

BRAZILIAN JOURNAL OF BIOMETRICS

ISSN:2764-5290

ARTICLE

Parametric time-to-event analysis to evaluate the germination of faba bean and lentil seeds¹

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(Received: August 21, 2024; Revised: January 13, 2025; Accepted: February 15, 2025; Published: September 03, 2025)

Abstract

This study aimed to evaluate the germination of faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medikus) seeds after disinfection with sodium hypochlorite and acetic acid, using parametric time-to-event analysis. The germination was evaluated under the effect of four concentrations (0.005%, 0.05%, 0.1%, and 0.5%) of sodium hypochlorite and acetic acid in addition to the control treatment (0%). Seed germination was counted daily for seven days. Seeds that did not germinate by the end of this period were considered censored. The main parametric models used in the time-to-event analysis, Exponential, Weibull, Log-normal, Log-logistic, Logistic, and Gaussian, were adjusted for each product and concentration, plus the control. The model selected was the Log-logistic, and the choice was based on Akaike and weighted Akaike information criteria. The adequacy of the selected model was verified by Cox-Snell residuals. The germination curve estimated by the Log-logistic model demonstrated an inverse relationship between the increase in concentrations and the percentage of germination. It is concluded that to maintain germination above 80% for both crops, the application of sodium hypochlorite should not exceed 0.1%. In the case of acetic acid, concentrations should not exceed 0.05% for *Vicia faba* and 0.005% for *Lens culinaris*. The parametric survival model effectively estimated the seed germination.

Keywords: Censored data, *Lens culinaris*; Seed vigor; Survival analysis, *Vicia faba*.

1. Introduction

Seed germination and emergence experiments are very common in agricultural sciences, whether for studies of physiological processes, dormancy, vigor, etc. The main characteristic of the data from these tests is that they are expressed as a percentage or as binary data. Another particularity is that they are evaluated over time, with repeated evaluations in the same experimental unit. The nature of these data can cause problems in statistical analyses, such as a serial correlation between the number of seeds counted on different dates in the same experimental unit (Onofri *et al.*, 2010), non-normal distribution, and heteroscedasticity of errors.

In addition to the problems reported, some viable seeds often do not germinate or rot during germination tests. This information, which is usually discarded, is known as censoring. A statistical technique used to overcome these problems is Survival Analysis. However, Onofri *et al.* (2022) report that the term “survival analysis” makes little sense in germination and emergence studies because we are not dealing with a survival process, and the most appropriate term should be time-to-event analysis.

Time-to-event methods refer to modeling the time until an event of interest occurs. The incorporation of censored data provides relevant information about the time of occurrence of the event, seeking more accurate information and allowing a more significant gain of pertinent information and interpretations (McNair *et al.*, 2012; Onofri *et al.*, 2019, 2022; Roesler *et al.*, 2023; Colosimo & Giolo, 2024). These techniques can be used to analyze germination time and estimate the probability of a seed germinating (Onofri *et al.*, 2010; Hsu *et al.*, 2024).

Some studies have demonstrated the application of time-to-event analysis in seed germination using non-parametric (Scott & Jones, 1982; Aerts *et al.*, 2006; Adegbola & Perez, 2016; Solarik *et al.*, 2016; Pérez & Kane, 2017; Barak *et al.*, 2018; Mamani *et al.*, 2018) and semi-parametric (Andersen *et al.*, 2016; Chhetri & Rawal, 2017; Cumming *et al.*, 2017) methods. These authors found that these techniques lead to valid inferences and reliable hypothesis tests. However, non-parametric methods do not allow the direct inclusion of covariates, and semi-parametric methods require proportional hazard assumptions, which may not always occur in agriculture (Onofri *et al.*, 2022).

Parametric models accommodate covariates and do not require proportional hazard assumptions. This approach allows for various statistical distributions for germination times. Based on the above considerations, this study aimed to evaluate the germination of faba and lentil seeds after disinfection with sodium hypochlorite and acetic acid using parametric time-to-event analysis methods.

2. Materials and Methods

2.1 Experiment

The data for this study came from an experiment conducted by Elezz & Ahmed (2021) and is available at <https://data.mendeley.com/datasets/k7vfsd4692/2>.

Seeds from two legume crops, faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medikus), were selected and disinfected with sodium hypochlorite 0.5% for two minutes, followed by rinsing three times with sterilized distilled water before applying treatments.

The treatments tested for seed disinfection consisted of different concentrations of sodium hypochlorite and acetic acid at 0.5%, 0.1%, 0.05%, and 0.005% of the active ingredient, and the control (0%).

The experiment was conducted in a 2x2x5 factorial scheme (2 crops vs. 2 products vs. five concentrations) using a completely randomized design, with three replicates of each treatment. Petri dishes (diam. 90 mm) with sterilized filter paper were used under laboratory conditions, considering that the seeds germinated when they reached a length of more than 2 mm. The germination test was carried out with daily counts for seven days. Seeds that germinated were considered failures, and those that did not germinate by the end of the experiment were considered censored.

2.2 Time-to-event analysis

The main parametric models used in time-to-event analysis Exponential, Weibull, Log-normal, Log-logistic, Logistic, and Gaussian models were fit for all treatments, and the

parameter estimates from these models were used to determine $S(t)$ survival functions (Table 1). Using the accumulated distribution function $F(t)$, through the relationship $F(t) = 1 - S(t)$, the probabilities of seed germinating up to a time t were calculated at each concentration of the products used. The germination curves were plotted based on the cumulative distribution function.

Table 1. Probability density functions and survival functions for the parametric models used to analyze the germination of *Vicia faba* and *Lens culinaris* under different concentrations of sodium hypochlorite and acetic acid

Distributions	Probability density function ¹	Survival function
Exponential $\lambda > 0, t \geq 0$	$f(t) = \lambda \exp\{-\lambda t\}$	$S(t x) = \exp\left\{-\frac{t}{\exp(\beta_0 + x'\beta)}\right\}$
Weibull $\gamma, \lambda > 0, t \geq 0$	$f(t) = \lambda \gamma t^{\gamma-1} \exp\{-\lambda t^\gamma\}$	$S(t x) = \exp\left\{-\left(\frac{t}{\exp(\beta_0 + x'\beta)}\right)^{\frac{1}{\gamma}}\right\}$
Log normal $\sigma > 0, t \geq 0$	$f(t) = \frac{1}{t\sigma\sqrt{2\pi}} \exp\left\{-\frac{1}{2}\left(\frac{\ln(t) - \mu}{\sigma}\right)^2\right\}$	$S(t x) = \Phi\left(\frac{-\ln(t) + \beta_0 + x'\beta}{\sigma}\right)$
Log logistic $\gamma, \lambda > 0, t \geq 0$	$f(t) = \frac{\gamma t^{\gamma-1} \lambda}{(1 + \lambda t^\gamma)^2}$	$S(t x) = \frac{1}{1 + \left(\frac{t}{\exp(\beta_0 + x'\beta)}\right)^{\frac{1}{\gamma}}}$
Logistic $\sigma > 0,$ $-\infty < \ln(t) < \infty$	$f(t) = \frac{\exp\left\{\frac{\ln(t) - \mu}{\sigma}\right\}}{\sigma \left(1 + \exp\left\{\frac{\ln(t) - \mu}{\sigma}\right\}\right)^2}$	$S(t x) = \frac{1}{1 + \exp\left\{\frac{t - (\beta_0 + x'\beta)}{\sigma}\right\}}$
Gaussian $\sigma > 0,$ $-\infty < t < \infty$	$f(t) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left\{-\frac{1}{2}\left(\frac{t - \mu}{\sigma}\right)^2\right\}$	$S(t x) = \Phi\left(\frac{-t + \beta_0 + x'\beta}{\sigma}\right)$

¹ Legend: \ln = natural logarithm; Φ = cumulative distribution function of the standard normal distribution.

The selection of the best model was based on the Akaike information criterion (AIC) (Akaike, 1974), favoring models with the least AIC values. Although the AIC is recognized as an essential measure, it does not have a meaning. Consequently, Akaike weights were also provided (Burnham & Anderson, 2002). These weights normalize the likelihood of the models, ensuring that they add up to 1. This normalization gives rise to the concept of Akaike weight or the "weight of evidence" for the model being the most suitable within the set, considering the recorded data:

$$w_i = \frac{\exp\left(\frac{-\Delta_i}{2}\right)}{\sum_{r=1}^R \exp\left(\frac{-\Delta_r}{2}\right)} \quad (1)$$

where $\Delta_i = AIC_i - \min AIC_i$.

The Akaike weights (w_i) is a value between 0 and 1, with the sum of w_i of all models in the candidate set being 1 and can be considered analogous to the probability that a given model is the best-approximating model (Symonds & Moussalli, 2011).

In order to evaluate the appropriateness of the chosen models, a method utilizing Cox-Snell Residuals (Cox & Snell, 1968) was employed. These residuals are characterized by:

$$\hat{e}_i = \hat{\Lambda}(t_i|x_i) = -\ln \hat{S}(t_i|x_i) \quad (2)$$

where $\hat{\Lambda}(\cdot)$ corresponds to the cumulative failure rate function obtained from the fitted model.

This residual serves as a valuable metric to assess the overall fit of the models. When the model is appropriate and the parameter estimates closely align with actual values, these residuals should resemble a censored sample from a standard exponential distribution. The visualization of survival curves for these residuals, generated using the Kaplan–Meier estimator and the standard exponential model, also aids in validating the model's adequacy. The closer the alignment, the better the model fits the data.

Time-to-event analysis was conducted utilizing the survival package (version 3.6-4). The Akaike weights were computed using the mvMORPH package (version 1.1.9), and survival time quantiles were calculated using the ciTools package (version 0.6.1). These analyses were performed within the R statistical environment (version 4.4.1) (R Development Core Team, 2024).

3. Results and Discussion

The results of the parametric time-to-event analysis, presented in Table 2, display the AIC, w_i values, and parameter estimates necessary for determining survival functions. AIC facilitated model ranking and identified the Log-logistic model as the optimal choice within the studied collection, evidenced by its lowest AIC value. However, AIC values do not have a specific meaning.

The use of w_i calculated from the AIC allows the results to be interpreted, indicating the probability of a model being the most appropriate model, given the experimental data and the set of models considered (Portet, 2020).

Table 2. Parameters estimates and standard errors (SE) of the models, Akaike information criterion (AIC), and Akaike weights (w_i) for *Vicia faba* and *Lens culinaris* seed germination

Parameters	Exponential		Weibull		Log-normal		Log-logistic		Logistic		Gaussian	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
β_0	0.7499	0.1007	0.8920	0.0502	0.6901	0.0545	0.6955	0.0499	1.9323	0.1383	2.0271	0.1663
β_1	0.0057	0.1542	0.0040	0.0790	-0.0001	0.0813	0.0041	0.0777	0.1534	0.2122	0.1635	0.2573
β_2	0.1287	0.1602	0.1393	0.0801	0.0404	0.0881	0.0023	0.0813	0.0633	0.2262	0.2098	0.2685
β_3	4.9286	0.7393	3.2012	0.3528	3.3110	0.3012	3.2232	0.2914	11.7226	0.8141	11.6799	0.8892
β_4	-0.1686	0.2398	-0.1865	0.1211	-0.0556	0.1298	-0.0144	0.1233	-0.1687	0.3383	-0.3546	0.4021
β_5	6.0953	2.1773	5.4720	1.2661	1.9197	0.8496	1.7261	0.8342	4.9213	2.2988	7.8034	3.2535
β_6	8.1221	2.4883	6.1108	1.2959	6.9356	1.3188	7.2189	1.2455	25.2203	3.8156	23.2078	3.9803
β_7	42.1928	9.7978	23.6310	4.8829	19.4140	3.4746	22.4001	3.8387	84.6250	10.9521	69.6645	10.6345
σ	1.0000	0.0000	0.4957	0.0191	0.5435	0.0479	0.3052	0.0248	0.8758	0.1981	1.6596	0.4456
ln L	-837.70	---	-721.86	---	-698.12	---	-694.12	---	-807.51	---	-829.89	---
AIC	1691.32	---	1461.72	---	1414.24	---	1406.22	---	1633.01	---	1677.78	---
w_i	0.0000	---	0.0000	---	0.0178	---	0.9822	---	0.0000	---	0.0000	---

Values in bold represent the best models.

Model: $\hat{y}_i = \ln(T) = \hat{\beta}_0 + \hat{\beta}_1 \text{crop} + \hat{\beta}_2 \text{dis} + \hat{\beta}_3 \text{conc} + \hat{\beta}_4 \text{crop} * \text{dis} + \hat{\beta}_5 \text{crop} * \text{conc} + \hat{\beta}_6 \text{dis} * \text{conc} + \hat{\beta}_7 \text{crop} * \text{dis} * \text{conc}$; $\Pr(\hat{\beta}_7 > z) < 0.0001$.

The Log-logistic model presented the lowest value for the AIC of 1406.22 and w_i of 0.9822, which means a 98.22% chance of being the most accurate model to describe the data. The Log-normal model had a w_i of 0.0178, which means it has a 1.78% chance of being the most accurate model to describe the data provided. Consequently, the log-logistic model adjusted for *Vicia faba* and

Lens culinaris seed germination in different sodium hypochlorite and acetic acid concentrations stands out as the best model.

Figure 1 illustrates the model's goodness of fit. The survival curves for the Cox-Snell residuals, obtained by the Kaplan-Meier method and the Standard Exponential model, demonstrate the adequacy of the Log-logistic model for the two legume crops.

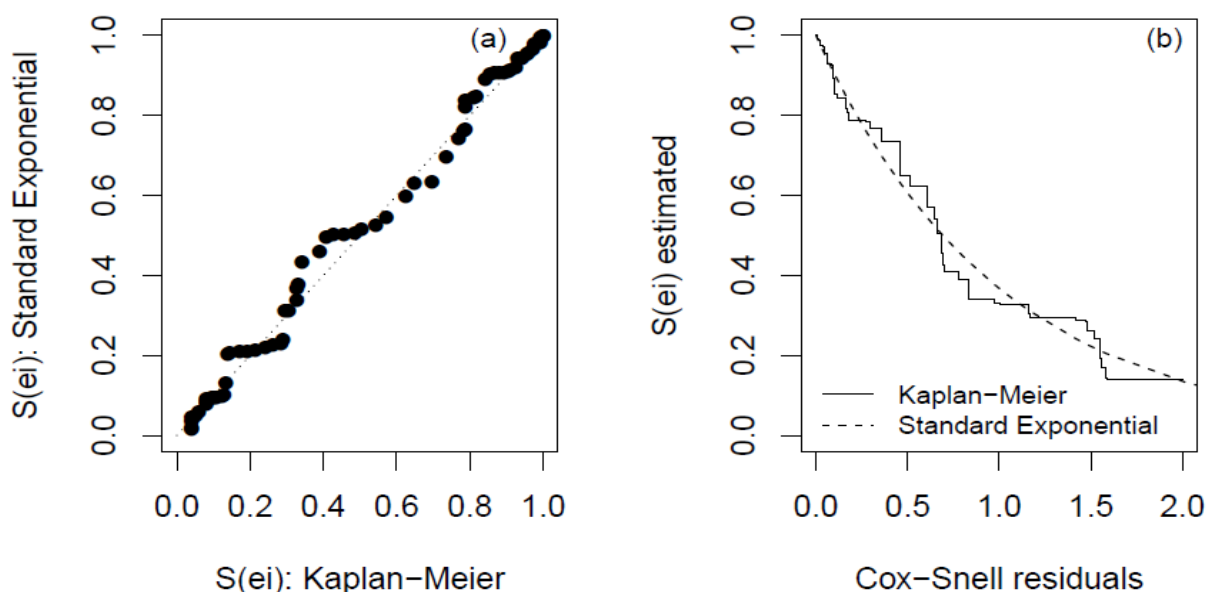


Figure 1. Survival of Cox-Snell residuals estimated by the Kaplan-Meier method and the standard exponential model (a) and respective estimated survival curves (b) adjusted for Log-logistic model for *Vicia faba* and *Lens culinaris* seed germination.

Time-to-event analysis, using the Log-logistic model, showed that at higher concentrations of the products used, there was a reduction in the probability of seed germination, as illustrated in Figures 2 and 3.

For *Vicia faba*, when sodium hypochlorite was used, it was found that only at the highest concentration of 0.5% did the seeds fail to reach 80% germination (Figure 2a). When acetic acid was used (Figure 2b) at a concentration of 0.1%, the seeds did not reach 80% germination, and no germination occurred at 0.5%. For both products, at the lowest concentrations, the time to germinate 80% of the seeds ($t_{0.80}$) increased as concentrations increased.

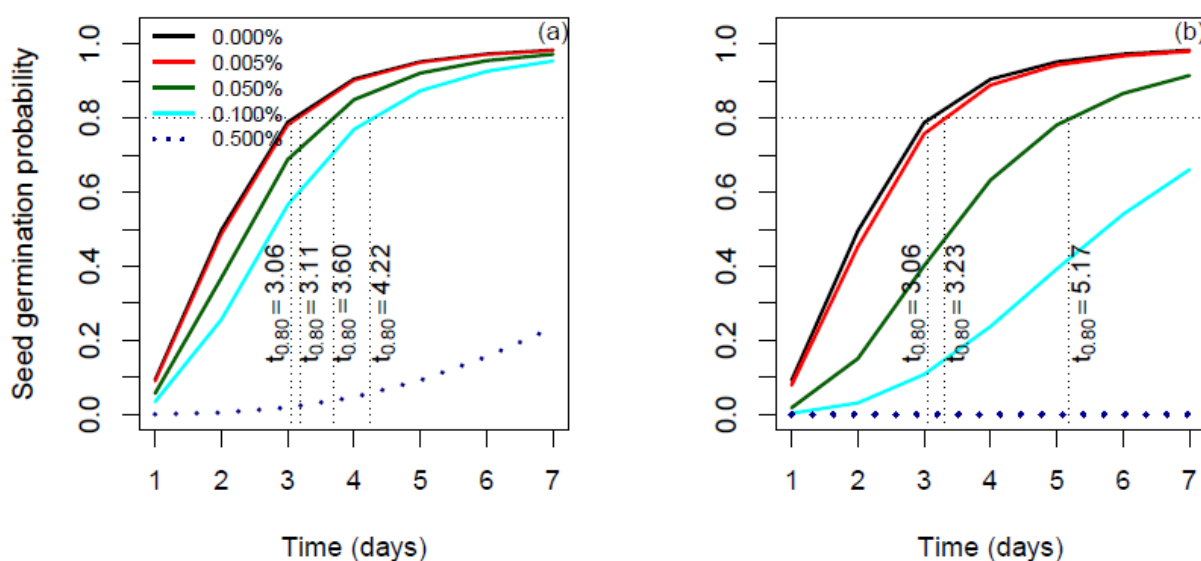


Figure 2. Seed germination probability estimated by Log-logistic model for *Vicia faba* exposed to different doses of sodium hypochlorite (a) and acetic acid (b). The time for 80% of the seeds to be germination is also presented.

For *Lens culinaris*, using sodium hypochlorite (Figure 3a), the results were similar to those for *Vicia faba*. However, the time to germinate 80% of the seeds ($t_{0.80}$) was slightly higher for the 0.1% concentration. It was also observed that this culture was more sensitive to acetic acid (Figure 3b), in which low germination can be seen at concentrations of 0.05, 0.1, and 0.5%.

Much research has shown various seed responses from different species to sodium hypochlorite and acetic acid, which can act positively or negatively. *Copaifera oblongifolia* seeds immersed in 3% sodium hypochlorite for 2 minutes did not have their germinability affected (Fernandes *et al.*, 2018), as did *Cabrlea canjerana* seeds, in which the 1% concentration for 2 minutes was efficient in asepsis, including when comparing with commercial fungicides (Aimi *et al.*, 2016). However, sodium hypochlorite can inhibit or delay seed germination, as occurred with Lettuce varieties (Mag *et al.*, 1995), as occurred with *Lens culinaris* and *Vicia faba* in this study.

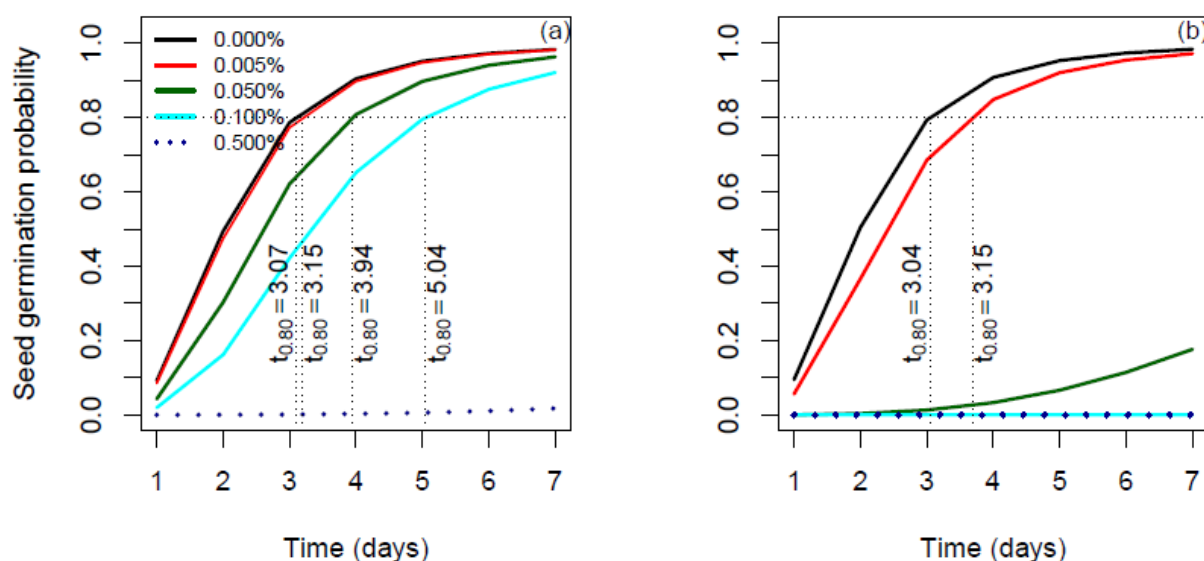


Figure 3. Seed germination probability estimated by Log-logistic model for *Lens culinaris* exposed to different doses of sodium hypochlorite (a) and acetic acid (b). The time for 80% of the seeds to be germination is also presented.

Sodium hypochlorite is a strong oxidizing agent. Thus, the constituent compounds of the seed coat or inside the seed with reducible carbon, such as enzymes, carbohydrates, lipids, and proteins, run the risk of being oxidized by NaOCl if they are exposed for long periods, damaging germination (Di-Tommaso & Nurse, 2004).

However, for some species with dormancy seeds, the highly reactive effect of sodium hypochlorite oxidizes substances that act as germination inhibitors in the seed or alter the composition of the integument, favoring germination (Severino, 2023).

Dorna *et al.* (2021) state that too high a concentration of acetic acid can be phytotoxic to seeds. These authors found that carrot seeds soaked in 0.5 and 1% acetic acid solutions had a higher germination capacity than untreated seeds. In comparison, the treatment of seeds with 2% acetic acid solution negatively affected their germination and vigor.

The phytotoxic effect of acetic acid at doses of 0.25 and 0.5% was verified by Pasini *et al.* (1997) in studies with rose seeds. Immersing zinnia seeds in acetic acid solution, regardless of concentration, decreased the total number of germinated seeds and germination capacity, prolonging germination time (Szopińska, 2013).

Despite these observations, some reports show that properly applied acetic acid treatment can improve seed germination and storability. El-Saidy & El-Hai (2016) found that soaking sunflower seeds in acetic acid increased the percentage and vigor of seed germination before and after six months of storage. Sholberg & Gaunce (1996), on the other hand, observed that canola,

corn, rice, and wheat seeds treated with 0.78 ml.kg⁻¹ acetic acid vapor showed improved germination. Acetic acid treatments had no adverse effect on the germination and vigor of onion seeds (Dorna *et al.*, 2023).

These results indicate that each crop has a degree of sensitivity to both sodium hypochlorite and acetic acid. This highlights the need for asepsis tests to define specific protocols for each species, which involve using different products and varying concentrations and soaking times of the seeds.

4. Conclusions

Based on the results obtained, it is concluded that the parametric model used here effectively estimated the germination of *Vicia faba* and *Lens culinaris* seeds exposed to different concentrations of sodium hypochlorite and acetic acid.

To maintain the germination probability of more than 80% of the seeds with a time of 7 days, the recommended doses of sodium hypochlorite should be less than 0.1% for both crops. In the case of acetic acid, the recommended doses for *Vicia faba* should be below 0.05%, and for *Lens culinaris*, 0.005%.

Acknowledgments

The authors express their sincere thanks to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes - 001) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support and the reviewers and editors for their suggestions.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: DUARTE, M. L., MARTINS FILHO, S. **Data curation:** MANHÃES SAINT'CLAIR, V., SILVA, M. A. **Formal analysis:** MANHÃES SAINT'CLAIR, V., DUARTE, M. L., SILVA, M. A., MARTINS FILHO, S. **Funding acquisition:** MARTINS FILHO, S. **Investigation:** MANHÃES SAINT'CLAIR, V., DUARTE, M. L., SILVA, M. A., ALMEIDA, L. S., MARTINS FILHO, S. **Methodology:** DUARTE, M. L., MARTINS FILHO, S. **Project administration:** MARTINS FILHO, S. **Software:** - **Resources:** MARTINS FILHO, S. **Supervision:** MARTINS FILHO, S. **Validation:** MANHÃES SAINT'CLAIR, V., DUARTE, M. L., SILVA, M. A., ALMEIDA, L. S., MARTINS FILHO, S. **Visualization:** DUARTE, M. L., ALMEIDA, L. S., MARTINS FILHO, S. **Writing - original draft:** MANHÃES SAINT'CLAIR, V., DUARTE, M. L., SILVA, M. A., ALMEIDA, L. S., MARTINS FILHO, S. **Writing - review and editing:** DUARTE, M. L., ALMEIDA, L. S., MARTINS FILHO, S.

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