

## Article

# Evaluation of *Trichogramma cacaeciae* (Hymenoptera: Trichogrammatidae) and *Blattisocius mali* (Mesostigmata: Blattisociidae) in the Post-Harvest Biological Control of the Potato Tuber Moth (Lepidoptera: Gelechiidae): Use of Sigmoid Functions

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**Abstract:** The potato tuber moth (PTM), *Phthorimaea operculella*, is the main potato pest in the world, having managed to adapt to a wide range of climatic conditions, and causing damage to stored tubers. The effectiveness of a biological control using the parasitoid *Trichogramma cacaeciae* and the predatory mite *Blattisocius mali* was evaluated in PTM under conditions that simulated those of tubers in storage. The number of adults found at the end of the trial was significantly higher in the control ( $181.75 \pm 11.00$  adults/container) than in treatments where *B. mali* ( $24.50 \pm 7.22$ ) or *T. cacaeciae* ( $102.00 \pm 14.61$ ) were released. The biological control's effectiveness using the Abbot formula was 86.52% for the predator and 43.88% for the parasitoid. In addition, the PTM population data was fitted by logistic models, and the best fit was found for the simple logistic function. Using the data generated from these functions, the control efficiencies for the mite and the parasitoid were 94.85% and 73.77%, respectively. *B. mali* showed greater potential for being incorporated into integrated PTM management programs than *T. cacaeciae*. Based on logistic functions, a novel way of estimating the effectiveness of predators and parasitoids is also presented and discussed.

**Keywords:** biological control; effectiveness; population dynamic; stored potato; pest

## 1. Introduction

The potato tuber moth (PTM) *Phthorimaea operculella* (Zeller) (Lep.: Gelechiidae) is a species native to the tropical, mountainous regions of South America, and is a pest species of the potato plant (*Solanum tuberosum* L.) and other Solanaceae [1]. Its distribution is cosmopolitan (CABI <https://www.cabi.org/isc/datasheet/40686#todistribution>, accessed on 7 March 2022) and of economic importance in crop cultivations, although it is especially severe in storage [2], due to its ability to reproduce and adapt to the different environmental conditions occurring in storehouses [3–8]. Under such conditions, the PTM infests potato tubers with its eggs, which are rarely detectable; later, the neonate larvae penetrate the tubers [4,8–10]. PTM damage lowers the commercial value of potatoes, and can cause losses of up to 100% when the temperature and storage period are not optimal, by favoring the development of several generations of the PTM [7,9,11,12].

PTM adults can live at temperatures between 10 °C and 35 °C without being affected by the relative air humidity [7,13]; furthermore, their immature states survive for brief periods

when exposed to temperatures between 4.25 °C and 13.5 °C, inducing quiescence [4]. These characteristics allow the PTM to easily adapt to the storage conditions used both for seed (2 °C to 4 °C) and consumption potatoes (8 °C to 12 °C), and, of course, in unrefrigerated storehouses, as occurs in some countries [11,14–16]. For all these reasons, the control of the PTM is complex, particularly in immature states.

Among the PTM management strategies used in storage are cleaning practices that prevent the entry and/or permanence of infected tubers [2,11], the use of hermetic storage conditions that prevent the entry of adult moths, the monitoring of tubers for signs of rot or larval damage [11], the use of potato varieties resistant to the moth [2], and chemical control [2], the latter being used only in tubers intended for planting. Biological control alone is recommended for potatoes destined for consumption and/or processing since there are no health risks, it does not harm the environment, and it does not induce resistance in pests, as insecticides do [4,11]. In this regard, the use of entomopathogens, such as viruses, bacteria, and nematodes, and the use of entomophages, such as parasitoids and/or predators, may be appropriate for the control of the PTM [2,4].

Among the entomophages, oophagous parasitoids of the genus *Trichogramma*, such as *T. evanescens* West., *T. cacaeciae* (Marchal), *T. achaeae* Nagaraja and Nagarkatti, *T. chilonis* Ishii, and *T. principium* Sugonjaev and Sorokina (Hymenoptera: Trichogrammatidae) have been evaluated in relation to PTM egg parasitism under field conditions, and also in storage [17–19]. Similarly, the mites *Blattisocius mali* (Oudemans) and *B. tarsalis* Berlese (Acari: Blattisociidae) have also been studied as potential predators of PTM eggs [16,20,21].

The techniques for evaluating the efficacy of entomophages can be carried out by direct or indirect methods, as reviewed by Mills [22]. Due to the diverse biological characteristics, direct evaluation is complicated in the case of parasitoid species (e.g., Van Driesche [23]), and even more so in the case of predatory species (e.g., Latham and Mills [24]). The question as to whether evaluation criteria can be used, and/or which of them would be most effective, has been debated at length, but is not yet clarified (van Lenteren et al. [25]).

The effectiveness of synthetic insecticides/miticides have been evaluated in the standard way by their corrected mortalities with respect to a control (Abbott's formula) [26] via laboratory and/or field bioassays (e.g., Robertson and Preisler [27], Matthews [28]). This has also been extended to evaluating entomopathogens (e.g., Navon and Ascher [29]). The Abbott's correction (or effective kill rate) is based on a multiple- (double-) decay life table over a single time interval (age) [30].

It should be noted that, firstly, in the case of insecticides/acaricides, such evaluation is problematic, and can lead to biased values with overestimations and/or underestimations of effectiveness (e.g., Fleming and Retnakaran [31], Rosenheim and Hoy [32]). Secondly, in the case of entomophages (predators and parasitoids) that do not act promptly, and whose responses are density dependent, the effectiveness evaluation seems inadequate, especially over the long term.

Within this context, it may be necessary to evaluate the effectiveness of an entomophage (predator or parasitoid) with the help of mathematical models. Thus, Berryman [33] considers that there are two basic trends in the modeling of interactions between predators and prey. One arises from logistic equations, which describe the growth of a consumer population living in a finite environment, and the other, from the Lotka–Volterra equations, describes the physics of interacting predator and prey populations—thus, different conclusions can be drawn about the biological control practice depending on the approach.

Based on the above, the objectives of this work were twofold—firstly, to try to improve the effectiveness evaluation of entomophages by fitting it to mathematical models, and secondly, to study the effectiveness of a PTM control that uses two biological control agents, *T. cacaeciae* and *B. mali*, to combat the PTM under storage conditions.

## 2. Materials and Methods

### 2.1. Evaluation of the Effectiveness of *T. cacaeciae* and *B. mali* Used in PTM Control under Storage Conditions

A completely randomized univariate experimental design was employed with a single factor or treatment. This factor presented 3 levels: (1) the release of the predatory mite *B. mali*, (2) the release of the parasitoid *T. cacaeciae*, and (3) the control with no release of entomophages. There were 8 repetitions for each treatment.

The trial was carried out following the methodology described by Saour [17] and Arthurs et al. [34]: 15 PTM adults (8 females + 7 males, 2 days old) were released into each cylindrical plastic container (diameter: 28 cm, height: 23 cm, volume: 12 L) and covered with a fine mesh (30 × 30 threads per cm<sup>2</sup>), into which 13–14 potato tubers (≈2 kg) (Variety: Melody, category 1, size 45/80 mm) were placed onto a layer of vermiculite (1 cm) as a substrate for the pupae. At 48 h after the release of the PTM adults, the *T. cacaeciae* and *B. mali* were released at the following doses: 20 female parasitoids (2 days old) or 216 adult predator mites (2 days old) per container, according to the treatment; except for the control containers (the check) into which the entomophages were not released. The containers were left to evolve for 49 days (1–2 PTM generations) under controlled conditions (22.7 ± 4.0 °C and 52.2 ± 14% RH) and natural diffused light.

During the test, 4 containers from the 3 treatments were left to evolve until the end of the test; and another 4 containers were sampled twice a week (13 samples). In each sampling event, 4 potatoes (1 per container) were randomly selected and frozen (at −20 °C). At the end of the test, the contents of each container (that had not been sampled during the test) were also frozen.

Subsequently, all the frozen samples were inspected, the tubers dissected, and the PTM individuals counted (eggs, larvae, pupae, and adults). In addition, the potatoes damaged by the infestation, and the number of galleries found per tuber, were estimated. During the trial, the ambient temperature and relative humidity values were recorded daily, every 15 min, using a Data logger (model EBI 20-TH, Ebro<sup>®</sup> Xylem Analytics Germany Sales GmbH and Co. KG ebro, Ingolstadt, Germany).

The values of PTM's new adults obtained at the end of the trial were statistically analyzed by means of generalized linear models (GZLM) with the normal distribution and the identity link function using IBM SPSS version 26 software; the mean values were compared in pairs using the Wald test, performed with the same software.

The effectiveness of the PTM control using both biological control agents, compared to the control, was calculated using the Abbott formula [26,30]:

$$EKR = \frac{P_{nat} - P_{trt}}{P_{nat}}$$

where *EKR* = effective kill rate, *P<sub>nat</sub>* = the proportion alive in the control, *P<sub>trt</sub>* = the proportion alive in the treatment, and (*P<sub>nat</sub>* − *P<sub>trt</sub>*) giving the proportion dead owing to the treatment alone.

### 2.2. Mathematical Model: Logistic Functions

The data obtained from the PTM (eggs, larvae, pupae, and adults) in the 13 samplings carried out during the trial were transformed into accumulated insect-days (AID) according to Ruppel [35]. This index has already been used to evaluate biological control under field conditions (e.g., Cabello et al. [36]).

The above data were fitted to the Verhulst–Pearl logistic function (single, double, and triple). In this case, the logistic differential equation is

$$\frac{dx}{dt} = rx \left( 1 - \frac{x}{K} \right)$$

the solution curve of which is

$$x(t) = \frac{K}{1 + \left(\frac{K}{x_0} - 1\right)e^{-rt}}$$

where  $x$  = the population density,  $x_0$  = the initial population density,  $r$  = the instantaneous growth rate, and  $K$  = the system support capacity. In the case of double and triple functions, the following adjustments were made to the sums of the previous equation:

$$x(t) = \frac{K_1}{1 + \left(\frac{K_1}{x_{01}} - 1\right) \cdot e^{-r_1 t}} + \frac{K_2}{1 + \left(\frac{K_2}{x_{02}} - 1\right) \cdot e^{-r_2 t}}$$

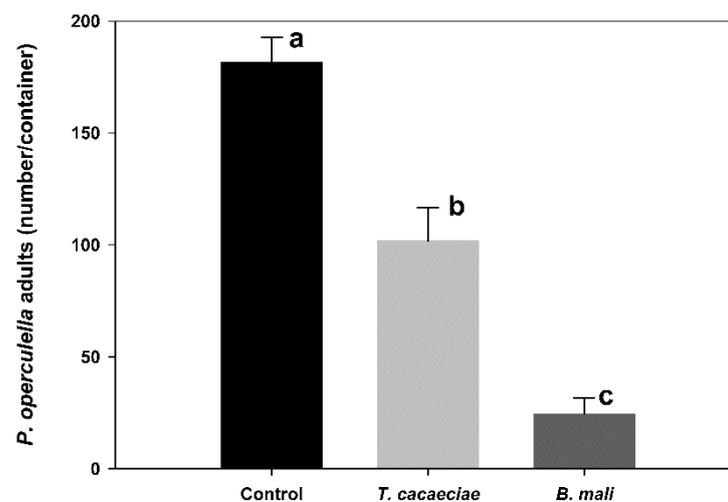
$$x(t) = \frac{K_1}{1 + \left(\frac{K_1}{x_{01}} - 1\right) \cdot e^{-r_1 t}} + \frac{K_2}{1 + \left(\frac{K_2}{x_{02}} - 1\right) \cdot e^{-r_2 t}} + \frac{K_3}{1 + \left(\frac{K_3}{x_{03}} - 1\right) \cdot e^{-r_3 t}}$$

The parameters are the same as before, but consider the double (2 sections) or triple (3 sections) logistic functions in each section. TableCurve 2D version 5.01 software was used for the logistic adjustments. The best fit to the model was evaluated by the lowest value of the corrected Akaike information criterion ( $AIC_c$ ). The effectiveness of each entomophage was calculated using the adjusted surfaces under the logistic curve for the predator and the parasitoid, with respect to the control, corrected for the estimated initial values ( $X_0$ ) (Carreño et al. [37]).

### 3. Results

#### 3.1. Effectiveness of *T. cacaeciae* and *B. mali* Used in PTM Control under Storage Conditions

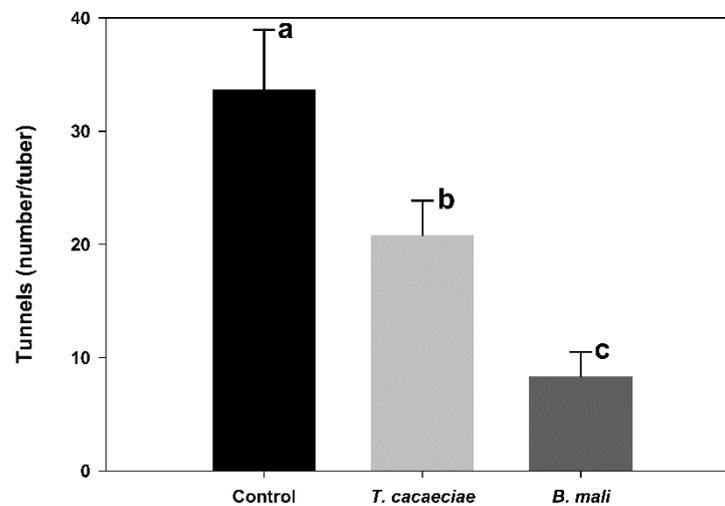
The number of adults that completed their development under tuber storage conditions are shown in Figure 1. In the statistical analysis, the model was significantly adjusted to the data according to the Omnibus test ( $\chi^2$  of the likelihood ratio = 29.478, d.f. = 2,  $p < 0.01$ ), and a high statistical significance was found in the pairwise comparison of the predator and parasitoid effect with respect to the control (Wald  $\chi^2 = 127.972$ , d.f. = 2,  $p < 0.01$ ). The mean values for specimens that were able to complete their development were  $181.75 \pm 11.00$  PTM adults in the control compared to  $24.50 \pm 7.22$  and  $102.00 \pm 14.61$  adults in the containers in which the mites or parasitoids were released, respectively.



**Figure 1.** Mean number of *P. operculella* adults (PTM) ( $\pm$ SE) after 49 days when an initial release of *B. mali* or *T. cacaeciae* was carried out, compared to the control, under storage conditions (values with different letters indicate significant differences at  $p = 0.01$ ).

The average number of galleries per tuber was significantly adjusted to the model according to the Omnibus test ( $\chi^2$  of the likelihood ratio = 33.407, d.f. = 2,  $p < 0.01$ ).

(Figure 2), and these values were significantly affected by the predators and the parasitoids with respect to the control (Wald's  $\chi^2 = 14.586$ , d.f. = 2,  $p < 0.01$ ).



**Figure 2.** Damage by *P. operculella*: mean number of tunnels ( $\pm$ SE) per potato tuber after 49 days, when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions (values with different letters indicate significant differences at  $p = 0.01$ ).

The effectiveness, or “effective kill rate” (EKR), according to the Abbott formula, was 86.52% for *B. mali* and 43.88% for *T. cacaeciae*.

### 3.2. Effectiveness Evaluation Using the Mathematical Model

Table 1 shows the adjusted AID values for the PTM in the three treatments performed. One can observe that, in each case, the best fit is the simple sigmoidal function, as it presented the lowest  $AIC_c$  values. This shows that only one PTM generation was present during the bioassay period (49 days) under the climatic conditions previously described. Figure 3A shows the simple logistic adjustments carried out in each treatment while, in turn, Figure 3B shows the functions derived from said adjustments.

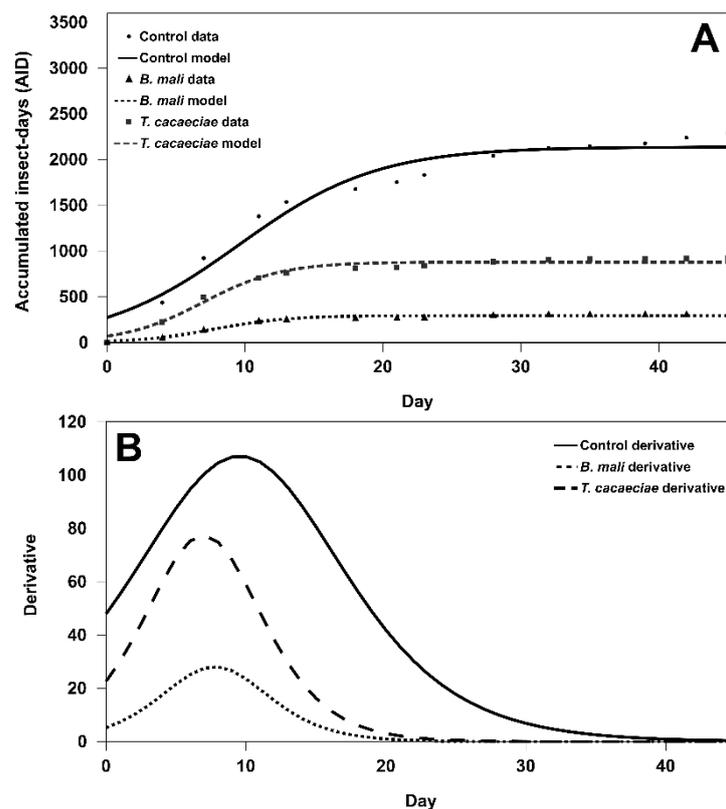
Confirming the values found in the previous section, one can see that the predatory mite was able to limit the PTM population to a greater extent than the parasitoid (Figure 3).

Using the results found in the previous logistic adjustments, we can estimate the effectiveness values for the predatory mite (94.85%) and for the parasitoid (73.77%) in both cases, compared to the control.

**Table 1.** Parameters and statistical significance for the logistic adjustments (single, double, or triple) of the total number of *P. operculella* individuals when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions.

Treatment	Function	Adjustment Parameters									Statistical Parameter
		X <sub>01</sub>	r <sub>1</sub>	K <sub>1</sub>	X <sub>02</sub>	r <sub>2</sub>	K <sub>2</sub>	X <sub>03</sub>	r <sub>3</sub>	K <sub>3</sub>	
Control	Single	6.80 ± 2.21	0.20 ± 0.03	2137.22 ± 65.94	-	-	-	-	-	-	190.51 *
	Double	14.78 ± 5.94	0.39 ± 0.07	1704.57 ± 88.47	9.32 × 10 <sup>19</sup>	1.96 ± 16.12	464.54 ± 95.44	-	-	-	206.54
	Triple	19.68 ± 6.60	0.49 ± 0.07	1510.97 ± 152.1	325.41 ± 1366.9	0.25 ± 0.2	660.06 ± 462.81	25,458,201 ± 1.39 × 10 <sup>11</sup>	0.24 ± 3.42	44,543.93 ± 2.49 × 10 <sup>11</sup>	245.65
<i>B. mali</i>	Single	19.41 ± 8.34	0.38 ± 0.05	294.20 ± 5.55	-	-	-	-	-	-	136.41 *
	Double	36.09 ± 8.63	0.52 ± 0.04	265.24 ± 4.55	5.2 × 10 <sup>23</sup> ± 3.7 × 10 <sup>26</sup>	2.32 ± 31.54	39.92 ± 5.05	-	-	-	152.29
	Triple	8.04 ± 1901.71	0.37 ± 15.67	298.6 ± 14,188.8	1.6 × 10 <sup>13</sup> ± 4.0 × 10 <sup>18</sup>	2.74 ± 22,096.5	-6.45 ± 14,198.23	-3.8 × 10 <sup>11</sup>	-2.06	-8.1 × 10 <sup>10</sup>	190.75
<i>T. cacaeciae</i>	Single	11.60 ± 3.86	0.35 ± 0.05	879.37 ± 15.11	-	-	-	-	-	-	165.42 *
	Double	17.67 ± 4.90	0.47 ± 0.05	801.93 ± 19.79	1.6 × 10 <sup>20</sup> ± 6.2 × 10 <sup>22</sup>	1.99 ± 16.63	104.38 ± 22.05	-	-	-	181.24
	Triple	16.60 ± 5.17	0.45 ± 0.05	812.82 ± 17.72	8.67 × 10 <sup>24</sup> ± 1.8 × 10 <sup>29</sup>	2.08 ± 774.62	97.08 ± 49.02	754,962 ± 3.52 × 10 <sup>8</sup>	0.33 ± 11.39	2.60 ± 82.00	220.24

Adjustments in each treatment are indicated by \* representing the best fits. n = 4.



**Figure 3.** (A) Adjustments to simple logistic functions and (B) their derivative functions from the accumulated insect-days (AID) of *P. operculella*/container when an initial release of the predatory mite *B. mali* or the parasitoid *T. cacaeciae* was carried out, with respect to the control, under storage conditions (whiskers represent the confidence limits at  $p = 0.05$ ).  $n = 4$ .

#### 4. Discussion

The conducted trial has made it possible to evaluate the effectiveness of two biological control agents under simulated potato tuber storage conditions, and to propose a new approach to estimate said effectiveness of the entomophages (predators and parasitoids) as control agents in a more reliable way, which does not require overly complex calculations—this now enables us to consider their potential application under real conditions in stored tubers.

Of the two ways to address the dynamics of the predator–prey or host–parasitoid population using mathematical models (as indicated in the Introduction), the use of logistic functions was chosen (Table 1). In this regard, it should be noted that the Lotka–Volterra equation has been applied more frequently, both theoretically and experimentally, in the biological control of pests compared to logistic equations (e.g., Gurr et al. [38], Barlow [39]).

The logistic functions were fitted using the AID values (Figure 3A). These were proposed by Ruppel [35] to evaluate the efficacy of insecticides and allow the magnitude of the number of insects, and their duration over time, to be combined in a single index. This index has been used for biological control evaluation under field conditions (e.g., Sanchez and Lacasa [40], Cabello et al. [36]) as well as in integrated pest management programmes [41]. In addition, these accumulated values allow for easy adjustment to single or multiple logistic functions. This has been achieved in the case of insect population dynamics (e.g., Carreño et al. [42]; Cabello & Carreño [43], Solano-Rojas et al. [44]), as well as in the epidemiology of phytopathogens [45–48]. Such an approach has been carried out in the present work (Figure 3A), allowing us also to obtain its derivative function (Figure 3B). In addition, these adjustments allow one to obtain an estimate of the instantaneous growth rate of the pest population ( $r$ ) in the three treatments (Table 1). As Birch [49] pointed

out, in a logistically growing population, the value of “ $r$ ” can be determined indirectly by calculating the appropriate logistic curve.

The best adjustment to simple logistic functions indicates that there was only one generation of the pest population during the test period in the three treatments (Figure 3B). Double or triple adjustments within the trial period could have indicated that either there were different cohorts developing at different rates (e.g., Solano-Rojas et al. [44]) or that there were two or more generations (e.g., Carreño et al. [42]), apart from overlapping populations.

It should be noted that a way to address the efficiency of entomophages has also been proposed using the “pest kill rate” ( $K_m$ ) [50,51] based on the calculation of life tables. Thus,  $K_m = \text{Ln}(K_0/T_k)$ , where  $T_k$  (the generation time) is the period of a generation that allows the predator to kill the prey, and  $K_0$  is the “net consumption rate”. This has been validated by evaluating different entomophagous species of the pest species *Tuta absoluta* (Lep.: Gelechiidae) [25,52]. We consider that this way of addressing the efficacy evaluation problem is highly suitable and quite well-founded. Regarding our proposal, both approaches for evaluating the effectiveness of entomophages are similar; both are based on life tables. The “net consumption rate”, according to the classification by Mills [22], is a direct method of evaluating entomophagous effectiveness. Conversely, in our case, it is an indirect method based on only assessing the effects on the phytophagous population.

As mentioned in the introduction, the predation/parasitism rates present calculation problems, especially under field conditions. Coupled to this is the fact that life table calculations under field conditions present greater practical difficulty than under laboratory conditions (e.g., Bellows and Van Driesche [53]; Southwood and Henderson [54]). Therefore, our proposal for evaluating entomophages may be less complex to carry out, especially under field conditions.

Returning to the effectiveness values found for *B. mali*, these were 86.52% using the Abbott formula and 94.85% using the new formula; for *T. cacaeciae*, the values were lower, 43.88% and 73.77%, respectively. In our view, the differences between the two methods of evaluating the effectiveness of both entomophages occur mainly because, when applying the Abbott formula, it only considers the initial and final values of the phytophagous populations in the trial. In contrast, the new calculation method considers all the intermediate values of the pest population, according to the number of samplings carried out over the trial in the different treatments. The initial application of this novel approach was proposed by Carreño et al. [37], although without applying logistic adjustments, and its validity was verified by evaluating the effectiveness of arthropod pests, phytopathogens, and weeds [55].

In the results obtained, *B. mali* presented an effectiveness of 94.85% in reducing the adult potato tuber moth population. This value is similar to that found by Gallego et al. [21], who reported that this mite species had a maximum PTM-control effectiveness of 92.51% (at the highest dose) under laboratory conditions and over the short term. In turn, for the species *B. tarsalis*, similar efficiencies of 98.86% have been cited [20] and 92.31% [16] in short-term trials under laboratory conditions.

In contrast, the parasitoid species *T. cacaeciae* presented an effectiveness of 73.77%, lower than that of the predatory mite. This value is in line with Gallego et al. [19] for the same species, with a parasitism percentage of 70.88% under laboratory conditions. These values were higher than those found for this species, as well as for *T. evanescens* and *T. principium*, by Saour [17], which never presented parasitization percentages of PTM eggs higher than 35%. Furthermore, under experimental conditions such as ours, Mandour et al. [18] only achieved reductions of 25.9% in the PTM infestation percentage, compared to the control, when they used the species *T. evanescens*. Conversely, Gallego et al. [19] reported parasitization percentage values for *T. achaeae* of 92.78%, which may indicate a greater effectiveness of this species, although the test was carried out in the laboratory.

## 5. Conclusions

We can conclude that, firstly, the use of PTM biological control agents in stored potatoes has proven a practical alternative, and that they can potentially adjust to warehouse conditions, particularly those that are not refrigerated, as has been indicated by other authors [16–18,21]. Secondly, we believe that the Abbott formula, even with the aforementioned drawbacks, is still useful in evaluating the effectiveness of pest control agents over the short term, especially in laboratory tests, with equal initial numbers of phytophagous between treatments, and for evaluations over hours or a few days. Finally, we consider that the proposed way of evaluating efficiencies, using logistic models, may be more appropriate for biological control agents (predators and parasitoids) than the standard methods (e.g., the Abbott formula). In addition, we believe it is especially appropriate when biological control is carried out by augmentation via inoculative releases. Accordingly, it is expected that the entomophagous populations released at low doses will increase over time, and can thus control the pest population—this can be achieved after several generations of the species involved (e.g., Vila and Cabello [56]).

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