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Research Application Summary

Effect of ethylene stimulant on the flow rate and biochemical properties of shea latex

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Abstract

The shea tree (*Vitellaria paradoxa*) is utilized extensively in the semi-arid savannah parklands from Senegal in West Africa where the sub species paradoxa is mainly found, to Sudan/Uganda in the East of Africa where the sub species nilotica is found. It is an important multipurpose tree and serves as the principal source of income for the local population in the Sahel region. The shea tree is a member of the *Sapotaceae*, a latex producing tree with beneficial properties. However, the flow rate of the latex is slow and minimal. An ethylene stimulant is used to stimulate the latex flow in the natural rubber tree *Hevea brasiliensis*. In this study, ethylene stimulant was applied to the shea tree in an attempt to increase the flow rate and quality of shea latex was investigated. Results indicated that the ethylene stimulant positively influenced the flow rate of shea latex and the biochemical quality of the shea latex in terms of proximate composition, ionic composition (metallic and non-metallic) and phytochemical constitution. Comparatively, the ethylene stimulated shea latex exhibited both physical and chemical properties which makes it suitable for confectionery and the gum industry. Properties such as good smell, appearance (colour) and chewiness, as well as testing negative for flavonoids and saponins for phytochemical composition, similar to an already exploited gum base (*Manilkara zapota* L.) suggested that it may have a role to play as a gum in the confectionery industry. The relatively high stickiness and adhesiveness of shea latex makes it ideal as a bio-adhesive for industrial purposes.

Key words: *Hevea brasiliensis*, rubber tree, *Sapotaceae*, *Vitellaria paradoxa*

Résumé

Le karité (*Vitellaria paradoxa*) est largement utilisé dans les parcs de savane semi-arides, du Sénégal en Afrique de l'Ouest, où se trouve principalement la sous-espèce paradoxa, et du Soudan/Ouganda en Afrique de l'Est, où se trouve la sous-espèce nilotica. Il s'agit d'un arbre important à usages multiples qui constitue la principale source de revenus pour la population locale dans la région du Sahel. Le karité est un membre de la famille des Sapotaceae, un arbre producteur de latex aux propriétés bénéfiques. Cependant, le débit du latex est lent et minime. Un stimulant de l'éthylène est utilisé pour inciter l'écoulement du latex dans l'arbre à caoutchouc naturel *Hevea brasiliensis*. Dans cette étude, le stimulant de l'éthylène a été appliqué au karité pour tenter d'augmenter le débit d'écoulement, et la qualité du latex du karité a été étudiée. Les résultats ont indiqué que le stimulant éthylénique a influencé positivement le débit d'écoulement du latex de karité et sa qualité biochimique en termes de composition générale, de composition ionique (métallique et non métallique) et de constitution phyto-chimique. Relativement, le latex de karité stimulé par l'éthylène a présenté des propriétés physiques et chimiques qui le rendent approprié pour la confiserie et l'industrie de la gomme. Des propriétés telles que la bonne odeur, l'apparence (couleur) et la mastication, ainsi que des tests négatifs aux flavonoïdes et saponines pour la composition phyto-chimique, similaires à une base de gomme déjà exploitée (*Manilkara zapota* L.) ont suggéré qu'il pourrait avoir un rôle à jouer comme gomme dans l'industrie de la confiserie. Le pouvoir collant et adhésif relativement élevé du latex de karité le rend idéal comme bio-adhésif à des fins industrielles.

Mots clés : *Hevea brasiliensis*, arbre à caoutchouc, Sapotaceae, Vitellaria paradoxa.

Introduction

The shea tree (*Vitellaria paradoxa*), is utilized extensively in the semi-arid savannah parklands from Senegal in West Africa, where the sub species paradoxa is mainly found, to Sudan/Uganda in the East of Africa where the sub species nilotica is found. Shea covers the entire northern Ghana, more than 78,000 km^2 (Maranz *et al*., 2004) with a potential annual yield of about 130,000 metric tons of shea products (Dogbevi, 2009). Hence, shea is an important multipurpose tree and serves as the principal source of income for the local population in the Sahel region. Shea products are good for rural poverty reduction in Northern Ghana since they are sources of livelihood for women in the rural areas. Shea butter fat is the current primary and most important product from the shea tree but utilization of additional hidden potentials of the shea tree is yet to be tapped (Quainoo *et al*., 2012).

The shea tree is a member of the Sapotaceae, a latex producing tree with potential beneficial uses (Fosu and Quainoo, 2013). The shea tree has a great untapped capacity for producing large amounts of latex that can constitute an important source of raw material for the gum and rubber industry which can create employment opportunities to millions of Ghanaians. Latex is an emulsion that contains water, proteins, and phytochemicals such as resins, tannins, and rubber in varying quantities (Cotter *et al*., 2009) that exudes from specialized canals when they are cut. Ethylene is used as a stimulant for latex production in natural rubber (*Hevea brasiliensis*) and this study seeks to determine the effect of ethylene on flow rate of shea latex and the biochemical properties of the latex. This may present the opportunity for the shea tree to receive the needed research attention for the total exploitation of the tree and open investment opportunities in the catchment area of the shea tree leading to poverty alleviation and improvement of the economies of the shea tree producing areas.

Materials and methods

Study Area and sampling of experimental sites. Experimental site was at Cheyohi, in the Kumbungu District in the Northern Guinea Savannah Agro-ecological zone of Ghana. It is located on latitude 90 25’ 45’’ and longitude 00 58’ 42’’ W. Rainfall is unimodal with annual average rainfall ranging between 900 mm and 1000 mm. The highest temperatures are normally recorded in March and can rise as high as 45 ^{0}C during the day (SARI, 2001). The first phase involved sampling to identify shea parkland area in the experimental site. Within the location, 50 adult trees were sampled for the experiment. Trees sampled were 22 m to 30 m apart to avoid selection of closely related individuals. Adult trees with diameters greater than 0.20 m, 1 m from the base of the tree were selected to ensure that stems were big enough for tapping. The shea trees were divided into two (25 trees each) with the first 25 trees treated with ethylene stimulant - Hevetex (SHS) and the second 25 trees without ethylene stimulant (SH) as control. Groves were made on the trees for tapping of latex early in the morning from August to December 2018.

Quantification of latex flow and proximate analysis. The flow rate of latex was measured with a graduated beaker and a stop clock and expressed as the capacity of fluid stored in a given time by the formula: $Q=C/t$, Where; Q is flow rate; C, is the capacity of fluid stored; t, is the time taken to flow.

Proximate analysis of latex was carried out for % moisture, % ash content, % crude fat, % fibre content, crude protein and % carbohydrate [Carbohydrates = 100 – (% moisture + % crude protein + % crude fat + % crude fiber + % ash)] (AOAC, 2000). Also, **t**he AAS method was used to determine inorganic metallic ions content for Pb, Zn, Mn, and Cu, while the inorganic non- metallic ion constituents of the latex samples were determined following the standard method of AOAC (2000). The latex samples were analyzed for nitrate, sulphate, phosphate, nitrate, chloride, and NH_3N. Further, Phytochemical Screening was done for alkaloids, flavonoids test, glycosides test, triterpenoids (Salkowski test), phytosterols test, saponins, reducing sugars, anthroquinones and soluble Starch.

Results and discussion

Physical properties of latex. Table 1 shows the properties observed for the different experimental shea latex samples observed and studied over the experimental period. The differences observed may have been due to the ambient weather conditions peculiar to the different tapping periods. The varying flow duration may be attributed to the different abilities of water stores expelled by the trees which are affected by the ambient climatic conditions including rainfall and drought, together with the age of tree, depth of cut and mode of stimulant application. Gunasekera *et al*. (2013) reported the major factors affecting the yield of latex at tapping may be attributed to the age of the tree, external factors such as stimulation, the depth and length of tapping cuts. Quainoo and Dugbatey (2016) also reported that latex flow decreases with increasing age of the tree. Tapping signifies a serious abiotic wounding stress for exploited trees - under regular tapping which necessitates latex cells to fully regenerate their cytoplasm after latex expulsion. Environmental and harvesting stress, as well as the metabolic activity necessary for latex regeneration between two tappings, will further lead to the production of reactive oxygen species (ROS). Excessive production and accumulation of ROS may lead to laticifer dysfunctions such as Tapping Panel Dryness (TPD). The browning colour of SH upon storage may be associated with enzymatic reactions of polyphenol oxidase (PPO) that is involved in the defense against pathogens, which is also responsible for latex browning.From this study, SHS did not show the brown colouration which may suggest that the presence of PPO may have been reduced as a result of the ethylene stimulation. This is in line with Li *et al*. (2014) who reported that PPO decreased in ethephon-treated latex serum down-regulated by ethylene and a decline in PPO accumulation in ethephon-treated sample may have hindered rubber particle aggregation which subsequently prolonged the latex flow.

Table 1. Physical properties exhibited by shea latex (non-stimulated) - SH and shea latex stimulated – SHS.

Sn.	Property	SH	SHS
1	Smell	No smell	No smell
2	Colour	White (changes to pale pinkish-red upon contact with inner back during tapping). Browning upon storage	White (changes to pale pinkish-red upon contact with inner back during tapping)
3	Flow duration	Relatively shorter period	Relatively shorter period
4	Elasticity	Relatively low	Relatively low
5	Stickiness	Relatively high	Relatively high
6	Coagulation	Relatively shorter period	Relatively shorter period
7	Chewiness	Very Good	Good
8	Thickness	Moderate	High
9	Viscosity	Moderate	High
10	Adhesiveness	High	High
11	Cohesiveness	Moderate	Moderate

Flow rate of latex (L/min). Significant differences as shown in Table 2 confirms the positive effects of ethylene stimulator on the flow rate of latex. According to Coupé and Chrestin (1989), treatment with ethylene increased the volume of exported latex by acting on membrane permeability leading to extended latex flow and kindles latex regeneration between tapings through general reformative metabolism. Quantitatively this method is said to increase latex production by 1.5- to 2-fold which was evident between SH and SHS. This confirms the ability of hevetex to migrate to tissues where it progressively enhances ethylene decomposition and acts on plant metabolism. This may occur with the increase in water uptake as a result of sucrose loading in laticifers increased latex flow. Thus, ethylene stimulator may increase membrane permeability and improved latex regeneration metabolism.

Table 2. Mean flow rate for shea latex (non-stimulated) - SH and shea latex stimulated – SHS.

Latex type	Mean flow rate (L/min)
SH	0.001433 ± 1.74E-05
SHS	0.002867 ± 4.61E-05
F pr.	<.001
Lsd (0.05)	0.000460

SH= ethylene stimulant; SHS= ethylene stimulant - Hevetex

Proximate analysis. Results presented in Table 3 show that except for crude protein, mean values recorded for proximate composition for the three latex samples were significantly different from each. Percentage moisture for SH was approximately double that of HV, whiles SHS was in-between. Percentage ash content recorded for SH was twice as that recorded for SHS, while HV recorded an intermediate value. The trend was the same for percentage fat content and percentage fibre content with either HV or SHS recording values in-between. For percentage carbohydrate, HV recorded the highest value which is approximately double for that of SHS and eight times that of SH. However, for crude protein, there was no significant difference between SHS and HV, where the value recorded for SHS is twice that of SH.

Table 3 further show that ethylene stimulation increased crude protein in latex and this might be attributed to increased nitrogen production and nitrogen precursor for protein metabolism. Pujade-Renaud *et al*. (1994) affirms that glutamine synthetase involved in nitrogen metabolism has been found in the cytosol of laticiferous cells after ethylene treatment paralleling the increase of latex yield. Percentage carbohydrate expressed a significant increase in SHS over SH and may be due to the increase in sucrose concentration which enhances the flow of latex after stimulation and may subsequently end up in the latex expelled from the trees. Ethylene stimulation during latex production results in high sugar flow from the surrounding cells of inner bark towards the latex cells. Application of ethylene on rubber tree has been reported to connect several metabolic responses and biochemical processes including nitrogen assimilation, carbohydrate transport and metabolism, sucrose and glucose loading and protein synthesis (Wang *et al*., 2015). Percentage fibre content was higher in SH and this may be attributed to the morphological and the physiological nature of the tree, which could have been due to climatic demands of the area. This result may favour the use of SH in producing dietary fibre supplements as it may contain complex polysaccharides such as lignin, hemicellulose and cellulose (Rao, 2003).

Moreno *et al.* (2005) reported that there is a tendency of increasing ash % triggered by low precipitation resulting in smaller availability of water in the soil, which in this study may have led to minor dilution of the latex. This is so because SHS which was sampled during the period of declining rainfall recorded the least value (Table 3). This may probably be due to the interaction of several factors including mineral ions which were either present or absent in latex.

Table 3. Proximate composition of shea latex samples

Latex type	% Moisture	% Ash	% Fat	% Fibre	% Crude protein	% Carbohy-drate
SH	66.240±0.587	4.187±0.081	15.920±0.469	6.420±0.310	2.220±0.022	5.020±0.558
SHS	48.820±0.076	1.650±0.071	14.420±0.203	4.750±0.088	4.950±0.568	25.400±0.401
F pr.	<.001	<.001	<.001	<.001	<.001	<.001
LSD (0.05)	2.337	0.219	1.056	0.652	1.144	2.424

Table 4. Mineral (metallic) ion composition of shea latex

Latex type	Pb (mg/L)	Zn (mg/L)	Mn(mg/L)	Cu (mg/L)
SH	0.028 ± 0.001	0.056 ± 0.001	0.146 ± 0.001	0.019 ± 0.000
SHS	0.016 ± 0.000	0.094 ± 0.000	0.132 ± 0.001	0.042 ± 0.001
F pr.	<.001	<.001	<.001	<.001
LSD (0.05)	0.003	0.003	0.004	0.002

Table 5. Mineral (non-metallic) ion composition of shea latex

Latex type	Nitrate (mg/L)	Sulphate (mg/L)	Prosphate (mg/L)	Nitrite (mg/L)	Chloride (mg/L)	NH_3-N (mg/L)
SH	3.567 ± 0.027	3.033 ± 0.027	0.031 ± 0.000	0.008 ± 0.000	8.617 ± 0.007	0.501 ± 0.000
SHS	4.400 ± 0.047	4.100 ± 0.047	0.028 ± 0.000	0.004 ± 0.000	6.447 ± 0.003	2.610 ± 0.005
Lsd (0.05)	<.001	<.001	<.001	<.001	<.001	<.001
F pr.	0.149	0.133	0.007	0.001	0.019	0.012

Table 6. Phytochemical composition of shea latex samples

Latex type	Alkaloids	Anthro quinones	Flavoids	Glyo sides	Phyto sterols	Reducing sugars	Saponin	Soluble starch	Triter penoids
SH	+	+	-	+	+	+	-	+	+
SHS	+	+	-	+	+	+	-	+	++

Tables 4 and 5 show the mineral composition of the latex samples categorized as metallic and non-metallic, respectively. The small amounts of ions recorded in the samples for the metallic and non-metallic ions confirm that Cu, Zn, Mn and Fe were present in traces but in amounts significantly different in the treated samples. This may be due to the differences in the trace mineral soil conditions in the two experimental sites and also abiotic factors such as rainfall and temperature. Table 6 shows that SH and SHS tested negative for flavonoids and saponins but positive for all phytochemicals tested which is in line with the findings by Mahajan and Badgujar (2008) that *Manilkara zapota* (L.) which belongs to the family Sapotaceae tested negative for flavonoids and saponins.

Conclusion

Application of an ethylene generator (Hevetex) positively influenced the flow rate of shea latex. The SHS exhibited both physical and chemical properties which makes it suitable for confectionery purposes. Properties such as good smell, appearance, chewiness, testing negative for flavonoids and saponins present a potential for shea latex in the gum industry. The relatively high stickiness and high adhesiveness of shea latex make it better option for bio-adhesive over natural rubber latex.

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