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# A field-applicable low-tech method for extracting capsaicinoids from red chili pepper

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ABSTRACT

Red chili pepper is a common crop found in southern countries often extracted to be use as animal repellent. It contributes managing human-wildlife conflicts in countries where this subject has become a major concern. Previous studies have highlighted the operating conditions which optimize the diffusion of capsaicinoids. However, they did not allow the precise identification of the realistic low-tech operating conditions which should be employed in the field. By the means of two Doehlert experimental designs, this work explored the impact of temperature, ratio solid/liquid and proportion of hydroalcoholic solution and soybean oil as extraction solvents on capsaicinoid content and yield, measured by HPLC and taken as an indicators of the repellent's strength. Kinetics was used to establish that 15 min was sufficient to obtain the best extract for both type of solvents, with a limited number of operation units. Surface response showed that on one hand hydroalcoholic extraction with a 1/10 ratio led to total yields < 50 % and capsaicinoid concentrations to 400 $mg{\cdot}L^{-1}$, which is 6-fold lower in capsaicinoids that conventional unleaded petroleum extract and so less efficient. However, the operation units are easier to deploy as lengthy evaporation times or filtration of a viscous extract are unnecessary. Moreover, the residue re-employable. On the other hand, oil extraction achieved yields between 40 % and 70 % and capsaicinoid concentrations between 600 and 3600 $mg{\cdot}L^{-1}$, although the extraction process involves viscous filtration which is a more time consuming. However, capsaicinoid content was 1.5 times higher than in conventional petrol extract. Schematized low-tech procedures have been proposed to farmers.

## 1. Introduction

Red chili pepper (*Capsicum annuum* L.) is cultivated all over the world, mainly in tropical regions but it also adapts to less favorable lowland climates when rainfall is sufficient (Hussain & Abid, 2011). In recent years, climatic conditions in Africa have led to the increase of its production in several countries, particularly in Eastern and Southern Africa where the main producers are Ethiopia, Tanzania, Sudan, Kenya, Malawi, Rwanda, Uganda and Zimbabwe (Waweru, Kilalo, Miano, Kimenju & Rukundo, 2019). Red chili pepper is well known for its high capsaicinoids content (Idrees, Hanif, Ayub, Hanif & Ansari, 2020), formed by the condensation of a fatty acid and vanillylamine. Capsaicinoids are increasingly studied for their health properties (X.-J. Luo, Peng & Li, 2011; Srinivasan, 2016). However, they are primarily valued for their sensory properties (N. Luo, Ye, Wolber & Singh, 2020) because they cause intense sensory reactions, characterized by burning or pain as well as a pronounced sensation of irritation resulting from their interactions with oral or nasal receptors. The representation of this pungent force is often represented by the Scoville heat unit (Scoville, 1912; Zhu et al., 2023).

These natural compounds present multiple benefits, such as their efficacy through combined modes of action (e.g., neophobia, irritation, conditioned aversion, and flavor modification). As a consequence, capsaicinoids are promising for uses as repellents, particularly against herbivores to protect crops and materials (Wang et al., 2021). This repellent action has been investigated on multiple case studies for example against mammals such as rodents (rats, squirrels, pocket gophers, rabbits etc.) (Bosland & Bosland, 2001; Fitzgerald, Curtis, Richmond &

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Dunn, 1995; Sterner, Shumake, Gaddis & Bourassa, 2005), horses (Aley, Adams, Ladyman & Fraser, 2015) or elephants (Montgomery et al., 2022). Given the global imperative for biodiversity preservation, the latter are the focus of major global conservation programs worldwide, particularly in Asia or Africa where agriculture relies on a multitude of smallholder farmers with limited financial, technical and scientific resources (Jayne, Mather & Mghenyi, 2010; Rapsomanikis, 2015; Vanlauwe et al., 2014). A prevalent method for deterring elephants involves brushing farm fences with various chili pepper-based product (Le Bel, La Grange & Czudek, 2016; Montgomery et al., 2022). Farmers often resort to quick and cheap solutions for repellent production such as using unleaded petrol (Le Bel, La Grange, & Drouet, 2015). However, the use of non-lethal and natural repellents to protect farms in these areas is of main importance (Schulte, 2016).

The extraction of capsaicinoids from red chili pepper is a fairly well-referenced subject (Lu, Ho & Huang, 2017). Numerous methods have been optimized for the extraction of these compounds, including enzymatic maceration (pectinase, carbohydrase, cellulase etc.), ultrasound-assisted extraction, microwave-assisted extraction, Soxhlet, supercritical or pressurized fluids. Overall, these processes are efficient, enabling capsaicinoids extraction rates to be increased to over 90 % yield. They also offer ecological and economic advantages, mainly through the reduction in energy required for extraction or the use of green solvents (Olguín-Rojas et al., 2024). Farmers in the southern countries who faced intense human-wildlife conflicts are unable to adopt this type of fine optimized processes and need more low-tech solutions (Francis, 2019; Le Bel et al., 2011). Low-tech process may be defined as a simple, appropriable and sustainable equipment and/or solution and are more and more considered because of their lower impact on society and environment. Farmers typically have access to basic extraction methods, including unleaded petrol, alcohol, various local oils as solvents, and equipment such as manual grinders (e.g., manual mortar and pestle) and tanks. Therefore, in addition to the extensive availability of data, specific application to this context is still lacking even though these regions constitute the main production areas for red chili pepper.

As shown by Peña-Alvarez, Alvarado and Vera-Avila (2012), the solvent polarity play a crucial role in the extraction of capsaicinoids which are predominantly hydrophobic, and so more soluble in non-polar solvents. Literature gives their an octanol-water partition coefficient of ~3.8 (Hanson, Newstead, Swartz & Sansom, 2015). Low polar solvents have been tested such as ethanol, acetone and acetonitrile even water in specific conditions (Bajer, Bajerová, Kremr, Eisner & Ventura, 2015; Chinn, Sharma-Shivappa & Cotter, 2011). However, a small proportion of these compounds can still be recovered using water as a solvent. Most of these works involved bioprocessing such as fermentation, for example in the production of Tabasco sauce (Farias, Araújo, Rocha, Garruti & Pinto, 2020). Theses bioprocessing are conduct over weeks on fresh material. They are not currently a practical option for African smallholders, although if they may become interesting in the future. Dong et al. (2014) optimized the hydroalcoholic extraction of capsaicinoids from red chili pepper using two orthogonal array designs, and varying four parameters: the ratio solvent to chili pepper (from 2 to 6 $mL \cdot g^{-1}$), the extraction temperature (from 60 to 180 °C), the concentration of ethanol in the solvent (from 10 to 70 %) and the time of extraction (from 90 to 150 min). Dong et al. (2014) obtained >50 % yield for a single extraction with optimized operating conditions which is promising. However, they worked on unfrozen fresh material, which is not adapted to the field. Moreover, the extraction yield and the concentration of active compounds were measured only at some discrete times and diffusion of capsaicinoids in the solvent may be faster that what they stated. The content of capsaicinoids extracted during oil maceration was also interesting for an application as repellent with values greater than 130 mg capsaicinoids·kg extracted $oil^{-1}$. It proved that oil may also be an efficient low-tech green solvent in this case study (Caporaso, Paduano, Nicoletti & Sacchi, 2013; Paduano, Caporaso, Santini & Sacchi, 2014). However, this study did a diffusion over 7 days without shorter kinetic.

Therefore, this work aims to study these limitations by focusing on determining the optimal conditions for capsaicinoids extraction under resource-constrained conditions (utilizing water, alcohol, and local edible oil) and with a greater correlation to available low-tech equipment. This work also aims to provide recommendations for farmers through a guide for repellent manufacturing optimized in terms of efficiency and durability.

## 2. Material and methods

### 2.1. Red chili pepper sampling

The samples used in the experiments were red chili peppers grown in Zimbabwe and harvested in May 2022. The fruits were solar dried, then ground using a stainless-steel mechanical grinder, as used by Zimbabwe merchandizer and packed in plastic bags. To understand the impact of the dried chili pepper powder heterogeneity on extraction, a part of the initial sampling (named thereafter “raw commercial”) was classed in two types of particles, the "flakes" and "seed parts". The whole dried chili samples were re-ground in the laboratory using a GM-200 Grindomix (Retsch, Germany) in 3 successive 10-second cycles at 10,000 rpm (thereafter “Grounded”).

Mass granulometry was performed on both raw (coarsely ground) and finely ground samples using a vibrating sieve shaker AS 200 (Retsch, Germany) equipped with eight sieves with mesh sizes of: 100, 200, 315, 500, 800, 1000, 1700 and 3150 μm.

### 2.2. Total capsaicinoids quantification

#### 2.2.1. From raw materials

Capsaicinoids extraction was adapted from Chinn et al. (2011). Approximately 1 g of ground red chili pepper was weighed using a balance PX323 (Pioneer, Japan) and 10 g ethanol was added. The tubes were placed in a water bath WNB7 (Memmert, Germany) under agitation at 70 °C (± 1 °C) during 45 min. Immediately after extraction, the tubes were cooled to ambient temperature using cold water. Separation of the liquid and solid phases was carried out using Aventi-JE centrifuge for 10 min, 30 000 g and 10 °C (Beckman coulter, USA). The supernatants were recovered and filtered at 0.45 μm using cellulose acetate syringe filters.

#### 2.2.2. From oil extracts

The oily extract was mixed with 10 mL of ethanol, shaked for 10 min and centrifuged using the Aventi-JE centrifuge (Beckman coulter, USA) for 10 min at 10 000 g and 10 °C. Then, the ethanol upper phase was collected, and 10 ml of fresh ethanol was added for a second extraction. After 3 successive extractions, the supernatants were pooled together, filtered at 0.45 μm using cellulose acetate syringe filters.

#### 2.2.3. High-performance liquid chromatography

Capsaicinoids were determined by high-performance liquid chromatography adapted from AOAC (1998) following Parrish (2020) and Tobolka, Škorpilová, Dvořáková, Cusimamani and Rajchl (2021). Analysis was performed on an HPLC 1260 Infinity II (Agilent Technologies, USA) equipped with a DAD detector and an Uptisphere $C_{18}$—HDO column 250×4.6 mm x 5 μm (Interchim, France). The injection was set at 20 μl and analysis was performed at 30 °C. The gradient elution was carried out using two phases, A (1 % formic acid) and B (pure acetonitrile), starting at 98 % of A at 0 min (and so 2 % of B), and then shifted to 80 % of A and 20 % B at 5 min, 60 % of A and 40 % of B at 20 min, 20 % of A and 80 % of B at 30 min, and a return to initial condition (98 % of A / 2 % of B) at 35 min maintained until 37 min. Standards of capsaicin (Sigma-Aldrich, USA), dihydrocapsaicin and nordihydrocapsaicin (Extrasynthèse, France), which are the capsaicinoids most commonly found in red chili pepper, were used for quantification. Standards ranged from 0 up to 1 g·L$^{-1}$. All extractions and quantifications were realized in triplicates.

### 2.3. Dry matter

Dry matter content was obtained by drying 3 g of ground pepper in an aluminum dish using an oven (Memmert, Germany) set at 105 °C for 30 h (i.e. to constant weight). Initial and final masses were determined with a precision balance Entris 2241 (Sartorius, Germany). Regarding the repeatability, the maximum standard deviation of the mass of was ± 0.17 %. All quantifications were realized in triplicates.

### 2.4. Extractions of reference

As references, we considered two limits, one as the least green-extraction which can be used on the field using petrol and the second as the best green option using water.

#### 2.4.1. Unleaded petroleum extraction

The extraction procedure was adapted as described by Le Bel et al. (2015) as commonly apply in field. A weight of 200 g of chili pepper was extracted with 1 L of petrol. The mixture was covered with aluminum foil and left to macerate at room temperature for 48 h. The solution was then filtered through cotton, and evaporated in a fume hood at ambient temperature to mimic local conditions until half the liquid evaporated. The time was adjusted according to the petrol evaporation rate, in this case this step lasted for 4 h. An equal quantity of soybean oil (500 mL) was then added to the petrol-based extract to formulate the final repellent, which was stored and protected from light to limit oxidation. For quantification of capsaicinoids, the petrol-based repellent was then evaporated using the rotavapor RV10 (IKA, USA) 3 h at 50 °C. The concentrated extract was free of petrol and consisted primarily of soybean oil.

#### 2.4.2. Water extraction

Approximately 0.5 g of red chili pepper was weighed and mixed with 10 mL of distilled water. The mixture was homogenized using an Ultra Turrax T10 (IKA, USA) and then subjected to one of two treatments: (1.) heated in a water bath at 95 °C under agitation for 30 min, or (2) left to macerate at room temperature for 6 days. Once hot extraction was complete, samples were immediately cooled using iced water. Experimentations were performed in hexaplicate ($n = 6$).

### 2.5. Experimental design

The experimental designs and the data processing (statistical etc.) were carried out using Statistica software (Statsoft, USA). The experimental design chosen were Doehlert as presented in the Table 1. The central point was always realized in triplicates to estimate the experimental error. Analysis of variance were realized using Tukey range test at $p < 0.05$ ($n = 3$). The results were presented as means with standard deviations in brackets.

#### 2.5.1. Ethanol extraction

As a preliminary step before the experimental design, the impact of the ratio was studied using 4 ratios: 1 g red chili pepper·20 g$^{-1}$ ethanol, 1/10, 1/5 and 1/2. The ratio was not included in the design, as its effect would have biased the interpretation of the fractionated experimental design (for example imbibition phenomenon that not happens with extraction using oil). The first experimental design had then two factors. The first factor was the ethanol relative content from 20 to 100 % with 5 levels of 20, 40, 60, 80 and 100 %. The second one was temperature from 33 to 67 °C with three levels of 33, 50 and 67 °C. Theses temperature were selected to be consistent with the practical capabilities of smallholder farmers, the lowest level representing the average ambient temperature on-site, and the highest reflecting the maximum temperature typically achievable with low-tech equipment while minimizing evaporation. Extraction was carried out according to the ethanol extraction protocol described previously (2.b) considering the variation described in Table 1. The time of reference was fixed at 45 min and the ratio at 1 g of dried chili pepper for 10 mL of ethanol.

In addition to the experimental design, 6 other kinetic extraction times were studied: 10, 30, 60, 80, 120 and 300 min. This longer time was interesting to estimate the stability of capsaicinoids in the extract. The main observation of the plan was the extraction yield of capsaicinoids in dried chili pepper, calculated as the ratio between the mass of capsaicinoids extracted on the mass of capsaicinoids in the raw material. The ethanol extracts were then filtered at 0.45 µm and transferred directly to vials prior to HPLC analysis.

#### 2.5.2. Soybean oil extraction

The second experimental design had 2 factors. The first one, was the ratio solid to solvent from 2 to 10 g chili pepper for 20 g of oil with 5 levels: 2, 4, 6, 8 and 10. The second one, was the temperature from 33 to 67 °C with three levels: 33, 50 and 67 °C. To do this, a quantity of chili powder as presented in Table 1 was mixed with 20 g of soybean oil which was placed in a water bath for 15 min to preheat the solvent at the desired temperature specified in Table 1. The mixture was then stirred during 45 min in a water bath WNB7 (Memmert, Germany) with a precision of ± 1 °C. Once extraction was complete, the mixture was immediately cooled on ice. The liquid and solid phases were then separated by filtration using a kitchen skimmer.

In addition to the experimental design, 4 kinetic points were realized at 15, 30, 60 and 120 min to understand the impact of time on the diffusion rates. The extraction of capsaicinoids from oily extract were realized as described in part 2.b. Here also, the response measured was the yield of capsaicinoids (capsaicins, dihydrocapsaicins and nordihydrocapsaicins).

**Table 1**
Coded and real values of the Doelhert experimental design used for ethanol and soybean oil extractions.

| | Coded values | | Real values | | | |
|---|---|---|---|---|---|---|
| | | | Experimental design 1 | | Experimental design 2 | |
| **Assay** | Ethanol % and solid to oil ratio $X_1$ | Temperature $X_2$ | Ethanol in water ( %) $X_1$ | Temperature $X_2$ | Solid to oil ratio (g·g$^{-1}$) $X_1$ | Temperature $X_2$ |
| **1** | 1 | 0 | 100 | 50 | 0.5 | 50 |
| **2** | −1 | 0 | 20 | 50 | 0.1 | 50 |
| **3** | 0.5 | 0.87 | 60 | 67 | 0.3 | 67 |
| **4** | −0.5 | −0.87 | 40 | 33 | 0.2 | 33 |
| **5** | 0.5 | −0.87 | 60 | 33 | 0.3 | 33 |
| **6** | −0.5 | 0.87 | 40 | 67 | 0.2 | 67 |
| **7** | 0 | 0 | 20 | 50 | 0.1 | 50 |
| **8** | 0 | 0 | 20 | 50 | 0.1 | 50 |
| **9** | 0 | 0 | 20 | 50 | 0.1 | 50 |

## 3. Results and discussion

### 3.1. Product homogeneity

The particle size distribution of commercial dried red chili pepper showed the predominance of large particles, with 67 % of particles bigger than 1000 µm and a $D_{90}$ of 2460 µm (Fig. 1). Particle size is one of the most important parameters influencing extraction efficiency, as it affects both the maximum extractable quantity of compounds and the extraction kinetics (Coats & Wingard, 1950; Prasedya et al., 2021). Reducing coarse particle size is therefore a prerequisite for extraction. In the farmers houses, conditions and resources do not allow to realize fine milling. Therefore, grounding is warried out using simple technologies (mortar-type or knife mills). The simple knife milling carried out in this study significantly reduced particle size to below 1000 µm, with a $D_{90}$ of 400 µm (Fig. 1). The mean dry matter content (DM) of all samples was 93.1 (0.3) g·100 $g^{-1}$ ($n = 12$) showing no difference between samples.

The commercial powder mainly consisted of two types of particles, "flakes" and "seed parts". To validate the positive impact of particle size reduction on extraction efficiency, capsaicinoids characterization were performed on each part, including the commercial powder and the grounded powder. These characterizations highlighted the heterogeneity of the different parts of the powder constituents with capsaicinoid contents of 3.17 mg ·$g^{-1}$ DM of flakes and 1.25 mg·$g^{-1}$ DM of the seed fragments (Table 2). Despite variations of 12.2 and 11.2 % for flakes and seed fragments respectively, there was a significant difference in capsaicinoids contents between the isolated particles. The capsaicinoids content of commercial dried red chili pepper was 2.83 mg·$g^{-1}$ DM, also reflecting its heterogeneity with a variation of approximately 4.4 %. The grounded product showed a similar capsaicinoids content of 2.79 (0.01) mg·$g^{-1}$ DM but with a much lower variation coefficient around 0.3 % (Table 2). Therefore, the first low-tech recommendation would be to ground the samples beforehand, to ensure greater homogeneity of the raw material and therefore of the subsequent extract. Moreover, it is known that the reduction in particle size during grounding inevitably have an impact on the extraction of bioactive compounds, up to a certain particle size, which depends on the matrix and the type of solvent used (Chemat et al., 2017; Chemat & Strube, 2015).

### 3.2. Extraction of reference (water & petrol)

All experimentations conducted in this work to optimize the migration of capsaicinoids from grounded red chili pepper by maceration in 100 % water led to null concentrations. Across all experiments ($n = 6$), no capsaicinoid diffusion in water was observed, regardless the temperature from ambient to 95 °C or extraction duration from 20 min to 6 days. This was probably due to their hydrophobicity. Turgut, Newby and Cutright (2004) proved that capsaicin must be dissolved, i.e. in a dispersible form, before be dispersed in water, which can explain this result.

**Table 2**
Capsaicinoids content in dried commercial and grounded red chili pepper. DM = Dry matter.

| Dried red chili pepper | Capsaicinoids mg·$g^{-1}$ DM | Standard deviation mg·$g^{-1}$ DM | % variation |
|---|---|---|---|
| **Flakes** | 3.17[a] | 0.39 | 12.24 |
| **Seeds** | 1.25[b] | 0.14 | 11.20 |
| **Raw commercial** | 2.83[a] | 0.13 | 4.44 |
| **Grounded** | 2.79[a] | 0.01 | 0.28 |

Means calculated using 3 repetitions. Same letters in column express non-significant difference between value group by means of ANOVA – Tukey test ($p < 0.05$).

Due to limited knowledge and of a lack of adapted local resources, many farmers use unleaded petrol as an extraction solvent to produce repellent. In addition to the danger for workers, this practice unreasonable and problematic in these protected environments has environmental impacts (pollution, non-sustainable fossil fuels, etc.). The performance of this conventional field method was evaluated and led to an average extraction yield of 66 %, which served as the reference point for the comparison of more eco-responsible and sustainable applications.

### 3.3. Hydroalcoholic extraction

#### 3.3.1. Preliminary test: effect of solid/liquid ratio

The extraction ratio is important to be consider when extracting compounds of interest. It is generally a critical factor limiting extraction yields. Fig. 2 shows both the capsaicinoids yield (i.e. the diffusion efficiency) and the concentration in the resulting ethanol (i.e. the final dilution of the repellent). In the case of hydroalcoholic extraction of capsaicinoids, the Fig. 2 shows that below a ratio of 1/5 (w/w), the extraction yields remained above 55 %. Yield is important for industrial application, as it optimize the production by minimizing losses and reducing overall operational costs. However, from a practical point of view, the concentration of active compounds in the extract is equally important. Indeed, maximizing yield may be less critical than obtaining a more concentrated extract, especially when raw material costs are low

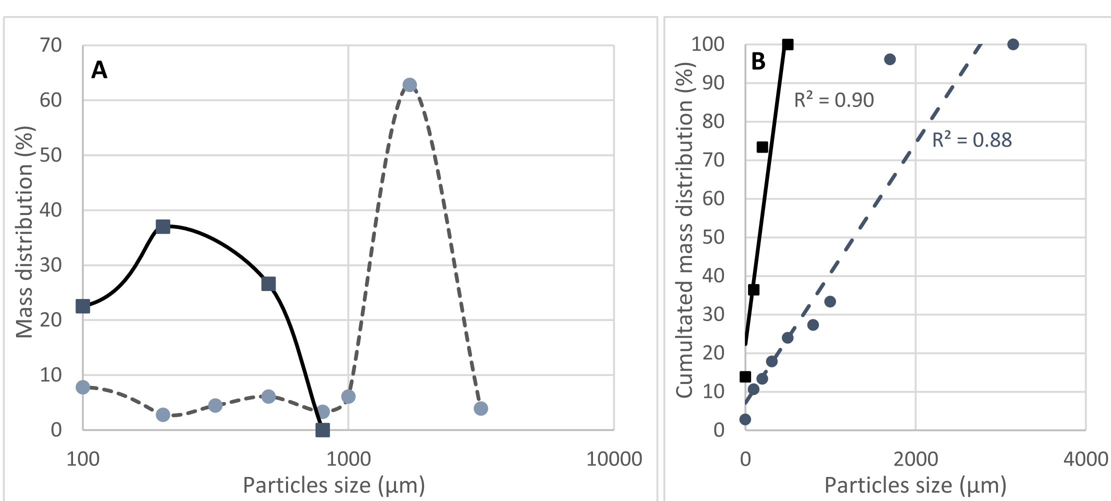


**Fig. 1.** A. Mass distribution (%) vs particle size of raw commercial dried chili pepper (dashed line) and grinded sample (solid line) and B. Cumulated mass distribution (%) vs particle size of raw commercial dried chili pepper (dashed line) and grinded sample (solid line).

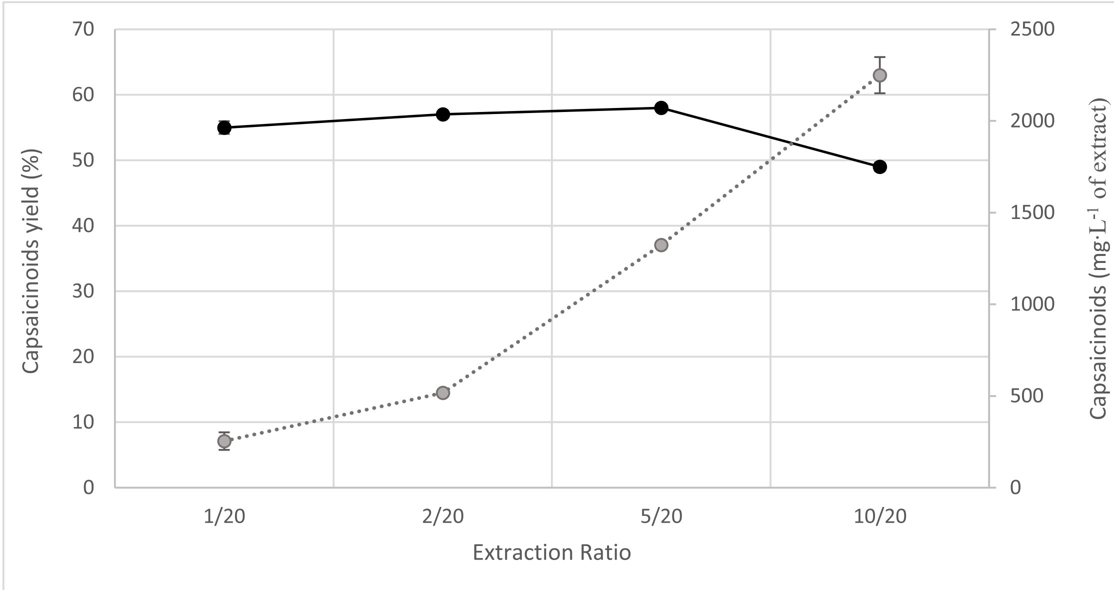


**Fig. 2.** Capsaicinoids yield (—◆— left axis) and content in mg·L of extract$^{-1}$ (···●··· right axis) for 4 extraction ratios (1/20, 2/20, 5/20 and 10/20 wt/weight) – ethanol 70 % at 70 °C for 45 min.

for farmers and higher potency is preferred for effectiveness. For our study, the choice of a 1/5 (w/w) yield seemed the most interesting in terms of maximizing extraction yield while ensuring maximum extraction of capsaicinoids in the solvent. However, another factor that needs to be taken into account is the imbibition of the solvent by the red chili pepper powder. In the case of the hydroalcoholic solvent, the samples absorbed an average of 2.5 mL solvent·g$^{-1}$ of powder, a part of the extract that is difficult to recover without specific syneresis methods. Therefore, beyond a ratio of 1/2 (w/w) the absorption of the extraction solvent by the dry chili pepper is too high and extract cannot be recovered by low-tech methods. At this ratio, the operation is no longer feasible, even if the capsaicinoids concentration did not appear to reach an upper limit (Fig. 2). Similarly, a ratio of 1/5 only recovered around 50 % of the extraction volume, whereas a ratio of 1/10 (which was

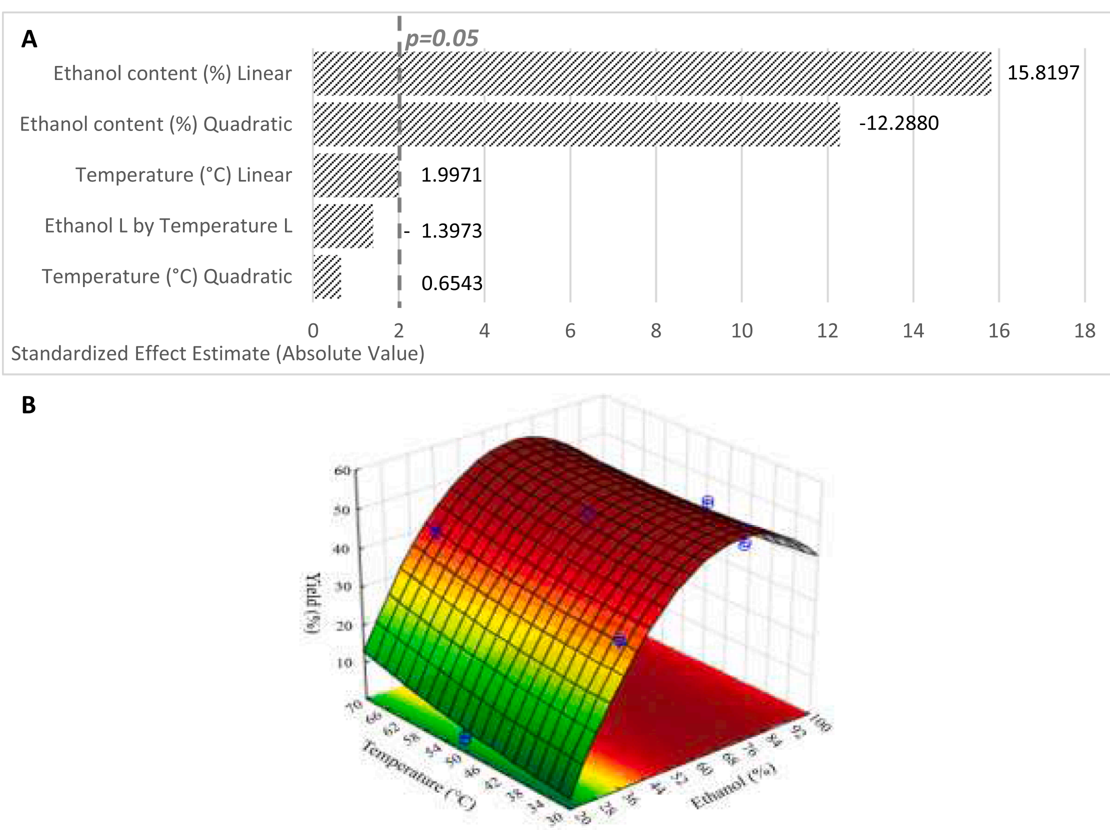


**Fig. 3.** A. Pareto chart of the experimental design at 2 factors (temperature and ethanol ratio) of capsaicinoids extraction in hydroalcoholic solvent and B. Response surface of capsaicinoids yield as a function of temperature and ethanol ratio. The blue circle shows the experimental points realized.

chosen for the experimental design) recovered over 75 % of the solvent volume added for extraction.

Interestingly, this ratio of 1/5 was the same used for the unleaded petroleum extraction, likely based on farmers' empirical observations regarding chili pepper extraction efficiency and the perceived effectiveness of the resulting extract as a repellent.

#### 3.3.2. Experimental design

With a linear regression coefficient of 0.97, the Pareto chart from Doehlert's screening design analysis showed a significant impact of ethanol content on capsaicinoids yield (Fig. 3). This factor, which had the greatest impact, was significant for both linear and quadratic effects. The temperature had no significant impact on capsaicin yield, as its standardized effect was below the 5 % threshold. Furthermore, there was no significant interaction between the two factors over the range of the operating conditions studied. The effects of the factors studied in the design on capsaicinoids yields were represented by response surfaces (Fig. 3). The highest capsaicinoids extraction yields (approximately 55 %) was observed for ethanol contents between 60 and 75 %. Beyond that, extraction yields dropped significantly, down to 40 % for 100 % ethanol. Similarly, reducing the proportion of ethanol in the extraction solvent significantly reduced the yield, down to <5 % at 20 % ethanol (limit of the experimental design), with nothing when full water was used in similar conditions. As shown in the Pareto diagram, temperatures between 30 and 70 °C had relatively little effect on extraction yield. Increasing temperature tended to improve yield slightly for temperatures below 70 °C.

#### 3.3.3. Diffusion kinetic

The screening design experiments were also carried out using kinetics with 7 extraction times between 10 and 300 min (10, 30, 45, 60, 80, 120 and 300 min). The aim of these measurements was to determine the importance of extraction time for practical application (Fig. 4). For each operating condition, defined by ethanol concentration and temperature, the maximum extraction yield appeared to be reached within the first few minutes, after which it stabilized and remained constant up to 300 min. The extract made with 20 % ethanol did not exceed a yield of 4 %. These results confirm that time is not a limiting parameter during this hydroalcoholic extraction, and that a practical protocol can be implemented in the field in just a short time (just a few minutes). Moreover, once the maximum yields were achieved for each operating condition, capsaicinoids concentrations remained stable even at a temperature of 67 °C. These observations support initial operational recommendations, as the observed yields differed by only 10 % compared to those obtained through conventional petrol extraction. In addition, unlike petroleum extraction with which the extraction residue cannot be reused, after ethanol extraction it may be advisable to reuse it for a second extraction. These results are both consistent and complementary with Dong et al. (2014) who also demonstrated the benefits of increasing ethanol concentration to extract more capsaicinoids, without however proving the drop in process efficiency beyond a critical ethanol content probably because they limited their ethanol content at max 70 %. Moreover, their study started with conditions above 60 min of extraction and 50 °C, which appears far too high for practical application, increasing the cost of the operation disproportionately to the potential gain in extraction yield.

### 3.4. Soybean extraction

#### 3.4.1. Experimental design

Extraction with soybean oil also enabled efficient extraction of capsaicinoids. With a linear regression coefficient of 0.94, the Pareto chart illustrating the standardized effects of operating conditions on extraction (Fig. 5) showed a significant impact of extraction ratio and temperature, with extraction ratio being by far the most influential parameter. The quadratic effects of the operating conditions were not significant. These conclusions are also supported by the surface response shown in the Fig. 5, with the yield showing a clear linear trend decreasing while extraction ratio increase. The maximum yield obtained with this extraction strategy was over 60 % from a solid/oil ratio (w/w) of 2. This yield varied by approximately 10 %, ranging from 60 % to 70 % (at a solid/oil ratio (w/w) of 2), depending on the temperature across the 30 °C to 70 °C range. The maximum yield was clearly achieved at the edge of the experimental range – corresponding to the lowest solid-to-oil ratio (2:20 w/w) and the highest temperature tested (70 °C). On the one hand, capsaicinoids seem to disperse better in oily media than in hydroalcoholic solvent. On the other hand, soybean oil extract is much more mechanically complex to separate, and some red chili pepper flakes are found in the oil after the solid/liquid separation steps. These flakes are more loaded with capsaicinoids than the rest of the sample (Table 2), and may therefore influence the measurements observed.

However, as previously described, yield cannot be the sole selection

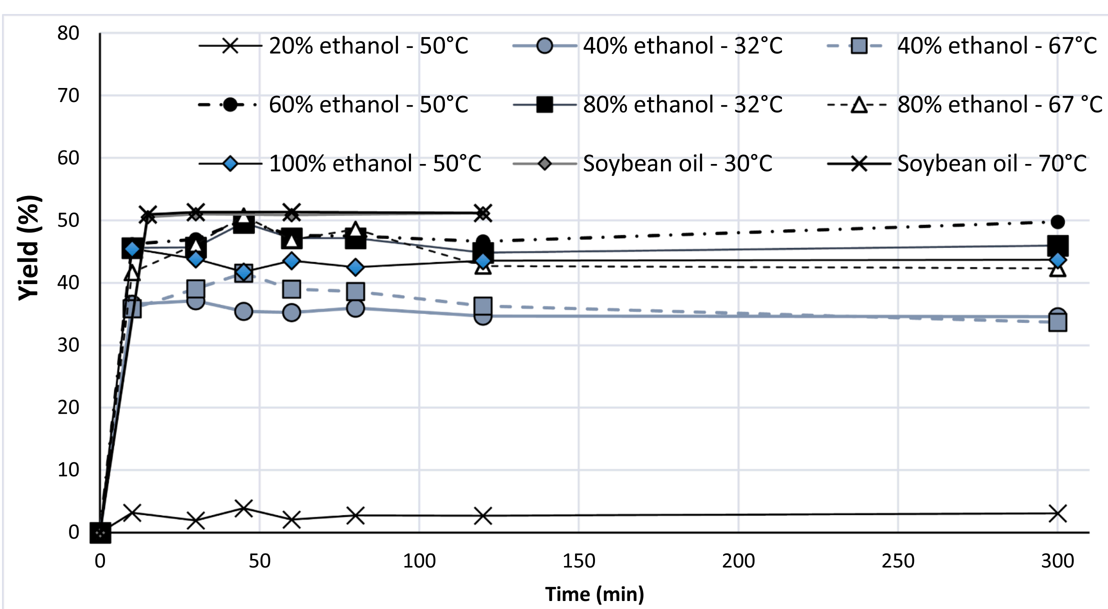


**Fig. 4.** Kinetics of yield of capsaicinoïds in % for each couple of operating condition used for the experimental design, all at the same ratio.

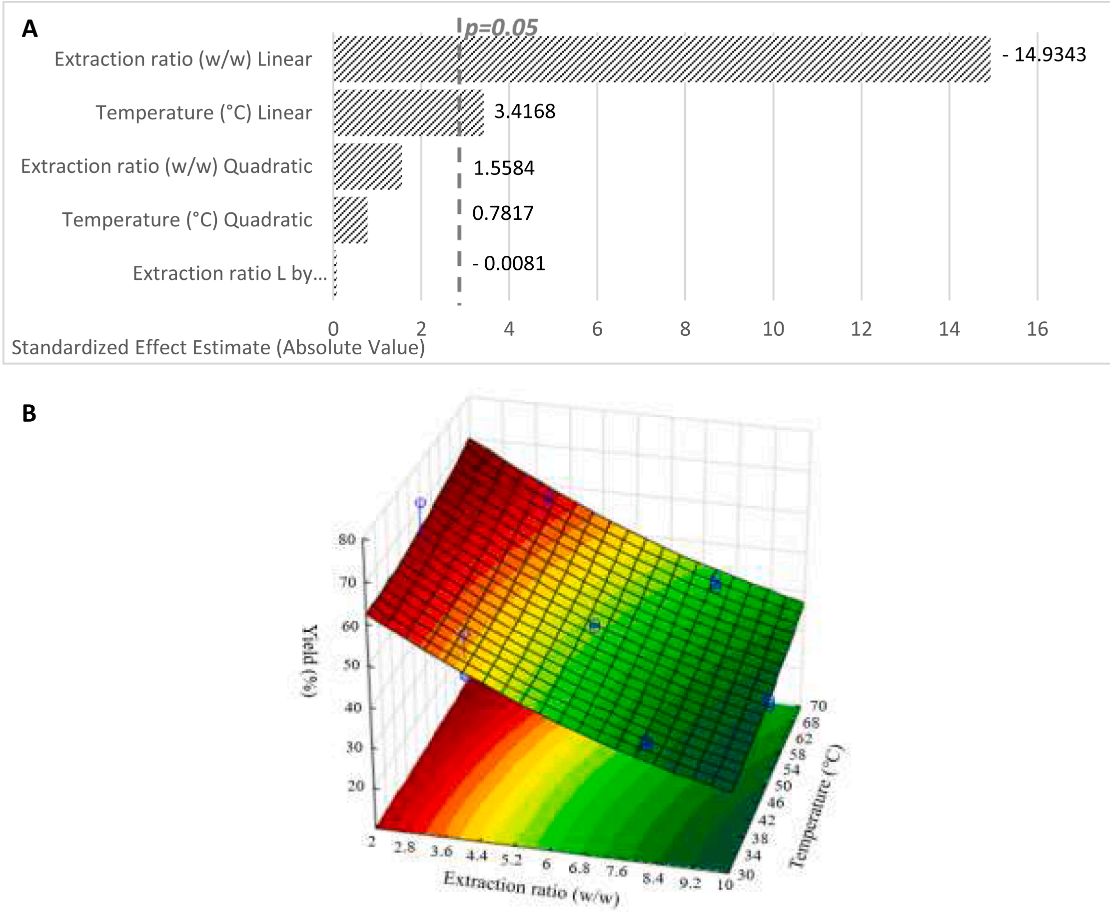


**Fig. 5.** A. Pareto chart of the experimental design at 2 factors (extraction ratio and temperature) of capsaicinoids extraction in soybean oil and B. Response surface of capsaicinoids yield as a function of extraction ratio and temperature using soybean oil. The blue circle shows the experimental points realized.

criterion for selecting optimal conditions. In contrast to ethanol extraction, oil imbibition by red chili pepper powder was not a function of extraction ratio, with a recovery of around 58.3 % (0.1) of the volume of oil added. Fig. 6 shows the relation between the concentration of capsaicinoids and the yield of the extraction and so illustrates the discrepancy between extraction yield and capsaicinoids concentration in

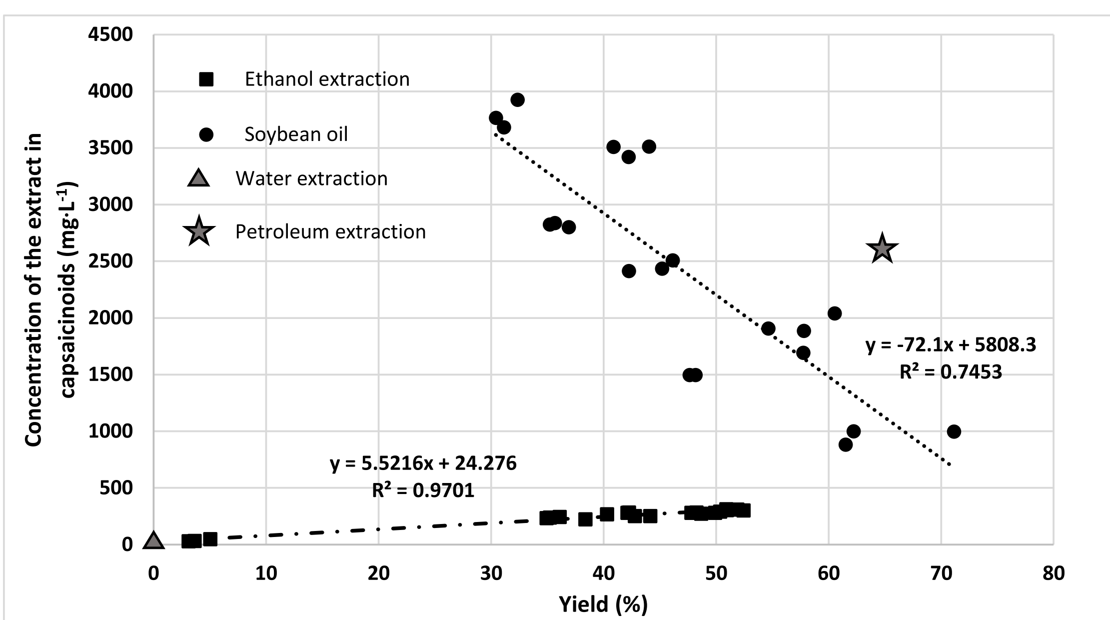


**Fig. 6.** Concentration of capasaicinoids (mg·L$^{-1}$) vs their corresponding yield after ethanol or soybean oil extraction.

the extract. On the one hand, for ethanol extraction under fixed-ratio operating conditions, there is a linear relationship between increasing yield and the concentration of active compounds in the extract. At this ratio of 1/10, reaching an amount of capsaicinoids close to the petroleum reference (2600 mg·L$^{-1}$) is not possible. However, the ratio might be increase at 1/5 to reach capsaicinoids content around 1400 mg·L$^{-1}$ (Fig. 2) with the sacrifice of more than half of the extraction solvent. This choice between processing costs or capsaicinoids concentration of the extract must be carefully done, after a rigorous study of the efficiency of the extracts as repellent. As a result, without more information on products efficiency, to maximize the repulsive power of the product at the ratio 1/10, the maximum yield (i.e. also maximum concentration) should be choosen. On the other hand, for soybean oil extraction (for which the ratio changes), the relationship between extraction yield and concentration is anti-correlated. The best extraction yields correspond to the lowest capsaicinoids content. However, content in oil extracted during 45 min were always superior to the content in the hydroalcoholic solvent, no matter the extraction ratio. So, maximizing yield will probably result in the least active products. It is therefore advisable to minimize the yield to obtain a strongest effect of the product.

### 3.4.2. *Diffusion kinetic*

The effect of extraction time was again evaluated outside the experimental design (Fig. 4), and no significant impact was observed between 15 and 200 min at both 30 °C and 70 °C. This indicates that the majority of the diffusive transfer occurs within the first 15 min. At a solid-to-oil ratio of 2 (w/w), the average extraction yields measured at five time points between 15 and 120 min were 72.63 ± 0.37 % at 30 °C and 73.02 ± 0.25 % at 70 °C."

## 3.5. *Technical recommandations*

The optimum points of this data set can be reported in practical technical data sheets to facilitate understanding by non-specialists, using simple tools accessible in local farming contexts (Fig. 7). Farmers thus have two efficient low-tech choices for gradually replacing unleaded petroleum in 15 min maceration. They can select the ethanol extraction method, that allow to produce with quick tools an extract for application on the fences, 6 times less rich in capsaicinoid than petrol extracts, but whose residue can be immediately reused to produce a second batch. Or they can choose the oil extraction method, which takes longer than ethanol because of viscous operations but is still easier and safer to use than petroleum, enabling concentrations up to 1.5 times higher than the latter.

## 4. Conclusion

These results obtain by the means of experimental designs, complement existing literature, and help to define optimal field-level operating conditions for producing repellent from locally sourced red chili peppers, tailored to the resources available. On one hand, if farmers have access to alcohol, the extraction process becomes simpler, particularly due to easier handling and filtration associated with hydroalcoholic solvents. Choosing a ratio of 1/5 achieved the highest possible yield (50 %) associated with the highest capsaicinoid content (≈ 400 mg·L$^{-1}$) under these conditions. On the other hand, the absence of alcohol is not a major limitation, as oil-based extraction remains a viable alternative. However, it was advised with this method to minimized the yield (30 %) to reach the highest content of capsaicinoid (≈ 3600 mg·L$^{-1}$), 1,5-fold better than unleaded petroleum one. All recommended methods achieve capsaicinoid extraction within 15 min, and need a griding step in order to ensure the best and fastest extraction possible. However, the question of comparative efficacy between the two extract types remains open. Hydroalcoholic and oily extracts may behave differently when applied to physical barriers (such as wooden fences), particularly in terms of immediate repellent effect, persistence over time, ease of storage, and resistance to rain or leaching. Field trials, conducted in collaboration with farmers, will be essential to evaluate these differences and complement the current biochemical findings.

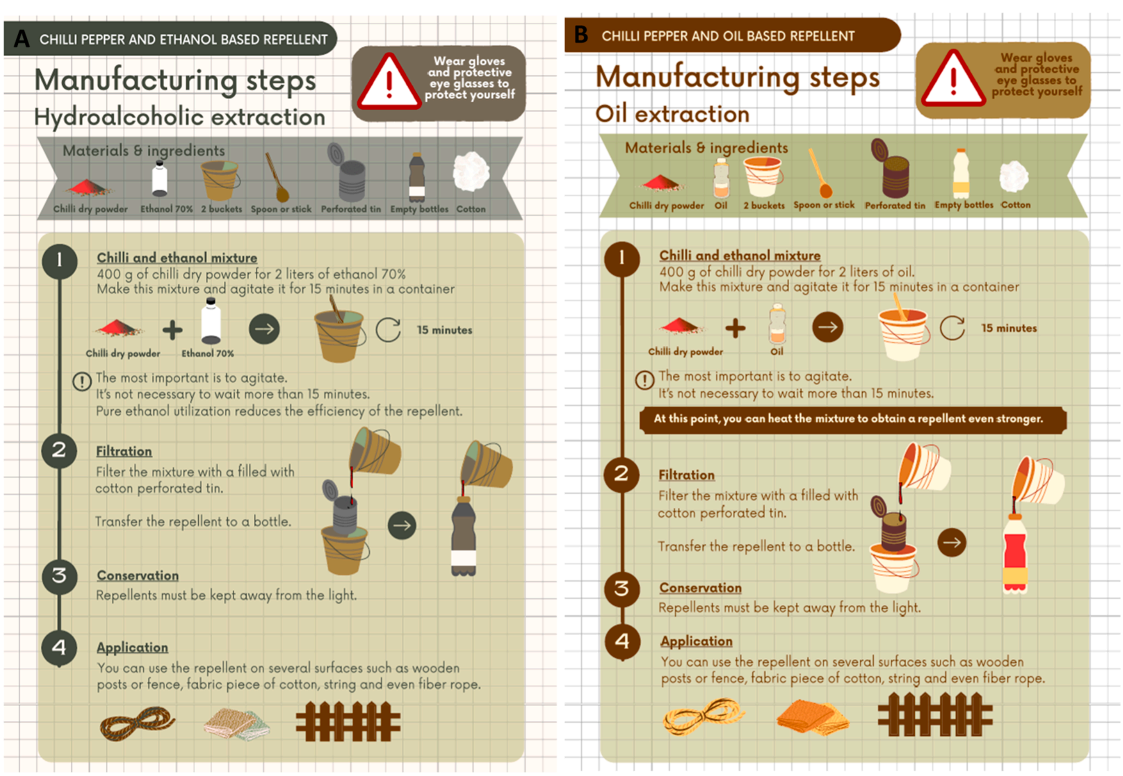


**Fig. 7.** Practical guide for farmers for the production of a repellent using A. hydroalcoholic solution and B. soybean oil as solvents.

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## Declaration of generative AI in scientific writing

Authors did not use AI in the submission.

## Ethical statement

No humans or animals was involved in this study.

## CRediT authorship contribution statement

**Adrien Servent:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nawel Achir:** Writing – review & editing, Supervision, Software, Methodology, Conceptualization. **Camille Vivas:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sebastien Le Bel:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Muhammad Faizan Usman:** Writing – review & editing. **Mathieu Weil:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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