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Nota técnica

NOVEL LEAF MACERATION TOOL FOR LABORATORY PROCEDURES

Nova ferramenta para maceração de folhas para procedimentos de laboratório

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Resumo: A maceração de folhas é utilizada em vários protocolos laboratoriais. O método mais comum é realizado com almofariz e pistilo. Essas ferramentas são milenares e executam satisfatoriamente o procedimento de maceração, embora seja trabalhoso e cansativo. O presente trabalho apresenta uma ferramenta e um método inovador para maceração foliar. Para esse fim, foi construído um macerador metálico, constituído por um cabo, um eixo, rolamentos e arruelas, que funciona como um mini rolo compressor. O processo de maceração consiste em rolar o macerador sobre um saco plástico contendo a amostra de folha e a solução extratora, apoiado sobre um pano. Para testar o equipamento, foram realizadas macerações em folhas de maracujazeiro com o método aqui proposto em comparação com o método tradicional, utilizando almofariz com pistilo. O tempo de maceração com o novo macerador foi em média 42,98% menor que o método tradicional, com almofariz e pistilo. Além disso, o processo de maceração é menos trabalhoso e permite a maceração de um maior número de amostras sem risco de contaminação.

Palavras-chave: extração, fitopatologia, fisiologia, método de maceração, novo equipamento.

Abstract: Leaf maceration is used in several laboratory protocols. The most common method is performed with a mortar and pestle. These tools are ancient and perform the maceration procedure satisfactorily, although it is laborious and tiring. This work presents an innovative tool and method for leaf maceration. For this purpose, a metal macerator was built, consisting of a handle, an axle, bearings and washers, which works like a mini steamroller. The maceration process consists of rolling the macerator over a plastic bag containing the leaf sample and the extracting solution, supported by a cloth. To test the equipment, macerations were performed on passion fruit leaves with the method proposed here in comparison with the traditional method, using a mortar and pestle. The maceration time with the new macerator was on average 42.98% shorter than the traditional method, with mortar and pestle. Furthermore, the maceration process is less laborious and allows the maceration of a greater number of samples without the risk of contamination.

Keywords: extraction, maceration method, novel equipment, physiology, phytopathology.

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In laboratory procedures, a mortar and pestle are used to macerate leaves and other parts of plants to quantify photosynthetic pigments (PETIBON & WIESENBERG, 2022), bioactive compounds (VONGSAK *et al.*, 2013), antioxidant enzymes and hydrogen peroxide (IBRAHIM *et al.*, 2023), in protocols for mechanical acquisition/transmission of plant viruses (SPADOTTI *et al.*, 2021), serological tests (BHAT & RAO, 2020) and crushing of samples for extraction of deoxyribonucleic acid (DNA) (MATISOO-SMITH *et al.*, 2016).

Although maceration using mortar and pestle is a well-established method in scientific procedures, the method is tiring and laborious when using a large number of samples because one needs to apply force in circular movements by pressing the samples against the mortar using the pestle, which results in friction between the hard surfaces of these two utensils. When used to acquire a solution with viral particles, the same utensil needs to be sterilized for subsequent use (BHAT & RAO, 2020). The present work reports a new method of faster and less tiring leaf maceration for plant physiology and phytopathology protocols.

A macerator was constructed using a 120 mm-long segment of stainless-steel tube measuring 31 mm in diameter and 3.5 mm in wall thickness, from the front shock absorber of a motorcycle; a 31 mm-long segment with 5 mm-thick welding electrode core; four roller bearings with external diameter of 11 mm, internal diameter of 5 mm and thickness of 4 mm; and eight 5x10 mm steel flat washers (Figure 1A-C). The electrode core served as an axle for the assembly of a mini roller-compressor made with the bearings and washers (Figure 1D).

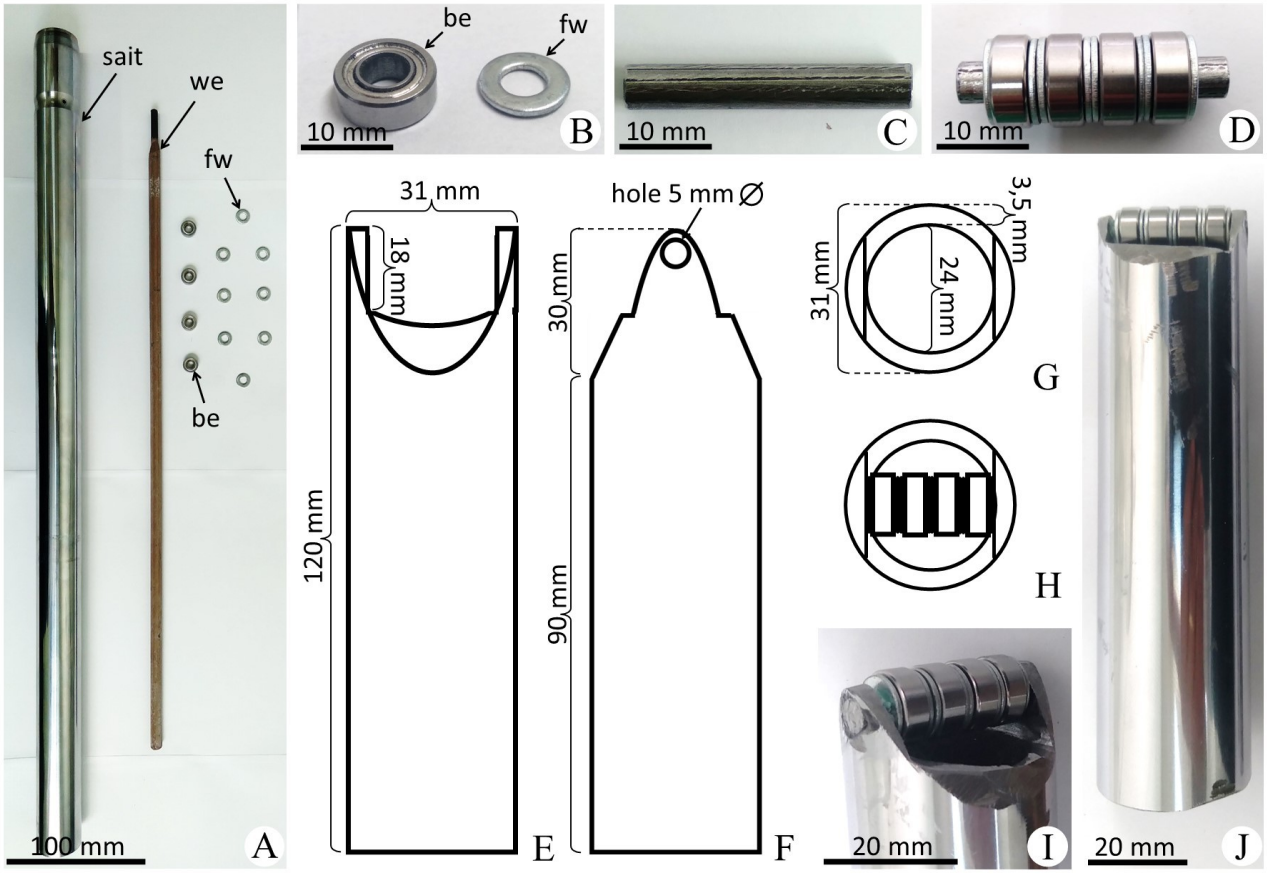

Figure 1. Construction of the macerator. (A) Materials. (B) Bearing and washer. (C) Welding electrode segment. (D) Mini roller. (E) Scheme of the handle made with the shock absorber rod in lateral view. (F) As in (E), but rotated 90°. (G) As in (E), but in top view. (H) As in (G), but with the mini roller installed. (I) Detail of the top of the macerator. (J) Whole macerator. Legends: be- bearing, fw- flat washer, sait- shock absorber inner tube, we- welding electrode.

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The stainless-steel tube segment was then molded to form a handle (Figure 1E-G) to hold the axle carrying the roller (Figure 1H-I) at a wedged end (Figure 1G-I) with a 5 mm diameter hole on each side (Figure 1F). The axle was fixed in the holes with superglue (Superbonder Loctite®) in such a way that only the bearings could rotate freely. The wedge shape of the handle allows the free rotation of the bearings even when holding the macerator at an angle. The final appearance of the macerator is shown in Figure 1J.

To use the macerator, the leaf sample was added to a polypropylene bag (10x15 cm) together with solvent or buffer. The bag was placed on a folded cloth and pressed with the macerator in a back-and-forth motion (Figure 2A-B).

The efficiency of the method (Figure 2A-B) was compared with that of the traditional method (mortar and pestle) (Figure 2C). 500 mg samples of passion fruit leaves (*Passiflora edulis* Sims) were macerated using 5 mL of water as a solvent. Ten repetitions were performed for each treatment. The samples were properly packed in polypropylene bags or in mortars containing the solvent, and then macerated until a satisfactory result was obtained to simulate virus extraction for inoculation (Figure 2D). The maceration was performed by the same person with experience in both methods, alternating each repetition of each treatment, where 20 repetitions were made in sequence. Each maceration procedure was timed using a Casio G-Shock dw5600 watch, considering only the seconds. The time data were subjected to analysis of variance using the SISVAR statistical software (FERREIRA, 2011), and means of each treatment were compared by the t-test.

Maceration times differed between the methods, with average of 46.3 and 26.4 seconds for mortar and pestle and macerator, respectively (Figure 2E). The use of the macerator reduced the maceration time by about 42.98 % compared with the traditional method using mortar and pestle, and provided similar results in terms of leaf material fragmentation (Figure 2D).

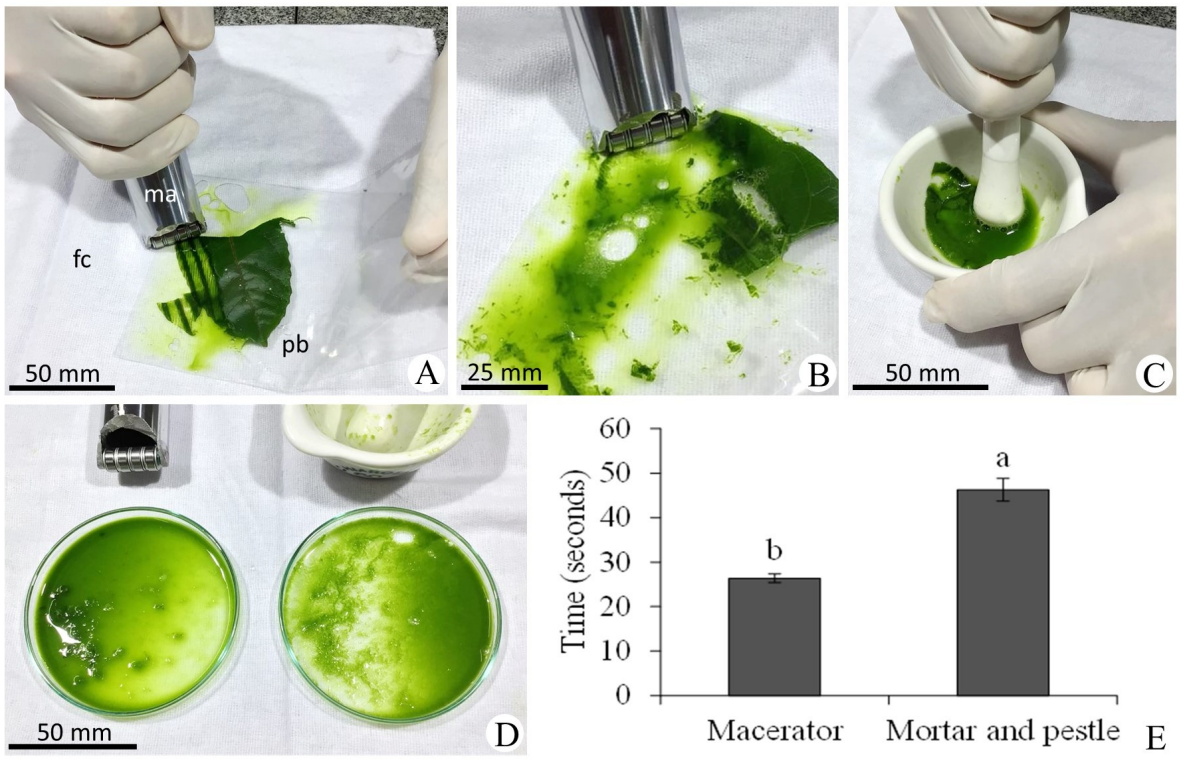

Figure 2. Comparative test of the marcerator. (A) Maceration with the macerator. (B) As in (A), but in advanced maceration process. (C) Mortar and pestle maceration. (D) Samples macerated by the macerator (left) and by the mortar and pestle (right); (E) Graph with average maceration times. Means accompanied by lowercase letters differ between maceration methods (t-test, $p < 0.01$). Legends: fc- folded cloth, ma- macerator, pb- polypropylene bag.

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The maceration method presented in this work proved to be faster, more comfortable and less tiring because the macerator slides smoothly over the plastic bag containing the sample. In addition, the back-and-forth motion for maceration is less strenuous than the circular movements required for maceration with a mortar and pestle. Therefore, we believe that if each treatment were tested with ten repetitions sequentially, the time difference would be even greater.

In the method developed here, samples are macerated in cheap, disposable plastic bags, thus allowing the maceration of numerous samples in a short space of time without risk of contamination. When building the macerator, the following considerations should be taken in account: metal tube with thick walls, small and narrow bearings with a small gap between the bearings and washers, no lateral vibration of the bearings, and an easy rotation.

The elaboration of the present work was another initiative aimed at working with science in an optimized, economic, and accessible way (LEITÃO & CORTELAZZO, 2008; WU *et al*., 2016; LEITÃO, 2018; LEITÃO & CAIRES, 2024).

This tool proved to be very useful for macerating leaves to perform serological tests and phytovirus transmission, being widely used in these laboratory practices. The method presented here is more efficient than the traditional method for maceration of leaves in solvent and is therefore an innovation for this procedure.

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