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Bacterial Nanocellulose: An Alternative To Increase The Mosquito Repellent's Duration. Natural Repellent Bifasic Spray. Community Experience in A Rural Area with A High Prevalence

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ABSTRACT

Bacterial nanocellulose (BNC) is a primary metabolite synthesized in the bacterial cell of Pseudomonas fluorescens. BNC possesses distinctive characteristics that modify its biological and physicochemical properties. Its ability to adhere to surfaces and skin without generating undesirable reactions makes it a unique model for the design of topical pharmaceutical formulations. Formulation studies developed in our laboratory demonstrate that, with the addition of measured proportions of BNC, it is possible to obtain liquid formulations that act as vectors for volatile substances. Due to its physical characteristics, it reduces the volatilization of substances, generating a thin film on the applied surface. In this work, we present the reformulation of a previously published natural mosquito repellent gel and its replacement with a biphasic spray emulsion, its implementation in the manufacture of a natural mosquito repellent, and a collaborative experience with a rural school and its community in Amberes, Monteros, Tucumán, Argentina. Epidemiological studies conducted in the community revealed alarming data on the prevalence of the dengue virus, which is endemic to the area due to its geographic location. For the selection of natural pharmaceutical active ingredients, aromatic plants from the area with high concentrations of citronellol and/or limonene were analyzed. Cymbopogon citratus was selected due to its abundance in the Antwerp area. Safety and persistence studies of the repellent action were conducted on 150 healthy volunteers from the Amberes Comune.

The main objective was to design a natural mosquito repellent bifasic spray, suitable for all ages, sustainable, and biodegradable, whose ease of application and persistence would represent a technological improvement over the previously designed gel repellent. To ensure the success of the technological design, the quality controls established by the Argentine Pharmacopoeia were carried out. In addition, the safety results, organoleptic characteristics, consumer acceptance, and persistence of the repellent action were evaluated.

Since there is no foreseeable risk from the use of BNC or any other ingredient in the designed repellent, it is possible and representative to assess the safety of its ingredients simply by evaluating the appearance of edema and/or erythema (screening for potential allergies). This study complies with the ethical and scientific standards for the design, conduct, recording, and reporting of studies involving human subjects, as stipulated by the Ministry of Health of Argentina (Resolution No. 1490/07). The redesigned formulation introduces sophisticated technological features, significantly increasing the repellent's duration of action, ease of application, and extending the age range for safe use. The selected plant species met the desired characteristics: a high concentration of limonene terpenes and abundance in the study area. The pH values obtained are adequate and contribute to maintaining skin health. Microbiological stability studies showed no growth of bacteria, fungi, or yeast. This indicates that preservatives suitable for natural products and without endocrine-disrupting effects are capable of preserving the product for at least six months. Studies in healthy volunteers showed an absence of edema and erythema within 24 hours of application. No skin dryness was observed among the 150 volunteers surveyed. The product's persistence on the skin and its repellent action lasted at least 8 hours in more than 80% of cases. The organoleptic properties were satisfactory. The product was described as smooth, with a pleasant aroma, rapid absorption, and a persistent cooling sensation.

The product is suitable for use as a mosquito repellent; its design is optimal for the area where the study was conducted, due to its climatic characteristics and living conditions. It is desirable to continue the studies by expanding the number of healthy volunteers and the area of influence of the Aedes aegypti mosquito in the province of Tucumán.

Keywords: Bacterial Nano Cellulose; Mosquito repelent; Topical formulation; Natural products

INTRODUCTION

Bacterial nano cellulose (BNC) is obtained through green methods, meaning they eliminate the environmental and ecosystemic damage generated by cellulose obtained from plant material. Even when dealing with plant waste, as in our province of Tucumán, Argentina, the damage continues during the process, since the traditional purification method, which persists in our country, uses a large volume of polluting substances with strong acids, generating a large amount of waste. [1] This waste is dumped into rivers and contaminates not only our waters but also those of neighboring provinces. It generates a large amount of environmental pollution.[2] Plant-based nanocellulose adds even more pollutants to the already complex and outdated process mentioned. It is obtained through enzymatic, mechanical, and chemical treatments of cellulosic waste from other processes. The most widely used precursors worldwide are cotton, wood, and annual plants. Other agricultural waste is also used; in our province, soybean hulls and sugarcane bagasse are common. BNC, on the other hand, involves a bottom-up process, quite the opposite of the traditional method.[3] It is biosynthesized using specific bacterial strains such as *Pseudomonas fluorescens*, *Gluconacetobacter xylinus*, and *Gluconacetobacter hansenii*, which produce cellulose as one of their metabolites.[4] BNC is a primary metabolite synthesized within the bacterial cell, which is deposited at the air-culture medium interface as a kind of bacterial defense mechanism.[5,6] It then intertwines into nanofibrils and is mechanically amplified to form microfibrils.[7,8] During the biosynthesis of cellulose chains, van der Waals forces and hydrogen bonds between the hydroxyl groups and oxygen of adjacent

molecules promote the parallel stacking of multiple cellulose chains, forming elementary fibrils. These fibrils then aggregate to form larger microfibrils.[9] BNC exhibits distinctive characteristics compared to nanofibers obtained from plant fibers. BNCs are lighter and have greater optical transparency. Their versatility lies in their ability to bind multiple functional groups to their chemically adaptable surface. This positively impacts the improvement of their mechanical properties. Their importance lies primarily in their size and fibrillar arrangement, which introduces modifications to their biological and physicochemical properties, such as biocompatibility and biodegradability. BNC fibers are optically transparent and lightweight, and their surface is chemically adaptable, allowing the bonding of multiple functional groups and improving their mechanical properties.[10]

DEET's mechanism of action involves interfering with mosquitoes' olfactory receptors, confusing their antennae and mouthparts, making it difficult for them to detect or orient themselves toward the odor of carbon dioxide and lactic acid emitted by humans. Instead of killing mosquitoes, this chemical compound acts as a repellent, deterring them from landing on and biting the skin. BNC is used in our laboratory as a prototype inactive pharmaceutical ingredient, but in this case, due to its characteristics, it will act as an active ingredient, as we will see in the results and discussion. As an Active Pharmaceutical Ingredient (API), it is capable of sustaining the repellent's mechanism of action and maintaining its effectiveness for a longer period. According to statistics published at kidshealth.org.[11] Commercial repellents contain between 10 and 30% DEET (N,N-Diethyl-meta-toluamide); the higher the concentration of DEET, the longer the duration of protection. In an interview conducted in our province with 100 parents of children between 3 months and 10 years old, it was found that during periods of high dengue prevalence, the recommended application intervals of 8 and 12 hours are not met. Parents are applying approximately six times more insect repellent to their children's clothing and skin to prevent dengue virus infection. This behavior is especially prevalent among children who have already had the viral disease. The possibility of encountering their infected children again compels them to act beyond recommended guidelines, significantly exceeding the therapeutic dose of a substance that is already naturally toxic. Within the framework of COP30,[12] natural products and environmental awareness are being discussed as we write this report. Highly toxic excipients and active ingredients used for decades in pharmaceutical formulations are now obsolete. The elimination of petroleum derivatives and endocrine disruptors from formulas is being prioritized. The new consumer seeks organic products, defined as those whose cultivation is free of agrochemicals, whose extraction is carried out with organic water or vegetable oils, and whose synthetic excipients do not exceed 5%.[13-15] New personal care products, such as mosquito repellents, will meet the criteria of natural personal care products: biodegradability and environmental sustainability, reconsidering formulas to replace our methods with natural processes. This is a comprehensive vision of formulation with new interpretations of the meaning of well-being. Prior to this work, our researchers designed a natural mosquito repellent focused on a rural area with a high prevalence of the insect.[16] The product was tested for 2 years on more than 1000 healthy adult volunteers. The product's duration of action was shown to range between 3 and 5 hours. Given the need to increase the retention time of the action to approach that of repellents containing DEET, cleaner production studies developed in our laboratory demonstrate that it is possible to increase the retention time of essential oils and plant extracts by adding small concentrations of BNC. This work presents our experience in redesigning the base formula for semi-solid gels and replacing it with a liquid emulsion, which allows application to skin and clothing. The design and production of the first batches were carried out in a joint

project with a school and a rural community in our province. Amberes is a rural town located 70 km from the capital of the province of Tucumán, Argentina (geographic coordinates: -27.267082, -65.488325).

Sixth-year students from the Amberes High School in Monteros, Tucumán, Argentina, contacted researchers from CONICET's LEfyBiFa (Laboratory of Biotechnology and Biology) after an epidemiological study conducted by professionals from the local Primary Health Care Center (CAPS). The study revealed alarming data, particularly regarding the prevalence of the dengue virus, which is endemic to the region due to its proximity to the Yungas forests of Tucumán and its high humidity levels. As part of a project-based learning project and studies conducted over the past two years in our laboratory using BNC, researchers from the National Scientific and Technical Research Council (CONICET) and the National University of Tucumán (UNT) collaborated with the rural educational community. The project utilized local flora treated with ultrasonics as the active ingredient, BNC as the aroma preservative, and water as the carrier. Aromatic plants from the area with high citronellol and/or limonene content were studied to select the natural active pharmaceutical ingredient. *Cymbopogon citratus*, also known as lemongrass, was selected for these trials due to its abundance in the area. The premise of this work was to provide the community with a 100% natural repellent to replace commercial repellents. DEET is especially dangerous for young children, who can suffer seizures if exposed to the chemical on their skin for prolonged periods.[17] Safety assessments and studies on the persistence of the repellent effect were conducted on 150 healthy volunteers from the municipality of Antwerp.
The main objective of this work was to design a natural, sprayable mosquito repellent emulsion. The desired characteristics were ease of application to skin and clothing, suitability for all ages, long-lasting action, sustainability, and biodegradability. To ensure the success of the technological design, quality controls established by the Argentine Pharmacopoeia were implemented, and training was provided to students, parents, and teachers at the rural school. In addition, safety results, organoleptic characteristics, consumer acceptance, and the persistence of the repellent effect were evaluated in 150 healthy volunteers from the community.

MATERIALS AND METHODS

Bacterial strains and culture conditions

A *Pseudomonas fluorescens* WS strain (wrinkly spreader) (donated by PhD Andrew Spears, School of Science, Engineering and Technology, Abertay University) It was grown in LB broth (Britania- C.A.B.A-Argentina) and kept at 25ºC.

BNC production process

King B Media (Britania- C.A.B.A-Argentina). For BNC production, static cultivation was used. All growing test were made on petri dishes with 50 mL of fresh medium 72h at 28±1°C. All cellulose pellicles obtained were washing with distillate water to remove medium. BNC was centrifuged 20 min at 8000rpm. Then 1mL of NaOH 2% v/v was added and autoclaved 15min at 121ºC, these procedure is made for detach some bacterial cell which could by immerse on BNC. Films were washed with distilled water until neutralization.[17, 18]

BNC dry weight

Dry weight was measured in dry films. The results were reported as ‘production’ and expressed in weight of dry cellulose per liter of King B medium (g/L).[17, 18]

Emulsion material

Pharmaceutical-grade distilled water, sweet almond oil, glycerin cosgard type preservative, *Cynbopogon citratus* essential oil, (Eiffel Química-Buenos Aires Argentina) *C. citratus* sonicated aqueous extract common name in the area lemon grass.

***Cynbopogon citratus* extract preparation**

The plant material was collected together with the students of the rural school in an organic cultivation field established in the Amberes comune (geographical coordinates:-27.256531,-65.510982). The aqueous extract was prepared with the clean and dry plant material, and macerated in 50º alcohol according to the provisions of the Argentine Pharmacopoeia, seventh edition. Then the extract was filtered using low porosity cotton canvas and was reserved until use.

Formulation design[17 – 22]

Formulation studies were carried out in order to evaluate pharmacotechnical aspects such as: pH, homogeneity, organoleptic properties, microbiological stability was developed as required on Administracion Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT) disposition Nº7667.

Homogeneity assays

Repelent bifasic emulsion designed (3g) was used for centrifugation assays (15 min, 3500 rpm) and separation in phases was controled.[17–22] The repelent bifasic emulsion homogeneity was qualitatively qualified as follows: very good (no phase separation), good (appearance of small volume of supernatant), regular (phase separation withslight appearance of clotted) and poor (separation of the phases with appearance of pellet).

pH measurements

The pH was measured in a pHmeter (Broadley James Corporation, Irvine, CA) by dipping the glass electrode into the gel.[17]

Microbiological stability[23]

Dilutions (1:9; formulations and controls) were inoculated on selective médiums Triptone soy agar for bacteria and Sabouraud agar for yeast and fungi (Britania) and inoculated for three days at 37°C for bacteria and seven days at 25°C for yeast and fungi. Mean result were expressed as CFU g^{-1} as required on Argentine Pharmacopoeia, seventh edition. Studies were conducted at 2, 4, and 6 months of study, in accordance with guideline ANMAT.

***In vitro* passive permeation and membrane retention studies**[24,25]

This assay was conducted using vertical type Franz diffusion cells having a receptor compartment capacity of 10 mL. Cellulose membranes (D9527 avg. flat width 43 mm (1.7 in.) Sigma Aldrich Chemical CO., St. Louis, MO) were mounted between the half-cells in contact with receptor fluid (0.9% NaCl) and were equilibrated for 1 h. The area available for diffusion was 1.8 cm^2. The fluid in the receptor compartment was maintained at 32 ± 0.5 ºC (skin temperature). Repelent bifasic emulsion designed (0.5 g) was placed in the donor compartment. The entire assembly was kept on a magnetic stirrer (100 rpm) and at each time four cells were removed from the system, aliquots (2 mL) of the receptor phase at specific time intervals (0,10, 20, 30, 40, 60 and 90 min). Limonene shows an absorbance peak at 207 nm due to isolated double bonds. The OD_{207} of the solution was measured in a UV spectrophotometer (Thermo Spectronic Genesys 10 UVRochester, NY).[26] Cumulative

amounts of limonene on *Cymbopogom citratus* extract that permeated the diffusion unit surface (cm^2) were plotted against time (min). The results were expressed as mean ± standard deviation (SD) (n=16).

Informed consent form development [27]

Informed consent sheet was designed by the protocols aproved for the World Health Organization[27] and the provisions of the national regulatory entity ANMAT Nº6677/10.[28]

Safety, efficacy and persistence and organoleptic caracteristics acceptance studies in healthy skin volunteers

No adverse health effects have been reported from the application of any of the components of the biphasic repellent formula. BNC is defined by the FDA as generally recognized as safe. Within this framework, it is possible and representative to evaluate the safety of its ingredients by monitoring for the appearance of edema or erythema (ruling out the appearance of potential allergies) in trials with healthy volunteers. The corrosion test on a healthy skin model was selected[25-27] as a methodology validated by ANMAT (Resolution No. 288/90)[28] in Argentina. This trial involved 150 healthy volunteers. The chosen skin surface was that recommended by the World Health Organization.[27] The guidelines for testing repellents on healthy volunteers suggest application to a limb. An approximate surface of 75 cm², a standard measurement of 5 cm x 15 cm for both men and women, was selected on the back of the right arm, based on the average height of the area. For data collection, three dimensions were required: the length of the treatment area and the proximal and distal limits of the treatment area. For the safety studies, the following parameters were considered:

a) absence of dermal reaction, edema, erythema, or dry skin.

b) mild reaction, described as slight edema and/or erythema; dry skin was considered present in the application area.

c) moderate reaction, described as the presence of edema or erythema in the application area.

For the efficacy and persistence studies, the following levels were considered:

a) at least 8 hours.

b) between 6 and 8 hours.

c) between 3 and 6 hours.

For the analysis of the results of the organoleptic evaluation, the absorption rate perceived by the healthy volunteer was considered as:

a) rapid absorption, less than 5 minutes.

b) medium absorption, from 5 to 10 minutes. c) Slow absorption, taking 10 minutes or more.

Other organoleptic characteristics achieved were those whose perception increases user comfort, such as a pleasant aroma and a refreshing sensation.[29, 30]

Ethical considerations

This study meets the ethical and scientists standards to design, conduct, recording and reporting studies that involve the participation of human beings stipulated by the Ministry of Health of Argentina, (Resolution Nº 1490/07).[31] They are based in the International Declarations of Human Rights and Ethics Research (Nuremberg, 1948)[32], Helsinki treated (1964 and updates of the World Medical Asociation)[33], the Operational

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Guidelines for Ethics Committees (WHO 2000 - World Health Organization)[34] and the International Ethical Guidelines for Health research Involving Human Subjects (CIOMS 2017 - Council for International Organizations of Medical Sciences).[35]

Statistics

The t-test was used for statistical analysis. $p<0.001$ was considered statistically significant.

RESULTS AND DISCUSION

BNC dry weight

The BNC dry weight per liter of King B medium (g/L) was 11.95 ± 0.22 g/L. For the pre-formulation studies in the laboratory, the school trials with students, teachers and the general rural community, a single batch of 80g of NCB was obtained.

Formulation design

Table 1 shows the formula of the mosquito repellent.

Formule and composition 100mL	
Cymbopogon citratus aqueous extract	6mL
Cymbopogon citratus essential oil	1mL
Glicerine	15mL
Vit E	1mL
Corgard preservative	5 drops
water	c.s.p

The natural mosquitoes biphasic repellent emulsion offers advantages over semi-solids, both creams and gels. These advantages include the possibility of applying the repellent over clothing, allowing for complete protection, since larger mosquitoes bite through clothing.[37-39] This was an observation made in previous studies conducted in 2023.[16] In this formulation, specific climatic factors of the Yungas region of Tucumán province were considered. The high humidity in summer and strong winds, along with airborne dust due to erosion caused by the large sugarcane monoculture plantations, were factors that influenced the effect. A skin softener, sweet almond oil, essential oil suitable for cosmetic use, and a natural preservative such as Cosgard were incorporated instead of parabens. Parabens are preservatives that act as estrogen-like endocrine disruptors.[17] The skin contains a large number of estrogen receptors across its entire surface. These considerations make our new design a conscious product that is easier to apply, lasts longer, offers better protection for humans, is suitable for use on pets, and is environmentally friendly.[15]

Aqueous extract preparation[40]

To prepare the extract, a thermostatically controlled ultrasonic bath (BioLab PN: LLAB0583 - Biolab Argentina) was used. Pharmaceutical-grade double-distilled water (Microfiltration Max. 0.2 µm - Cicarelli - Argentina) was used. The active pharmaceutical ingredient was selected from the local plant Cymbopogon citratus due to its abundance in the rural area of Antwerp. This also aligns with the findings published by Bossou et al. regarding homogeneity. Their studies demonstrated that the composition of the essential oil and extracts of *Cymbopogon citratus* is much more homogeneous, with geranial being the main constituent, regardless of the plant's origin. The chemical composition and the order of major components of the essential oil have been previously reported. The leaf extract of *C. citratus* revealed the concentration order of the main compounds: geranial, limonene, β-myrcene, citronellal, limonene oxide, geraniol, and linalool.[41]

Homogeneity assays[23]

When the homogeneity studies were carried out, no phase separation was observed after centrifugation at 3500 rpm. The homogeneity of the repellent bifasic emulsion was categorized as very good and without phase separation.

pH measurements

The pH measurements yielded a result within the expected range for a topical product applied to humans with healthy skin: 5.6 ± 0.12.[42] These values were reported to be within the acceptable range for topical cutaneous formulations in general. Previous studies by other authors reported that while pH is not constant throughout the body, this variation does not affect skin health, although it does affect skin hydration. Consequently, lower pH values are associated with greater dehydration in areas such as knees, elbows, and feet.[43] These values can also vary between 4.0 and 6.2 depending on race, age, sex, and environmental factors.[44] The importance of this parameter lies in the fact that acidic pH values on the skin surface regulate the homeostasis of the stratum corneum and the permeability of the skin barrier. This slightly acidic pH in the repellent formula promotes keratinocyte differentiation, maintains the balance of the normal skin microbiota, and stimulates the formation of epidermal lipids and the lipid envelope of corneocytes. The designed topical formulation is an extrinsic factor that has the added value of contributing to the maintenance of skin health.[44]

On the other hand, an increase in pH value tending towards neutrality negatively affects inflammatory processes. Also afects the synthesis of enzymes essential for ceramides and proteases production. It is also associated with skin inflammatory diseases of varying severity, including keratosis, atopic dermatitis, and even rosacea. Normalizing the pH by acidification through topical products helps to establish a physiological microbiota, repair the skin barrier, induce epidermal differentiation, and reduce inflammation.[45]

***In vitro* passive permeation and membrane retention studies**[25]

Since our main objective was to increase the repellent's residence time on skin and clothing fabrics, an in vitro membrane retention study was conducted. The versatility of the equipment patented by our research group allows for the measurement of both the percentage of limonene retained on the membrane and the amount of active ingredient that penetrates the synthetic membrane simulating skin. To measure non-permeation retention, membrane samples were taken and immersed for 60 minutes in the same solution as the ampoule. The addition

of BNC generated an adhesive layer on the synthetic membrane, holding the sonicated limonene extract to the surface. This is a desired reaction in our technological design. When the amount of limonene accumulated on the membrane (µg/cm²) was tested over time (min) in all analyzed samples, no *in vitro* permeation values were found. It is known that excipients used in topical formulations can significantly influence the rate and extent of drug penetration into the skin. In this case, the BNC concentration significantly increased the theoretical retention of the two-phase spray on synthetic skin. This was our fundamental objective in the formulation design. *In vitro* passive permeation studies were primarily conducted to verify the retention of the active ingredient in the carrier matrix. Retention studies in healthy human volunteers are necessary to determine the effectiveness and retention time of the bifasic spray repellent.

Microbiological stability[23, 46]

For non-sterile topical formulations in Argentina microbiological stability studies must comply with ANMAT regulations, including hygienic control according to the acceptable limits for each route of administration and the effectiveness test of the preservative system. Studies were conducted at 0, 2, 4, and 6 months of use, following guideline 4061/2023.[46] In the studies performed on Tryptone soy agar (Britania-Argentina) for bacteria, a 1:9 dilution was prepared in triplicate, as per regulations. The triplicates were incubated and inoculated for three days at 37°C. No colony-forming units (CFU) were found in any of the samples tested at 0, 2, 4, and 6 months of use. For yeast and fungi, Sabouraud agar (Britania-Argentina) was used at 25°C for seven days. No CFUs were found in any of the studies conducted for fungal or yeast counts at 0, 2, 4 and 6 months after the manufacture of the two-phase spray repellent. Microbiological stability studies showed no growth of bacteria, fungi, or yeast. This indicates that preservatives suitable for natural products and without endocrine-disrupting effects are capable of preserving the product for at least six months.

Safety studies in healthy skin volunteers

Safety studies conducted on 150 volunteers with healthy skin. Evaluated parameters were the likelihood of allergies, represented by the appearance of edema, erythema, or a sensation of dryness on the skin of the volunteers surveyed. For better interpretation of the results, the following categories were selected:

a) absence of dermal reaction, with no edema, erythema, or dryness;

b) mild reaction, described as a slight presence of edema and/or erythema; dryness was considered present in the application area;

c) moderate reaction, described as the presence of edema or erythema in the application area. No allergies in the form of edema, erythema, or dryness were recorded. Previous studies by other authors have shown that the use of BNC in pharmaceutical formulations does not present side effects or genotoxicity in vitro.[47] The absence of toxins from BNC nanofibers was demonstrated in vitro through cell viability and in flow cytometry assays in mouse cells in vivo.[48] BNC showed no adverse effects on cultured human umbilical vein endothelial cells, fibroblasts, or chondrocytes.[49] *In vitro* analysis also reveals that 95% of mesenchymal stem cells accumulate in the cellulose membrane.[50] Furthermore, modified hydrophobic celluloses are GRAS materials widely used as excipients in the pharmaceutical industry.[51, 52] This would explain the absence of undesirable effects in the designed biphasic mosquito repellent spray emulsion.

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Efficacy and persistence studies in healthy skin volunteers

The results of the effectiveness and persistence studies were analyzed. An approximate area of 75 cm^2, a standard measurement of 5 cm x 15 cm for both men and women, was selected on the anterior portion of the right arm, based on the average height of the area. For the effectiveness and persistence studies, the following levels were considered: a) at least 8 hours, b) between 6 and 8 hours, and c) between 3 and 6 hours. In the survey conducted 24 hours after application of the mosquito repellent to the 150 healthy volunteers surveyed, it was found that 81% of the volunteers perceived the product's effectiveness and persistence for 8 hours or more, reaching up to 12 hours in some cases. 9% of the respondents reported the product's persistence between 6 and 8 hours after application, but in all cases, the respondents reported a noticeable aroma and good persistence. Only 10% of the volunteers reported persistence between 3 and 6 hours. These values are consistent with the currently used DEET-based commercial repellent, whose application instructions suggest repeating the application approximately every 8 hours.[53] As previously mentioned, the toxicity of commercial repellents is based on two issues. First, DEET is a toxic substance, and misuse exacerbates the situation. DEET has a toxicity classification that considers it harmful if ingested, a skin irritant, and a cause of serious eye irritation. It is harmful to aquatic life with long-lasting effects. Another problem is the repeated application outside of the recommended intervals. According to surveys conducted during this study, volunteers reported applying the commercial repellent every time they left an enclosed space, such as their home or workplace, sometimes repeating the application up to 6 times within an 8-hour period. This behavior, combined with the product's natural toxicity, significantly increases the risks of poisoning, the appearance of skin eczema, overexposure reactions, respiratory and skin allergies, etc. The natural repellent designed and presented in this work exhibits good persistence over time and very good repellency results. The main objective of ensuring that most of the repellent's active ingredient remains on the skin's surface was achieved. Variability in persistence could be due to factors such as skin type (oily, combination, or dry) and genetic factors. Mosquitoes respond to human emanations with varying degrees of attraction. This attraction depends not only on factors related to mosquito behavior but also on the emanations produced by the host. Attraction is also partly influenced by bacteria present on the skin, diet, and exercise.[54]

Organoleptic acceptance studies in healthy skin volunteers

For the analysis of the organoleptic characteristics, the absorption rate perceived by the healthy volunteer was considered as: a) rapid absorption time as less than 5 minutes, b) medium absorption time as 5 to 10 minutes, and c) slow absorption time as 10 minutes or more. Other positive organoleptic characteristics achieved with this new formula relate to the ease and comfort perceived by the user. These variables were described as "pleasant aroma" and "refreshing sensation." Of the 100% of volunteers surveyed, 62% perceived rapid absorption of the product in less than 5 minutes. 38% perceived absorption of the product in a period of between 5 and 10 minutes. As previously mentioned, the area around the town of Antwerp is a high-prevalence mosquito zone within our province. Its climatic characteristics require a high absorption rate of the product to prevent the evaporation of the volatile repellent agents. This represents a technological advantage in this area with high humidity during the summer months. Furthermore, the rapid drying time is desirable, as it has been reported to increase the ease of application and complement the action of commercial aerosol repellents without the need to increase the concentration of volatile excipients in the formula. The natural, two-phase spray repellent was

described by the healthy volunteers involved in this study as gentle, with a pleasant aroma, rapid absorption, and a lasting cooling sensation.

CONCLUSIONS

DEET's mechanism of action involves interfering with mosquitoes' olfactory receptors, confusing their antennae and mouthparts, making it difficult for them to detect or orient themselves toward the odor of carbon dioxide and lactic acid emitted by humans. Instead of killing mosquitoes, this chemical compound acts as a repellent, deterring them from landing on skin and biting. However, while its mechanism of action is undeniably beneficial, its toxicity to humans and its potential for misuse pose a problem that needs addressing. The novel formulation presented in this work, using BNC as an active ingredient that generates a light layer of biphasic natural repellent on skin and clothing, represents a natural solution to this problem. The selected plant species met the desired characteristics of high concentrations of limonene terpenes and their abundance in the study area. Aqueous extractions using physical methods allow for a wider age range of use, as none of the formula's components are toxic or irritating. The resulting pH values are suitable for topical products and help maintain skin health by contributing to keratinocyte differentiation, maintaining the balance of the skin microbiota, and stimulating the formation of protective lipids. Studies in healthy volunteers showed no edema or erythema in the 24 hours following product application. No skin dryness was observed among the 150 volunteers surveyed. The product's persistence on the skin and its repellent action lasted at least 8 hours in 81% of cases. The organoleptic properties were satisfactory. The product was described as smooth, with a pleasant aroma, rapid absorption, and a persistent cooling sensation. The product is suitable for use as a mosquito repellent, and its formulation is optimal for the area where the study was conducted, given its climatic characteristics and living conditions. It is advisable to continue the studies by expanding the number of healthy volunteers and the area of influence of the Aedes aegythi mosquito in the province of Tucumán.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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