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Effective homemade V8 juice formulation as alternative suitable medium for culturing *Phytophthora infestans*

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Abstract

A suitable growth medium is essential for isolating, studying, and managing pathogens. To mitigate the importation dependence and availability of commercial V8 growth medium, three V8 juice formulations (F1, F2, and F3) were developed and tested in this study for their potential to stimulate the growth and sporulation of *Phytophthora infestans* at different growing temperatures in comparison with commercial V8 juice formulation. The results showed that the three V8 juice forms successfully induced the growth and sporulation of *P. infestans* at varied temperature regimes. However, V8 juice F2-based medium significantly caused quicker growth of *P. infestans* and increased in number and size of sporangia as well as the commercial formulation (FC) of V8 juice. In addition, the pathogen successfully grew at 15 ± 2 °C, 20 ± 2 °C, and 25 ± 2 °C; however, the plates incubated at 20 ± 2 °C displayed faster growth of the colony as compared to those incubated at the other two temperatures. Furthermore, more spores were obtained on the plates incubated at 15 ± 2 °C compared to the plates incubated at 20 ± 2 °C and 25 ± 2 °C. Overall, the V8 F2-based media was more suitable for *P. infestans* growth and sporulation. This finding is critical since it helps to lessen reliance on V8 juice imports and increases available V8 juice to culture or mass produce the pathogen. Consequently, further research is encouraged for sustainable management of *P. infestans*.

Article highlights

- Homemade V8 alternative formulations support *P. infestans* growth and sporulation.
- However, F2 formulation performs similarly to the commercial with quicker growth and increased number and size of sporangia.
- The pathogen grew best at 20 °C, but produced more spores at 15 °C.
- Local V8 alternatives can minimize dependency on imports and support future research on managing the pathogen.

Keywords Formulation protocol, *Phytophthora infestans*, Late blight disease, *Solanum tuberosum*, *Solanum lycopersicum*

1 Introduction

Since 1845, the plant pathogen *Phytophthora infestans* (Mont.) de Bary- causal agent of late blight disease, has remained a severe threat to global potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) production [1]. This disease has been responsible for catastrophic tomato yield loss of up to 100% in East Africa if management intervention is delayed [2–5]. Economically, it is estimated that the pathogen contributes to annual potato loss of up to 12 billion US$ annually [6]. Effective control of *P. infestans* requires a deep understanding of its growth requirements, including media and environment. *Phytophthora infestans* can grow on a variety of media, including Potato Dextrose Agar (PDA) as a non-selective medium, rye-based media with agar, Pea Broth, Unclarified V8/Lima bean Agar, selective media, corn meal agar (CMA), soybean agar, carrot agar, oat meal agar (OMA), and V8 agar medium [7–10]. However, V8 agar medium was reported to be the most suitable medium for *P. infestans* growth [9].

The use of V8 juice in the field of pathology for media formulation was first reported by Caten and Jinks in 1968 [1, 9]. Since then, numerous researchers have emphasized the potential of V8 juice media based for the isolation, identification, and study of fungal pathogens such as *Pythium* spp., *Phytophthora* spp., and *Botrytis cinerea* [1, 11–13]. Despite the popularity of V8 agar medium in the studies of *P. infestans*, the unavailability of commercial V8 juice as a key constituent of this medium limits major research approaches of this pathogen, not just in East Africa but also in other regions of the world [14]. There is therefore a strong need to search for alternative growth media that could effectively substitute V8 juice to fill this research gap in exploring sustainable management of this major plant pathogen.

Historically, the name V8 juice refers to a cocktail produced from a blend of eight vegetables (V8). It was first initiated in Chicago, USA by Frank Constable, an employee of William Gilbert Peacock in 1933 [15]. The juice gained popularity as the year progressed due to its nutritional benefits, although its use in Africa is limited. Currently, commercial V8 juice used in Africa is mainly imported from Italy, the UK, USA, and Australia, making its availability and affordability difficult in East Africa region. Several researchers, including Hardina et al. [16] highlighted the basic constituents of the juice, although, their quantities/proportions remain unrevealed. Furthermore, comparative analyses between various standardized V8 juice formulations and commercially available alternatives remain unexplored. Consequently, this study aimed to optimize homemade V8 juice formulations to improve local availability and reduce import reliance, while maintaining effective *P. infestans* growth conditions and supporting sustainable management strategies of the pathogen.

2 Materials and methods

2.1 Study area, ingredients, and tools used

The study was conducted at the Arthropod Pathology Unit (APU) laboratory of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville campus, Kasarani, Kenya. All the eight vegetables used in V8 juice formulation were locally available in Kenya and which were organically grown. Six vegetables including tomato, carrot, celery, beetroot (red), parsley, and spinach were purchased in Githurai market (Nairobi, Kenya), while lettuce (romaine/Slada) and watercress were obtained in Zucchini Greengrocers

Limited (Village market, Nairobi, Kenya) (Fig. S1). Salt, lemon and garlic were purchased in the nearest shop (Duka), while distilled water was obtained in the APU laboratory.

2.2 Quantities and proportions of ingredients for the various formulations

For V8 juice ingredients, it is important to choose the organically grown, fresh ones that have not overstayed in the market. Tomatoes should be well ripened in red color, carrot and beet root should also be in orange and red color, respectively. The V8 juice formulations were based on various homemade V8 juice formulation tutorials, since little or limited information is available on the formulation of commercial one. The first formulation (F1) was made of tomato (650 g), carrot (182 g), celery (68 g), lettuce (40 g), spinach (36 g), parsley (20 g), watercress (8 g) and beetroot (6 g), garlic (2 g), salt (1 g) and one teaspoon of lemon juice extract. The second formulation (F2) was made of tomato (890 g), carrot (60 g), celery (20 g), lettuce (10 g), spinach (10 g), parsley (5 g), watercress (2 g) and beetroot (2 g), garlic (1 g), salt (1 g), and one teaspoon of lemon juice extract. The third formulation (F3) was made of tomato (672 g), carrot (88 g), celery (4 g), lettuce (2 g), spinach (2 g), parsley (2 g), watercress (1 g) and beetroot (1 g), garlic (1 g), salt (1 g) and one teaspoon of lemon juice extract. The final volume of the V8 formulations was 1 L.

2.3 V8 juice formulation protocol

To prepare the juice, start by using a knife to remove the top ends of the carrots and beetroot. Next, wash all the ingredients thoroughly with distilled water and place them on paper towels laid over aluminum foil to drain any excess water. Afterward, measure each ingredient using an accurate weighing balance with a precision of ± 0.001 g. Once measured, transfer the ingredients into a blender, add 100 ml of distilled water, and blend them until well blended homogenous mixture is obtained. Following this, set a sieve in a funnel and position the funnel over a container/bottle. Gradually, pour the blended mixture into the sieve, adding small quantities step by step while compressing the mixture with gloved hands. Once the blending and sieving process is completed, add 1 gram of salt and one tablespoon of lemon juice into the extracted liquid. Thereafter, securely cover the container/bottle and shake it thoroughly to ensure all the ingredients are well mixed. Finally, store the juice at a temperature of at least 4 °C in the refrigerator or freezer to extend its shelf life.

2.4 Effect of the various V8 juice formulations on the mycelial growth and sporulation of *Phytophthora infestans*

A previously isolated *P. infestans* isolate with GenBank accession no. KC677793.1 and MN422922.1 was obtained at *icipe*'s APU germplasm and maintained on Pea agar medium made of 120 g of fresh peas, 7 g crystallized sucrose and 15 g technical agar as described by Gamboa et al. (2019). To prepare V8 agar media, 200 mL of either commercial V8 juice (Campbells Soup Co.) from South Africa (FC: Control/Standard) or the three newly prepared V8 juice formulations (F1, F2, and F3) were mixed with 3 g of calcium carbonate ($CaCO_3$), 15 g of technical agar, and 800 mL of distilled water (H_2O). The media were agitated with a magnetic stirrer inside 500 mL Erlenmeyer flask before being autoclaved for 15 min at 121 °C, 17 psi, and allowed to cool to 45–50 °C. The media were then dispensed onto 90 mm Petri dish plates within a laminar flow hood and allowed to

cool and solidify overnight. The following day, using a sterile corn borer, 5 mm mycelial disks were cut from a pure culture of *P. infestans* grown on pea agar medium as described above and placed upside down on the center of Petri dish plates containing the various treatments of V8 agar media. Four Petri dishes were used per treatment, and the experiment was repeated four times overtime to validate the results. The Petri dish plates were parafilm-sealed and labelled according to the V8 juice formulations used. The various treatment plates were incubated in complete darkness in three different incubators, which were set at 15 ± 2 °C, 20 ± 2 °C and 25 ± 2 °C, and the experimental treatments were arranged in a completely randomized design (CRD) as described by Gomez and Gomez [17].

The ability of different V8 juice formulations in promoting the growth of *P. infestans* was assessed every day for two weeks post-inoculation by measuring the radial growth of the colony from the center of the plate towards its edge using a ruler [18]. Following that, the stock suspension solution was prepared by flooding the cultures with 1 mL of Triton water (0.05% (w/v) Triton X-100 (EMD Millipore Corporation, USA) and scraping their surface using a sterile spatula to remove the sporangia from the mycelia [19]. The collected sporangial suspension was mixed with 9 mL of sterile distilled water containing 0.05% (w/v) Triton X-100 (EMD Millipore Corporation, USA) in a universal bottle and vortexed for 5 min at about 700 rpm to produce homogenous sporangial suspensions. Sporangial counts were performed with an improved Neubauer Hemacytometer, according to Inglis et al. [20]. Furthermore, the effect of V8 formulations on the sporulation of *P. infestans* was also assessed by counting number of spores available in one microliter of stock suspension solution using improved Neubauer Hemacytometer under a binocular microscope at x 40 magnification following the approach described by Inglis et al. [20].

2.5 Data analysis

Before undertaking data analysis, the Shapiro-Wilk test was used to check the normality of the data. Non-normally distributed data such as data on the radial growth and sporulation of *P. infestans* grown on the various V8 agar media based on the different formulations of V8 juice and the effect of the temperatures on the radial growth and sporulation of the pathogen were analyzed using a generalized linear model (GLM) package of R version 4.3.1. 1 [21]. Afterwards, non-normally distributed data were transformed and back-transformed into original units to obtain the real treatment means after analysis. To separate the treatment means, the Student Newman Keuls (SNK) test was applied, and the statistical significance was determined at P value ($P < 0.05$).

3 Results

3.1 Effects of the new V8 juice formulations on *Phytophthora infestans* growth under different temperature regimes

Phytophthora infestans growth performance on the four V8 juice formulations media based and at 15 ± 2 °C, 20 ± 2 °C, and 25 ± 2 °C incubation temperatures were shown in Fig. 1. There were significant differences ($\chi^2 = 29.25$, df = 3, $P = 0.001$; $\chi^2 = 38.26$, df = 3, $P < 0.001$; $\chi^2 = 53.56$, df = 3, $P < 0.001$) among *P. infestans* colonies grown on the four different V8 juice formulations media based and incubated at 15 ± 2 °C, 20 ± 2 °C, and 25 ± 2 °C, respectively, 1 week post-incubation (Fig. 2A). However, no significant

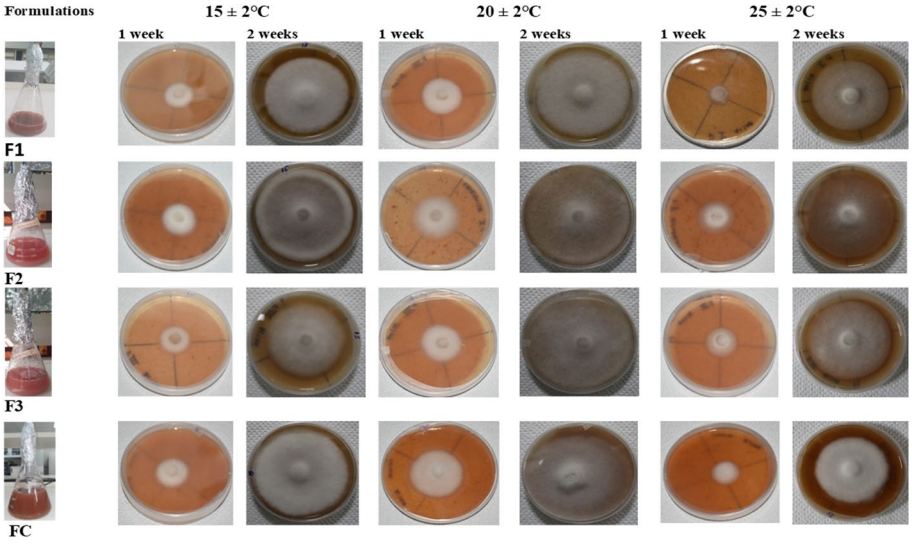

Fig. 1 Colony morphology of *Phytophthora infestans* grown on the various formulations of V8 juice media based and temperatures at one and two weeks after incubation

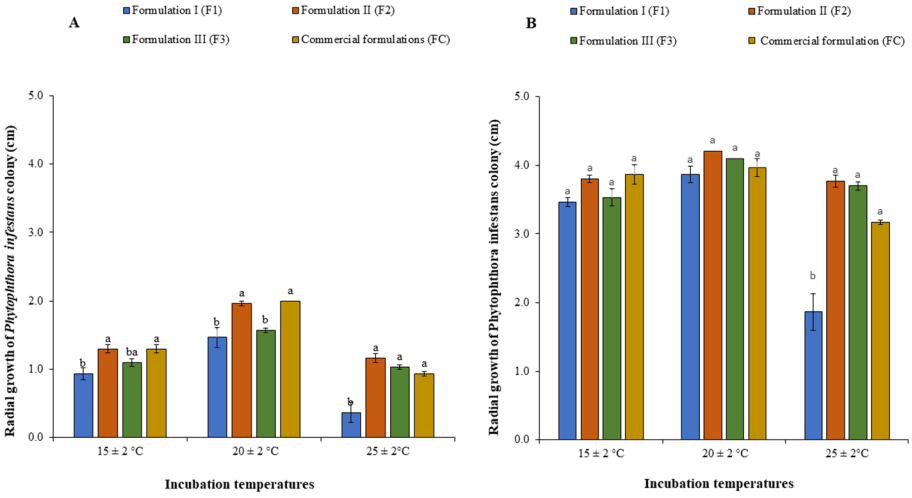

Fig. 2 Mean length ± std error of the *Phytophthora* infestans colonies on V8 agar media in different incubation temperatures: **A** a week after inoculation, **B** 2 weeks after inoculation

variation was observed in the colony growth performance of *P. infestans* grown on formulation 2 (F2) and the commercial formulation (FC) at the three different growth temperatures after 1 week of incubation (Fig. 2A). Furthermore, a significant difference ($\chi^2 = 20.387$, df = 3, $P < 0.001$) was observed among *P. infestans* colonies grown on the four different V8 juice formulations media based and incubated at 25 ± 2 °C at the second week after incubation (Fig. 2B). However, there were no significant differences ($\chi^2 = 3.5$, df = 3, $P = 0.673$; $\chi^2 = 8$, df = 3, $P = 0.46$) observed in colonies of *P. infestans* grown on the four V8 juice formulations media based at 15 ± 2 °C and 20 ± 2 °C, respectively (Fig. 2B). Although *P. infestans* successfully grew on all the assessed temperatures, the 20 °C was the best preferable temperature as it favored faster growth of this pathogen (Figs. 1 and 2).

3.2 Effects of new V8 juice formulations on the sporulation of *Phytophthora infestans* under different temperature regimes

The microscopic examination demonstrated that all the V8 juice formulations were able to induce sporulation of *P. infestans* on V8 agar media (Fig. 3). However, the number and size of produced sporangia differed depending on the type of V8 juice formulation used. For example, numerous sporangia of small size, ranging from 24.6 to 31.7 µm in length were observed in V8 juice formulation 1 (F1) media based, when displayed under microscope. In addition, the produced sporangia in F1 of V8 juice were detached to hyphae in all the three evaluated temperatures. On the other hand, formulation 2 (F2) of V8 juice formulation induced the formation of more sporangia of big size ranging from 32.5 to 57.9 micrometers in length. Additionally, the sporangia in F2 of V8 juice were not adhered to hyphae except at 15 ± 2 °C, where spores were detached to hyphae (Fig. 3).

The V8 juice formulation 3 (F3) media based resulted in few spores of large sizes in the range of 2.59–40.2 micrometers, which were attached to hyphae. A similar observation was seen in the commercial formulation (FC) of V8 juice media based, where the length of sporangia was in the range of 30.3–42.4 micrometers. In contrast to other formulations, the commercial formulation (FC) resulted in very thick-dark spores clustered together in a group of two or more sporangia. Furthermore, there were significant differences ($\chi^2 = 80.258$, df = 3, $P = 0.001$; $\chi^2 = 10.667$, df = 3, $P = 0.05$; $\chi^2 = 106.67$, df = 3,

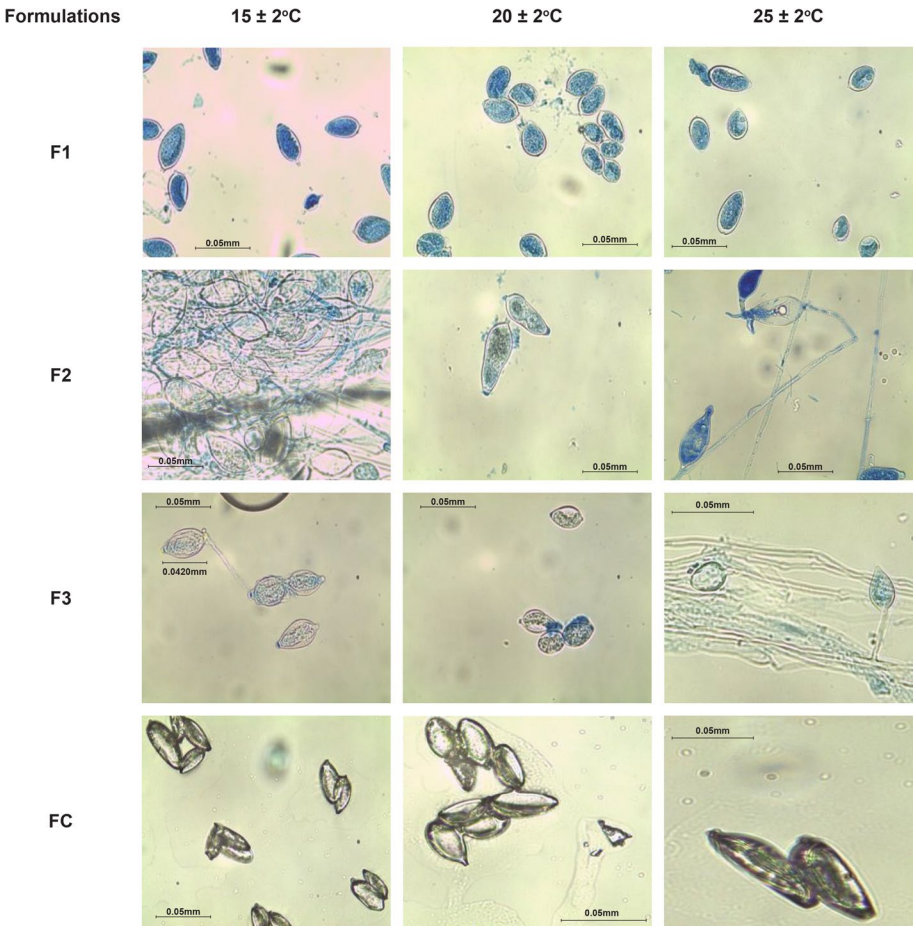

Fig. 3 *Phytophthora infestans* sporangia morphology under the various formulations of V8 juice media based and temperatures

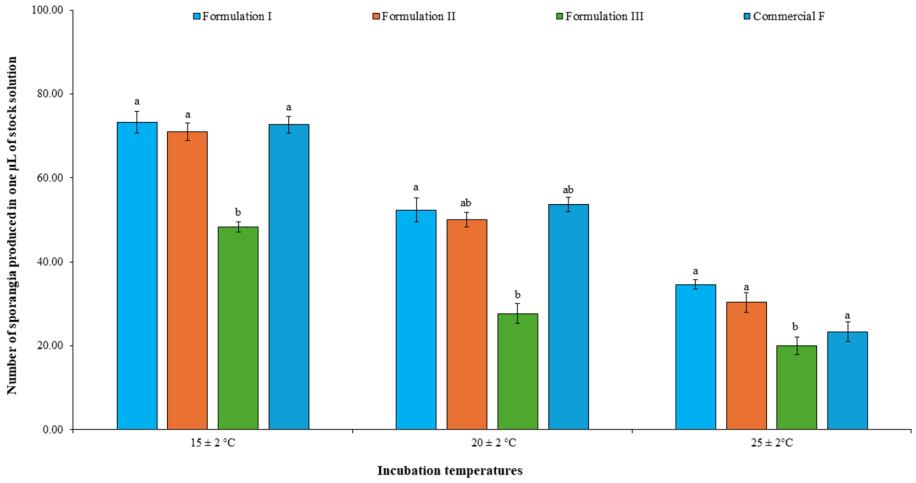

Fig. 4 Mean number ± std error of *Phytophthora infestans* sporangia produced on V8 agar media based on various V8 juice formulations and at different incubation temperatures

$P = 0.001$) in number of spores produced on various formulations of V8 juice media based at 15 ± 2 °C, 20 ± 2 °C, and 25 ± 2 °C incubation temperatures, respectively (Fig. 4).

4 Discussion

The present study aimed at finding out the proper, cheap and effective homemade formulation of V8 juice, establishing its production protocol for improved availability and reduced reliance on imports by using locally available ingredients. In reference to commercial formulation, our findings revealed that the three V8 juice formulations developed were able to favor the growth and sporulation of *P. infestans.* In addition, we observed no significant difference in the colony growth of *P. infestans* when cultured on the V8 juice formulation F2 compared to the commercial formulation (FC). Similarly, the colony growth of *P. infestans* on the V8 juice formulation F3 showed no significant difference when compared to the commercial formulation (FC). On the other hand, formulation F1 did not result in faster growth of *P. infestans* colony as compared to commercial formulation. We hypothesized that the slow growth of *P. infestans* on formulation F1 might be caused by the low amount of tomato as the basic ingredients of V8 juice, since its quantity was the lowest compared to the two other formulations. This finding is in line with previously reported results, which indicated that tomato should account for more than 80% of all active components used in the production of V8 juice [15]. Furthermore, we found that the three V8 juice formulations were able to induce sporulation of *P. infestans* in their respective V8 agar-based media. However, F1 resulted in the production of a huge number of small -sized sporangia compared to other formulations. Additionally, the results of our study showed that the sizes of sporangia were in the range of 0.0246–0.059 mm in length. This finding agrees with the earlier study, which reported an average sporangia length of 0.0605 mm [16].

Besides, the findings of this study demonstrated that *P. infestans* can grow at 15 ± 2 °C, 20 ± 2 °C, and 25 ± 2 °C temperatures. Similar findings have also been reported by other studies [4, 7, 9, 16]. Although the above three incubation temperatures favored the growth and sporulation of the *P. infestans*, our findings revealed that 20 ± 2 °C was the most suitable temperature to favor better radial growth of *P. infestans*, while 15 ± 2 °C was best for the pathogen sporulation. This finding concurred with the previous studies

that highlighted that the optimum temperatures for the sporulation of *P. infestans* range between 8 and 15 °C [9, 22].

Furthermore, the newly formulated V8 juice was enhanced with garlic to improve its selectivity. While garlic has been added to the new V8 juice formulation for its antimicrobial properties [23–25], further optimization of the V8 agar medium remains necessary to ensure better selective pathogen isolation. Several studies have reported that selective media suppress competing microorganisms and encourage the growth of only target pathogens, which help in identifying and studying them accurately [26–30]. For *Phytophthora infestans*, selective media that contain antibiotics (like rifampicin, pimaricin, and ampicillin) and fungicides (such as nystatin and benomyl) prevent bacteria and saprophytic fungi from growing [29]. This allows *P. infestans* to survive without contamination. This selectivity is therefore crucial for reliable pathogen characterization, virulence tests, and fungicide resistance evaluation [28]. Without this specificity, co-occurring might cloud the results and lead to misinterpretations in managing pathogens or diseases. Therefore, refining selective media protocols plays a direct role in the accuracy and consistency of *P. infestans* research studies. Further studies are therefore warranted to enhance or improve on the newly developed formulations for their specificity or selectivity in *P. infestans* isolation, culture and other related research aspects of the pathogen.

5 Conclusion

In this study, we successfully developed three V8 juice formulations using locally available and accessible ingredients for effective growth (culture and mass production) and sporulation of *P. infestans*. Although the three V8 juice formulations favored the activities of this pathogen, formulation II (F2) outperformed the other formulations in reference to the commercial formulation (FC) of V8 juice. Furthermore, we confirmed that *P. infestans* can grow at 15 °C, 20 °C, and 25 °C incubation temperatures; however, our results also demonstrated that the selection of incubation temperatures should depend on the purpose of culturing this pathogen. For example, our findings indicated that, for the production of abundant sporangia, *P. infestans* should be incubated at 15 ± 2 °C, while for quick/faster growth of *P. infestans* colony, 20 ± 2 °C would be the best incubation temperature. Additionally, we found that the size of spores increases with an increase in the incubation temperatures. Overall, we encourage a potential use of the newly developed V8 juice formulation II (F2) as a substitute for the commercial V8 juice for its reduced cost, availability challenges, and reliance on imports. However, further studies are needed on key physicochemical parameters, such as pH, nitrate content, salts, starch, sugars (especially glucose), water activity, and specific gravity. Additionally, basic QA/QC tests should be developed to ensure batch-to-batch consistency, considering factors like plant material variety, growing conditions (e.g., organic or conventional), agroecological zones, and seasonality. These improvements would help refine significantly the formulations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42452-025-07495-z.

Supplementary Material 1

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Author contributions
Conceptualization: KSA and FMK, Funding acquisition: KSA, Experimental design: MCM, KSA and FMK, Software: MCM and KSA, Investigation: MCM, Visualization: MCM, FMK and KSA, Resources: KSA and FMK; Supervision: ESN, WW and KSA, Project administration: KSA, writing—original draft preparation: MCM, Writing—review and editing: MCM, KSA, FMK, ESN and WW. All authors have read and approved the manuscript.

Data availability
All relevant data are within the paper and supplementary materials.

Declarations

Ethics approval
The experimental research and field studies on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation. The appropriate permissions and/or licenses for collection of plant or seed specimens were obtained for the study as approved by the National Commission of Science, Technology and Innovations, Kenya (License No: NACOSTI/P/23/23608). This article does not contain any studies with human participants performed by any of the authors.

Competing interests
The authors declare no competing interests.

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