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RESEARCH ARTICLE

LOWERING THE LATEX PH OF HEVEA BRASILIENSIS WITH ACID TO REDUCE ITS COAGULATION TIME: A METHOD OF PREVENTING YIELD LOSSES DUE TO RAIN

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ABSTRACT

Frequent heavy rains are a major hindrance for rubber harvests in the coastal zone of Cameroon, due to the pouring of latex out of cups as a result of water overflow. Lowering the pH of latex with acid to accelerate its clotting could be a solution. A factorial experiment *in vitro* was conducted to assess the pH and the corresponding coagulation times of different treatments obtained from three volumes of latex (40, 80 and 120 ml), four rubber clones (GT1, PB217, PR107 and RRIC100), and seven doses of formic acid (0, 1, 2, 3, 4, 5 or 6 ml). The three volumes of latex were tested pure (no addition of rain water) or diluted (with an addition of 120 ml of rain water). A RCBD experiment *in vivo* was also carried out to assess the percentage of trees with coagulated latex in their harvest cups 5 hours and 30 min after addition of acid. Both experiments were repeated weekly during four months in the rainy season. The use of formic acid had a highly significant effect ($P<0.001$) on lowering the pH and accelerating the coagulation of latex *in vitro* and no significant effect ($P>0.05$) *in vivo*. An adjustment of the doses of formic acid to be used in the field might be necessary for a successful acidification of latex.

INTRODUCTION

With 3,284.54 ha of trees under exploitation and 1,183.64 ha of trees yet to be exploited (Anonymous, 2015a), SAFACAM S.A is one of the major actors in the production of natural rubber in Cameroon. Meanwhile, for years, the agro-industry is faced with many constraints; one of which is rains during harvests. Morning rains, coming after tapping or just prior to it, are conducive to the run off of rain water down the bark till the tapping notch. As a consequence, latex is washed away till the ground. During heavy downpours, harvest cups are filled with water; and latex, due to its lower density, pours out on the ground. In order to reduce losses of production pertaining to rains, many trials have been carried out in the past within SAFACAM S.A. It has been the case of "Rain-guard" trials and the case of "Cup-over" trials. It was about protecting the tapping notch and the harvest cup against rain water. Both equipments, although allowing a relative increase of yields, have not been adopted, due to their very expensive final cost. SAFACAM has decided to approach the problem differently. It is about acidifying latex after tapping during the rainy season in order to significantly cut short its coagulation time and then to significantly reduce losses by overflow of cups.

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As a matter of fact, once the latex has coagulated, rains could no longer do it any harm. It should be clearly understood that compared to coagulated latex only fluid latex gives the finest quality rubber materials after the manufacturing processes. That is why latex is kept fluid as long as possible after harvest. Adding about 4 g of water ammonia per liter of latex helps to maintain it fluid until it is processed (Delabarre and Serier, 1995; Delabarre and Eschbach, 2002). Coagulating latex soon after harvest and before manufacturing processes could be an acceptable method of preventing yields losses due to rain, for coagulated latex is far better than no fluid latex at all. However, things are not that easy, for many questions about that technique need to be answered. For instance: what should be the latex pH after adding acid? What quantity of acid should be put in the cup after tapping? Should that quantity have to be the same for all the clones? What must be the time duration prior to latex coagulation after a particular dose of acid has been added? The overall objective in this study is to suggest clear steps in using formic acid to lower the pH of latex and to shorten its coagulation time as a method for preventing production losses after tapping.

MATERIALS AND METHODS

Study area: The trial was carried out within the SAFACAM S.A rubber estates in Dizangué between July and December 2015. Dizangué in Cameroon is located within the subdivision

of the same name (Dizangué), in the Sanaga Maritime division, and the Littoral region. Thus, Dizangué is located in the coastal zone of Cameroon, inside a wide plain between 3°40' and 3°55' of latitude North and between 9°33' and 10°05' of longitude East, at an altitude of 0-80 m. The climate is typically equatorial; with a one-mode rainfall pattern (with a single dry season and a single rainy season). The proximity with the sea and a dense hydrography are conducive to more than 142 days of rain per year, totaling close to 3000 mm rainfall per year. Temperatures vary annually between 22 to 30 °C, with 26 °C as the mean annual temperature. Soils range from clayey to sandy, depending on the situation in lowlands or plateaus; they are acidic under plantations, with pH between 4.5-5.5 (Baert and Debersaques, 2006).

MATERIALS AND METHODS

As plant materials, four of the most productive clones exploited in SAFACAM S.A were used in the trial (GT1, PB217, PR107 and RRIC100). Studying more than one clone was important for comparison purposes and for broadening the scope of the results. Chemicals and tools used in the trials are listed (Table 1).

Formic acid is the simplest of all carboxylic acids. Its chemical formulation is CH_2O_2 or HCOOH. Its name comes from *formica*, a latin word that signifies "ant"; because the first time it was obtained was from distillation of the bodies of these insects. Nowadays, it is industrially synthesized (Wikipédia, 2015). Formic acid has many uses in different types of industries, one of which is coagulation of latex in the field and in the factory in rubber production (Anonymous, 2015b). Formic acid should be handled with an extreme care for it can be very harmful when in direct contact with the skin or by inhalation. It should be kept locked under strict vigilance and only very experienced workers must manipulate it (Anonymous, 2011).

Experimental design

The trial has been realized in two phases. The first phase was *in vitro* (measurements of latex pH and coagulation time were done inside test cups); while the second phase was *in vivo* (the percentage of trees of which harvest cups had coagulated latex was assessed directly on the field). For the trial phase *in vitro*, the experimental design was a factorial experiment; three experiment factors (doses of acid, volumes of latex and types of clones) were combined in it. There were seven doses of formic acid (0, 1, 2, 3, 4, 5 and 6 ml), three volumes of latex (40, 80 and 120 ml) and the aforementioned four clones (GT1, PB217, PR107 and RRIC100). The experiment encompassed 4 repetitions (R1, R2, R3 and R4) each time it was run. In one

Table 1. Chemicals and tools used in the trials

Materials	Use
Stimulation paste	For the application onto barks to stimulate trees
Formic acid	For the latex acidification
Iragon	For the coloration of the acid to make sure it can be easily differentiated from water and that latex has truly been acidified
pH paper	For the solution pH measurement before and after the addition of acid
Graduated glass test tubes	For the measurement of small volumes of acid, latex and water
Rain water	For the dilution of latex
Test cups	For stocking the latex to be tested in vitro or in vivo

Table 2. Factorial experiment *in vitro* with pure latex

Repetitions	Clones	Volumes of latex and doses of formic acid (in ml)		
		40 ml of latex	80 ml of latex	120 ml of latex
	GT1	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
R 1	PB217	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
	PR107	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
	RRIC100	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
	GT1	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
R4	PB217	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
	PR107	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
	RRIC100	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7

Table 3. Factorial experiment *in vitro* with diluted latex

Repetitions	Rain water	Clones	Volumes of latex and doses of formic acid (in ml)		
			40 ml of latex	80 ml of latex	120 ml of latex
R 1	120 ml	GT1	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
		PB217	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
		PR107	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
		RRIC100	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
.		.	.	.	.
.		.	.	.	.
.		.	.	.	.
		GT1	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
R4	120 ml	PB217	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
		PR107	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7
		RRIC100	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7	0, 2, 3, 4, 5, 6, 7

aspect of the trial phase *in vitro*, the three volumes of latex were used pure (no addition of rain water); making a total of 84 experimental units (or test cups). In another aspect of this trial phase, the three volumes of latex (40, 80 and 120 ml) were diluted in 120 ml of rain water; that is 1/3, 2/3 and 3/3 volumes of pure latex diluted in one volume of rain water, respectively; making again a total of 84 experimental units (or test cups).

A summary of the experimental design *in vitro* is illustrated with pure latex (Table 2) and diluted latex (Table 3). The experiments were repeated weekly during 4 months (between July and November 2015) in the rainy season. Response factors were the latex pH and the latex coagulation time for each experimental unit. For the trial phase *in vivo*, a RCBD experiment was carried out in the field; two experiment factors (doses of acids and types of clones) were combined in it. For practical purposes, only three clones (PB217, PR107 and RRIC100) out of the previous four used in the trials *in vitro* could be tested; with, however, the same seven doses of formic acid as before. This phase of the experiment was laid out in the field in three blocks. Five lines of seven trees were selected in each clone for the measurements. Each tree was an experimental unit with a single dose of formic acid added in the harvest cup. Each harvest cup at full capacity could contain 2000 ml. The response factor was the percentage of trees in each treatment that had the content of their harvest cups coagulated 5 hours and 30 min after harvest. A summary of the experimental design *in vivo* is illustrated (Table 4).

Table 4. RCBD experiment *in vivo*

Blocks	Clones	Lines of trees	Doses of formic acid (ml)
Block X	PB217	1	0, 1, 2, 3, 4, 5, 6,7
		.	.
		.	.
		7	0, 1, 2, 3, 4, 5, 6,7
		1	0, 1, 2, 3, 4, 5, 6,7
	PR107	.	.
		.	.
		7	0, 1, 2, 3, 4, 5, 6, 7
		1	0, 1, 2, 3, 4, 5, 6, 7
	RRIC100	.	.
		.	.
		7	0, 1, 2, 3, 4, 5, 6, 7

X: Block number (X = 1, 2 or 3)

Experiment carrying out

For the trial phases *in vitro*, latex was collected separately for each clone in the field. In order to boost their production, trees were stimulated with Ethrel at two different periods; at first before the beginning of tapping, then four weeks later. The latex collected was carried right away back to the laboratory and distributed in sets of cups of three volumes of latex: 40, 80 and 120 ml for each clone. A graduated test tube was used in the process to ensure a correct measurement of the volumes (Figure 1). Prior to its use in the tests, formic acid was diluted in water; in the proportions of 0.25 l of formic acid and 9.75 l of water. That mixture had as an advantage to render the acid less corrosive and less nauseating. To make sure that tapping workers would not confuse acid and water, 50 g of inorganic coloring (blue Iragon) were added to the acid-water mixture. The final solution was of a dark blue color with a pH of 3 (Figure 2).

Figure 1. Distribution of latex in test cups

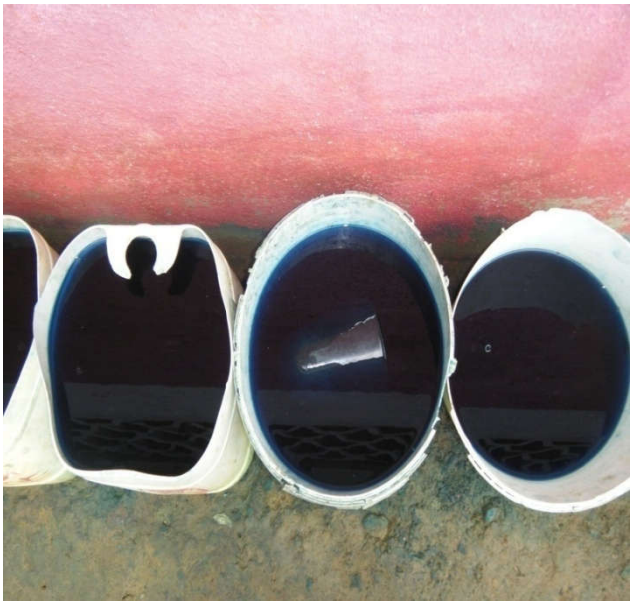

Figure 2. Acid and rain water solution colored in dark blue with Iragon

Experiments on pure and diluted latex were done simultaneously to avoid any bias. However, concerning the diluted latex, the three volumes of pure latex were diluted with an addition of 120 ml of rain water. Upon completion of the distribution of latex in the cups, be it pure or diluted, the seven doses of the acid (0, 1, 2, 3, 4, 5, 6, or 7 ml) were added; one dose per cup of latex. It is worth mentioning that 0 ml of acid (or no acid added) was the test control. The time of the addition of the acid was immediately noted. The pH was measured in each cup with a pH-paper immediately after the addition of the acid (Figure 3) and the texture of the latex was checked every 30 minutes until the entire coagulation was obtained. Latex was considered totally coagulated when none of its drops fell on the ground after the cup was tilted or even turned upside down (Figure 4). For the trial phase *in vivo*, the experiments were carried out in the field where loss of latex naturally takes place as trees are directly exposed to rain (Figure 5). The same seven doses of acid as in the trial phase *in vitro* were used, one per tree. The addition of acid in harvest cups was performed two hours after the tapping of trees.

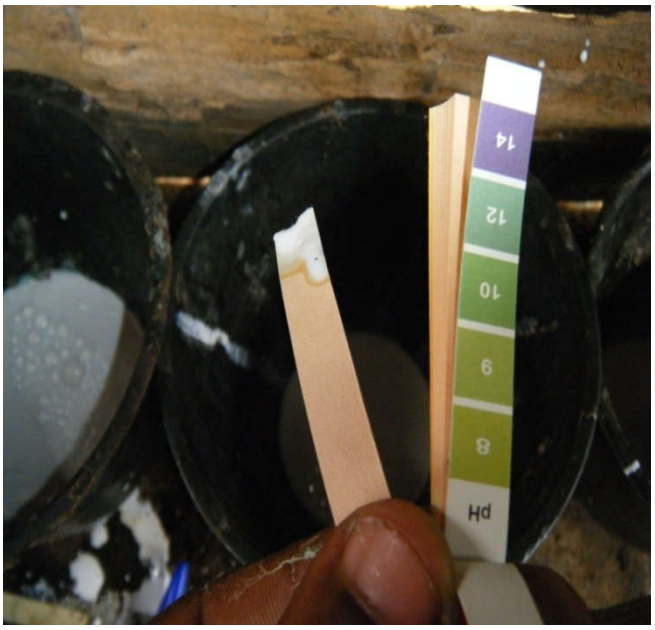

Figure 3. Measurement of the latex pH

Figure 4. Clotting of latex after its acidification

The texture of the latex in cups was observed 5 hours and 30 minutes later (which is usually the time to complete in the field all the daily operations pertaining to rubber tapping). The proportion of trees in each treatment that had the content of their harvest cups coagulated 5 hours and 30 min after the onset of the tapping was noted.

Data collection and analysis

The response variables were:

- The pH of pure latex;
- The coagulation time of pure latex (in hours);
- The pH of diluted latex;
- The coagulation time of diluted latex (in hours);
- The percentage of trees with coagulated latex in harvest cups.

The Analysis of variance (ANOVA) has permitted to compare the four clones, the seven doses of acids and the three volumes of latex, pure or diluted, on the basis of the response variables. The test of Duncan has allowed separating means in case of a significant difference. The Genstat 9.2 software was used for data analysis and the EXCEL version 2007 software was used to build tables and draw graphs.

RESULTS

pH values of pure latex after addition of formic acid in vitro

After the seven doses of formic acid (0, 1, 2, 3, 4, 5 and 6 ml) were added respectively to the three volumes of pure latex (40, 80 and 120 ml) in all the four clones (GT1, PB217, PR107 and RRIC100), the pH values of pure latex were measured in all the test cups. An increment in formic acid doses by 1 ml has led to a regular decrease in the latex pH, no matter which clones were tested. Also, an increment of formic acid doses by 1 ml always decreased the pH by 0.5. Identical results were found in the three volumes of pure latex (Figure 6).

Figure 5. Loss of latex on the ground due to rain

An analysis of variance (ANOVA) was run to find out if the different experiment factors (doses of formic acid, volumes of latex and types of clones) and their interactions had a significant effect on the pH lowering of pure latex. Results are summarized (Table 5). It came out of the analysis that doses of formic acid had a highly significant effect ($p < 0.001$) on the lowering of the pH of pure latex. It was also seen that types of clones had a significant effect ($p = 0.048$). However, volumes of latex showed no significant effect. The analysis has also shown that there has been a highly significant effect ($p < 0.001$) of the interaction between doses of formic acid and types of clones. The separation of the means of formic acid doses based on the pH values of pure latex was performed with the test of Duncan (Table 6). Adding 6 ml of formic acid lowered the most the pH of pure latex (to an average of 4.54, all types of clones taken into consideration).

The separation of the means of types of clones based on the pH values of pure latex was also performed with the test of Duncan (Table 7). The two clones GT1 and PR107 reacted identically to the addition of doses of formic acid into their latex and the two clones PB217 and RRIC100 equally reacted identically to this addition. However, clones GT1 and PR107 appeared significantly different from clones PB217 and RRIC100.

Coagulation times of pure latex after addition of formic acid in vitro

After the seven doses of formic acid (0, 1, 2, 3, 4, 5 and 6 ml) were added respectively to the three volumes of pure latex (40, 80 and 120 ml) in all the four clones (GT1, PB217, PR107 and RRIC100), different coagulation times were observed. An increment in formic acid doses led to a decrease in the coagulation times of pure latex for three clones (GT1, PR107 and RRIC100). Identical results were found in all the three cases of 40, 80 and 120 ml of pure latex (Figure 7). However, increasing formic acid doses by 1 ml did not give a regular decrease in the time of latex coagulation, as it was the case for the decrease in the latex pH. Increasing formic acid doses in clone PB217 led to the same coagulation time (2.30 hours); which was the lowest coagulation time in overall. An analysis of variance was run to find out if the different experiment factors (doses of formic acid, volumes of pure latex and types of clones) and their interactions had a significant effect on the time of pure latex coagulation. Results are summarized (Table 8). It came out of the analysis that doses of formic acid had a highly significant effect ($p<0.001$) on the pure latex coagulation time. It was also seen that types of clones had a highly significant effect ($p<0.001$). Meanwhile, volumes of latex showed no significant effect. A highly significant effect ($p<0.001$) of the interaction between doses of formic acid and types of clones was also revealed. The separation of the means of formic acid doses based on the time of latex coagulation was performed with the test of Duncan (Table 9). Adding 6 ml of formic acid reduced the most the coagulation time of pure latex (to an average of 4.74 hours, all types of clones taken into consideration). However, no significant difference could be found between the additions of 4, 5 and 6 ml of formic acid. The separation of the means of types of clones was also performed based on the pure latex coagulation time with the test of Duncan (Table 10). All the clones reacted differently from each other. Clone PB217 was the one of which the pure latex coagulation time was significantly reduced the most (2.42 hours in average).

pH values of diluted latex after addition of formic acid in vitro

The three volumes of pure latex (40, 80 and 120 ml) were, this time, diluted with the use of 120 ml of rain water in all the four clones (GT1, PB207, PR107 and RRIC100), and the seven doses of formic acid were distributed to all the test cups. Once again, pH values were measured in all the test cups. And here again, it was noticed that an increment in formic acid doses led to a decrease in the latex pH values, no matter which clones were tested. Results found with 40 ml of diluted latex (Figure 8) differed from those found with 80 and 120 ml of diluted latex; the latter being both identical (Figure 9). An analysis of variance (ANOVA) was run to find out if the different experiment factors (doses of formic acid, volumes of latex and types of clones) and their interactions could have a significant effect on lowering the pH of diluted latex. Results are summarized (Table 11). The analysis has revealed that doses of formic acid, volumes of diluted latex and types of clones, all had a highly significant effect ($p < 0.001$) on the lowering of the pH of diluted latex. It was also seen that the interaction between volumes of latex and types of clones had a significant effect ($p = 0.022$), the interaction between volumes of latex and doses of formic acid had equally a highly significant effect ($p < 0.001$), and finally the interaction between types of clones and doses of formic acid had a significant effect ($p = 0.029$). However, no significant effect could be found ($p = 0.108$) due to the interaction between all the three experiment factors taken together.

The separation of the means of formic acid doses on the basis of the pH values of diluted latex was performed with the test of Duncan (Table 12). Adding 6 ml of formic acid lowered the most the pH of diluted latex (to an average of 5.01, all types of clones taken into consideration). The separation of the means of volumes of latex based on the pH values of diluted latex was also performed with the test of Duncan (Table 13). The lowest pH in average (6.01) was recorded with 40 ml of latex, the highest pH in average was recorded with 80 ml of latex (6.24) and the pH next to the highest was recorded with 120 ml (6.22). However, no significant difference could be found between 80 and 120 ml of latex. The separation of the means of types of clones based on the pH values of diluted latex was also performed with the test of Duncan (Table 14). Clone PB217 had the lowest values of latex pH (with an average of 6.02) and clone RRIC100 had pH values next to the lowest (with an average of 6.12). No significant difference could be established between clone GT1 (average pH = 6.36) and clone PR107 (average pH = 6.20).

Coagulation time of diluted latex after addition of formic acid in vitro

The seven doses of formic acid (0, 1, 2, 3, 4, 5 and 6 ml) were distributed to all the test cups after the three volumes of pure latex (40, 80 and 120 ml) were diluted respectively in 120 ml of rain water in all the four clones (GT1, PB207, PR107 and RRIC100). Once again, coagulation times were observed in all the test cups. And here again, an increment of 1ml in formic acid doses led to a decrease in the times of latex coagulation, no matter which clones were tested. However, results found were the same in all three volumes of diluted latex (Figure 10). Besides, these results did not differ from those observed with the coagulation times in pure latex. No wonder an analysis of variance run to find out whether or not the different experiment factors (doses of formic acid, volumes of latex and types of clones) and their interactions had a significant effect on the time of diluted latex coagulation has led to identical results as for those obtained with pure latex coagulation times. Seemingly, diluting volumes of latex tested in this experiment (40, 80 and 120 ml) into 120 ml of rain water has conferred no difference.

Proportion of trees with coagulated latex in harvest cups in vivo

The proportion of trees with coagulated latex in harvest cups 5 hours and 30 minutes after adding formic acid varied, depending on formic acid doses and types of clones.

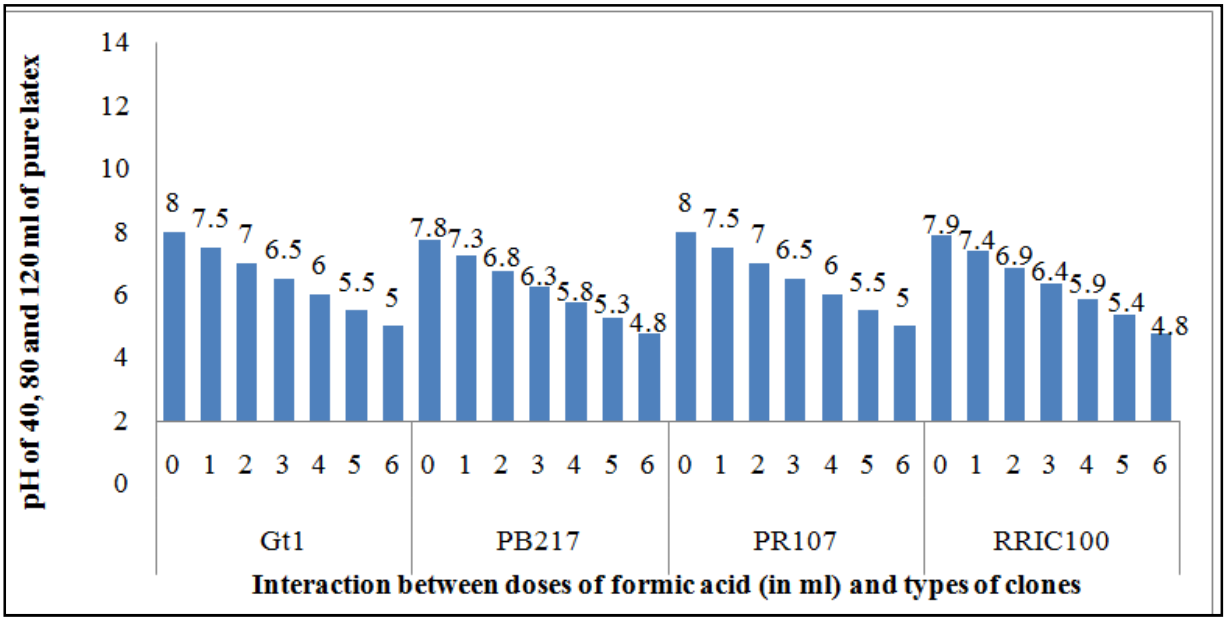

Figure 6. pH of 40, 80 and 120 ml of pure latex after addition of seven doses of formic acid in four clones

Table 5. ANOVA of formic acid doses, volumes of latex and types of clones and their interactions on the basis of pH values of pure latex

SV	DF	SS	MS	F	P<F
Repetitions (A)	3	9.723214	3.241071		
Volumes of latex (B)	2	0.000000	0.000000		
Error residual	6	0.000000	0.000000	0.00	
Types of clones	3	3.723214	1.241071	3.00	0.048*
Volumes of latex ×Types of clones (C)	6	0.000000	0.000000	0.00	1.000
Error residual	27	11.169643	0.413690	185.33	
Doses of formic acid	6	340.540179	56.756696	25427.00	<.001**
Volumes of latex × Doses of formic acid	12	0.000000	0.000000	0.00	1.000
Types of clones × Doses of formic acid	18	0.120536	0.006696	3.00	<.001**
Volumes of latex × Types of clones × Doses of formic acid (D)	36	0.000000	0.000000	0.00	1.000
Error residual	216	0.482143	0.002232		
Total	335	365.758929			

Table 6. Separation of the means of formic acid doses

Doses of formic acid	0	1	2	3	4	5	6
Means	7.54^{a}±0.01	7.24^{a}±0.01	6.54^{b}±0.01	6.24^{bc}±0.01	5.54^{cd}±0.01	5.24^{d}±0.01	4.54^{e}±0.01

N.B.: numbers followed with the same letter are not significantly different

Table 7. Separation of the means of types of clones

Types of clones	GT1	PB217	PR107	RRIC100
Means	6.30^{a}±0.07	6.15^{b}±0.07	6.30^{a}±0.07	6.22^{b}±0.07

N.B.: numbers followed with the same letter are not significantly different

Table 8. Analysis of variance of formic acid doses, volumes of latex and types of clones and their interactions on the basis of times of pure latex coagulation

SV	DF	SS	MS	F	P<F
Blocks (A)	3	20.0625	6.6875		
Volumes of latex (B)	2	0.0000	0.0000		
Error residual	6	0.0000	0.0000	0.00	
Types of clones	3	1413.6696	471.2232	31.87	<.001**
Volumes of latex × Types of clones (C)	6	0.0000	0.0000	0.00	1.000
Error residual	27	399.2411	14.7867	49.27	
Doses of formic acid	6	440.7054	73.4509	244.76	<.001**
Volumes of latex × Doses of formic acid	12	0.0000	0.0000	0.00	1.000
Types of clones × Doses of formic acid	18	102.8304	5.7128	19.04	<.001**
Volumes of latex × Types of clones × Doses of formic acid (D)	36	0.0000	0.0000	0.00	1.000
Error residual	216	64.8214	0.3001		
Total	335	2441.3304			

%CV(A): 4,5; %CV(B): 0,0; %CV(C): 23,4; %CV(D): 8,8.; **: highly significant (P<0.01)

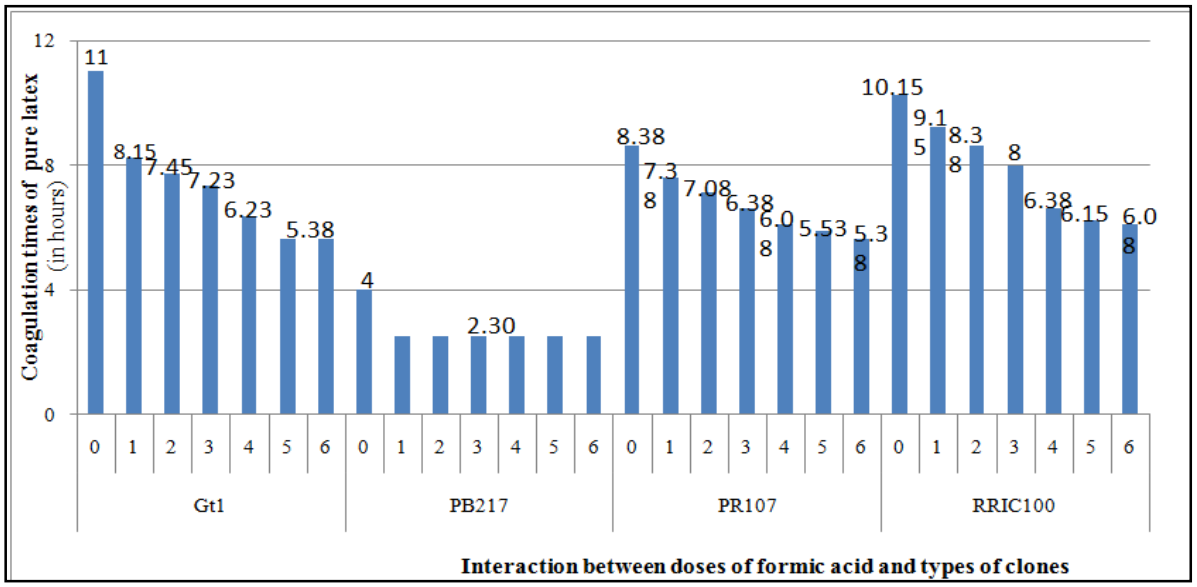

Figure 7. Coagulation times of 40, 80 and 120 ml of pure latex after addition of seven doses of formic acid in four clones

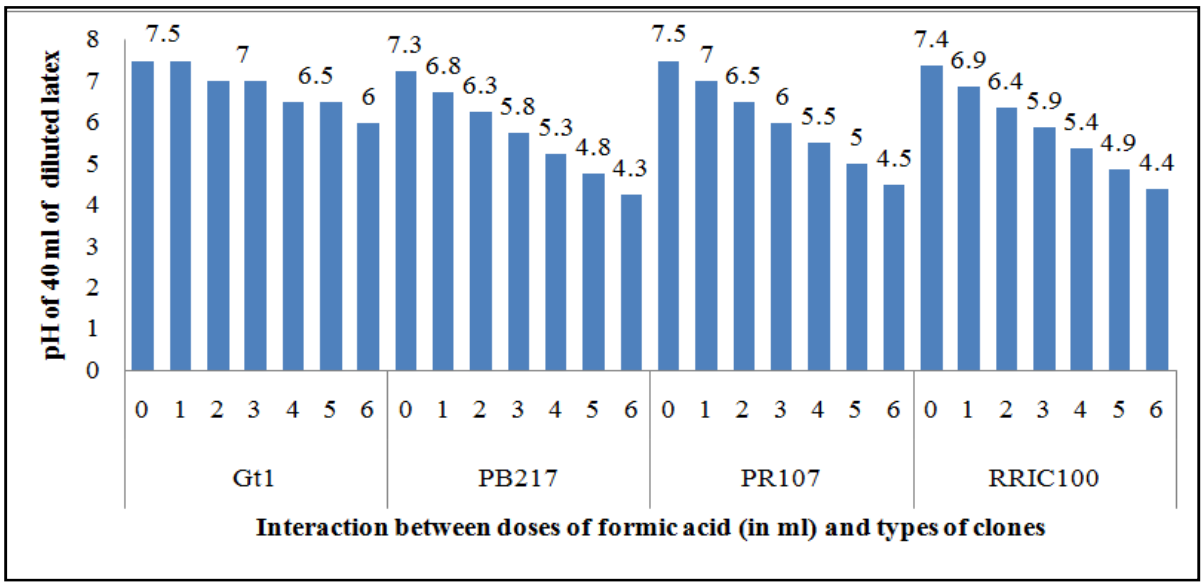

Figure 8. pH of 40 ml of latex diluted in 120 ml of rain water after addition of seven doses of formic acid in four clones

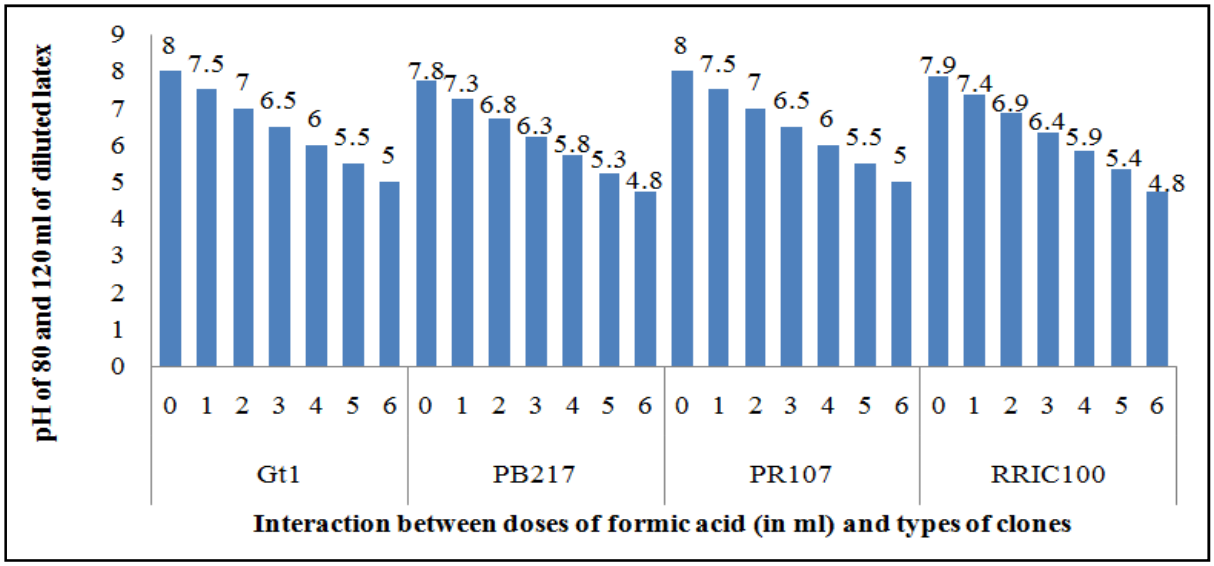

Figure 9. pH of 80 and 120 ml of latex diluted in 120 ml of rain water after addition of seven doses of formic acid in four clones

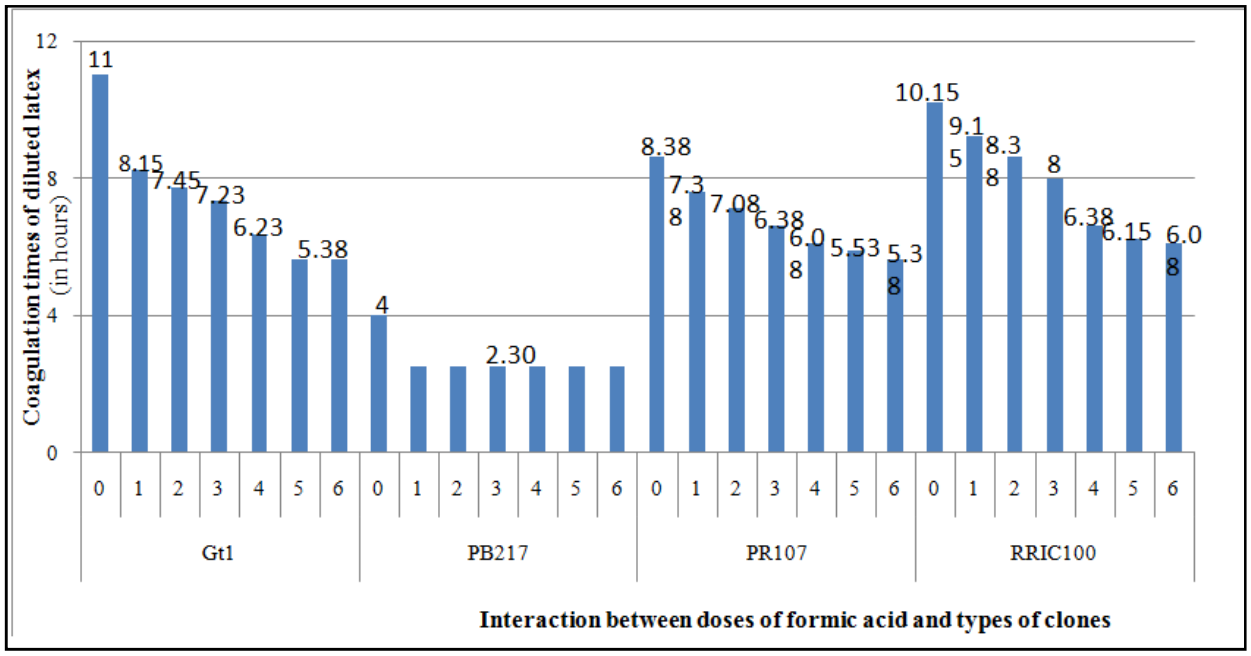

Figure 10. Coagulation times of 40, 80 and 120 ml of latex diluted with 120 ml of rain water after addition of seven doses of formic acid in four clones

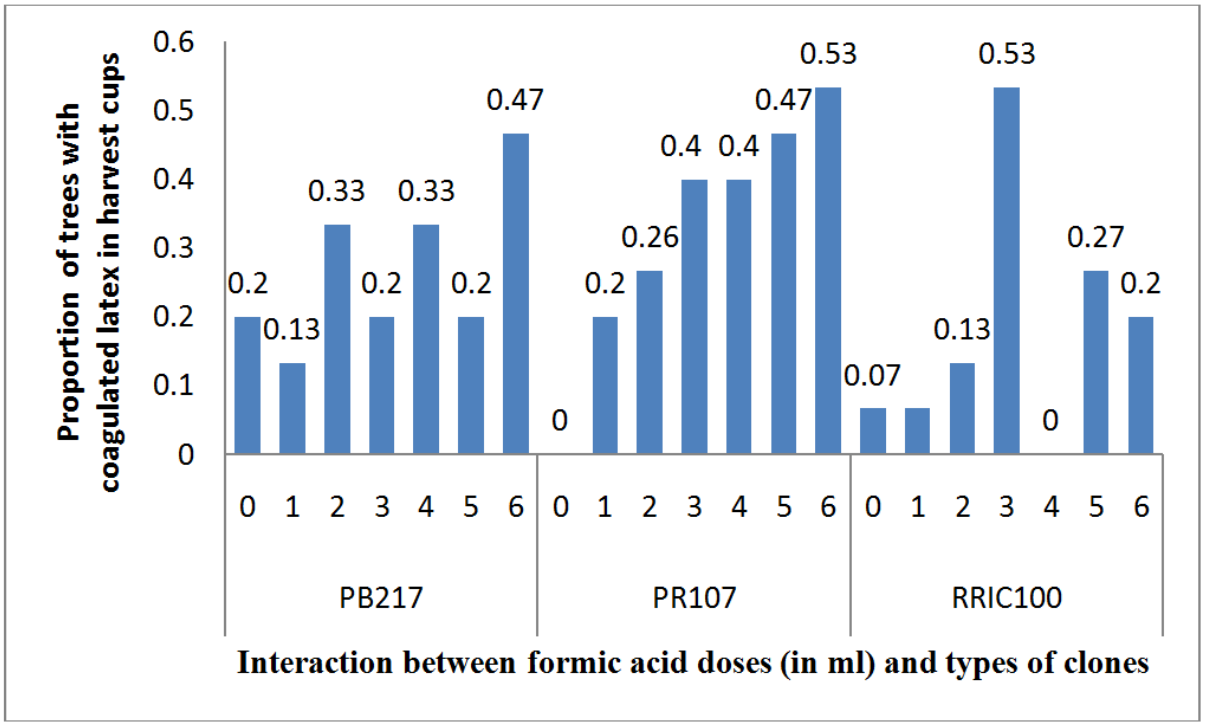

Figure 11. Proportion of trees with coagulated latex in harvest cups *in vivo*

Table 9. Separation of the means of formic acid doses

Doses of formic acid	0	1	2	3	4	5	6
Means	8.24^{a}±0.11	6.54^{b}±0.11	6.30^{b}±0.11	6.06^{bc}±0.11	5.24^{cd}±0.11	5.04^{d}±0.11	4.74^{d}±0.11

N.B.: numbers followed with the same letter are not significantly different

Table 10. Separation of the means of types of clones

Types of clones	GT1	PB217	PR107	RRIC100
Means	7.24^{a}± 0.59	2.42^{b}±0.59	6.48^{c}±0.59	7.54^{d}±0.59

N.B.: numbers followed with the same letter are not significantly different

Table 11. ANOVA of doses of formic acid, volumes of latex and types of clones and their interactions on the basis of the pH values of diluted latex

SV	DF	SS	MS	F	P<F
Blocks (A)	3	11.57143	3.85714	64.00	
Volumes of latex (B)	2	5.35863	2.67932	44.46	<.001**
Error residual	6	0.36161	0.06027	0.11	
Types of clones	3	15.01786	5.00595	9.17	<.001**
Volumes of latex × Types of clones (C)	6	9.82589	1.63765	3.00	0.022*
Error residual	27	14.74554	0.54613	6.07	
Doses of formic acid	6	282.74851	47.12475	523.44	<.001**
Volumes of latex × Doses of formic acid	12	3.44345	0.28695	3.19	<.001**
Types of clones × Doses of formic acid	18	2.88839	0.16047	1.78	0.029*
Volumes of latex × Types of clones × Doses of formic acid (D)	36	4.33036	0.12029	1.34	0.108
Error residual	216	19.44643	0.09003		
Total	335	369.73810			

%CV(A): 3.4; %CV(B): 0.7; %CV(C): 4.4; %CV(D): 4.8; *: significant (P<0.05); **: highly significant (P<0.001)

Table 12. Separation of the means of formic acid doses

Doses of formic acid	0	1	2	3	4	5	6
Means	7.42^{a}±0.04	7.14^{a}±0.04	6.42^{ab}±0.04	6.17^{bc}±0.04	5.48^{c}±0.04	5.18^{d}±0.04	5.01^{e}±0.04

N.B.: numbers followed with the same letter are not significantly different

Table 13. Separation of the means of latex volumes

Volumes of latex	40	80	120
Means	6.07^{a}±0.02	6.24^{b}±0.02	6.22^{b}±0.02

N.B.: numbers followed with the same letter are not significantly different

Table 14. Separation of the means of types of clones

Types of clones	GT1	PB217	PR107	RRIC100
Means	6.36^{a}±0.08	6.02^{b}±0.08	6.20^{a}±0.08	6.12^{d}±0.08

N.B.: numbers followed with the same letter are not significantly different

Table 15. ANOVA of doses of formic acid and types of clones and their interaction on t he basis of proportion of trees with coagulated latex in harvest cups

SV	**DF**	**SS**	**MS**	**F**	**P<F**
Blocks	2	1.24571	0.62286	7.17	
Types of clones	2	0.21714	0.10857	1.25	0.0297
Doses of formic acid	6	0.73651	0.12275	1.41	0.0234
Types of clones × Doses of formic acid	12	0.68063	0.05672	0.65	0.784
Error	40	3.47429	0.08686		
Total	62	6.35429			

%CV: 67

An increment in the doses of formic acid by 1 ml has led to a fluctuating state of the proportion of trees in clones PB217 and RRIC100; however, it has led to a constant increase of the proportion of trees in clone PR107. The highest dose of formic acid (6 ml) generally provoked the highest proportion of trees with coagulated latex in harvest cup; 47% in clone PB217 and 53% in clone PR107. However, the highest proportion of trees (53%) was caused by the dose of 3 ml of formic acid in clone RRIC 100. An ANOVA was run to find out if the experiment factors (doses of formic acid and types of clones) and their interactions could have a significant effect on the proportion of trees with coagulated latex in harvest cups. Results are summarized (Table 14). The analysis has revealed no significant effect of none of these factors. A possible explanation is that the doses of formic acid tested *in vivo* could have been too small to acidify more important volumes of latex (about 2000 ml) obtained in larger harvest cups in the field. An adjustment of doses of formic acid to use in the field might be necessary for a successful acidification of latex.

Effects of acids on the pH and coagulation time of latex in rubber

Latex in most rubber clones is naturally basic; thus with its pH values above 7 (Woo, 1973; Gomez and Moir, 1979). Adding enough acid to latex, it was demonstrated in this study with formic acid, will lower its pH. Lowering latex pH has as a consequence the reduction of its coagulation time. That has been verified in this study. Previous studies reported by Archer and Cockbain (1969), Hanower and Mathew (1976) and D'Auzac *et al.* (1991) admitted those facts. According to

D'Auzac *et al.* (1991), lowering latex pH between 4 and 6 in most clones will considerably reduce the time of coagulation.

Effects of clones on the pH and coagulation time of latex in rubber

The study has revealed that significant differences do exist between rubber clones concerning pH values and coagulation times of their latex. Some authors think that this is, among other factors, a consequence of different latex physiological compositions (Delabarre and Eschbach, 2002; Koffi *et al.*, 2005). Many years back, Ribailler (1968) already had pointed out that the structural stability of latex in rubber was related to the types of clones. According to him, clones with a few lutoïds in their latex might necessitate more time to coagulate. Satchuthananthavale and Satchuthananthavale (1971) came to the conclusion after studying latex in several clones that latex coagulation time in certain clones could be shortened simply by adding sugar in latex.

Effects of latex dilution in water on its time of coagulation

Proportions of $1/3^{rd}$, $2/3^{rd}$ and $3/3^{rd}$ of latex were diluted in rain water in the trial phase *in vitro* of this study. Results have shown that rain water did not have any significant effect on reducing the pH and coagulation time of latex. That finding is seemingly contrary to what was said by some authors in the past. According to them, it all depends in the quality of water, and not in its quantity. As a matter of fact, many years back, Cook and McMullen (1951) and Bouychou (1962) had demonstrated that when rain water was dripping along the bark, it was accumulating some chemicals like tannins, sugars, cations (most often Ca, K and Mg), etc. Once in contact with latex through rain water, these chemicals would cause premature latex coagulation. However, according to Hanower *et al.* (1976), rain water will increase latex time of coagulation if latex is considerably diluted.

Latex pH and time of coagulation in vitro versus in vivo conditions

From what has preceded, it should be understood that the acid effects in experiments carried out *in vitro* might somewhat differ from those in experiments carried out *in vivo*. Sethuraj and Mathew (1992) and Hanower *et al.* (1976) before them, all had agreed that latex acidification was depending on some parameters (temperature, oxygen, bacteria, enzymes, etc.) which are not the same in both conditions. Satchuthananthavale and Satchuthananthavale (1971) had demonstrated that when latex flew into laticiferous vessels, it was sterile; and if it was collected in sterile conditions, it would remain stable for a longer period of time. But if a tree underwent tapping and latex was collected into harvest cups in the field, that latex would be immediately contaminated with several microorganisms like bacteria and yeasts. Depending on the contaminating bacterial species, latex coagulation could be accelerated or decelerated. Yeang (2005) had pointed out that stimulation in rubber had an impact on latex coagulation in the field and that the longer the duration of latex flow, the latter the start of latex coagulation.

Conclusion

Loss of rubber latex is very important in the field in Cameroon during the heavy rainy season. Rain water washes away latex from notches and mostly from harvest cups. Shortening the coagulation time of latex by adding acid in fresh latex could help reducing its loss. Doses of formic acid, volumes of latex and types of clones were combined to find the right combinations to reach that purpose. In the experiments *in vitro*, adding increasing doses of formic acid to latex has lowered its pH and reduced its coagulation time progressively. The highest acid dose (6 ml) always provoked the lowest latex pH and generally the lowest latex coagulation time. Using pure or diluted latex *in vitro* made no difference. Also, no significant difference was observed when diluting $1/3^{rd}$, $2/3^{rd}$ or $3/3^{rd}$ of latex in rain water. However, significant differences were observed depending on the clone types. In the experiment carried out in the field, the doses of formic acid and the types of clones tested, and their interactions had no significant effects on the proportion of trees with coagulated latex in their harvest cups. A possible explanation is that these doses of formic acid tested *in vivo* could have been too small to acidify more important volumes of latex (about 2000 ml) obtained in larger harvest cups in the field. An adjustment of doses of formic acid to be used in the field (*in vivo*) might be necessary for a successful acidification of latex.

Competing Interest: The authors declare that they have no competing interest.

Authors' contributions: This work was carried out in collaboration between both authors. BMBF managed the literature searches, carried out the daily activities in the field and collected the data, performed part of the statistical analysis and helped in writing the first draft. NF designed the study, wrote the protocol, supervised the data collection, did most of the statistical analysis, realized the presentation of data through the use of figures and tables, wrote the second draft of the manuscript and edited the final document.

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