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Evaluation of lead and arsenic content of *Azardirachta indica* seed oil and *Citrus sinensis* peel oil creams as mosquito repellent

Godfred Yaw Boanyah*[1], Ruth Brenyah[1], Precious Bondzie-Quaye[2]

[1]*Department of Clinical Microbiology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China*

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Abstract

There is an increase preference for plant-based repellents due to their effectiveness, environmentally friendliness and biodegradable nature. It is therefore necessary to ascertain the safety of these repellents by analysing their heavy metal content. This study has shown that lead and arsenic content of *Azadirachta indica* seed oil cream and *Citrus sinensis* peel oil cream as mosquito repellent is insignificant and therefore very safe for use according to the Ghana Standard Authority specification. These results provide new insight into the safety of these natural mosquito repellents.

***Corresponding Author:** Godfred Yaw Boanyah ✉ goboanyah@gmail.com

Introduction

The use of repellent is an effective and reliable method used in breaking Human-vector transmission cycle of pathogens (Hazarika *et al.*, 2012). Additionally, the application of repellent on clothing and bed nets have shown excellent results in Africa, thus, conferring protection to the individual using them (World Health Organization., 2017; Njumkeng *et al.*, 2019). Moreover, topical repellent is one of the most dependable ways of controlling outdoor biting of mosquitoes especially in rural and farming communities (Wilson *et al.*, 2014).

However, the heavy metal constituent of naturally manufactured repellent creams could be toxic and in turn defeat their overall safety purpose. Exposure of the skin to arsenic can cause a variety of benign skin lesions including hyperpigmentation and hyperkeratosis according to the findings of Bernstam *et al.* (2002). It was indicated in a recent research that developing countries have high environmental pollution of Lead which may be absorbed by plants from which these natural repellents are made from. Organic Lead may be absorbed directly through the skin and its effects are devastating. High levels of lead in repellents when inhaled can result in decreased performance in some tests of cognitive performance that measure functions of the nervous system (Wani, Ara and Usmani, 2015).

In the last two decades, an intensive effort has been made by several researchers to investigate the safety of diethyltoluamide (DEET), one of the most widely used and reliable insect repellents available(Goodyer and Behrens, 1998). However, many users of repellent are still concerned about the safety use of DEET (Shukla, Wijayapala and Vankar, 2018). Furthermore, the easily biodegradative nature of plant-based repellents and friendliness to the environment has added to their preference by customers in recent years (Ketkar and Ketkar, 2005; Tripathi *et al.*, 2016) but the toxicity of some heavy metals in these natural topical repellents have not been given much attention.

This study seeks to evaluate the Arsenic and lead content of *Azadirachta indica* seed oil Cream and *Citrus sinensis* peel oil cream as mosquito repellent and as as well compare them to the standard maximum allowable concentration requirement of these metals in topical repellents.

Materials and methods

Study Design

This experimental study was conducted from January to February, 2020 at the Soil Science Laboratory of KNUST. The *Azadirachta indica* seed oil and *Citrus sinensis* peel were purchased from Agape Moringa Processing in Tamale, Ghana.

Sample materials and extraction of oils

The neem (*Azadirachta indica*) seed oil and sweet orange (*Citrus sinensis*) peel oil were purchased from Agape Moringa Processing in Tamale Northern Region of Ghana. The seed and the peels were collected from Tamale as well. Coloured rinds of Citrus sinensis of two oranges were put into Clevenger apparatus and distilled water of 100 cm^3 is added. The flask containing the peels is heated just below 100°C and distillation took place steadily. Distillate of 50 cm^3 is collected in a measuring cylinder and the oil layer is removed with pipette completing the steam distillation method (Limonene, Bunsen and Receiver, 2009).This was done till 200ml of oil was obtained.

Azadirachta indica from Tamale were dried and milled into powder. Oil was extracted from the powder (458.65 g) using Petroleum ether (2.2 L) with the Soxhlet apparatus for 4 days. The extract was concentrated using the rotary evaporator at temperature 45°C and a percentage yield of 43.6% v/w obtained. The oil was then stored in an amber bottle at a cool dry place until ready for use.

Yield of extract obtained

$$= \frac{Volume\ of\ neem\ seed\ oil\ x\ 100\%}{Weight\ of\ plant\ material\ taken}$$

Formulation of Repellent Creams

Three creams were formulated from the two oils (Neem oil, orange oil and Combined Neem and Orange Oil) at 30% concentrations respectively. A volume, 6mls of oil was added to 20g of Aqueous cream which was stirred and later homogenized to form the repellent creams. For the combined cream, 3mls of Neem seed Oil and 3mls of Orange peel oil were used.

Heavy Metal Analysis on the Creams

Heavy metal analysis was conducted on all the three natural creams at the Soil Science laboratory of Faculty of Agriculture, KNUST using the Buck Scientific Atomic Absorption Spectrophotometer. Prior to the analysis 1g of each sample was digested using hydrochloric and nitric acid in the ration 3:1 respectively. For Heavy metals, according to the Ghana Standard's Authority guidelines (catalogue code: GS 10902017) only Lead and Arsenic levels in repellents are tested for.

Results

Table 1. Results of heavy metal content of creams.

Sample Name	As(mg/kg)	Pb(mg/kg)
Orange peel oil cream I	0.00629	0.0039
Orange peel oil cream II	0.00809	0.0041
Orange peel oil cream III	0.00869	0.0040
Neem seed oil cream I	0.00225	0.0036
Neem seed oil cream II	0.00210	0.0036
Neem seed oil cream III	0.00270	0.0035
Neem seed oil and orange peel oil cream I	0.00150	0.0029
Neem seed oil and orange peel oil cream II	0.00132	0.0030
Neem seed oil and orange peel oil cream III	0.00165	0.0030

The values shown on Table 1 were obtained for the heavy metal Lead and arsenic content of on all the three natural creams namely; neem seed oil Cream, Orange peel oil cream and the neem seed oil and orange peel oil cream in three replicates each.

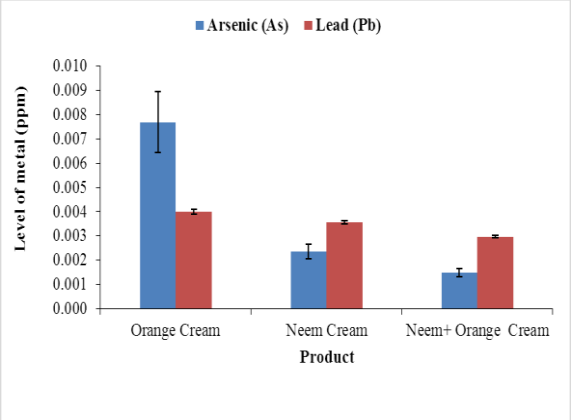

Fig. 1. Comparison of Arsenic and lead content of Neem seed oil Cream, Orange peel oil Cream and the combination of neem seed oil and orange peel oil cream.

Discussion

The level of Arsenic (As) recorded was highest in orange cream (0.008 ± 0.0012mg/kg), followed by neem cream (0.002 ± 0.0003mg/kg) with neem and orange cream recording the least (0.001 ± 0.0002mg/kg). There was a statistically significant difference between the level of Arsenic (As) in Orange cream vs. Neem cream ($p < 0.001$), and Orange cream vs. Neem and orange cream ($p < 0.001$).

With the level of Lead (Pb), both orange cream and neem cream had a concentration of 0.004 ± 0.0001mg/kg while neem and orange cream recorded 0.003 ± 0.0001mg/kg as shown in Fig 1. Generally, there was a statistically significant ($p < 0.001$) difference between the levels of Lead (Pb) recorded in the three creams (Table 2).

Statistical analysis for heavy metals

Table 2. One-way ANOVA between Arsenic (As) and Lead (Pb) concentrations in the three creams.

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Arsenic	Between Groups	0.000	2	0.000	60.270	0.000
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
Lead	Between Groups	0.000	2	0.000	145.400	0.000
	Within Groups	0.000	6	0.000		
	Total	0.000	8			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Product	(J) Product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Arsenic	Orange	Neem	0.00534000*	0.00061188	0.000	0.0034626	0.0072174
		Neem + Orange	0.00620000*	0.00061188	0.000	0.0043226	0.0080774
	Neem	Orange	-0.00534000*	0.00061188	0.000	-0.0072174	-0.0034626
		Neem + Orange	0.00086000	0.00061188	0.396	-0.0010174	0.0027374
	Neem + Orange	Orange	-0.00620000*	0.00061188	0.000	-0.0080774	-0.0043226
		Neem	-0.00086000	0.00061188	0.396	-0.0027374	0.0010174
Lead	Orange	Neem	0.00043333*	0.00006086	0.001	0.0002466	0.0006201
		Neem + Orange	0.00103333*	0.00006086	0.000	0.0008466	0.0012201
	Neem	Orange	-0.00043333*	0.00006086	0.001	-0.0006201	-0.0002466
		Neem + Orange	0.00060000*	0.00006086	0.000	0.0004133	0.0007867
	Neem + Orange	Orange	-0.00103333*	0.00006086	0.000	-0.0012201	-0.0008466
		Neem	-0.00060000*	0.00006086	0.000	-0.0007867	-0.0004133

*. The mean difference is significant at the 0.05 level.

It was observed that Arsenic (As) and Lead (Pb) concentrations in the combined Neem and orange cream was the least for both metals. This is as a result of half of the initial volume of 6mls used that is 3mls each of Orange and Neem oils in the cream formulation.

Both the levels of Arsenic (As) and Lead (Pb) recorded in all the creams were below the Ghana Standard Authority (GSA) guideline value of 5.0mg/kg and 1.0mg/kg respectively. The result from the Heavy metal analysis shows that all the creams in comparison to the standard maximum concentration requirement was less than 0.2% and 0.5% for both Arsenic and Lead respectively. This proves that neem and orange peel oil creams are very safe to be applied on the human skin (Fig 1). There were no adverse effects observed during and after the experiment.

Conclusion

It can be concluded that the heavy metals, lead and arsenic content of *Azadirachta indica* seed oil cream and *Citrus sinensis* peel oil cream as mosquito repellent is significantly lower than the maximum allowable requirement and hence safe topical repellent.

Reference

Bernstam L. 2002. 'Effects of arsenic on human keratinocytes: Morphological, physiological, and precursor incorporation studies', *Environmental Research*. Academic Press **89(3),** pp. 220-235.

Goodyer L, Behrens RH. 1998. The safety and toxicity of insect repellents. The American journal of tropical medicine and hygiene **59(2),** pp.323-324.

Hazarika S. 2012. 'Repellent Activity of Some Essential Oils Against Simulium Species in India', Journal of Insect Science. Oxford University Press (OUP) **12(5),** pp. 1-9.

Ketkar CM, Ketkar MS. 2005. 'Soap production from mixtures of neem oil with other non-edible or edible oils', in The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA pp. 549-554.

Limonene C, Bunsen G, Receiver C. 2009. 'Extracting limonene from oranges by steam distillation', Advancing the Chemical Science, (Vii) pp. 41-44.

Njumkeng C. 2019. 'Coverage and usage of insecticide treated nets (ITNs) within households: Associated factors and effect on the prevalance of malaria parasitemia in the Mount Cameroon area', BMC Public Health **19(1),** p. 1216.

Shukla DK, Wijayapala S,Vankar PS. 2018. 'Effective mosquito repellent from plant based formulation', Population dynamics of Aedes mosquito larvae from peridomestic water bodies **5(1),** pp. 19-24.

Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR. 2009. A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and phytotherapy **1(5),** pp.52-63.

Wani AL, Ara A, Usmani JA. 2015. 'Lead toxicity: A review', Interdisciplinary Toxicology **8(2),** pp. 55-64.

Wilson AL. 2014. 'Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis', Malaria Journal **13(1),** p. 446.

World Health Organization. 2017. Report of the twentieth WHOPES working group meeting. Geneva. Available at: http://www.who.int/whopes/en