latex sludge in the compounds while gradual increasing tendency is observed for the surface coated sludge filled compounds. This may be due to the availability of excess unreacted stearic acids that acted as a plasticizer in the NR compounds. Due to the lubricating effect created by un-reacted stearic acid, sludge particles could slide over one another during stretching the matrix which resulted in an extra extension.

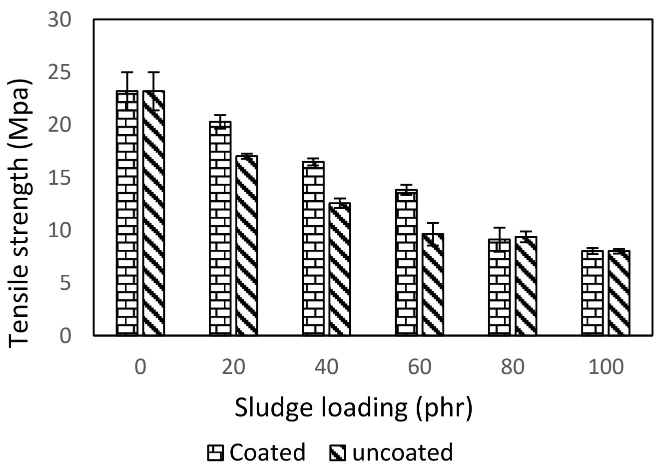


Fig. 9. Tensile strength of stearic acid coated and uncoated sludge containing vulcanizate

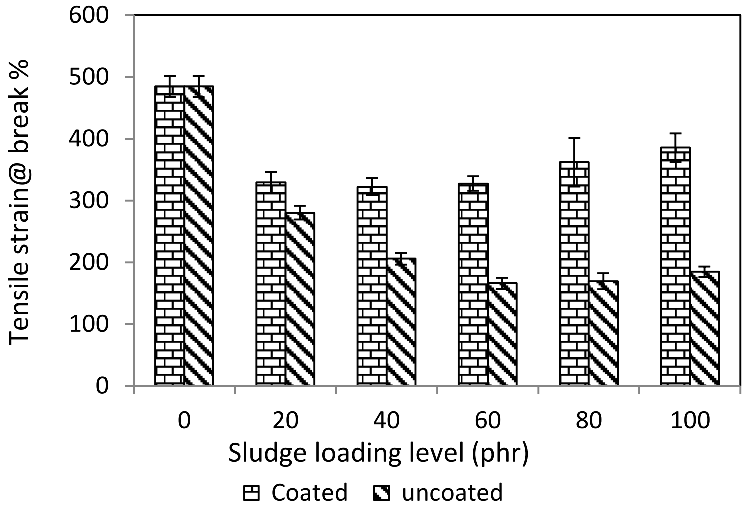


Fig. 10. The effect of filler loading on % elongation at break of stearic acid coated and uncoated sludge containing compound

Stress at 100% elongation related to degree of filler to rubber interaction and the dispersion of the filler in elastomer matrix. Table 3 shows the effect of filler loading on modulus at 100% elongation. Modulus of the filled compound increased with an increase of filler loading. When further increase of latex sludge loading, the rubber content decreases resulting in increase in the stiffness of the composites. As there is no adequate rubber phase to wet the filler particles, the composite shows properties of a lightly bonded weak inorganic material yielding low modules values at higher filler loading. The lower modulus values registered for coated composites may be again due to the presence of free stearic acids in the composites. They also follow the same variation of modulus with the loading as uncoated composites.

Table 3. *The effect of filler loading on 100% moduless of stearic acid coated and uncoated sludge containing compound*

Filler loading (sludge) (%)	**Moduless @ 100% (Mpa)**	
	Un-coated	**Coated**
0 (unfilled)	1.707	
20	4.003	3.418
40	4.924	3.886
60	5.399	3.34
80	5.109	2.314
100	4.036	1.736

Table 4. *Mechanical properties of un-coated and coated sludge containing composites*

Filler loading (sludge) (%)	Hardness (IRHD)		Resilience (%)		Compression Set (%)		Abrasion as volume loss (mm^3)	
	Un-coated	Coated	Un-coated	Coated	Un-coated	Coated	Un-coated	Coated
0(unfilled)	53.4		77.0		8.29		57.0	
20	64.1	60.5	71.5	72	10.93	8.57	77.85	197
40	67.75	66.6	70.5	70	11.26	11.17	216.6	234
60	72.58	63.9	69	62	13.96	11.56	258.8	300
80	70.35	56.8	63	51	13.4	27.8	275.2	367
100	65.39	55.1	54	49	26.6	32.5	313.4	360

Both vulcanisates, it has shown that hardness (IRHD) is increased at lower loading levels of latex sludge while it decreases gradually when the loading level is high. However, at any given sludge loading level, coated sludge filled vulcanisate shows a lower value than that of uncoated sludge filled vulcanisate. This is attributed to the fact that unreacted stearic acid present in the rubber matrix acts as a plasticizer, resulting in reduced hardness.

There is no considerable reduction of resilience up to 60 phr level indicating persistence of bouncing effect. It is evident from the results that there is no significant difference in compression set percentage in both coated and uncoated sludge incorporated compounds up to 60 phr level. Above 60 phr, there is a huge increment in percentage of compression set in both USC and CSC compounds. It could also be noted that at higher filler loading, percentage of compression has become higher in stearic acid coated sludge containing compounds when compared with uncoated sludge containing compounds.

In high filler loading levels such as 80 and 100 phr, agglomeration of filler is higher owing to poor filler dispersibility as well as the percentage of non vulcanisable materials in the composites. Consequently, lower cross linking density in the composites. It creates low strain recovery when the compression load relived resulting in high percentage of compression set. Higher compression set is exhibited in stearic acid coated sludge containing higher filler loading due to presence of higher quantity of free fatty acids which increase lubricating effect. Fillers could slide over one another when compressed resulting low recovery on relieve due to stearic hindrance of stearic acid. (Nik Nur Azza NikAdik *et al*., 2016). This may also lead to increase of percentages of compression set get higher at high loading levels.

Abrasion loss has significantly increased with incorporation of the sludge for both uncoated and coated composites. It has gradually increased with increasing sludge loading. Modified sludge containing compounds however, shows relatively lower abrasion resistance over the uncoated counterpart. Lubricating effect of the stearic acid discussed earlier may be responsible for this observation too.

Conclusions

Studies on chemical composition and structural characterization revealed that processed latex sludge has a structure resembling the crystalline material known as struvite. In addition to magnesium salts and phosphates, it contains rubber and other inorganic materials trapped within the latex sludge. Inorganic latex sludge is successfully converted into organo-philic by surface modification with stearic acid treatment. Unmodified latex sludge accelerates the vulcanization reaction of NR compounds as shown by cure rate index data. However, surface modification of latex sludge with stearic acid neutralizes the curing acceleration created by latex sludge and consequently curing characteristics of NR compounds are not affected by surface modified latex sludge.

Surface modification of latex sludge with stearic acid improves the uniform dispersion of sludge within the rubber composite and, moreover, enhances the compatibility between the two components. As a result, failure strength characteristics under tensile deformation of coated sludge filled composites (CSC) is improved compared to that of uncoated sludge filled composites (USC). Although surface treatment of sludge has a significant impact to prevent latex sludge agglomeration and enhances in sludge dispersion within the NR composite material, at higher loading levels of coated sludge, negatively affected on mechanical properties of composite due to higher amount of remaining free stearic acid in coating process. Therefore, this surface modified latex sludge material could be used in NR compounds as a semi reinforcing filler for different applications depending on the required vulcanizate properties.

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Impact of tapping quality and harvesting practices on the sustainability of the rubber industry in Sri Lanka

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Abstract
Maintaining the tapping quality in rubber fields is the key requirement to obtain potential yield with minimum harm to the tree. It also determines productivity and the economical lifespan of 30 years and sustainable rubber industry. The length of the tapping cut, the depth, the thickness of the bark shaving, and the slope of the tapping cut are the main factors determining the tapping quality. Use of a stencil according to the intended tapping frequency, i.e. d2, d3, d4, etc., to mark the guidelines to maintain the proper tapping angle of 30 degrees to the horizontal, and the bark consumption allowed for one year, are all equally contribute to maintaining the tapping quality. About 5000 ha. was surveyed to assert the tapping quality and to make recommendations. The average bark consumption rate per panel was four years deviating from recommended six years under d2 tapping. The monitory loss per hectare under this bark consumption rate is Rs.2.5 million at a productivity level of 1000 kg/ha/y and Rs.350.00 per kg of rubber. Trees affected by tapping panel dryness varied and was over 70% in some extreme cases. Introducing a quarter cut on the upper opposite panel of the tree ceased the situation to a greater extent.

Key words: bark audit, excessive bark consumption, harvesting, *Hevea*, panel dryness, rubber

Introduction
The extent under rubber cultivation in Sri Lanka has come down from around 200,000 ha. in the 1970s to about 130,000 ha. at present (Anon, 2017). But, the rubber production in Sri Lanka was on an increasing trend until 2016, mainly owing to the usage of high yielding clones and the adoption of improved agro-management practices recommended by the Rubber Research Institute of Sri Lanka. However, from 2016 onward there is a marked decrease in rubber production owing to many reasons such as diversifying rubber lands to other crops mainly oil palm, abandoning productive lands due to low productivity and poor price, *etc*.
The economical life span of the rubber tree is about 30 years as both the virgin

and renewed panels are used up in about 24 years under every other day tapping. This can be extended under low-frequency harvesting. The latex production period of about 24 years can be divided into two phases based on the bark tapped, *i.e.* virgin bark and renewed bark. The first virgin bark, panel BO-1 is tapped for 6 years at every other day tapping. The opposite virgin panel, *i.e.* BO-2, is tapped from 7 to 12 years. The tapping of the first renewed bark, *i.e*. BI-1 should commence from the initial height of the opening of the virgin panel after 12 years and tap from 13 to 18 years. Then the second renewed panel is started from 19th year and at this time quarter upward cuts are opened above the E panel. This is called 150% intensity and this is gradually increased up to 400% followed by uprooting the trees for replanting at the end of 24 years of harvesting (Anon, 2016/03).

As far as the clones recommended for planting is concerned, Rubber Research Institute of Sri Lanka has produced the best clones having high yield and important secondary characteristics like disease resistance, high timber volume *etc.* The clones recommended for planting under Smallholder rubber farmers are in Group I of the recommended list of clones. For the Regional Plantation Companies clones of all three groups I, II, and III along with the newest clones in group IV, as collaborative trials with RRISL, are recommended (Anon, 2013). Among the other unique characteristics such as disease resistance, high timber volume, *etc*. almost all the clones recommended in the list are capable of giving 2500-3000 kg of rubber per hectare per year. The recommended density of rubber planting at present is 516 trees per hectare, but due to various reasons such as damages due to white root disease incidences, lightning strikes, wind, fire, animal attacks, *etc*. the tree stand tends to decrease over the years and due to that reason though the bark is available sometimes the clearing becomes uneconomical.

However, in order to harvest the maximum potential yield from any clone or a tree, many factors needed to be fulfilled. Among them, the growth and the physiological condition of the tree, agro management practices, weeding, manuring, branch induction, *etc*. especially during the immature period, harvesting practices, mainly the frequency and quality, are the most important.

Irrespective of the clone, the growth condition of the tree is important as the vegetative growth or the girth of the tree determines the number of latex vessel rings and thereby the amount of latex that can be harvested from a tree (Nugawela, 2001). Growth which is determined by the growth rate is directly correlated to the quality of the planting material and the maintenance of the clearing during the immature period, mainly the first five years of planting. What is highlighted in this article is the importance of adopting correct harvesting practices in obtaining potential yields from rubber trees.

The tree is tapped for latex once it has reached the tappable girth which is a minimum of 50 cm girth measured at 120 cm from the graft union. If a clearing has more than 70% of such trees, the clearing becomes tappable and this condition should generally be achieved in 5-6 years of planting. Some Plantation Companies and smallholder farmers tend to postpone the tapping by another year or so and the crop is higher due to higher girth of such trees. Tapping, as described by Ridley in 1889, is excision of bark without damaging the cambium tissue and is a skilled job. To perform this controlled wounding, a special tool called a tapping knife is used. Mainly two types are used for tapping but in Sri Lanka push knife or the 'Michie Goledge' knife is popular than the pull knife called 'Jebong knife'. The bark consumption is generally higher with the Jebong knife.

The ideal harvesting method is expected to cause the least damage while giving out the potential yield of the tree at the lowest possible cost. Accordingly, an ideal harvesting method should remove only about $\frac{1}{20}$" (1.25 mm) thick bark shaving, cut open as many latex vessels as possible without damaging the cambium while maintaining the correct angle of 30 degrees to the horizontal and marked from high right to low left. A slope of a few degrees should also be maintained on the tapping cut towards the tree to prevent latex spillage. Marking 'Neththi Kanu' and 'Poi Kanu' by correctly dividing the tree into two halves with the use of a measuring tape and use of a stencil according to the intended tapping frequency, *i.e*. d2 or d3 are important practices. Even if the stencil is used if it is not properly placed on the tree, the angle of 30 degrees cannot be achieved. Therefore, the correct angle and the guidelines for the tapper on bark consumptions allowed per quarter should be marked annually to control bark consumption. Therefore, a new clone is generally released along with a harvesting method, especially the harvesting frequency. This is more important for high yielding clones as if a high yielding clone is harvested at a higher frequency than the recommended, trees become very stressed and eventually dry within a short period of about 6-12 months (Eschbach *et al*., 1989). This condition is generally termed as "brown bast" or tapping panel dryness where the bark is live but latex vessels are dry. Tapping panel dryness is observed in almost every rubber clearing but in an accepted level and generally an increase is observed with the advancement of the panels from BO-1 to BI-2. During the first 5-6 years of tapping, only about 5% tapping panel dryness is generally accepted. The quality of tapping and the tapping frequency determine the productivity and the productive lifespan of rubber cultivations. Low yielding clones planted early days could withstand daily tapping but the high yielding clones that are planted at present should be tapped every other day, *i.e.* d2 frequency and some clones once in every three days, *i.e*. d3 frequency.

The main objective of the survey conducted was a proper bark auditing on tapping quality and field wise remedial measures to arrest the condition of poor intakes and high brown bast incidences.

Methodology

Based on the information received from Regional Plantation Companies (RPCs) in 2009 on poor yields, poor intake per tapper, and a high number of dry trees in rubber fields, the survey was carried out during the year 2010 covering all RPCs having rubber estates. To schedule the field visits, information on estates, divisions, fields, extents, age, and years under tapping were collected in advance through emails. Fields tapped on D panel were excluded in many occasions. As the general norm of tapping is 300 trees, data collection was done in about 10% of the trees representing each field. Blocks were selected to represent each tapper and at least 30 trees were selected in a Zig-Zag pattern across the field and in some cases every 11th tree was used to collect data. The format used to collect data contained, name of the estate, division, extent, year of planting the field, year of opening for tapping, clones planted, number of tapping blocks, opening height of tapping, tapping system, estate representation and date of inspection. To calculate the bark consumption rate, the actual panel position was measured with a measuring tape and the number of years in tapping was recorded from their records available at the Estate Office.

For the tapping quality, angle of tapping, angle of the cut, depth of tapping, bark consumption, and length of the cut was recorded for 30 trees per block and percentage correctness was calculated.

Estimation of percentage correct tapping angle

$$\%\ CA = \frac{TC}{m} X\ 100$$

where, "TC" is the number of trees with correct tapping angle and "m"is the total inspected trees in the tapping block. Then, percentage of trees with correct tapping angle of a clearing ($\%CA_c$) was estimated as,

$$\%CA_c = \frac{\sum_{i=1}^{n} \%CA_i}{n}$$

where, $\%CA_i$ is the percentage correct tapping angle in the ith block of the clearing and "n" is the total number of tapping blocks in the clearing.

Estimation of percentage correct tapping depth

Percentage of trees with correct tapping depth of a tapping block ($\%CD$) was estimated as.

$$\%CD = \frac{TD}{m} X\ 100$$

Where, TD is the No. of trees with correct tapping depth in the tapping block and "m" is the total inspected trees in the tapping block. Then, the percentage of trees with correct tapping depth of a clearing was calculated as,

$$\%CD_c = \frac{\sum_{i=1}^{n} \%CD_i}{n}$$

where, $\%CD_i$ is the percentage of trees with correct tapping depth in the ith block of the clearing and the "n" is the number of blocks in the clearing.

Estimation of correct tapping length

Percentage of trees with correct tapping length of a tapping block ($\%CL$) was estimated as,

$$\%CL = \frac{TL}{m} X\ 100$$

Where, "TL" is the no. of trees with correct tapping depth in the tapping block and "m" is the total inspected trees in the tapping block. Then, the percentage of trees with correct tapping depth ($\%CL_c$) of a clearing was calculated as,

$$\%CL_c = \frac{\sum_{i=1}^{n} \%CL_i}{n}$$

where, $\%CL_i$ is the percentage of trees with correct tapping depth in the i[th] block of the clearing and the n is the number of blocks in the clearing.

Estimation of bark consumption

With the data collected, the correct panel was estimated and then the total excessive bark consumption, excessive bark consumption per year and the life span of each A, B, C and D panels were calculated as follows.

Let bark consumption of a tapping block (BC) is defined as

$$BC = \frac{\sum_{j=1}^{m} \boldsymbol{h_j}}{\boldsymbol{m}}$$

where"$\boldsymbol{h_j}$" is the bark consumption of j^{th} tree in the tapping block and “m” is the total number of tree in the tapping block. Then the bark consumption of a clearing (BC_c) was estimated as

$$BC_c = \frac{\sum_{i=1}^{n} \boldsymbol{BC_i}}{\boldsymbol{n}}$$

where BC_i is the bark consumption of i[th] tapping block and”$\boldsymbol{n}$" is the number of tapping blocks in the clearing. Using calculated “BC_c" and total duration under tappingin years of the clearing (T), annual bark consumption [$ABC(cmy^{-1})$] was estimated using the following formula.

$$ABC(cmy^{-1}) = \frac{BC_c}{T}$$

The life span of a tapping panel of a given clearing ($LoTP_c$) can be estimated using the following formula.

$$LoTP_c = \frac{H}{ABC}$$

where, H is the opening height of the panel.

In order to calculate the percentage of trees with dry panels, a count of dry trees was taken. Determining the dry trees is difficult as it happens gradually over a period of time. When the yield was very poor they were considered as dry in this study.

About 5000 ha. under the regional plantation companies were assessed to collect data. Only the fields that were tapped at S/2d2 system were selected initially. The clones represented were mainly RRIC 121, RRIC 100 and RRIC 102.

Simultaneously, all Field Officers of the estates, Rubber Development Officers of the Rubber Development Department and the Rubber Extension Officers of the RRISL were trained at the RRISL by the Officers of RRISL on bark auditing. Training was done on every Tuesday for a period of about six months and around 600-700 people were trained.

The Regional Plantation Companies and rubber estates, where the survey was carried out are presented in Table 1.

Results

Though the list of estates was given in the methodology, the identity of the estates was confidential and therefore, estates are identified as 1, 2, 3, *etc.* without divulging the names of the estates.

Table 1. *The Regional Plantation Companies and rubber estates, where the survey was carried out*

Plantation Company	Estate
1. Pussellawa	Pussella
	Pambegama
	Penrith
	Siriniwasa
	Salawa
	Halpe
	Sunderland
	Durampitiya
	Eheliyagoda
	Elston
	Ayr
2. Kelanivalley	Dewalakanda
	Ganepalla
	Lavent
	Urumeewala
	Panawatta
	Kiriporuwa
	Edarapola
	Kelani
	Weoya
	Kalupahana
3. Agalawatta	Ambetenna
	Clyde
	Culloden
	Doloswella
	Kiribathgala
	Kiriwanaketiya
	Mohomedi
	Niriella
	Niriwatta
	Noragalla
	Peenkande
	Pimbura
	Watapotha

Plantation Company	Estate
4. Horana	Neuchatel
	Hillstream
	Mirishena
	Halwathura
	Dumbara
5. Kotagala	Eduragala
	Delkeith
	Padukka
6. Balangoda	Millawitiya
	Mahawela
	Galathura
	Palmgarden
	Rambukkanda
	Matuwagala
7. Lalan	Udabage
8. Kahawatta	Ekkerella
	Houpe
	Hunuwella
	Opatha
	Pelmadulla
	Poronuwa
	Rilhena
	Wellandura
8. Watawala	Nakiyadeniya
	Homadola
	Thalangaha
9. Kegalle	Atale

Tapping quality

The results indicated that the percentage of correct adoption has deviated from the recommended criteria in most of the cases. The percentage of 100% adoption, 100% deviation, and the average adoption rate for each criterion, the angle of tapping, the depth of tapping, and the length of the tapping cut are given in Table 2.

The reasons for the results given in the Table 2, on common errors are shown in Fig. 1a, b, c, and d.

Table 2. *The percentage of 100% adoption, 100% deviation, and the average adoption rate for each criterion, the angle of tapping, the depth of tapping, and the length of the tapping cut*

	Angle of tapping (%)	Depth of tapping (%)	Length of tapping cut (%)
Percentage of blocks with 100% accurate tapping	4.57	20	38.28
Percentage of blocks with 100% wrong tapping	6.2	6.2	3.42
Average accuracy of tapping per block	46.85	54.16	75.42

(a) **(b)** **(c)** **(d)**

Fig. 1. (a). No marking of 'Poi Kanu' or 'Neththi' kanu' and thereby indiscriminate use of panels. The base panels have totally been consumed and the current panel is marked above the base panels and again downward tapping is done on the higher panels and minimum yield is obtained.

(b). Incorrect angle of less than 30 degrees which has resulted very low yields due to coagulation of latex on the cut due to slow latex flow.

(c). Angle of more than 30 degrees which leads to wasting of a bigger potion of panel along with other disadvantages.

(d). Nodule formation on the renewed panel due to poor tapping done on the virgin panel.

Bark consumption

Data collected on bark consumption and the bark consumption rate showed that they have exceeded the recommended rate of frequency of tapping in almost every clearing, irrespective to the panel. Data on year of planting, age of the clearing, year of commencement of tapping, number of years in tapping, current panel in tapping, expected panel and remarks for all the fields of one estate belongs to one of the RPCs are given in Table 3. As it is clear from the data of Table 3, the age of the clearings varies from 15 to 27 years and none of the clearings have any bark left on the trees to harvest any crop. Though this was an extreme case, there were fields of this kind in other estates too.

Average values of actual and recommended bark consumption for nine estates of Plantation Company 1 are given in Figure 2a. As it is clear from the Figure 2a, the actual bark consumption is higher than the recommended or the estimated bark consumption.

Table 3. *Year of planting, age of the clearing, year of commencement of tapping, number of years in tapping, expected panel, actual panel in tapping, and remarks for the clearings in one of the estates surveyed*

Year of planting	Age	Year of tapping commenced	Number of years in tapping	Expected panel	Actual panel (Intensity %)	Remarks
1985	27	1991	21	D3	No bark (400%)	Highly intensified (no bark)
1986	26	1992	20	D2	No bark (400%)	Highly intensified (no bark)
1987	25	1993	19	D1	No bark (400%)	Highly intensified (no bark)
1988	24	1994	18	C6	No bark (400%)	Highly intensified (no bark)
1989	23	1995	17	C5	No bark (400%)	Highly intensified (no bark)
1990	22	1996	16	C4	No bark (400%)	Highly intensified (no bark)
1991	21	1997	15	C3	D (400%)	Highly intensified (no bark)
1993	19	1999	13	C1	D (400%)	Highly intensified (no bark)
1994	18	2000	12	B6	D (400%)	Highly intensified (no bark)
1995	17	2001	11	B5	D (200%)	Highly intensified (no bark)
1996	16	2002	10	B4	D (400%)	Highly intensified (no bark)
1997	15	2003	9	B3	D (400%)	Highly intensified (no bark)

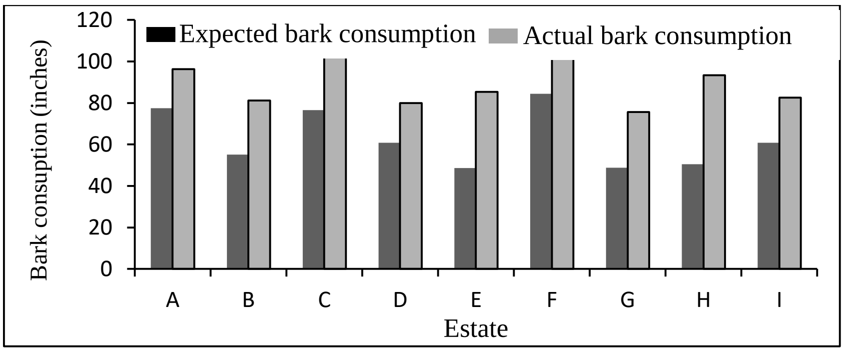

Fig. 2a. Average values of expected and actual bark consumption for nine estates of Plantation Company 1.

Percentage of the excess consumption calculated for all fields with compared to the recommended panel position for the same estates of Plantation Company 1 is shown in Figure 2b. The percentage values vary from about 18% to 85% among the estates as seen from Figure 1b. The actual and recommended bark consumption for eight estates of Plantation Company 2 are given in Figure 3a.

Percentage of the excess consumption with compared to the recommended panel position for the Plantation Company 2 is shown in Figure 3b. A variation from 40% to 110% bark consumption is seen the estates under Plantation Company 2.

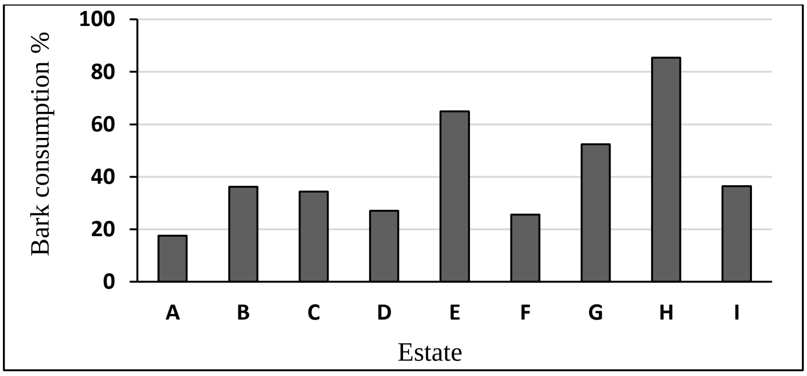

Fig. 2b. Percentage of the excess consumption with compared to the recommended panel position for the nine estates of the Plantation Company 1

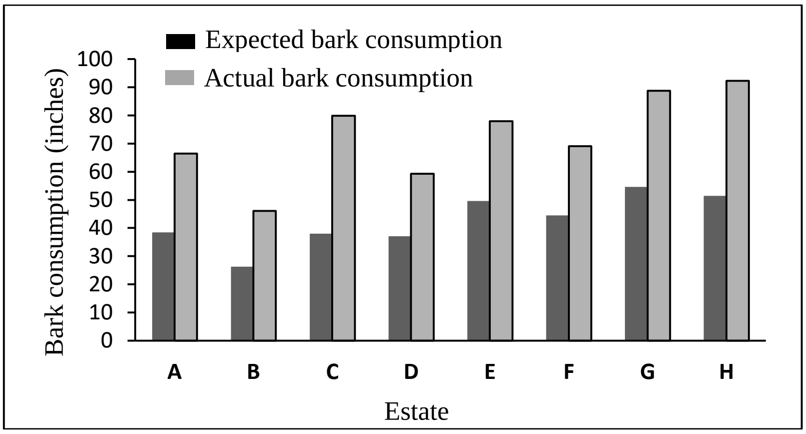

Fig. 3a. The expected and actual bark consumption for eight estates of Plantation Company 2

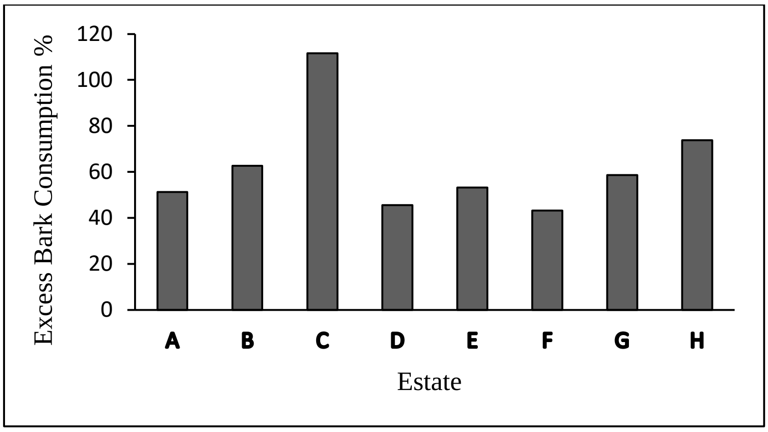

Fig. 3b. Percentage of the excess consumption with compared to the recommended panel position for the eight estates of Plantation Company 2

The actual and recommended bark consumption for estates of Plantation Company 3 are given in Figure 4a. Percentage of the excess consumption with compared to the recommended panel position for the Plantation Company 3 is shown in Figure 4b. The percentage vary from 30% to 80% among the estates.

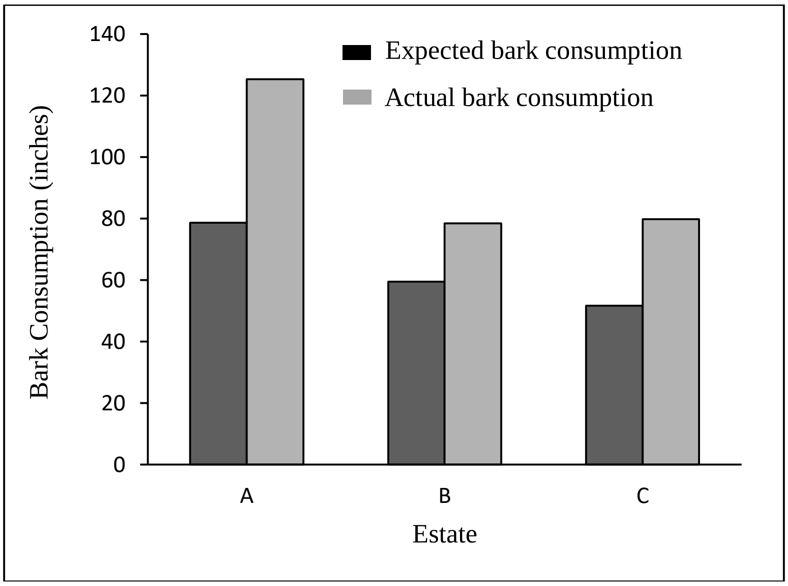

Fig. 4a. The expected and actual bark consumption for the three estates of Plantation Company 3

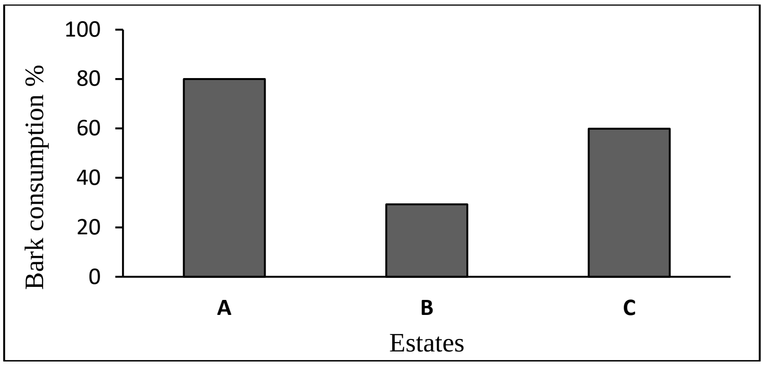

Fig. 4b. Percentage of the excess consumption with compared to the recommended panel position for the Plantation Company 3

Bark consumption was always higher in all the estates and therefore results for individual estates or plantation companies were not discussed in this paper. But detailed reports were provided for each estate.

The summary of the average life span of the two virgin panels, A and B, for all fields of all the estates as per the rate adopted is shown in Figure 5. For this figure, data collected from about 5000 ha. representing many RPCs and estates have been used and therefore is considered a reliable information representing the entire sector. As the expected lifespan of two virgin panels under d2 tapping is 12 years, only about 15% of the extent is within that as per the Figure 4. About 5% of the area shows less than 5-6 years for panels A and B. Also an area close to 60% has consumed the two virgin panels in less than nine years and it was an alarming situation.

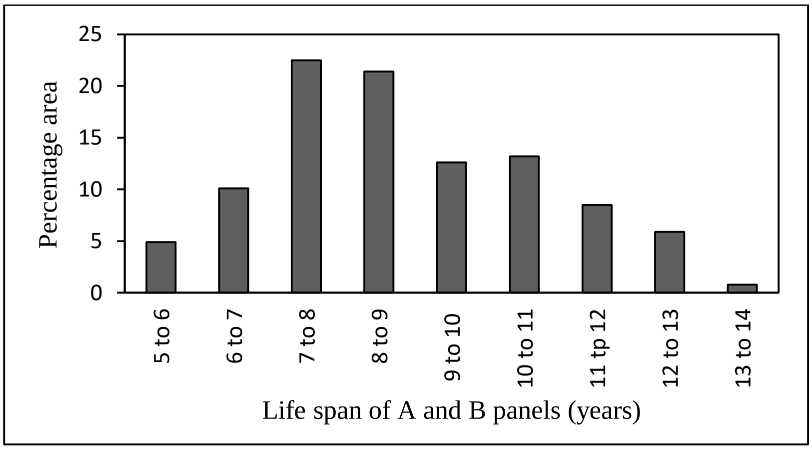

Fig. 5. The summary of the average life span of the panels of all fields of all the estates as per the rate adopted

The age wise distribution of expected and actual panel positions for all the fields in one estate were calculated to see any correlation between the age of the clearing and the bark consumption. Actual panel position and the expected panel positions for all fields in one estate are shown in Figure 6. Percentage of bark consumption indicates high variation among fields, some exceeding 100%, but no correlation was seen and young clearings too showed very high excessive bark consumption.

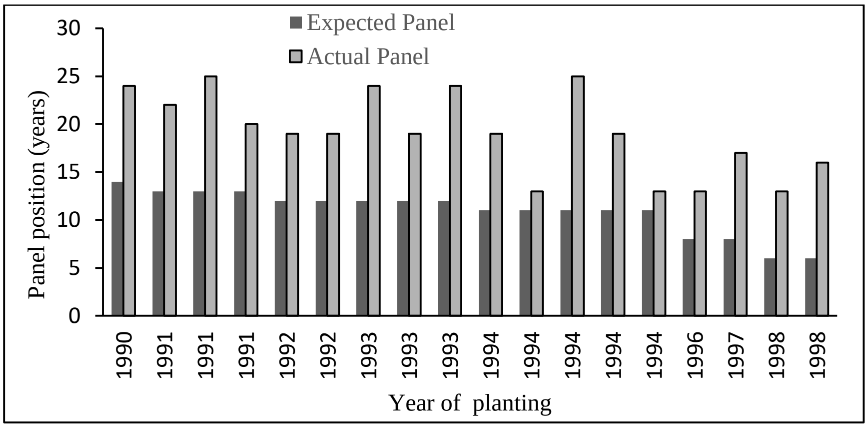

Fig. 6. Actual panel positions and the expected panel positions for all fields in one estate

However, according to some of the managers of RPCs, the excessive bark consumption was partly due to the unskilled tappers. It can be true to a certain extent but, many tappers had performed so well during the bark audit, and could remove a shaving of 1.25 mm or less, without causing any damage to the tree. Figure 7 shows a rare incident of very thick shavings and estates are generally very attentive on such tappers. It is a general practice of the tappers to remove a thicker bark shaving after many absent tapping days.

Fig. 7. A very thick bark shaving of about 5 mm

Tapping Panel Dryness

The brown bast or the condition of tapping panel dryness (TPD) of the fields in one of the estates belongs to one of the RPCs surveyed is shown in Figure 8. Though there is no clear pattern between the percentage of TPD trees and the age of the field, the three fields on panel BO-1 or the first virgin panel shows no trees with tapping panel dryness. However, the fields have been in tapping only for 1-3 years when data were collected.

A correlation could be seen between the age and the bark consumption of the fields of an estate and the recommended and the actual bark consumption of all fields of this estate in Kaluthara region is given in Figure 9a. The percentage of excessive bark consumption of fields are given in Figure 9b. The percentage of brown bast or tapping panel dryness incidences for the area shown in Figure 9c.

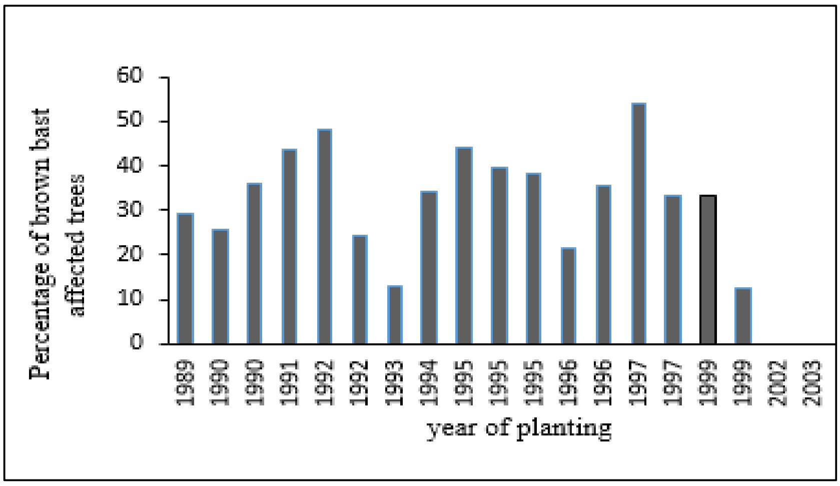

Fig. 8. The percentage of brown bast condition of the fields in one of the estates belongs to one of the RPCs

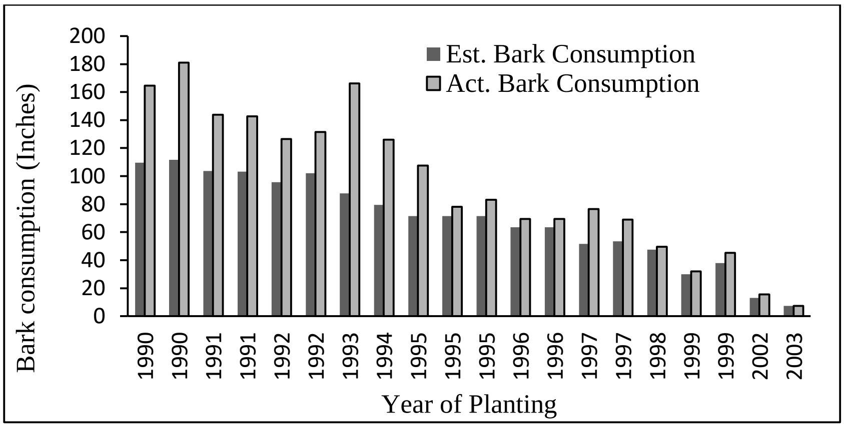

Fig. 9a. The recommended and the actual bark consumption of all fields in one estate in the Kaluthara region

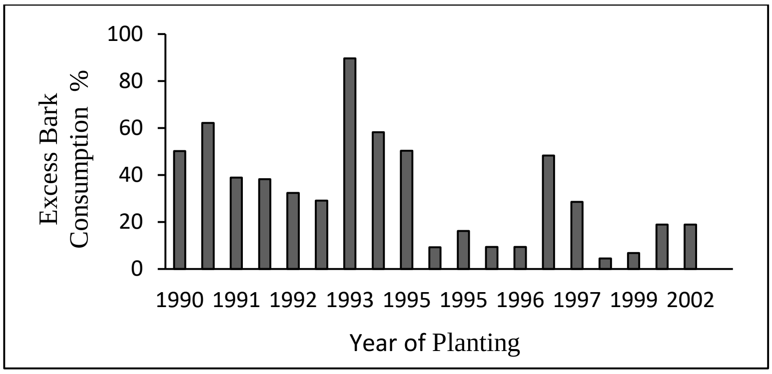

Fig. 9b. The percentage of excess bark consumption of the same fields

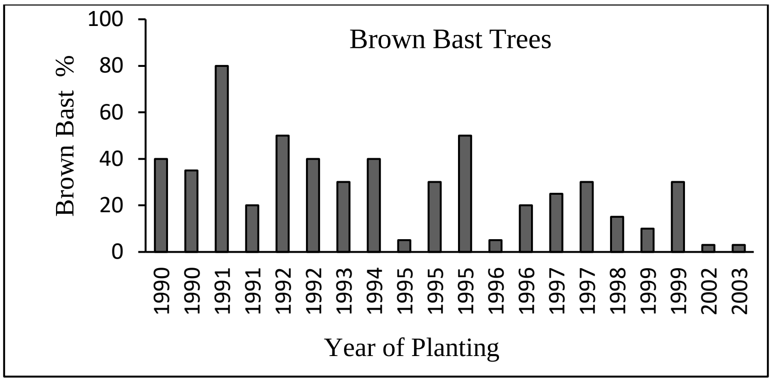

Fig. 9c. The percentage of brown bast (BB%) incidences for the same fields

The correlation between the excess bark consumption and the tapping panel dryness for the fields of this particular estate is shown in Figure 10.

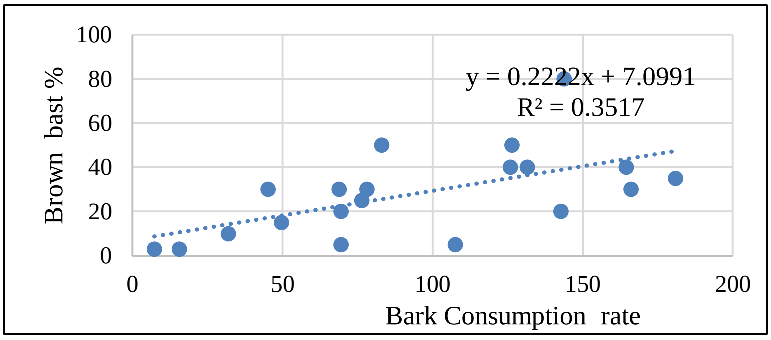

Fig. 10. Correlation between the bark consumption rate and the tapping panel dryness in the tapping fields in the particular estate shown in Figures 9a, b and c

The year of planting, year and extent audited, estimated and actual panel position and the tapping quality of the estates audited from 2013 to 2020 are given in Table 4. It is clear from the data, the gap between the actual and the estimated panel positions vary from field to field. Also, it is clear when the taping quality is very good or good, the gap between the estimated and the actual panel positions are lower when compared to gap where the tapping quality is poor and very poor.

Table 4. *The year of planting, year audited, extent audited, estimated panel position, actual panel position and the tapping quality of the estates audited from 2013 to 2020*

Year of planting	Year audited	Extent audited (ha.)	Estimated panel position	Actual panel position	Tapping quality
1989	2013	7.8	C5	C&D	++++
1989	2013	2.7	C1	B6+C	+++++
1989	2013	4.1	C3	B+C	+++++
1989	2013	9.1	C3	D	++
1989	2013	3.0	C6	D2	++
2007	2013	4.5	A4	B2	+
2007	2013	6.1	A6	B6	+
1999	2013	6	B1	C4	+
1999	2013	2	B2	D2	+
1999	2013	2.3	B2	D	+
2007	2016	6.42	A3	A3 & A4	+++++
2006	2016	11.6	A5	A5 & A6	+++++
2004	2016	10.6	A7	B1	++++
2008	2016	1.22	A2	A2 & A3	+++++
1988	2016	11.46	D	Not clear	+
1981	2016	4.51	D2	Not clear	+
1989	2016	4.1	D	Not clear	+
1987	2016	9.47	D	Not clear	+
1999	2016	5.64	B4	Not clear	+
1999	2016	12.78	B3	B8	++
1994	2017	7.1	C	E & F	+
1995	2017	13.1	C	E & F	+
1996	2017	5.1	C	D & E	+
1997	2017	12	C	D & E	+
1997	2017	4	C	D & E	+
2000	2017	1	B	C,D&E	+
2003	2017	9.0	B	C & D	+
2004	2017	7	A	B	+
2005	2017	6.9	A7	B4	+
2006	2017	7.6	A6	B1	++
1984	2018	5	C2	D	+

Year of planting	Year audited	Extent audited (ha.)	Estimated panel position	Actual panel position	Tapping quality
2004	2018	20	B1	B4	++
2005	2018	30	A6	B6	+
2006	2018	30	A5	B6	+
2007	2018	29	A5	A&B	++
2009	2018	2	C4	E	+
2000	2018	1.3	C1	D4	+
2000	2018	5	C1	D	+
2008	2018	10	C4	D&E	+
2008	2018	6	C3	E	+
2008	2019	3.2	C4	D&E	+
1989	2019	4.6	C2	D&E	+
2001	2019	5	B4	C	++
2008	2019	11.9	B3	B	++++
1999	2019	15.8	B2	C	++
1989	2019	2	C2	D&E	+
1988	2019	6	C3	D&E	+
1988	2019	10.8	C3	D&E	+
1989	2019	6.8	C2	D&E	+
2007	2019	10	A3	A4	++++
1989	2020	5	C2	D	+
1989	2020	8	C3	D	+
2008	2020	4.6	C6	D	+
2009	2020	5.6	C4	D	+
2008	2020	14.4	C4	D	+
2001	2020	5.9	B6	C6	+
2008	2020	7	C4	D	+
1989	2020	4.3	C3	D	+
1989	2020	6.3	C4	D	+
1988	2020	5	D	D	+

Key for tapping quality
+++++ - very good; ++++ - good; +++ - acceptable; ++ - poor; + - very poor

Ad hoc tapping practices

The general practice adopted in many estates when the tapping panel dries partially or fully was to switch over to the opposite panel. However, various kinds of ad hoc tapping were seen, and the tappers' explanation was most of the time was to avoid dry areas of the tapping cut (Figs. 11a and b). Tapping quality, as it is seen in Figures 10a and b is also very poor and nodule formation is very high.

(a) **(b)**

Fig. 11. (a). Changing the tapping cut in order to avoid the dry areas and **(b).** a new cut opened above the base panel due to drying up of the original base panel

Discussion

Though harvesting is the most important agro management practice on an estate, so many malpractices are seen including tapping of under-girth-trees which are not reported in the results. The main concern throughout the survey was to stop immediately the excessive bark consumption rate which was far too high and if continued would totally ruin the rubber industry. However, the approach was timely, correct and straight forward which resulted the expected outcome in almost all the estates. While giving the required recommendations, all the field staff and the management were clearly educated on the situation and convincing was easy due high brown bast percentages experienced already.

As recommended, when only 1.25 mm thick bark shaving is removed at each tapping, each panel can be consumed for about 6 years at every other day tapping. Bark consumption rate should be controlled by guidelines marked on the panel every year using a proper stencil. When the tapping quality is good, the panel above the tapping cut will be smooth and even, with no nodule formation due to damaged cambium.

In order to obtain the potential yields economically, each panel should be exploited for at least 6 years *i.e.* under every other day tapping generally termed as "d2 tapping". But the average bark consumption rate, according to the data gathered so far is about 4 years per panel. In a situation like this, the crop loss is huge and can be calculated as follows.

When a panel that should be tapped for 6 years is consumed in 4 years, the crop loss on the two virgin panels will be equal to 4 years crop (two years on each panel). If the average yield per hectare is 1000 kg, this is about 4000 kg of rubber. When two virgin panels are consumed 4 years earlier than the recommended period of 12 years, the renewed panels cannot give expected yields as they are only partially renewed. Further, if the same high frequency is practiced on the renewed panels as well, then another 4 years are lost on renewed panels.

Therefore, the total crop loss will be a crop for 8 years and lower crop due to tapping on partially renewed panels. This amount will be equal to about 9000 kg of rubber and even at a moderate price of Rs.350/=, this will be over 3 million rupees per hectare.

As the high-frequency tapping leads to drying up of trees, the effect will be even higher which has not been taken into consideration for the above calculation. A gradual increase in "brown bast" with the increase of the age of the clearing is acceptable, to be around 5-10%. But, in clearings where the tapping frequency is very high, the percentage of brown bast affected trees has well exceeded the accepted levels. Therefore, this condition should properly be understood by the management of estates and remedial actions should be taken with no further delay. Many observations on tapping panel dryness with local clones under different conditions have been reported by Seneviratne *et al.* (2007).

One reason for this situation seems to be the wrong estimates or rather "crop targets" which can never be achieved or realized from present-day clearings that are on estates. Further, the only strategy adopted to achieve the targets is to increase the number of tappings. These extra tappings are called "recovery tappings" which leads to daily tapping of the trees eventually. Recovery tappings are recommended when normal tappings are disturbed due to rain interferences or lack of tappers but with strict guidelines *i.e.* 2 per week and 6 per month. Under daily tapping conditions, the crop will be reduced gradually. When the crop is low more tappings will be needed to cover the estimates. This will go on and on until the tree becomes dry.

Michels *et al.* (2012) have reported a similar exercise in which the life span of plantations had been diagnosed using the amount of virgin bark consumed and the number of tapping years that remained. They have validated that in a sample of 25 smallholder plantations in Cameroon, where they have characterized eight tapping management systems reflecting different levels of tapping intensity. The assessment of the respective share of each tapping practice on virgin bark consumption has revealed major effects of tapping frequency and of shaving thickness.

They have used the information gathered through this for decision making which can increase remaining tapping years and as useful support for the participatory development of innovating tapping management schemes involving both technicians and smallholders Lacote, *et al.* (2013).

Atminingsih and Darojat (2019) have studied the effect of the direction of the cut, panel height, and the frequency of tapping on the shaving thickness of the bark. Tapping frequency effect was investigated in a trial plot using the frequency of once in three days (d3), four days (d4), five days (d5), six days (d6), and eight days (d8) on the basal panel (B0-2). Bark thickness has been measured directly using a digital caliper. They have observed that the frequencies d5, d6, and d8 had resulted in insignificantly thicker bark shavings per tapping compared to d3 and d4 which had a lower thickness of bark shaving. The recent trend in rubber exploitations everywhere is adopting a low-frequency tapping system to reduce labor costs consequently to the low rubber price in recent years. They have indicated that though low-tapping frequency applications would increase daily bark shaving thickness, the annual total would be lower due to less tapping days per year.

Their observations and results have also indicated that upward tapping had higher bark shaving thickness than downward tapping. In downward tapping, the lower the tapping position, the higher the shaving thickness would be, whilst in upward tapping, shaving thickness had increased along with the panel height. In this study too a higher bark consumption was observed in the upper-cuts which is anyway unavoidable.

Opening a downward cut above the dry panel is an unsuccessful attempt as the drainage area will soon be over making no crop. If the upper-cut was an upward cut and if not the tree is fully stressed, a reasonable crop could have been obtained from such trees. In the report it was advised to this kind of tapping with immediate effect and upward cuts were introduced most of the time a quarter cut.

Atminingsih and Darojat (2019), have classified panel heights into < 50 cm, 50 – 100 cm, and 100 – 130 cm in downward tapping and 130 – 150 cm, 150 – 170 cm, and >170 cm in upward tapping. In downward tapping, the lower tapping position, the higher the bark shaving would be, whilst in upward tapping, bark shaving thickness has increased along with the panel height. Our observations are on par with their conclusions.

In the present survey, for about 90% of the fields, the recommendation was to stop harvesting the panel on tapping and to open an upward ¼ spiral cut on the opposite panel, which is generally termed as CUT or controlled upward tapping. Soon after the visit to the estates for data collection, calculations were done and a report consist of the current issues and the remedial actions to be taken to address the issues were sent to the management of each estate with a copy to the CEO of the RPC, for implementation. The response was very positive and the recommendations were implemented in almost all the cases.

As seen in figure 9 of the results section, a correlation exists between the tapping panel dryness and the excessive bark consumption. But it would be more pronounced if the variables such as location, clone, tapper *etc.*, could be excluded, which was not possible in this exercise.

The damage to the tree due to over-exploitation is permanent. Therefore, if this rate continues, the rubber production in the country is severely affected while the rubber industry, in particular, the local consumers compelled to import more and more rubber to the country to maintain the production in their factories. From 2013 onwards, bark auditing was done only on the requests received from estates. Also, the Ready-Reckoner chart developed to read the correct bark position by measuring the current panel position was an important invention (Silva *et al*., 2012).

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Effect of nanoZnO over conventional ZnO on preservation of concentrated natural rubber latex

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Abstract
An attempt was made to replace conventional ZnO with nanoZnO in low ammonia, tetramethylthiuramdisulphide (TMTD)/Zinc oxide (ZnO) natural rubber latex preservative system (LATZ). Different percentages of TMTD and nanoZnO dispersions containing equal portions were prepared and used to preserve the latex. Centrifuged natural rubber latex (CNRL) thus prepared were tested for VFA (volatile fatty acid) number and MST (mechanical stability time) periodically over a period of 75 days. Mechanical properties of vulcanized latex thin films made out of CNRL preserved were measured. Development of VFA number in CNRL samples preserved with nanoZnO was lower than the sample prepared with the conventional ZnO (control). Both the VFA number results and surface plots of the matured CNRL samples showed that preservative action of the modified preservative system was mainly governed by the amount of nanoZnO in the system. MST values of CNRL preserved with nanoZnO substituted systems were significantly high when compared to the control. Tensile and tear properties of the vulcanized films prepared using CNRL preserved with nanoZnO were almost comparable with those of the vulcanized films prepared using control system. The statistical analysis indicated that CNRL preserved with modified preservative system of 10% and 15% of TMTD/nanoZnO could be stored up to 55 and 75 days respectively, without exceeding VFA value above 0.02.

Key words: centrifuged natural rubber latex, MST, nanoZnO, preservative system, TMTD, VFA

Introduction
Natural rubber latex (NRL), being a natural polymer obtained from *Hevea brasiliensis* possesses certain important unique characteristic properties owing to its high molecular weight polymer chains, and chemical structure of the polymer backbone. These characteristic features have enabled natural rubber to be among one of the highly consumed elastomers in the world with over 40,000 products (van Beilen and Poirier, 2007). However, the presence of non-rubber substances in latex, such as proteins and carbohydrates leads to spontaneous coagulation and putrefaction of field NRL, which is regarded as one of the major constraints in the NRL industry (Blackley, 1997).

In order to suppress the spontaneous coagulation and putrefaction and also to improve the stability of the latex for long term storage, field NRL need to be preserved. It is considered one of the most important steps in latex processing as it plays a vital role in keeping the properties of field NRL suitable for further processing. The effectiveness of a preservative system depends mainly on the ability to (a) suppress the activity and the growth of microorganisms, (b) enhance the colloidal stability of latex, and (c) deactivate trace metals ions (Blackley, 1997). In addition, a preservative system should be harmless, environmentally safe, odorless, commercially viable, low cost, and should not interfere with production processes.

The widely used preservative systems for CNRL include high ammonia (HA) latex preservation system and low-ammonia latex preservative system in tetramethylthiuramdisulphide (TMTD)/ Zinc oxide (ZnO). Ammonia, being a volatile and irritating substance, use of HA system has become a great challenge in NRL industry (Chaikumpollert, 2008). In LA system, ammonia is used as the primary preservative agent, while TMTD and ZnO are used as the secondary preservatives, thus called LATZ system (Nadarajah, 1979). For the industrial applications where HA system cannot be applied, LATZ is the commonly employed alternative preservative system used in the NRL industry. However, LATZ system is inherited with its own drawbacks; generation of carcinogenic nitrosamines during product manufacture and contamination of natural water resources (Lee, Chon and Kim, 2005; Miao *et al.*, 2010; Sheth and Desai, 2013; Rajput *et al.*, 2018).

As such, during past two decades, the researchers have carried out a considerable amount of research to find less risky, commercially viable alternative preservative system for field NRL and CNRL (Chaikumpollert, 2008; Loykulnant *et al.*, 2012; Wang *et al.*, 2015). However, to date, none of these studies have been able to come up with an industrially viable preservative system to replace the currently used LATZ preservative system. The recent advancement of nanomaterials, which involves the materials with the size of 100 nm or less in one or more dimensions (Espitia *et al.*, 2012), have shown their potential use in enhancing the antibacterial properties. Many researchers have studied the performance of inorganic nanomaterials, especially the nanoparticles of metal oxides, which are found to be effective and possess excellent antibacterial properties in comparison to their conventional micro and macro counterparts (Espitia *et al.*, 2012; Dizaj *et al.*, 2014). Among these metal oxides, nanoZnO has been widely studied in different preservative systems in a variety of applications owing to its enhanced antibacterial properties, low cost, excellent stability and less complex manufacturing processes (Wang *et al.*, 2003; Padmavathy and Vijayaraghavan, 2008; Dizaj *et al.*, 2014; Kołodziejczak-Radzimska and Jesionowski, 2014). It has been further shown that nanoZnO is more effective against certain bacteria when compared to some commonly used other metal oxide nanoparticles such as CuO, and Fe_2O_3 (Dizaj *et al.*, 2014). The ZnO is considered a comparatively less toxic material to human health as it is used as an additive in textile and rubber applications, which directly come in contact with human skin (Dizaj *et al.*, 2014). According to Dizaj *et al.*, the enhanced antibacterial activity of nanoZnO is attributed to its photocatalytic activity, high stability, and effectiveness against Gram-positive/Gram- negative bacteria, and their highly resistant pores. However, the same study reports that the antimicrobial activity of nanoZnO is largely depend on its particle size and concentration (Dizaj *et al.*, 2014).

Even though the antibacterial activity of nanoZnO has been widely reported, only a very few have reported about the antibacterial effect of nanoZnO in natural rubber. Rathnayake *et al*., have recently shown that nanoZnO incorporated natural rubber latex foam possesses enhanced antimicrobial properties (Rathnayake *et al.*, 2014). A recent study carried out by Anand *et al*., highlighted that pre-vulcanized latex films containing nanoZnO showed better antifungal properties when compared to latex films containing micro ZnO (Anand, Varghese and Kurian, 2015). Recently, Lv *et.al.*, reported that ZnO/ natural rubber nanocomposites have shown excellent antibacterial properties against *Escherichia coli* colonies (Lv *et al.*, 2014).

Despite having studied many novel preservative agents for NRL in recent years, none of the studies have focused on the use of nanoZnO as a preservative agent in LATZ system. The main objective of this study is therefore to reduce the amounts of secondary preservatives used in LATZ preservative system for CNRL. In this study, potential of nanoZnO and TMTD as secondary preservative system for CNRL at different loading is investigated. In addition, film properties of the CNRL preserved with nanoZnO and the CNRL preserved with conventional LATZ preservative system were compared.

Materials and Methods

Field latex with 30% (w/w) dry rubber content (DRC) was obtained from Dartonfield Estate of Rubber Research Institute of Sri Lanka. Industrial grade chemicals required for the preparation of centrifuged latex and vulcanized latex films, including ammonia, tetramethylthiuramdisulphide (TMTD), conventional zinc oxide (ZnO), lauric acid and phosphoric acid [for the preparation of di-ammonium hydrogen phosphate (DAHP)], nanoZnO (98.6% w/w), potassium hydroxide (KOH), sulphur, zinc diethyldithio carbamate (ZDEC), and phenolic -type antioxidant, were purchased from a general chemical supplier. All the analytical grade chemicals needed for latex testing were purchased from Sigma-Aldrich, GMBH, Germany.

Characterization of conventional ZnO and nanoZnO by using XRD, SEM and particle size distribution data

Bruker D8 Advance Eco X-ray powder diffractometer (XRD) with CuKα (λ = 1.54060 Å) radiation was employed to obtain X-ray diffraction patterns of ZnO samples in order to determine the purity of samples. The instrument was operated from 5-80° on 2θ scale at the scanning rate of 0.001θ/s.

The average crystallite size, L of both types of ZnO samples were calculated by Scherrer equation (Patterson, 1939; Monshi, Foroughi and Monshi, 2012),

$$L = \frac{K\lambda}{\beta \cos\theta} \qquad \text{Eq 01}$$

where, λ is the weighlength of X-ray in nanometer (nm), β is the peak width of the diffraction peak profile at half maximum height in radians, and K is a constant related to crystallite shape ($K = 0.9$).

The morphologies of conventional ZnO and nanoZnO powders were obtained by scanning electron microscope images by using a Zeiss Evo LS15 Scanning Electron Microscope (SEM) operated at the accelerating voltage of 5.0kV. Quorum SC7620 Mini Sputter Coater system was employed to apply a gold/palladium alloy coat on powder samples before performing the SEM analysis.

The particle size distributions of both powders were determined in Malvern NanoS 90 particle size analyzer by using polycarbonate cuvettes at 25 ºC. The powder dispersions were made by using de-ionized water. Here, the refractive index and absorption of both conventional and nanoZnO were taken as 1.96 and 0.010, respectively.

Preparation of centrifuged natural rubber latex (CNRL) samples preserved with different preservative systems

CNRL samples were prepared using field latex preserved with different combinations of TMTD/ZnO along with 0.2% (w/w) ammonia as shown in Table 1. TMTD/ZnO dispersions were prepared by vigorous grinding of powder mixture at room temperature with the addition of water and dispersion agents in a ball mill for three days.

Table 1. *TMTD/ZnO compositions used*

Dispersion System	Chemical combination			
	TMTD % (w/w)	**Conventional ZnO % (w/w)**	**Nano ZnO % (w/w)**	**Percentage of total dispersion of TMTD/ZnO (%)**
Control	12.5	12.5	-	25
Nano-1	12.5	-	12.5	25
Nano-2	10.0	-	10.0	20
Nano-3	7.5	-	7.5	15
Nano-4	5.0	-	5.0	10
Nano-5	2.5	-	2.5	5

CNRL samples were prepared as mentioned elsewhere (Tillekeratne, Nugawela and Seneviratne, 2003). In brief, soon after collecting field latex, all the samples were first preserved with the addition of 0.2% (w/w) ammonia on latex using a 10% (w/w) ammonia solution. Then TMTD/ZnO dispersion systems were added as shown in Table 1. Here, if the strength of the dispersion is X, X% TMTD/ZnO is added at the rate of 1 L for 1000 L of field latex, so the final strength of TMTD/ZnO would be 0.00X% by weight on latex. Subsequently, 10% (w/w) ammoniumlaurate, 15% (w/w) DAHP were added and allowed to strand for 24 hours to settle magnesium ions in the latex. Then the sludge was removed and the latex samples were then centrifuged using a laboratory scale latex centrifuge machine with the rotational speed of 7000 to 10,000 rpm to obtain CNRL. After centrifugation process, 10% (w/w**)** ammonia solution and respective TMTD/ZnO dispersions were topped up assuming 50% of these chemicals were removed with skim latex during the process of centrifugation. In order to ensure the accuracy and precision of data, latex samples were prepared in triplicate. In this study, the control sample was prepared using 25% (w/w) TMTD and conventional ZnO dispersion as practiced by the CNRL manufacturers in the industry to meet the preservative strength of 0.025% on latex.

Characterization of CNRL samples

Raw rubber properties of CNRL including, total solid content (TSC), alkalinity and DRC of each sample were evaluated as per ISO 124, 125 and 126 standard test procedures, respectively**.**

Determination of VFA number

The VFA numbers of the CNRL samples preserved with different preservative combinations were determined according to ISO 506 after keeping the samples for 15, 35, 55 and 75 days subsequent to centrifugation process.

Determination of Mechanical Stability Time (MST)

Mechanical stability of CNRL samples were determined as per ISO 35, after 20 and 30 days from the date of centrifugation.

Preparation of compounded latex films and evaluation of properties

Each CNRL sample was compounded according to latex compounding formula shown in Table 2, and latex films casted on glass plates were oven dried at 70 °C for 24 hrs to obtain thin vulcanized latex films with ≤ 1 mm thickness.

Table 2. *Formulation of compounded latex films*

Ingredient	Dry parts per hundred rubber (phr)
60% CNRL	100.0
10% KOH	4.0
20% Potassium laureate	2.0
50% Sulphur	1.0
50% ZDEC	1.5
50% Phenolic type Antioxidant	2.0
50% ZnO	0.5

Determination of tensile and tear properties of compounded latex films

Tensile and tear tests were performed in a universal tensile machine (Model: Instron 3365) as per ISO 37 and 34. Tensile properties in terms of tensile strength, modulus at 100% elongation and 300% elongation, elongation at break were determined in all the samples using dumbbell-shaped films with a 5 kN load cell at a crosshead speed of 500 mm/min at ambient temperature (28 ^{0}C). Crescent-shaped films were obtained from compounded latex films by using a standard cutter and were used to measure tear strength at a crosshead speed of 500 mm/min.

Statistical analysis

Each system was prepared in triplicate. A statistical model was designed by reducing both TMTD and nanoZnO in equal amounts as mentioned in Table 1. Then the response-surface analysis was performed using MINITAB 17 by plotting ZnO and TMTD with respect to different levels of VFA number. Analysis of variance (ANOVA) was employed to analyze VFA number data of the six treatment levels (Control, Nano-1, Nano-2, Nano-3, Nano-4 and Nano-5) using SAS software. Mean separations were determined by Duncan's multiple range tests for the durations of 15, 35, 55, and 75 days at the alpha value of 0.05.

Results and Discussion

SEM analysis of conventional ZnO and nanoZnO powders

The SEM images of ZnO samples (Fig.1) show the morphological difference between nano and conventional ZnO particles. It could be seen in Fig.1a, that nanoZnO sample contains ZnO agglomerates, which are formed by the clusters of needle-like nano-rods. However, most of the conventional ZnO particles exist as agglomerates having particle size of more than 200 nm in all dimensions. Moreover, Fig.1c, which is a magnified part of Fig.1b, reveals the conventional ZnO particles are hexagonal-shaped crystals.

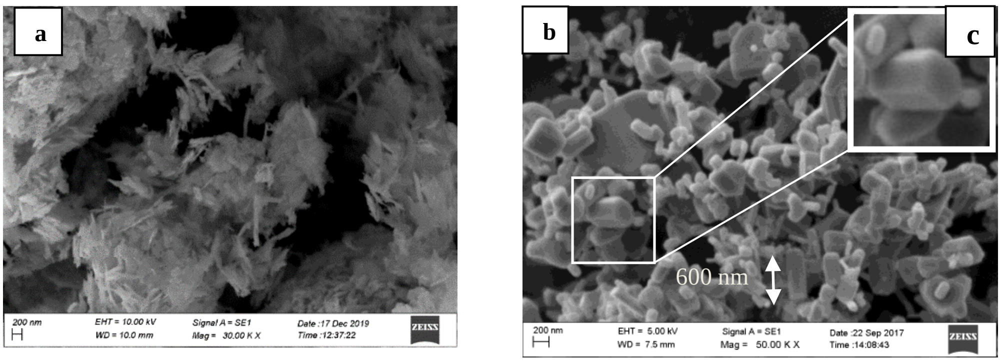

Fig. 1. SEM image analysis of **(a)** nanoZnO crystals, **(b)** conventional ZnO crystals, and **(c)** hexagonal-shaped ZnO crystal

XRD analysis conventional ZnO and nanoZnO powders

XRD is one of the most important and widely employed techniques in recent studies to determine the crystalline structure, purity and particle size of crystals especially when developing nanomaterials (Mantilaka *et al.*, 2014; Somarathna *et al.*, 2016). In our study, XRD patterns confirmed that the dominant crystalline phase is ZnO in both nano and conventional samples.

According to XRD pattern of nanoZnO (Fig. 2), the 2θ values of 31.7º, 34.4º, 36.2º, 47.5º, 56.6º, 62.8º, 66.4º and 69.0º confirm the presence of zincite as the crystalline form of nanoZnO (ICDD card No. 01-071-6424). The 2θ values of 31.7º, 34.4º, 36.2º, 47.5º, 56.6º, 62.9º, 68.0º and 69.1º indicate the presence of ZnO as the main crystalline substance (ICDD card No.1-082-3143) in conventional ZnO sample.

According to Scherrer equation, the average crystallite sizes of conventional ZnO and nanoZnO are 45.16 nm and 13.69 nm, respectively (Patterson, 1939; Monshi, Foroughi and Monshi, 2012). These results indicated that average crystallite size nanoZnO is smaller than that of conventional ZnO as seen on the SEM images.

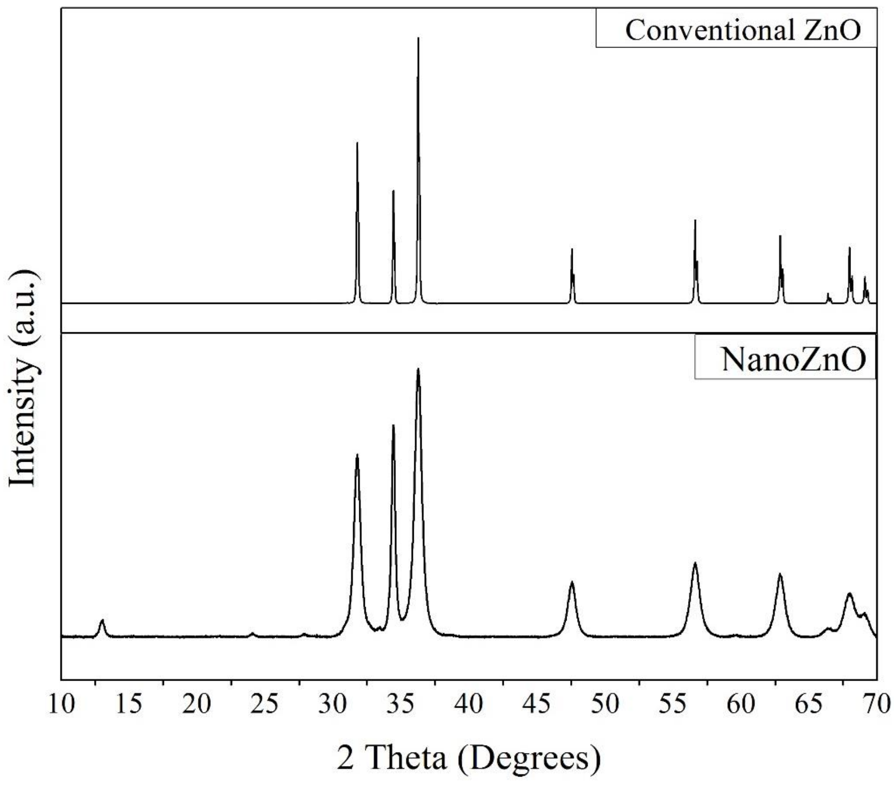

Fig. 2. XRD patterns of conventional ZnO and nanoZnO

Particle size distribution analysis of conventional ZnO and nanoZnO powders

The dynamic light scattering (DLS) technique is employed to obtain the particle size distribution data of two types of ZnO powders (Fig. 3). The DLS technique is widely employed to study average particle size and their agglomerations in colloidal systems (Fissan *et al.*, 2014). In our case, it is clear that the average particle distribution of conventional ZnO is broader than that of nanoZnO. This could be attributed by the presence of more particle agglomerations in conventional ZnO. It is noted that average particle size of nanoZnO is more than 200 nm, which may be due to the presence of crystal agglomerations as seen in SEM data. However, the narrow distribution of the particle sizes inferred that colloidal system of nanoZnO is more stable than that of conventional ZnO.

VFA number development in CNRL samples

The bacterial action on the carbohydrates present in latex mainly attributes to the formation of various volatile fatty acids. Volatile fatty acids formed in NRL is mainly composed of acetic acid, while formic and propionic acids also contribute to its composition as minor components (Pendle and Gorton, 1984; Blackley, 1997). VFA number is the key parameter that reflects the degree of bacterial action and hence the effectiveness of the preservation of CNRL. Therefore, lower the VFA number; better the state of preservation of latex.

According to VFA development results shown in Fig. 4, the preservation systems with nanoZnO yielded low levels of VFA development, when compared to that of the control sample. It was also revealed that the rate of VFA development increases with reduction of nanoZnO loading in the LATZ over the time period studied. The lowest VFA development was found in the Nano-1, which contains the highest dosage of TMTD/ nanoZnO, whereas the highest VFA number was observed in the sample, which contains the lowest amount of TMTD/nanoZnO over the entire period of the study.

Both Control and Nano-1 samples contain same quantities of TMTD (12.5 parts) along with 12.5 parts of conventional ZnO and nanoZnO, respectively. It is interesting to note that there is a significant difference between VFA developments in these two samples throughout the storage period studied. Replacement of conventional ZnO with nanoZnO in Nano-1 system has resulted in a significant reduction in VFA number suggesting that the latter has been the major contributor towards the enhancement of the antibacterial properties of CNRL.

It has been reported that the antimicrobial activity of nanoZnO is mainly attributed to its increased surface area/volume ratio (Zhang *et al.*, 2007; Jones *et al.*, 2008). This may be the reason for enhanced antibacterial properties of samples that contain nanoZnO compared to that of the control. Another reason for enhanced antimicrobial action might be the rod-shaped morphology of nanoZnO, which is more likely to achieve better dispersion in the aqueous phase than the bulky conventional ZnO.

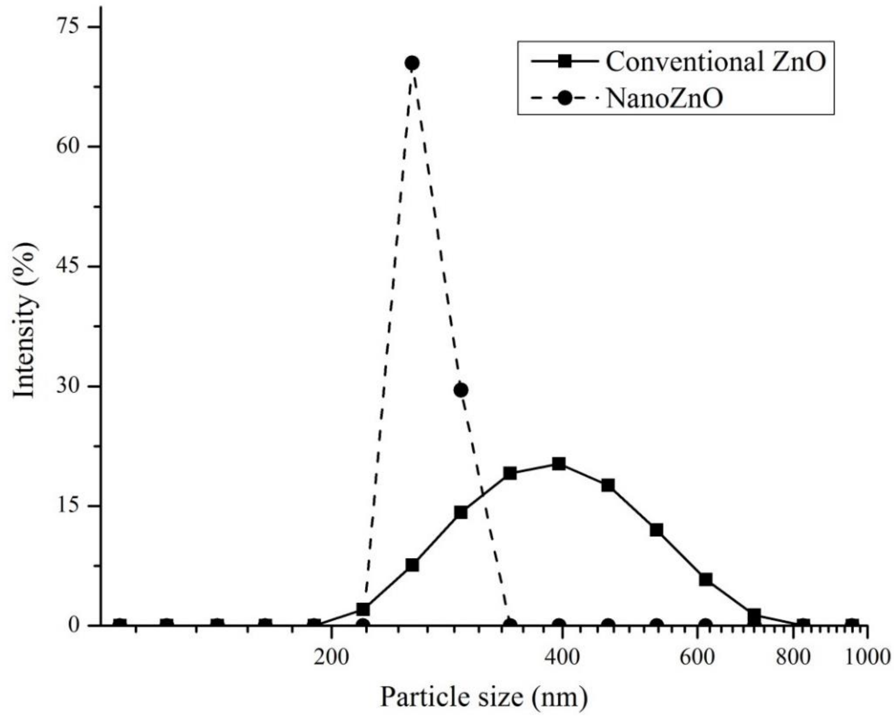

Fig. 3. Particle size distribution of conventional ZnO and nanoZnO by their intensities

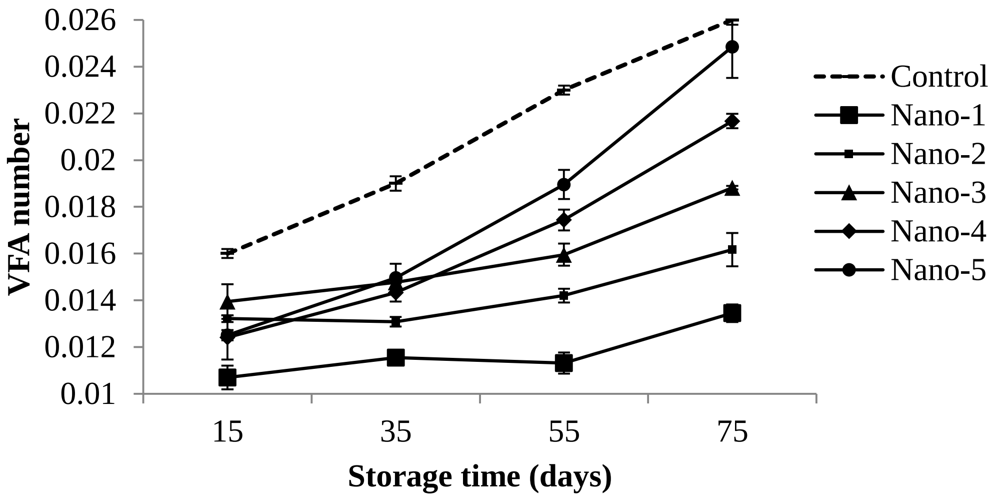

Fig. 4. Variation of VFA number of centrifuged latex samples with storage time

MST results in CNRL samples

MST is an important parameter of CNRL that measures the ability of latex to retain its colloidal stability against mechanical agitation during pumping, transportation, concentration, compounding, processing *etc.* As per the MST development illustrated in Fig. 5, all samples with nanoZnO/TMTD showed higher MST values than that of control sample after 20 days of storage time. It is further noted that MST of CNRL has shown a general trend of decreasing MST with the reduction of nanoZnO/TMTD with the exception of Nano-4 sample.

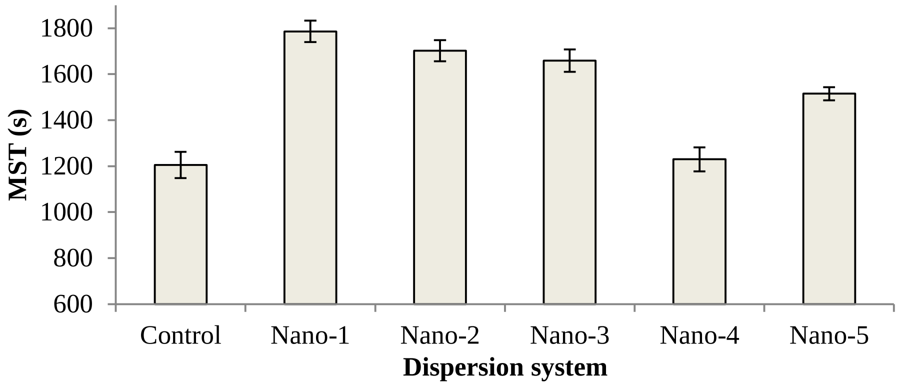

Fig. 5 Variation of MST after 20 days of storage time in CNRL samples

It is interesting to note that the MST of all the samples with nanoZnO registered significantly higher values compared to that of Control after 30 days (Table 3). A study conducted on the mechanical stability of natural rubber latex has suggested that the key factor that reduces the MST was the increase of ionic strength result in compressing the electrical double layer of the latex particles (Pendle and Gorton, 1984). Moreover, the same study proposed that the increase of VFA number may also be attributed due to the enhancement of ionic strength in the aqueous phase in latex. In our study, during first 35 days of centrifugation, the rate of VFA development in the systems with nanoZnO is very low resulting low rate of ionic strength development, thus maintaining the colloidal stability with time. Another recent study carried out on particle size and zeta potential of ZnO has shown that the alkaline pH with about 60-100 nm sized ZnO particles have negative zeta potential values (Marsalek, 2014). Similarly, the negative zeta potential values on nanoZnO may have reduced the ZnO thickening effect, thus enhancing the colloidal stability of latex resulting remarkably enhanced MST values in latex samples preserved with TMTD/nanoZnO.

Mechanical properties of vulcanized NR films

The suitability of CNRL samples for production of latex products are assessed by the mechanical properties of the vulcanized latex films made out of preserved latex films.

Table 3. *MST of CNRL samples after 20 and 30 days of centrifugation*

Sample name	MST (seconds)	
	After 20 days	After 30 days
Control	1205	1446
Nano-1	1786	> 1800
Nano-2	1702	> 1800
Nano-3	1659	> 1800
Nano-4	1230	> 1800
Nano-5	1515	> 1800

According to the results shown in Table 4, the properties including modulus at 300% elongation and elongation at break of Nano-1 to Nano-4 samples were almost comparable with those of control. It is further noted that the tensile strengths of Nano-1 to Nano-4 have slightly decreased with decreasing the loading of nanoZnO. This may be caused by the enhanced activity of nanoZnO as an activator in sulfur vulcanization, resulting more cross-links formation in rubber compounds with more nanoZnO (Sahoo *et al.*, 2007; Panampilly and Thomas, 2013). Further, modulus at 100% elongation values are also slightly higher in the samples preserved with nanoZnO when compared to control. At this strain, nanoZnO may have contributed to enhance the modulus by acting as filler in rubber compounds (Panampilly and Thomas, 2013), thus showing resistance to stretch.

Tear strengths, which depend on the crack propagation and the average molecular weight of the polymer, do not show any regular trend in all the samples (Sreeja and Kutty, 2000). In summary, the mechanical test results indicate that the nanoZnO quantities that we used in this study have very little effect on the molecular weight and cross linking densities of rubber vulcanizates. Therefore, it could be concluded that preservation of CNRL with nanoZnO does not adversely affect the final mechanical properties.

Statistical analysis of VFA number

A statistical model was developed to determine the best treatment levels to produce CNRL from nanoZnO based systems based on their VFA number variations. The surface plots revealed (Fig. 6) the effect of both TMTD and nanoZnO concentrations on VFA number after 15, 35, 55 and 75 days of centrifugation.

Table 4. *Mechanical properties of the vulcanized latex films*

Sample	Tensile strength (MPa)	100% modulus (MPa)	300% modulus (MPa)	Elongation at break (%)	Tear strength (MPa)
Control	20.22 ± 4.11	1.25±0.18	9.68±1.74	362.64±32.30	52.26±8.36
Nano-1	23.62 ± 1.96	1.59±0.11	7.00±2.60	402.71±29.99	46.48±4.19
Nano-2	21.77 ± 1.30	1.70±0.17	9.75±2.98	369.96±20.49	47.83±2.44
Nano-3	20.71 ± 2.55	1.55±0.25	8.90±2.67	372.75±25.17	53.18±5.20
Nano-4	19.48 ± 1.79	1.77±0.45	9.72±1.61	374.70±19.85	51.33±5.83
Nano-5*	-	-	-	-	-

**Mechanical properties of Nano-5 could not be determined due to the poor quality of the preserved latex*

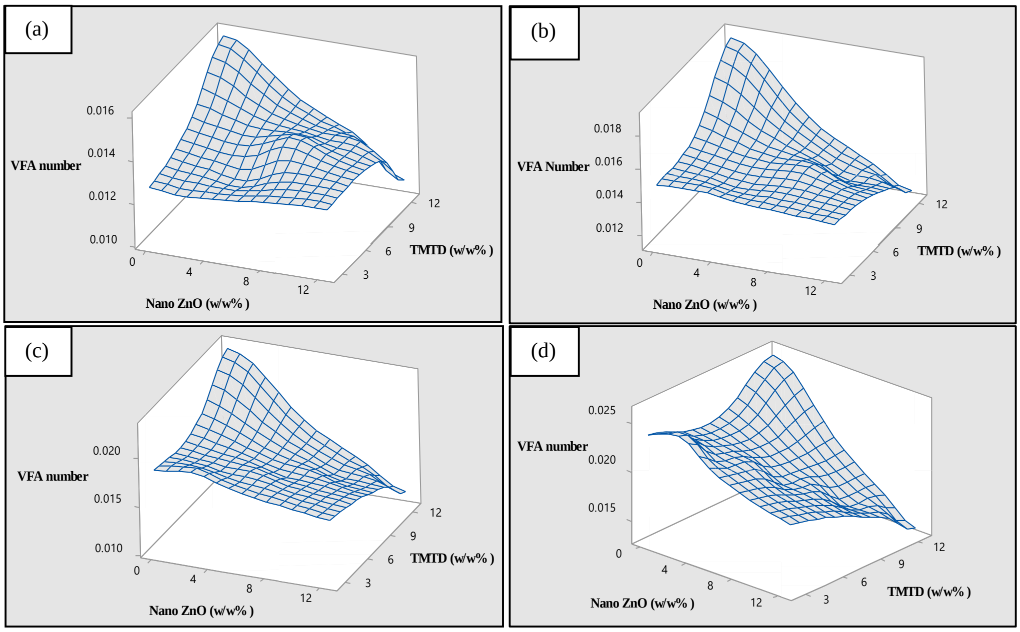

Fig. 6. Surface plots of VFA number variation with TMTD and nanoZnO after **a)** 15 days, **b)** 35 days, **c)** 55 days and **d)** 75 days of centrifugation

As per the surface plots, it is clearly seen that VFA levels are rapidly reduced with increasing both TMTD and nanoZnO amounts except in Fig. 6a. Further, VFA values are higher in the absence of nanoZnO in all the cases. The plots (a, b, c, and d) also reflect that the VFA number can be maintained less than 0.020 in the presence of only nanoZnO at 12.0 w/w %. Therefore, it could be inferred that the effect of nanoZnO is more significant than TMTD, when the preservation of CNRL is concerned.

The effects of ZnO/TMTD on VFA levels at each time interval (15, 35, 55 and 75 days) were compared using one-way ANOVA. Out of four time intervals, except for15-day time interval, the rest of the time periods showed significant differences among treatments at the probability level, $p < 0.05$.

Subsequently, the Duncan's multiple range test was employed to compare the means of VFA number values of systems at different time intervals with the exception of 15-day interval, as it was not significantly different at the probability level, $p = 0.05$. The test revealed that the Control has a higher mean VFA value when compared to mean VFA values of the rest of the systems at 35-day interval. At 55-day interval, mean VFA values of Control and Nano-5 systems were higher when compared to that of the other systems, among which, Nano-4 had the lowest amounts of ZnO and TMTD. In 75-day interval, mean VFA values of Control, Nano-5 and Nano-4 were higher than those in the rest of the systems, among which Nano-3 used the lowest amounts of ZnO and TMTD for the preservation. Therefore, based on this analysis, Nano-4 system might be optimum system to be used if the latex is stored up to 55 days, while Nano-3 can be recommended if the latex is stored up to 75 days until it reaches the production facility.

Conclusions

This study investigated the effect of nanoZnO as a component of a preservative system for CNRL along with TMTD and low ammonia. The characterization techniques including XRD confirmed both conventional ZnO and nanoZnO were in zincite crystalline form. The SEM data revealed that the nanoZnO crystals were in needle-like shape, while conventional ZnO were hexagonal-shaped crystals. VFA studies confirmed that nanoZnO has a significant contribution as a preservative for CNRL, when compared to conventional ZnO. This may be due to the higher surface area/volume ratio of nanoZnO resulting enhanced active surfaces against bacterial reactions. The rate of VFA development reduced with the increase of nanoZnO/TMTD concentration. Systems with nanoZnO had higher MST values than the control system, which may be due to combination of small particle size along with alkaline pH resulting negative zeta potential values in nanoZnO. Mechanical properties *i.e*. tensile and tear properties of vulcanized films prepared using CNRL preserved with conventional and modified preservative systems showed no significant difference. Therefore, it could be concluded that the effect of amounts of nanoZnO use in the experiment on mechanical properties of CNRL was insignificant. Surface plots indicated that nanoZnO was more effective than TMTD on preservation of CNRL. Thus, the use of TMTD could be reduced when nanoZnO is employed as the secondary preservative agent. Statistical analysis suggested that Nano-3 and Nano-4 systems could be employed, if the CNRL is stored up to 75 and 55 days, respectively. In summary, nanoZnO can be used as an effective preservative agent while lowering the usage of both toxic TMTD and ZnO for the preservation of low ammonia CNRL.

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