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Smoke of *Capsicum baccatum* L. var. baccatum (Solanaceae) repels nymphs of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae)

[Humo de *Capsicum baccatum* L. var. baccatum (Solanaceae) repele las ninfas de *Triatoma infestans* (Klug) (Hemiptera: Reduviidae)]

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Abstract: Control of the Chagas disease vector, *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) with synthetic pesticides in Bolivia has become increasingly inefficient due to the development of resistance in the insects. In the Chaco region of Bolivia, guaraní populations have approached the problem by fumigating their houses with the smoke of native plants. Through interviews and field work with local guides, the main plant used by the guaraníes was collected and later identified as *Capsicum baccatum* L. var. *baccatum* (Solanaceae). In choice bioassays, filter papers exposed to the smoke of the plant repelled nymphs of *T. infestans*. Activity remained significant after storing the exposed filter papers for 9 days. Chemical analysis of smoke and literature data suggested that capsaicinoids present in the smoke were responsible for the repellent effect. The data presented provide a rationale for the use of *C. baccatum* var. *baccatum* to control the Chagas vector bythe guaraní populations.

Keywords: Chagas; Vector control; Capsaicinoids; Repellent effect; Guaraní

Resumen: El control del vector de la enfermedad de Chagas, *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) con plaguicidas sintéticos en Bolivia se ha vuelto cada vez más ineficiente debido al desarrollo de resistencias en los insectos. En la región del Chaco de Bolivia, las poblaciones guaraníes han abordado el problema fumigando sus casas con el humo de las plantas nativas. A través de entrevistas y trabajo de campo con guías locales, se recogió la principal planta utilizada por los guaraníes y posteriormente se identificó como *Capsicum baccatum* L. var. *baccatum* (Solanaceae). En bioensayos selectos, los papeles de filtro expuestos al humo de la planta repelieron a las ninfas de *T. infestans*. La actividad siguió siendo significativa después de almacenar los papeles de filtro expuestos durante 9 días. El análisis químico del humo y los datos de la literatura sugieren que los capsaicinoides presentes en el humo eran responsables del efecto repelente. Los datos presentados proporcionan una justificación para el uso de *C. baccatum* var. *baccatum* para el control del vector Chagas por las poblaciones guaraníes.

Palabras clave: Chagas; Vector control; Capsaicinoids; Repellent effect; Guaraní.

INTRODUCTION

Chagas disease affects some 6 to 7 million people in the world. Although it is considered a poverty disease endemic to 21 countries of America, increased movement of populations between countries has spread the disease worldwide (WHO, 2016). In endemic regions of Latin America, prevalence of Chagas disease is highest in Bolivia (6.10%) (Guhl, 2017), with 4 million people at risk (PAHO, 2019). Similarly, in five countries of Europe the Bolivian community is the group with the highest prevalence among migrants (18.1%) (Requena-Méndez *et al*., 2015).

Bolivia is considered the epicenter of expansion of *Triatoma infestans* (Klug) (Hemiptera; Reduviidae) (vinchuca, timbuco), the main vector of Chagas disease, towards other Latin American countries (Gorla & Noireau, 2017). The Bolivian Chaco, the area occupied by the Guaraní indigenous nation, is one of the most affected areas (Fernandez *et al*., 2015). Seroprevalence in this area has been reported at 51.7% in the population as a whole (Samuels *et al*., 2013) with 19.8% in the 2-15 age group (Hopkins *et al*., 2019), 72.7% in the 15-30 age group, and 97.1% in the >30 age group, in spite of large-scale vector control campaigns (Samuels *et al*., 2013).

Vector control in Bolivia is based on fumigation with organochlorine, carbamate and pyrethroid insecticides (Dias *et al*., 2002; WHO, 2006; Breniére *et al*., 2012; Buitrago *et al*., 2016). For example, the fumigation campaign in 2002 reduced infestation by *T. infestans* to 1% in the Bolivian Chaco; nevertheless, subsequent fumigation campaigns lost their effectiveness, showing a reinfestation in homes from 3% in 2003 to 58% in 2008 (Samuels *et al*., 2013), indicating that continuous use of insecticides promotes resistance in the population of *T. infestans* making its elimination difficult (Breniére *et al*., 2012; Buitrago *et al*., 2016).
The unsuccessful attempt to control the vector with recurrent doses of a small range of synthetic insecticides that are no longer effective opens a space for alternative ways to control *T. infestans*. Cultural and ancestral knowledge of ethnic groups cohabiting with the vector for a long time has allowed them to achieve the capacity to use local plants to repel the insect. For example, native plants are used as repellents and insecticides for the control of disease-transmitting vectors (Villavicencio *et al*., 2010; Moretti *et al*., 2015); in particular, the local population of Nazca (Ica), Peru, uses the smoke of *Capsicum* spp. to control vector infestation (Cabrera *et al*., 2003).

Bolivia is a plurinational state with 36 indigenous nations making up more than 41% of the population. These native communities treasure a vast repertoire of ancestral ethnobotanical knowledge, including the use of native plants for a wide variety of purposes. The Guaraní indigenous community in Bolivia inhabits the Chaco area of southeastern Bolivia. They use a wide variety of plants as repellents and insecticides for vectors that transmit diseases (Villavicencio *et al*., 2010; Moretti *et al*., 2015). In this study, we approached five Guaraní communities and enquired about the plants they use to drive away and kill insects from their homes, and studied the repellent effect of one of these plants towards *T. infestans*, the main vector of Chagas disease in the area.

MATERIALS AND METHODS

Study site

The study was performed at five Guaraní communities, Tentamí, Isipotindi, Ñancaroinza, Estación Macharetí and Tati, all belonging to the Machareti municipality in the Chaco area of Chuquisaca (Figure No. 1). Initial meetings were held with the *Capitán Grande* (Guaraní chief) of each community, to earn the trust and acceptance of potential participants in the study. A presentation was made explaining the objectives of the research and the importance of rescuing the ancestral knowledge they possess, in particular their use of plants to control the Chagas vector. Thereafter, a questionnaire was submitted to potential participants which contained the following questions: 1) Do you know the vinchuca or timbuco (common name of *T. infestans*)?, 2) Do you use any medicinal plant to eliminate the vinchuca or timbuco?, 3) Who taught you this method of elimination?, 4) Could you teach me how to use that method of eliminating vinchuca or timbuco?, and 5) Could you provide me the plant you use in that method?. Volunteers who filled the questionnaire (n=84) signed an agreement specifying the use to be given to the information provided.

Collection and identification of plant material

The use of a pepper plant whose smoke repels the vector from their home and surroundings was singled out by participants. With the help of local Guaraní guides, the relevant plant material (400 g of fruit and 1000 g of aereal tissue including leaves, stems, flowers and fruits) was collected in the community of

Isipotindi (S 20°37'2.9", O 63°18'59.2"). An herborized sample was later identified as *Capsicum baccatum* L. var. *baccatum* (Solanaceae) (ají de zorro) by the Herbarium of the South of Bolivia (HSB).

Figure No. 1
Sampling sites. Five localities were visited (Isipotindi, Ñancaroinza, Tati, Estación Macharetí and Tentamí) at the rural Municipality of Macharetí in the Chaco region of the Chuquisaca Department

Management of insects

Fifth-instar nymphs of *T. infestans* of the CIPEIN strain were used to perform bioassays. Nymphs were kept in groups of 5 individuals in plastic flasks (10 cm diameter x 7 cm height) whose lid was perforated and whose floor was lined with filter paper. Containers were kept at 26°C, 50% relative humidity and 12:12 photoperiod.

Behavioral assays

Dry tissue of *C. baccatum* var. *baccatum* (200 mg with a proportion of fruit, leaves and stems similar to that used by the guaraní people) were burned in a 250-mL beaker placed on a hot plate (SP46920-26, Barnstead Thermolyne Corp., Dubuque, IA, USA). A circular piece of filter paper (12-cm diameter) was cone shaped and positioned over the top of the beaker thereby exposing it to the smoke emanating from the beaker. After 3 minutes of exposure to smoke, the circular pieces were cut in halves and stored in Petri dishes to be used in the behavioral bioassays. To analyze the effect of smoke over time, i.e. its residual effect, smoked semicircles were stored for different time periods: 0, 3, 6 and 9 days (storage times).

The arena consisted in two paper semicircles (one exposed to smoke and one not exposed to smoke) placed on a 20x20 cm glass plate forming a circle 12-cm in diameter. One fifth-instar nymph was placed at the center of the circle and a glass cylinder (12-cm diameter x 5-cm height) positioned above the circle. The location of the nymph on the filter paper arena was recorded for 20 min with a video camera

(C920, Logitech).

A nymph was placed in the arena containing two untreated paper semicircles and its activity recorded (control for experimental design). The nymph was then removed from the arena and introduced in the plastic flasks used to maintain them in the lab. The semicircles were replaced by a recently smoked treated one (day 0) and a new untreated one. The same nymph was then introduced in the arena and its behavior recorded. The same procedure was subsequently repeated with semicircles stored for 3, 6 and 9 days. Twenty-five replicates were performed with different individuals.

Data analysis

The analysis of the videos was performed with the software Jwatcher Version 1.0. The time spent by each insect in the treated semicircle and in any one of the untreated semicircles in the control experiment were used as raw data. Values between 0 and 10 min indicate a repellent effect; values close to 10 indicate no effect.

Dependent 2-group Wilcoxon signed rank tests were performed on raw data for the four treatments and the control to assess a repellent effect of the smoke. The residual effect of the smoke was assessed by analyzing the variation along storage time of the time spent on the sprayed side of the paper using a generalized linear mixed model (GLMM) with Gamma error structure and log link in which insect group was included as a random factor. The model was fitted through the *glmer* function of the *lme4* package in the R software (R Core Team, 2020). Overall significance of the effect of storage time on time spent on the sprayed semicircle was tested using a likelihood ratio test, while differences with respect to day 0 were tested with t-tests.

Collection and analysis of volatile compounds

A beaker containing plant material (200 mg of dry fruits, leaves and stems of *C. baccatum* var. *baccatum* in the proportion used by the guaraníes) was placed on a hot plate (SP46920-26, Barnstead Thermolyne Corp., Dubuque, IA, USA) inside a glass bell jar which had an inlet for synthetic air and an outlet connected to a Porapak Q filter, a suction pump (Dymax 30, Charles Austen Pumps Ltd., Byfleet, Surrey, UK) and a flow regulator. When the desired temperature was reached in the hot plate (400°C), air flow was started and kept at 0.5 L/min for 3 min. The volatile compounds retained by the Porapak Q filter were eluted with dichloromethane to a final volume of 1 mL. The solvent was evaporated on a hotplate (SP46920-26, Barnstead Thermolyne Corp., Dubuque, IA, USA) at 40°C under a constant flow of nitrogen.

Samples were reconstituted for analysis in 20 µL of dichloromethane 2 µL of which were analyzed in a gas chromatograph with mass detector (GCMS-QP 2010 Ultra; Shimadzu, Kyoto, Japan) equipped with an Rtx-5MS capillary GC column (30 m length, 0.25 mm I.D., 0.25 µm film thickness; Restek, Bellefonte, PA, USA). The GC was used in the splitless mode. Helium was the carrier gas at a flow of 1.3 mL/min. The mass spectrometer was used in the electron impact ionization mode at 70 eV. The temperatures of the injection port, ion source and transfer line were set at 225°C, 250°C and 250°C, respectively. The GC oven was programmed to remain at 90°C for 1 min and then to increase at 5°C/min to 290°C (Saha *et al*., 2015). Main compounds in the chromatogram were identified based on comparisons of their retention index and mass spectrum with data in the NIST14 database and that reported in the literature.

RESULTS

Behavioral analysis

T. infestans nymphs remained significantly more time in the non-smoked than in the smoked filter paper semicircle in the day 0 and day 3 experiments (day 0: V= 300, p=0.000227; day 3: V= 288, p=0.000329), while in the control, day 6 and day 9 experiments they remained equal times on both filter papers (Control: V = 129, p=0.388; day 6: V= 223, p=0.1073; day 9: V= 221, p=0.120). A significant effect was found of storage time of the smoked semicircle on the average time spent by nymphs on the smoked semicircle (χ^2= 21.419, p<0.0001, Figure No. 2). After 3 days of storage, the average time spent on the smoked side increased 10.5% with respect to day 0, but this difference was not significant (t= 1.415, p=0.157). The time increased 40.0 % in day 6, (t= 4.775, p>0.0001) and 47.4% in day 9 (t= 5.505, p<0.0001) with respect to day 0.

Chemical analysis of smoke

Main peaks in the chromatogram of the smoke of *C. baccatum* var. *baccatum* were identified on the basis of their retention index (RI) and mass spectrum (Table No. 1); retention indexes were within 1% of published data and mass spectra similarity indexes were above 90% in relation to spectra in the NIST2014 database.

Figure No. 2

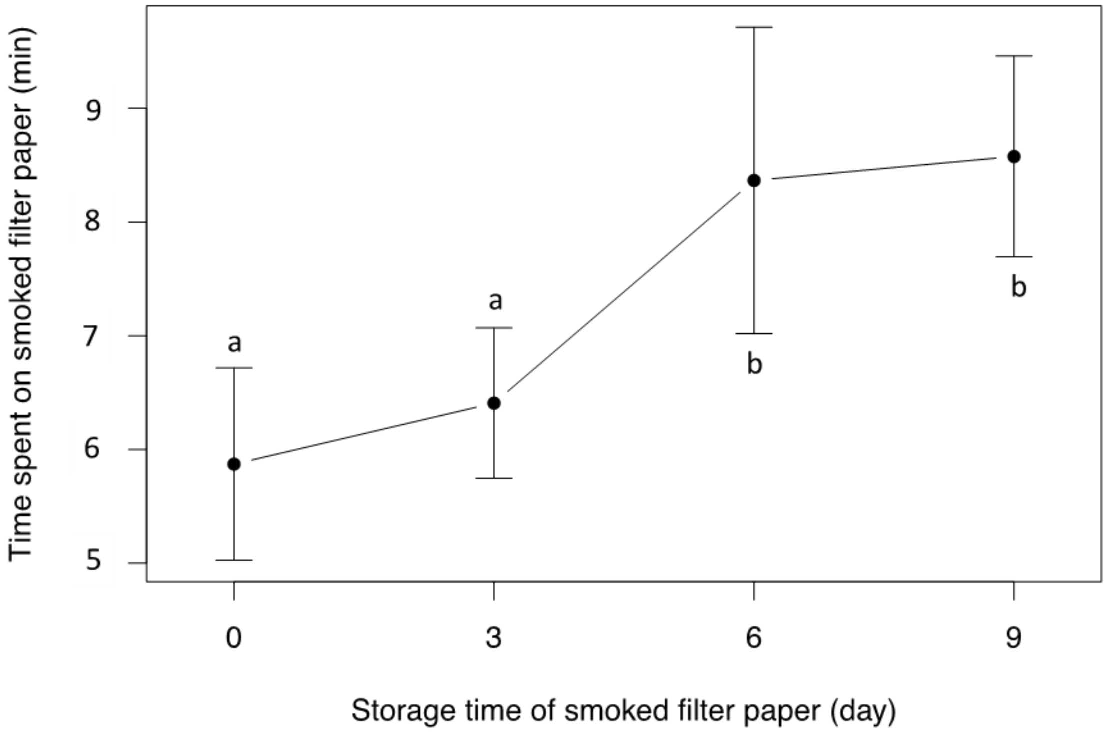

Figure No. 2
Time spent by fifth-instar nymphs of *T. infestans* on a filter paper exposed to the smoke of *Capsicum baccatum* var. *baccatum* and stored for different times. Different letters denote significant statistical differences (p<0.0001)

Table No. 1
Compounds identified in the smoke of *C. baccatum* var. *baccatum*

Retention index	Compound	Similarity index (%)
1840	Neophytadiene	95
1845	(*7E*)-2-Methyl-7-octadecene	91
1925	Palmitic acid, methyl ester	90
2093	Linoleic acid, methyl ester	94
2119	Phytol	95
2572	Capsaicin	97
2596	Dihydrocapsaicin	95
2840	Squalene	95
2899	*iso*-Nonacosane	94
3138	Tocopherol	91

DISCUSSION

The bioassays performed showed that the smoke of *C. baccatum* var. *baccatum* repelled nymphs of *T. infestans*, thus providing a rationale for the traditional use of this plant in repelling the Chagas vector at homes. Chemical analysis of the smoke showed the presence of several compounds frequently found in plant material and two compounds which are characteristic of the genus *Capsicum*, capsaicin and dihydrocapsaicin. Repellency by *Capsicum* is most likely explained by the presence of these two compounds, given their wide range of activities against insect pests (Tomita & Endo, 2007; Tunca *et al*., 2016; Li *et al*., 2019). Moreover, capsaicin has been shown to decrease responsiveness to heat stimuli, to increase preference for lower temperatures, and to negatively affect orientation in space of the triatomid bug, *Rhodnius prolixus* (Hemiptera; Reduviidae) (Zermoglio *et al*., 2015).

The search for plant extracts or plant essential oils with insect repellent activity is a common research topic aimed at developing alternatives to synthetic chemicals for pest control (Lobo-Echeverri *et al*., 2016; Kim *et al*., 2017; Mangang *et al*., 2020; Nwanade *et al*., 2020; Rosa *et al*., 2020; Yarou *et al*., 2020). The repellent activity of these natural compounds has shown favorable results, as numerous studies have reported repellency and even death to insects after the application of plant-derived compounds (Ojo *et al*., 2020; Saraiva *et al*., 2020; Sharaby *et al*., 2020; Ya-Ali *et al*., 2020). Among these studies, plants of genus *Capsicum* have also shown pest control activity (Lale, 1992; Antonious *et al*., 2006; Castillo-Sánchez *et al*., 2012; Vasconcelos *et al*., 2014).

Residual effect is defined as the ability of a preparation to maintain, for a period of time, the ability to repel or to eliminate a target organism after application (Pontes *et al*., 2005). In general, the residual repellent effect is case-dependent and may depend not only on the concentration of the repellent organic compound but also on the exposure time of the insect and the type of insect tested (Bett *et al*., 2017). The present results show that repellent effect of the smoke of *C. baccatum* var. *baccatum* towards nymphs of *T. infestans* decreased over time. The demonstrated effectiveness of the traditional use of the plant suggests that the present bioassays do not fully mimic the conditions of such use.

The Guaraní populations at the Machareti municipality share an ancestral knowledge on the use of natural insect repellents. Their use involves a sequence of operations, i.e. collecting plant material, drying it, setting up a fire, and burning the plant material inside the houses until they are completely consumed. All these steps are carried out in a precise predefined way to obtain the desired end result. The Guaraní people as well as other native groups continue to use medicinal and repellent plant preparations, but are doing so at ever decreasing frequency (Nolan & Robbins, 1999; Begossi *et al*., 2002; Bueno *et al*., 2005; Quinlan & Quinlan, 2007; Karunamoorthi *et al*., 2009a; Karunamoorthi *et al*., 2009b; Giovannini *et al*., 2011; Morris, 2011; Kujawska *et al*., 2017; Youmsi *et al*., 2017). Hence, it is important to rescue this knowledge before it disappears. It is also important to find scientific rationales for the effects of the plants used and employing this additional knowledge to design improved methods for the control of harmful pests.

CONCLUSIONS

The present study shows that the smoke of *C. baccatum* var. *baccatum* smoke repels nymphs of *T. infestans,* at least until the third day after application. This knowledge may be useful to encourage the periodic use of smoke of *C. baccatum* var. *baccatum* to flush-out the Chagas vector and maintain houses free of it.

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