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# Survival Analysis of the Green Lacewing, *Chrysoperla externa* (Hagen) Exposed to Neem-Based Products

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**Abstract:** Brazil is the country which has produced the most coffee for over 150 years, and to achieve high productivity, pesticides are the most common control measure for pests. Due to the need to adopt less impactful control practices, natural enemies or insecticides of botanical origin have been studied as alternatives to synthetic insecticides. However, botanical pesticides can negatively affect some natural enemies, and the effect depends on the formulation and concentration. The objective of this study was to estimate the survival of green lacewing larvae, *Chrysoperla externa* (Hagen), exposed to different doses of neem-based products, whose active ingredient is azadirachtin (Azamax®: 0 (control treatment with distilled water), 12, 24, 36, 48, 60, 72, 84, 96, and 108 mg a.i. $L^{-1}$; Organic neem®: 0, 3.3, 6.6, 9.9, 13.2, 16.5, 19.8, 23.1, and 26.4 mg a.i. $L^{-1}$; Natuneem®: 0, 3.8, 7.5, 10.5, 15.0, 18.8, 22.5, 26.3, 30.0, and 33.8 mg a.i. $L^{-1}$), using a parametric approach. Predator larvae were exposed to neem-based insecticides and evaluated for 20 days. Survival curves estimated by the models—Log-logistic for Azamax®, Weibull for Organic neem®, and Log-normal for Natuneem®—demonstrated an inverse relationship between increasing doses and survival time. These concluded that the application dose should be less than 84 mg a.i. $L^{-1}$ for Azamax®, 19.8 mg a.i. $L^{-1}$ for Organic neem®, and 26.3 mg a.i. $L^{-1}$ for Natuneem® to keep 50% of the green lacewings alive for 13 days, which is the average time for the larval cycle of *C. externa*.

**Keywords:** azadirachtin; biological control; biopesticides; failure-time analysis; survival models



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## 1. Introduction

Coffee (*Coffea arabica* L. and *Coffea canephora* Pierre ex Froehner) is a crop of global importance that sustains a multibillion-dollar global industry [1] in an extended value chain, which includes the producer, the industry, and the consumer. On the world stage, Brazil stands out as the largest producer of coffee, reaching about 44.85 million bags in 2022 [1]. Many developing countries grow coffee as their primary source of income [2,3].

However, the incidence of pests and diseases in the coffee crop compromises production, causing significant losses to the market of hundreds of millions of dollars annually [4–7]. The coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) [8–10], the red mite *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) and scales Coccidae (*Coccus viridis Green*) and Pseudococcidae (*Planococcus citri Risso*) [11] are the main pests of coffee in Brazil.

In Brazil, the most common control measures for coffee pests are pesticides [11]. However, because these are often broad-spectrum and highly toxic, many concerns are associated with pesticide applications, such as adverse effects on human health and the environment, pest resistance, the emergence of secondary pests, and damage to beneficial insects [12–16]. All these examples show the need to develop alternative strategies and

discover new sustainable pest controls as an alternative to synthetic insecticides [11], such as using natural enemies and/or insecticides of botanical origin.

One example of the use of natural enemies is the green lacewing (*Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae)), which is a voracious predator found in several crops of agricultural importance, being able to feed on a large variety of prey. The larvae feed on aphids, whiteflies, eggs and small caterpillars of Lepidoptera, different mealybugs, and mites. Recently, *C. externa* was reported to prey on the coffee berry borer in Brazil [17]. Additionally, members of the family Chrysopidae prey on the coffee leaf miner immature stages, mites, and scales [18–20].

Another alternative for the chemical control of several insect pests is the use of botanical insecticides. An example is azadirachtin, a tetranortriterpenoid derived from the plant *Azadirachta indica* A. Juss, known as neem, which is the most widely used botanical pesticide in world agriculture, interfering mainly as a phagoinhibitor and in the development of insects [21]. This tree has been known for over 2000 years in India and countries in South Asia, and its leaves, fruits, seeds, oil, and roots are used to control insect pests. Azadirachtin A, azadirachtin B, deacetylsalanin, and salanin are the main compounds with insecticidal, anti-food, and putative antimicrobial properties in neem. Some research has recommended neem extract as a potent crop protectant. However, the wide variation in azadirachtin content (0.01%–0.9%) in neem extract is a problem for its commercialization [21]. These variations may be due to ecotypes and/or extraction conditions.

A reduction in leaf miner oviposition was observed when coffee seedlings were treated with neem oil (0.125–2.5%) or with neem leaf extract (20–40%) [22]. Coffee seedlings sprayed with 0.025–0.1 g $L^{-1}$ of azadirachtin prevented the development of mines [23]. Neem seed extract has a systemic and translaminar effect that permeates the leaves, stops leaf miner development, drastically reduces pupation, and prevents adult emergence [23]. Plants treated with neem products are expected to have a lower coffee leaf miner infestation, either because treated plants repel egg-laying females or because coffee leaf miner development is adversely affected by neem. Azadirachtin concentrations above 0.065 g $L^{-1}$ reduced the population growth rate of *O. ilicis* [23]. Despite these advantages, some natural enemies can be negatively affected by neem, and the effect will depend on the formulation and concentration used [23,24].

In this way, the compatibility of the use of neem-based products and biocontrol agents, such as lacewings, in pest management has emerged as a viable strategy in sustainable agriculture, requiring, however, the development of research to study this association. One way to carry out this compatibility is to evaluate the survival time of these predatory insects after being exposed to the product. The survival statistical analysis technique can be performed for this type of study. Survival analysis is a set of methods and statistical models used to analyze experiments in which the response variable is the evaluated time until the occurrence of an event of interest, called the failure time [25]. The presence of censored observations is the main characteristic of this methodology. Censoring occurs when the event of interest is not observed for some individuals, either because the study ended without the event of interest or because the experimental unit died for a different cause from the one studied. This means that the information about the response boils down to the knowledge that the time until the failure occurs is longer than the observed one.

Classic statistical techniques become unfeasible in the presence of censoring because they do not allow the use of partial observations. In statistical analyses, all observations from a study (full and partial) should be used [25]. Even if incomplete, the censoring provides relevant information about the time of the event, and their omission from the calculation of the statistics of interest can lead to biased conclusions [25]. Therefore, statistical methods are needed to make it possible to incorporate the information contained in the complete observations and the censoring in the analysis.

In survival analysis, values can be estimated using non-parametric and parametric techniques. A widely used non-parametric method is the Kaplan–Meier estimator, which enables the estimation of the survival function and the construction of graphs to facilitate

the visualization of these functions. The curve is characterized by steps, each representing one or more events. Parametric or probabilistic models are also widespread in survival analysis to estimate the survival function and are often used by researchers in practical problems. The most widely applied are the exponential, Weibull, and Log-normal models. The adjustment of these models also makes it possible to perform the likelihood ratio test, Akaike's information criterion, to proceed with the selection of models and estimate the survival time for any percentile of interest.

Some studies have demonstrated the application of non-parametric survival analyses to evaluate insecticides' effects [26–34], although parametric methods still need to be used. Based on the previous considerations, this study aimed to estimate the survival of larvae of *C. externa* exposed to different doses of three neem-based products using parametric models.

## 2. Materials and Methods

### *2.1. Chrysoperla Externa Rearing and Neem-Based Products*

Adults of *C. externa* used in the experiment originated from a rearing in the entomology laboratory of EPAMIG Sudeste, Viçosa, state of Minas Gerais, Brazil (20° 45′ 14″ S; 42° 52′ 55″ W), maintained under the following conditions: temperature: 25 ± 2°C, relative humidity: 70 ± 10%, and photophase of 14 h. PVC tubes (8 × 11 cm) covered with nylon gauze were placed in a Petri dish (15 cm in diameter) and used as cages to keep the individuals. Their diet consisted of yeast and honey (1:1) offered on a parafilm stripe hung inside the cage. Water was provided on a soaked piece of cotton and placed inside a 10 mL vial. Food and water were replaced twice a week. The predatory insects used in the experiments were obtained from eggs collected from the cages by cutting their pedicel and transferring them to glass tubes (2.5 × 8.5 cm). The rearing followed the methodology described by Venzon et al. [35].

The neem-based products evaluated were Azamax (emulsifiable concentrate formulation 12 g Azadirachtin A and B $L^{-1}$) (UPL do Brasil Indústria e Comércio de Insumos Agropecuários S.A., Ituverava, São Paulo, Brazil), Organic neem (emulsifiable concentrate formulation 3.3 g Azadirachtin A and B $L^{-1}$) (Dalquim Industria e Comércio, Itajaí, Santa Catarina, Brazil), and Natuneem (emulsifiable concentrate formulation 1.5 g Azadirachtin A and B $L^{-1}$) (Natural Rural Industria e Comercio de Produtos Orgânicos e Biológicos Ltd.a, Araraquara, São Paulo, Brazil).

### *2.2. Bioassays*

In carrying out the experiments, the arenas were composed of pepper leaf discs (3.0 cm in diameter) placed inside Petri dishes (3.5 cm in diameter) on a layer of 10% solution of carrageenan ($n$ = 10 discs per treatment). The discs were sprayed in Potter's tower [36] under a pressure of 5 lb $pol^{-2}$ with a volume of 2.5 mL with different concentrations of the three commercial neem-based products. After drying, a newly emerged larva of *C. externa* was transferred to each disc. The larvae were kept on the discs for six days until the beginning of the deterioration of the leaf discs. Subsequently, they were transferred to glass tubes (2.5 × 8.5 cm) and kept until adult emergence in an acclimatized room (temperature: 25 ± 2 °C, relative humidity: 70 ± 10%, and photophase of 14 h). During the larval period, they were fed an ample supply of eggs of the flour moth *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) [27]. The experimental design was completely randomized, with ten replications for each tested concentration; the experimental unit consisted of one larva.

The tested concentrations for each product were obtained by calculating responses to lower and upper limits in a previous bioassay to evaluate *C. externa* mortality. These concentrations corresponded to no mortality or 100% mortality responses. The tested concentrations were: (**a**) Azamax®: 0 mg a.i. $L^{-1}$ (control treatment with distilled water), 12 mg a.i. $L^{-1}$, 24 mg a.i. $L^{-1}$, 36 mg a.i. $L^{-1}$, 48 mg a.i. $L^{-1}$, 60 mg a.i. $L^{-1}$, 72 mg a.i. $L^{-1}$, 84 mg a.i. $L^{-1}$, 96 mg a.i. $L^{-1}$, and 108 mg a.i. $L^{-1}$; (**b**) Organic neem®: 0 mg a.i. $L^{-1}$, 3.3 mg a.i. $L^{-1}$, 6.6 mg a.i. $L^{-1}$, 9.9 mg a.i. $L^{-1}$, 13.2 mg a.i. $L^{-1}$, 16.5 mg a.i. $L^{-1}$, 19.8 mg a.i. $L^{-1}$, 23.1 mg a.i. $L^{-1}$, and 26.4 mg a.i. $L^{-1}$; and (**c**) Natuneem®: 0 mg a.i. $L^{-1}$,

3.8 mg a.i. $L^{-1}$, 7.5 mg a.i. $L^{-1}$, 10.5 mg a.i. $L^{-1}$, 15.0 mg a.i. $L^{-1}$, 18.8 mg a.i. $L^{-1}$, 22.5 mg a.i. $L^{-1}$, 26.3 mg a.i. $L^{-1}$, 30.0 mg a.i. $L^{-1}$, and 33.8 mg a.i. $L^{-1}$.

### *2.3. Statistical Analysis*

Individual mortality was evaluated every 24 h for 20 days during the larval period. After this time, insects still alive were censored, and dead insects were considered failures. The main parametric models used in the survival analysis—exponential, Weibull, Log-normal, Log-logistic, Logistic, and Gaussian models—were fitted for each neem-based product, and the parameter estimates from these models were used to determine the survival functions (Table 1).

**Table 1.** The probability density functions and survival functions for the models used in the analysis of *Chrysoperla externa* exposed to neem-based products.

| Models | Probability Density Function [1] | Survival Function |
|---|---|---|
| Exponential | $f(t) = \lambda \exp\{-\lambda\, t\}$ | $S(t\|x) = exp\left\{-\frac{t}{\exp(\beta_0+\beta' x)}\right\}$ |
| Weibull | $f(t) = \alpha\lambda t^{\alpha-1} exp\{-(\lambda t)^{\alpha}\}$ | $S(t\|x) = \exp\left\{-\left(\frac{t}{\exp(\beta_0+\beta' x)}\right)^{\frac{1}{\sigma}}\right\}$ |
| Log-normal | $f(t) = \frac{1}{t\sigma\sqrt{2\pi}}\, exp\left\{-\frac{1}{2}\left(\frac{ln(t)-\mu}{\sigma}\right)^2\right\}$ | $S(t\|x) = \Phi\left(\frac{-ln(t)+\beta_0+\beta' x}{\sigma}\right)$ |
| Log-logistic | $f(t) = \frac{\alpha t^{\alpha-1}\lambda}{(1+\lambda t^{\alpha})^2}$ | $S(t\|x) = \frac{1}{1+\left(\frac{t}{exp(\beta_0+\beta' x)}\right)^{\frac{1}{\sigma}}}$ |
| Logistic | $f(y) = \frac{exp\left\{\frac{y-\mu}{\sigma}\right\}}{\sigma\left(1+exp\left\{\frac{y-\mu}{\sigma}\right\}\right)^2}$ | $S(t\|x) = \frac{1}{1+exp\left\{\frac{t-(\beta_0+\beta' x)}{\sigma}\right\}}$ |
| Gaussian | $f(t) = \frac{1}{\sigma\sqrt{2\pi}}\, exp\left\{-\frac{1}{2}\left(\frac{t-\mu}{\sigma}\right)^2\right\}$ | $S(t\|x) = \Phi\left(\frac{-t+\beta_0+\beta' x}{\sigma}\right)$ |

[1] ln = natural logarithm; Φ = cumulative distribution function of the standard normal distribution; y = ln(t).

The best model choice was based on the Akaike information criterion (AIC) [37]. This measure uses the log-likelihood estimate (*LL*), but adds a penalizing term associated with the number of model parameters (*p*). It is well known that by adding variables, one can improve the fit of models. Thus, the AIC tries to balance the goodness of fit versus the inclusion of variables in the model. Smaller values indicate better model fitting. The AIC is computed as follows:

$$AIC = -2(LL) + 2p \quad (1)$$

Although the AIC is recognized as an essential measure, it does not have a meaning. For this reason, Akaike weights [38] were also presented. The model likelihoods can be normalized so that they sum to 1. This normalization yields the definition of the Akaike weight or "weight of evidence" of model i for being the best model of the collection given the data recorded:

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

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

The $\min_i AIC_i$ is the AIC value of the best model in the collection. $AIC_i$ is the score for the particular model i. The AIC difference, $\Delta_i$, estimates the information loss when using model i rather than the estimated best model. Hence, the larger the $\Delta_i$, the less plausible model i is.

The Akaike weight, $w_i$, of model i can be interpreted as the probability that model i is the best (approximating) model given the experimental data and the collection of models considered. Hence, the smaller the weight, $w_i$, the less plausible model i is. We can consider a single best model i if $w_i$ > 0:9.

The Akaike weight ($w_i$) 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 [39].

Assessing the adequacy of an adjusted model is essential in data analysis. Several residuals have been proposed in the literature to assess the adequacy of parametric survival models. Cox–Snell residuals [40] were used to assess the selected models' adequacy. These are defined by:

$$e_i = \hat{\Lambda}(t_i|x_i) \quad (3)$$

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

This residual is a useful measure for examining the models' overall fit. If the model is adequate and the parameter estimates are close to actual values, these residuals should look like a censored sample of a standard exponential distribution. The graph of the survival curves of these residuals, obtained by the Kaplan–Meier estimator and the standard exponential model, also helps to verify the model's quality. The closer they are, the better the model fits the data.

Survival analyses were performed using the *survival* package [41]. The *mvMORPH* package [42] was used to calculate the Akaike weights, and the *ciTools* package [43] was used to calculate the survival time quantiles. All analyses were performed in the R statistical environment [44].

## 3. Results and Discussion

The parametric models used in this study for survival analysis were adjusted for each neem-based product. Table 2 presents the AIC, $w_i$ values, and estimates of the parameters of these models, which were necessary to estimate the survival functions. The AIC enabled the ranking of candidate models and was able to select the best model within the collection studied. For the Azamax® product, the model chosen was Log-logistic; for Organic neem®, it was the Weibull model; and for Natuneem®, it was the Log-normal model. The best model presented the lowest AIC values. However, the actual values of AIC have no meaning.

**Table 2.** Parameter estimates and standard errors (SE), Akaike information criterion (AIC), and Akaike weights ($w_i$) for the models used in the analysis of *Chrysoperla externa* exposed to neem-based products.

| Parameters | Exponential | | Weibull | | Log-Normal | | Log-Logistic | | Logistic | | Gaussian | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimate | SE | Estimate | SE | Estimate | SE | Estimate | SE | Estimate | SE | Estimate | SE |
| **Azamax®** | | | | | | | | | | | | |
| $\beta_0$ | 4.0726 | 0.2992 | 3.4303 | 0.1288 | 3.2001 | 0.1241 | 3.2202 | 0.1079 | 22.5068 | 1.4292 | 22.2546 | 1.5065 |
| $\beta_1$ | −0.0158 | 0.0042 | −0.0089 | 0.0018 | −0.0078 | 0.0019 | −0.0084 | 0.0016 | −0.1143 | 0.0205 | −0.1055 | 0.0227 |
| $\sigma$ | 1.0000 | 0.0000 | 0.3853 | 0.0450 | 0.5270 | 0.0564 | 0.2725 | 0.0333 | 3.6114 | 0.4361 | 6.4241 | 0.6961 |
| Log L | −212.30 | — | −190.30 | — | −190.90 | — | −187.70 | — | −196.40 | — | −196.90 | — |
| AIC | 428.67 | — | 386.56 | — | 387.76 | — | **381.42** | — | 398.74 | — | 399.87 | — |
| $w_i$ | 0.00 | — | 0.07 | — | 0.04 | — | **0.89** | — | 0.00 | — | 0.00 | — |
| **Organic neem®** | | | | | | | | | | | | |
| $\beta_0$ | 4.2724 | 0.3264 | 3.6093 | 0.1632 | 3.3696 | 0.1618 | 3.3835 | 0.1441 | 24.1767 | 1.6901 | 23.8370 | 1.7897 |
| $\beta_1$ | −0.0788 | 0.0181 | −0.0485 | 0.0090 | −0.0453 | 0.0096 | −0.0459 | 0.0084 | −0.5847 | 0.0967 | −0.5540 | 0.1069 |
| $\sigma$ | 1.0000 | 0.0000 | 0.4425 | 0.0548 | 0.6297 | 0.0703 | 0.3320 | 0.0429 | 3.8997 | 0.4950 | 7.0087 | 0.7936 |
| Log L | −188.00 | — | −172.70 | — | −174.70 | — | −172.80 | — | −180.40 | — | −181.10 | — |
| AIC | 379.98 | — | **351.45** | — | 355.50 | — | 351.54 | — | 366.73 | — | 368.23 | — |
| $w_i$ | 0.00 | — | **0.48** | — | 0.06 | — | 0.46 | — | 0.00 | — | 0.00 | — |
| **Natuneem®** | | | | | | | | | | | | |
| $\beta_0$ | 4.9722 | 0.3716 | 4.0718 | 0.2205 | 3.8558 | 0.1939 | 3.7988 | 0.1880 | 28.5799 | 2.0402 | 28.8093 | 2.0433 |
| $\beta_1$ | −0.0867 | 0.0148 | −0.0549 | 0.0087 | −0.0563 | 0.0083 | −0.0538 | 0.0081 | −0.6350 | 0.0855 | −0.6355 | 0.0874 |
| $\sigma$ | 1.0000 | 0.0000 | 0.5041 | 0.0603 | 0.6591 | 0.0712 | 0.3754 | 0.0453 | 4.1101 | 0.4918 | 7.0062 | 0.7705 |
| Log L | −198.00 | — | −185.70 | — | −183.80 | — | −184.30 | — | −195.40 | — | −194.40 | — |
| AIC | 399.91 | — | 377.45 | — | **373.62** | — | 374.51 | — | 396.80 | — | 394.73 | — |
| $w_i$ | 0.00 | — | 0.08 | — | **0.56** | — | 0.36 | — | 0.00 | — | 0.00 | — |

Values in bold represent the best models.

Using $w_i$ computed from the AIC enables the interpretation of results by providing the probability that a model is the best model given the experimental data and the set of considered models [45]. Thus, the Log-logistic model adjusted for Azamax® is the best model with a $w_i$ of 0.89, which means that there is an 89.0% chance that it is the model which best approximates the described data. The $w_i$ values for the Weibull model for Organic neem® and Log-normal for Natuneem® were 48.0% and 56%, respectively.

The goodness of fit of the selected models is illustrated in Figure 1. The survival curves for the Cox–Snell residuals obtained by the Kaplan–Meier method and by the standard exponential distribution, in general, indicate the overall goodness of fit of (a) Log-logistics, (b) Weibull, and (c) Log-normal models for Azamax®, Organic neem®, and Natuneem® products, respectively. In panel (a), a slight deviation can be seen at the end of the two curves. Klein and Moeschberger [46] reported that departures from the exponential distribution may be partially due to the uncertainty in estimating parameters. This uncertainty is the largest in the right-hand tail of the distribution and for small samples.

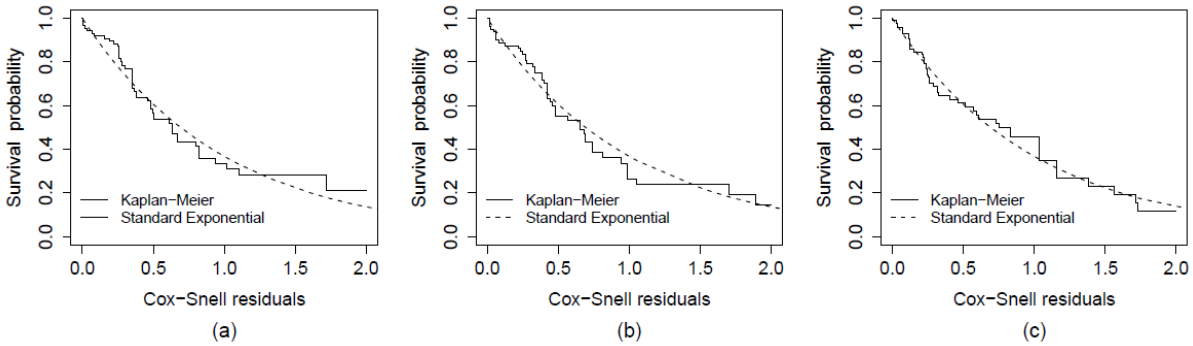


**Figure 1.** Cox–Snell residuals from the (**a**) Log-logistic model for Azamax®, (**b**) Weibull model for Organic neem®, and (**c**) Log-normal model for Natuneem®.

Survival curves estimated by the models—Log-logistic for Azamax®, Weibull for Organic neem®, and Log-normal for Natuneem®—demonstrate an inverse relationship between increasing doses and survival time (Figures 2–4). For insects without exposure to neem-based products (control treatment), the survival rates were 69.5%, 77.9%, and 90.4% at the 20-day evaluation for Azamax®, Organic neem®, and Natuneem®, respectively, and the survival rates with the maximum dose were 7%, 1.1%, and 5.7% at the end of the 20 days of evaluation for Azamax®, Organic neem® and Natuneem®, respectively. Therefore, it is concluded that azadirachtin harms *C. externa* and that bioinsecticides should not be exempt from the risk assessment [26,47].

Considering that the larval stage of *C. externa* has an average duration of approximately 13 days [48], and that this is the stage when it is viewed as a predator, inferences can be made about the doses of the studied products and their survival time. Thus, to survive 75% and 50% of the individuals, the recommended Azamax® doses should be lower than 48 mg a.i. $L^{-1}$ and 84 mg a.i. $L^{-1}$, respectively (Figure 2). Additionally, for the survival of 25% of the green lacewings, all tested doses can be used.

The same considerations can be accomplished for the other two studied products. For Organic neem®, considering the Weibull model, dosages need to be below 13.2, 19.8, and 26.4 mg a.i. $L^{-1}$ to achieve 75%, 50%, and 25% survival, respectively (Figure 3). For Natuneem® and the Log-normal model, the dosages must be less than 15 (or equal), 26.3, and 33.8 mg a.i. $L^{-1}$ (Figure 4).

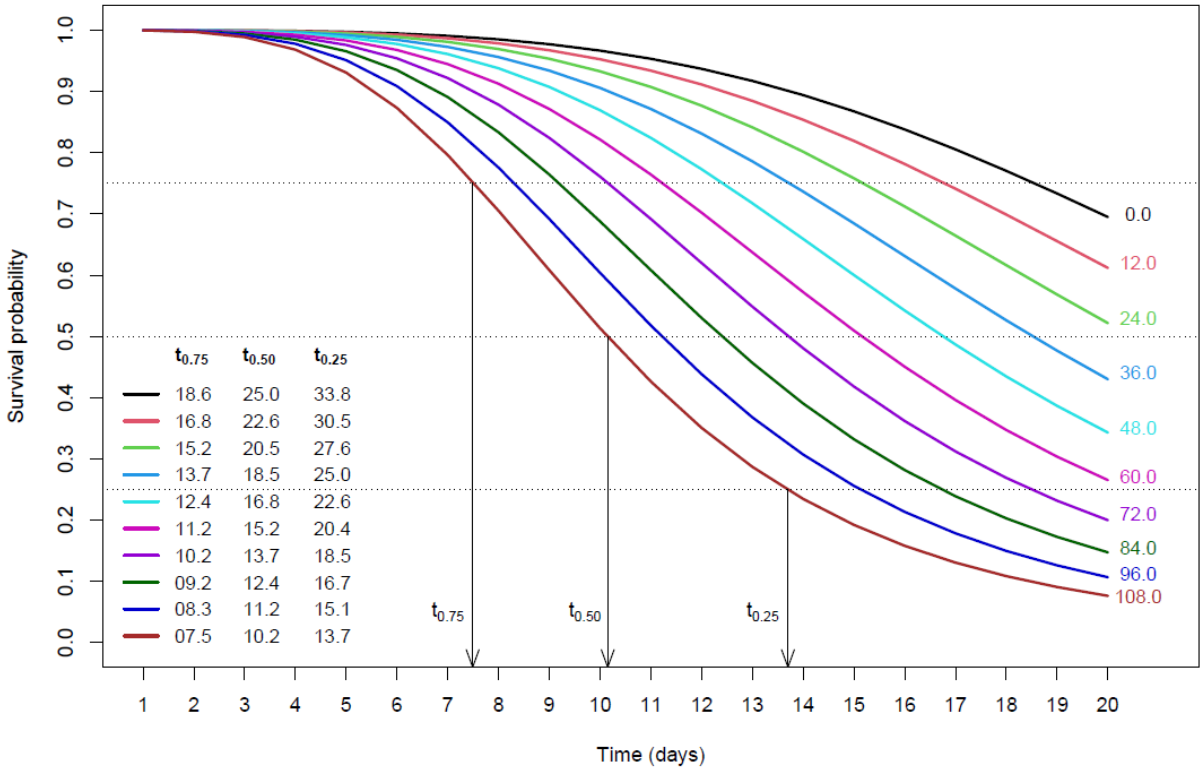


**Figure 2.** Survival curves estimated by Log-logistic model for *Chrysoperla externa* exposed to different doses (mg a.i. $L^{-1}$) in Azamax®. The times for 75%, 50%, and 25% of the individuals to be alive are also presented.

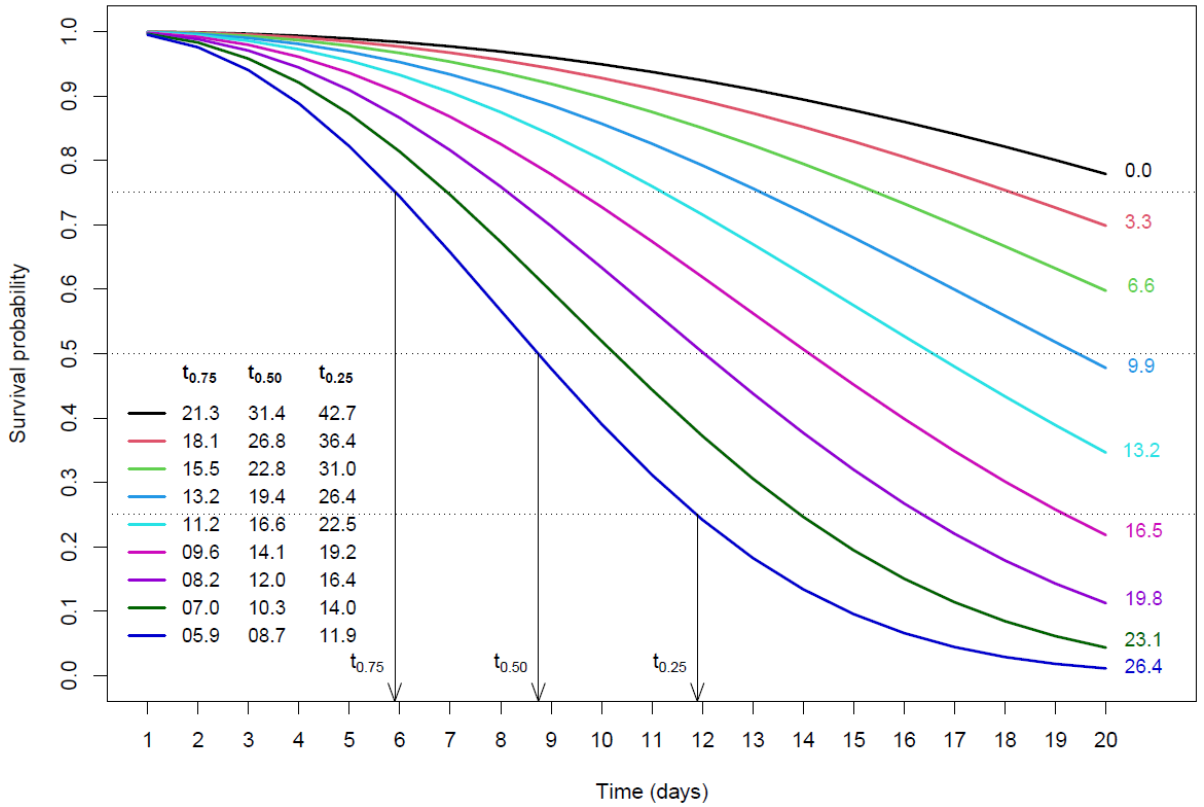


**Figure 3.** Survival curves estimated by the Weibull model for *Chrysoperla externa* exposed to different doses (mg a.i. $L^{-1}$) in Organic neem®. The times for 75%, 50%, and 25% of the individuals to be alive are also presented.

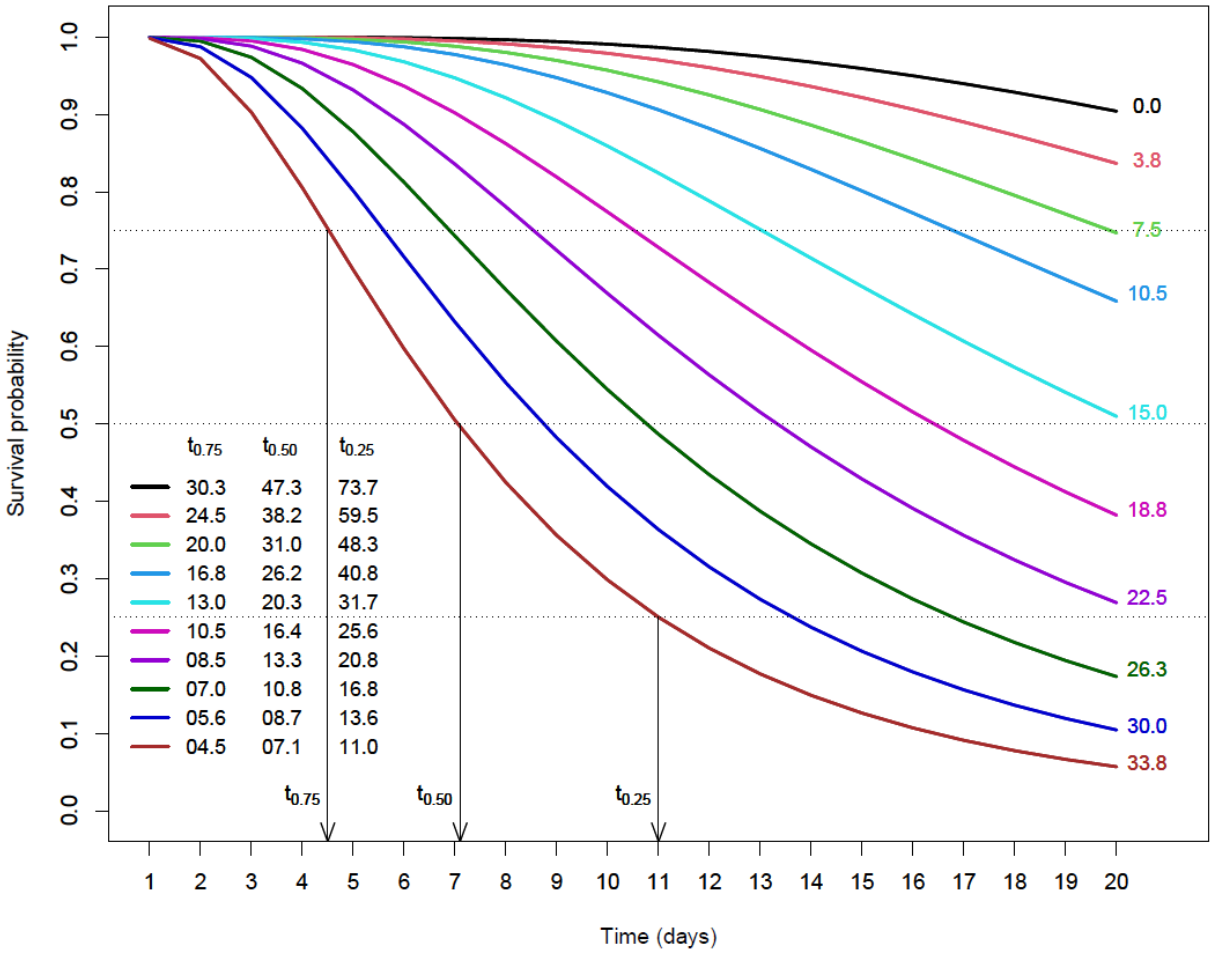


**Figure 4.** Survival curves estimated by the Log-normal model for *Chrysoperla externa* exposed to different doses (mg a.i. $L^{-1}$) in Natuneem®. The times for 75%, 50%, and 25% of the individuals to be alive are also presented.

The most important neem composition includes azadirachtin, nimbolide, salannin, nimbin, deacetylnimbin, mahmoodin, epoxy-azadiradione, deacetylgedunin, and gedunin [21,49,50]. Thus, the differences found among the results, in addition to the concentrations of azadirachtin, can be explained by the composition of each product. Venzon [51] reports that the efficacy of botanical insecticides using neem or azadirachtins as the main active ingredient is expected to vary among commercial products. Furthermore, each product should be evaluated separately, and any generalization regarding neem-based products can be misleading.

The recommended dose of Azamax® to control *L. coffeella* and *H. hampei* in coffee plants can vary from 24–30 mg a.i. $L^{-1}$ and 72–96 mg a.i. $L^{-1}$, according to the level of infestation [52]. According to the adjusted model, if these dosages are applied to control these pests, 84–81% and 55–37% of green lacewings would be alive, considering the average survival time of 13 days, respectively (Figure 2). High concentrations of neem-based products to control the coffee berry borer have negative impacts on *C. externa* and other natural enemies [26], as well as on some pollinators [29]. Azadirachtin is a broad-spectrum insecticide, which affects the neuroendocrine, reproduction, anti-feeding, cellular, and molecular activity of insects, and its toxicity varies between insect orders [21]. These adverse effects on beneficial insects can be minimized if the formulation and concentration of the product to be applied are carefully chosen based on research data and technical information [23,24].

Miranda [53], using non-parametric survival methods, evaluated the survival of *C. externa* exposed to Organic neem® and Natuneem® products, and observed that for 13-day survival, doses below 16.5 mg a.i. $L^{-1}$ and 18.6 mg a.i. $L^{-1}$, respectively, kept 50% of the

green lacewings alive. Organic neem® and Natuneem® products still need to be registered for any crop in Brazil [52]. However, their uses are common in research, such as side effects on bees [29] and control of mites [51,54,55].

*C. externa* is one of the main predators of coffee pests; therefore, management must be adopted between dosages of neem-based products and the conservation of green lacewings in crops. This practice can add benefits and promote the balance of the agroecosystem, making pest control more effective. Thus, we suggest further research using different products and concentrations to obtain a safe dose for the predator. Likewise, new studies under field conditions should be conducted to study the effects of neem-based insecticides on other insect predators and pollinators. This strategy used in coffee crops would provide subsidies for integrated pest control.

## 4. Conclusions

Based on the results obtained, it is concluded that the parametric models used here effectively estimated the survival of *C. externa* exposed to different neem-based products. To maintain the survival of more than 50% of the individuals, with a time of 13 days, the recommended doses must be lower than 84 mg a.i. $L^{-1}$ for Azamax®, 19.8 mg a.i. $L-^{1}$ for Organic neem®, and 26.3 mg a.i. $L^{-1}$ for Natuneem®.

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