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# MATHEMATICAL PROBIT AND LOGISTIC MORTALITY MODELS OF THE KHAPRA BEETLE FUMIGATED WITH PLANT ESSENTIAL OILS

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ABSTRACT. In the current study, probit and logistic models were employed to fit experimental mortality data of the Khapra beetle, Trogoderma granarium (Everts) (Coleoptera: Dermestidae), when fumigated with three plant oils of the gens Achillea. A generalized inverse matrix technique was used to estimate the mortality model parameters instead of the usual statistical iterative maximum likelihood estimation. As this technique needs to perturb the observed mortality proportions if the proportions include 0 or 1, the optimal perturbation in terms of minimum least squares  $(L_2)$  error was also determined. According to our results, it was better to log-transform concentration and time as explanatory variables in modeling mortality of the test insect. Estimated data using the probit model were more accurate in terms of  $L_2$  errors, than the logistic one. Results of the predicted mortality revealed also that extending the fumigation period could be an effective control strategy, even, at lower concentrations. Results could help in using a relatively safe and effective strategy for the control of this serious pest using alternative control strategy to reduce the health and environmental drawbacks resulted from the excessive reliance on the broadly toxic chemical pesticides and in order to contribute safeguard world-wide grain supplies.

1. Introduction. Grain crops are considered as one of the main food sources for human in the majority of developing countries. Recent estimates declared that losses in these crops caused by insects and other pathogens reached 20-30% in the tropical and temperate zones [21]. The Khapra beetle, *T. granarium* (Everts) is one of the most destructive pests of stored grains worldwide [15]. Nowadays, it is considered as a quarantine pest, hence very strict legislation are being taken by many countries so as to prevent the introduction of this pest with agricultural products [9]. The problem of preventing the beetle's spread is further compounded by its ability to survive for several years in the larval stage with little or no food, and its habit of hiding in cracks and crevices [16]. Control of this pest using conventional insecticides and fumigants led to toxic hazards to non-target organisms, environmental drawbacks

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and development of insect resistance [2, 6, 20]. For the possibility of producing good quality foodstuffs, it is necessary to reduce the risks associated with the excessive application of chemical pesticide in primary agricultural production. The current trend is the search for newer insecticides, which will have to meet entirely different standards. They must be pest specific, non-phytotoxic, nontoxic to warm-blooded animals, degrade rapidly and do not persist in soil or leach into groundwater, do not leave toxic residues in food products, less prone to pesticide resistance, less expensive, and locally available [11]. In this context, the use of botanical pesticides to protect plants from pests is very promising because these natural products fulfill many of the previous requirements [12]. Plant essential oils are among the most efficient alternative strategies as pest control agents with minimal side effects, especially when tested against stored grain insects [13, 17–19, 22, 23]. In this regard, mathematical models can provide a relatively fast, accurate and inexpensive way to project the consequences of different assumptions about the merits of various pest management options [14, 25]. The usefulness of such models depends on generating or estimating the values of certain key parameters [25]. In our study, these parameters include mortalities of a stored grain insect under various pesticide doses and time period. Estimating parameters based on measured empirical data is a critical issue in biosecurity models. These models based on estimating and integrating various parameters related to different sub-models representing different key biological processes. However, there are many problems of quantitative inference in biological research concerning the relation between a stimulus (e.g. oil fumigation) and a binomial response (e.g. mortality). A binomial generalized linear model, with a link function such as the probit or logistic functions, is usually used to analyze the empirical biological data. Normally, maximum likelihood estimation is applied to fit the parameters of such probit models. However, in such models the probit is a linear function of parameters or metameter (e.g. log) of parameters and the corresponding equation values with respect to the parameters, which form an over determined linear system. We also used a generalized inverse matrix method to find the least-squares solution of the regularization equations. This method has advantages over other methods if we only need to estimate parameters without other statistical information such as significance or confidence intervals for the estimates: it is simple with only one key command, provides a more accurate estimate of parameters, and even if the coefficient matrix of the over-determined linear system is not numerically (column) full ranked, it will still work and yield a solution with a minimum error in the  $L_2$  (the least square error) norm sense [3]. In the present study, we employ probit and logistic models for the development of viable, precise and long-term strategy to support the management of T. granarium funigated with plant oils from three species of the genus Achillea.

## 2. Methodology and models.

2.1. Maintenance of the test insect. A culture of the Khapra beetle, *T. granarium* was established depending on an original culture reared for several generations at Stored-Product Pests Department, Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt. Wheat grains, *Triticum aestivum* (6.5% moisture content) were used as media for insects. Seeds were previously sterilized by freezing at 5  $^{\circ}C$  for one week to kill any prior insect infestation before use. Emerged adults were maintained in 1 L capacity glass jars, each contained 100-150 adults and about 250 g sterilized wheat grains. Jars were covered with

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muslin cloth held with rubber bands and maintained in the laboratory under rearing conditions of  $28 \pm 2 \ ^oC$  and  $68 \pm 5 \ \%$  r.h. We use the adult stage for biological experiments as it is the most destructive for grains.

TABLE 1. Observed Mortality for various concentrations and times (A. biebersteinii).

Concentrations	2  days	4 days	8 days
1.56	0.043	0.150	0.210
3.13	0.142	0.242	0.492
6.25	0.215	0.325	0.703
12.5	0.313	0.483	0.836
37.5	0.590	0.746	0.961
50.0	0.692	0.830	1.000

2.2. Collection of the test plants and extraction of essential oils. The aerial parts of Achillea biebersteinii, A. santolina and A. millefolium were collected from different locations of Alamain desert and Sinai Peninsula, Egypt at the flowering period. Plant samples were identified and authenticated by the Botanists of Botany Department, Faculty of Science, Kafrelsheikh University. The fresh plant samples were air dried in the shade for 5 days at environmental temperature and the dried parts were powdered mechanically by using an electric blender, then sieved through a mesh size of 0.5 mm. Plant powders were subjected to hydrodistillation using a modified Clevenger-type apparatus to produce the plant oils. The extraction condition was 50 g powders; 500 mL distilled water, 6 h distillation and the process was repeated several times. Anhydrous sodium sulphate was used to remove water after extraction. The oil yield (%v/w) was calculated on a dry weight basis.

TABLE 2. Observed Mortality for various concentrations and times (A. santolina).

Concentrations	2 days	4 days	8 days
1.56	0.000	0.076	0.171
3.13	0.080	0.172	0.297
6.25	0.160	0.241	0.489
12.5	0.214	0.415	0.685
25.0	0.355	0.522	0.789
37.5	0.421	0.654	0.860
50.0	0.457	0.708	0.951

2.3. Fumigation bioassay. The fumigant toxicity of the plant oils was determined against adults of T. granarium [17, 18]. Filter papers (Whatman No. 1, cut into 4 cm diameter pieces, 12.56  $cm^2$ ) were impregnated with 25  $\mu$  L of each oil in *n*-hexane at doses calculated to obtain equivalent fumigant concentrations of 50.0, 37.5, 25, 12.5, 6.25, 3.13, and 1.56  $\mu$  L/L air or *n*-hexane only (control). After evaporating the solvent, the filter paper was attached to the undersurface of the screw cap of 50 mL volume glass vial. Test insect was transferred to the vials in groups of twenty unsexed 1 week-old adults. The vials were covered with fine steel gauze secured with adhesive tape. Six replicates of each treatment and control were set up. Exposure of insects were continued for 24 h, then, insects were transferred to clean vials, as groups of twenty insects, with culture media and kept under the

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same rearing conditions. Mortality counts were made 2, 4 and 8 days post treatment.

TABLE 3. Observed Mortality for various concentrations and times (A. millefolium).

Concentrations	2 days	4 days	8 days
1.56	0.000	0.054	0.161
3.13	0.068	0.110	0.292
6.25	0.098	0.215	0.405
12.5	0.194	0.312	0.488
25.0	0.269	0.435	0.650
37.5	0.341	0.495	0.701
50.0	0.417	0.571	0.802

2.4. **Probit model.** Probit models were introduced earlier by Bliss [4] as a fast method for computing maximum likelihood estimates. It is a type of regression where the dependent variable can only take two values, for example survived or not survived. Its name is composed of the first four letters from "**prob**ability" and the last two letters from "unit". The purpose of the model is to estimate the probability that an observation will fall into a specific one of the categories. Probit analysis is used to analyze many kinds of binomial response experiments in a variety of fields especially, analysis of dose-response, mortality of inspects and quantitative genetics. It is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. The probit link function  $\phi(P) = Y - 5$  is the inverse cumulative distribution function (CDF) associated with the standard normal distribution [4, 10].

$$P = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{Y-Z} e^{\frac{-u^2}{2}} du$$
 (1)

Where z = 5, P is the actual mortality (proportion that died,  $0 \le P \le 1$ ) and  $Y = \phi(P) + 5$  is the probit transformed mortality. Note that, adding five to  $\phi(P)$  just ensures all Y values are positive in practice [25]. The probit mortality Y may be depends on time t, concentration C and their interaction (*i.e.* Ct) to get a four parameter probit model

$$Y = a + b_1 \log(t) + b_2 \log(C) + b_3 \log(t) \log(C)$$
(2)

Without the interaction between C and t, we get three parameter probit model in the form [10]:

$$Y = a + b_1 \log(t) + b_2 \log(C) \tag{3}$$

In the case of a fixed time t on a range of concentration C or a fixed C on a range of t whereas, independent data do not depend on C or t separately, but on the product Ct, the parameters  $b_1$  and  $b_2$  can be merged into a single parameter b to get the two probit model:

$$Y = a + b \log(Ct) \tag{4}$$

2.5. Logistic model. The canonical logit link function for logistic models [1] is

$$Y = ln \frac{P}{1 - P} \tag{5}$$

Eqs (2-4) still valid with the new link function (5)

Concentrations		1.56	3.13	6.25	12.5	25.0	37.5	50.0
Observed		0.210	0.492	0.703	0.836	0.950	0.961	1
2 Parameters	Probit	0.235	0.448	0.677	0.853	0.949	0.976	0.987
	Logit	0.233	0.451	0.688	0.856	0.941	0.966	0.977
3 Parameters	Probit	0.327	0.507	0.684	0.827	0.920	0.953	0.969
	Logit	0.323	0.511	0.695	0.833	0.916	0.945	0.960
4 Parameters	Probit	0.241	0.442	0.658	0.832	0.935	0.967	0.981
	Logit	0.242	0.447	0.671	0.837	0.928	0.957	0.970

TABLE 4. Observed and predicted mortality for various concentrations at fixed time t = 8 days using 2, 3 and 4 parameter probit models (A. biebersteinii)

2.6. Generalized inverse matrix approach. The concept of a generalized inverse [3] was firstly known as "pseudoinverse". When fitting the above Probit or Logit model, we usually get an over-determined system of linear equations with respect to the parameters  $a, b_1, b_2, b_3$ . Let  $\{Y_i; t_i; C_i\}_{i=1}^N$  be N of observed data, where  $Y_i$  is the link function,  $t_i$  is the time and  $C_i$  is the concentration, then the over-determined system linear equations may be in the form:

$$Y_i = 1.a + (log t_i).b_1 + (log C_i).b_2 + (log t_i)(log C_i).b_3, \ i = 1..N$$

Or, in the matrix form:

$$\begin{pmatrix} 1 & log(t_1) & log(C_1) & log(t_1)log(C_1) \\ 1 & log(t_2) & log(C_2) & log(t_2)log(C_2) \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ 1 & log(t_N) & log(C_N) & log(t_N)log(C_N) \end{pmatrix} \begin{pmatrix} a \\ b_1 \\ b_2 \\ b_3 \end{pmatrix} = \begin{pmatrix} Y_1 \\ Y_2 \\ \cdot \\ \cdot \\ Y_N \end{pmatrix}$$

Let Ax = b be the matrix form of the over-determined system of linear equations, where x is the model parameter vector, b is  $\{Y_i\}_{i=1}^N$ , A is  $N \times p$  matrix,  $p \leq N$  is a parameter (in our case p = 4). When we calculate the model estimator parameters, the Least Square solution [29] is the solution which minimizes the least square error:

$$L_{2} = \sum_{i=1}^{N} (\tilde{Y}_{i} - Y_{i})^{2}$$

where  $Y_i$  is the observed link function, and

$$Y_i = a + b_1 \log(t_i) + b_2 \log(C_i) + b_3 \log(t_i)\log(C_i), \ i = 1..N$$

is the predicted link function. The Least Square method used to solve regularized systems Ax = b (*i.e.* A is nonsingular square matrix). If A is singular or rectangular matrix, then  $A^T A x = A^T b$  hence  $x = (A^T A)^{-1} A^T b$  or  $x = A^+ b$  where  $A^+ = (A^T A)^{-1} A^T$  is the generalized inverse (or Moore-Penrose pseudo-inverse) matrix of A. Normally, one can notice that if A is nonsingular square matrix then  $A^+ = A^{-1}$ . Even when A is not column full ranked [3] there is still exist  $A^+$  where the system has a solution with minimum  $L_2$ .

TABLE 5. Observed and predicted mortality for various concentrations at fixed time t = 2 days using 2, 3 and 4 parameter probit models (A. santolina)

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Concentrations		1.56	3.13	6.25	12.5	25.0	37.5	50.0
Observed		0.000	0.080	0.160	0.214	0.355	0.421	0.457
2 Parameters	Probit	0.045	0.085	0.146	0.233	0.342	0.414	0.466
	Logit	0.054	0.090	0.146	0.228	0.339	0.414	0.470
3 Parameters	Probit	0.019	0.049	0.108	0.208	0.348	0.443	0.513
	Logit	0.030	0.059	0.113	0.206	0.345	0.444	0.518
4 Parameters	Probit	0.037	0.074	0.132	0.217	0.327	0.400	0.455
	Logit	0.046	0.079	0.132	0.211	0.321	0.397	0.455

2.7. Perturbation technique. If the observed data starts with 0 or ends with 1, the link functions of the probit or logit model do not defined, then these values must be changed. Authors usually change 0 to 0.0001 and 1 to 0.9999, but it is not the case that the smallest changes of 0 or 1 the smallest  $L_2$  error, and so, we let 0 to be  $\epsilon$  (a small perturbation value), and 1 to be  $1-\epsilon$  and then  $L_2$  becomes a function of  $\epsilon$ . To get the appropriate value of  $\epsilon$  which minimizes  $L_2$ . In our study, we apply the well-known Newton-Raphson technique to solve the equation  $L'_2(\epsilon) = 0$  using the iterative formula

$$\epsilon_{i+1} = \epsilon_i - \frac{L_2'(\epsilon)}{L_2''(\epsilon)}$$

Where,  $L_{2}^{'}(\epsilon)$  and  $L_{2}^{''}(\epsilon)$  are the first and second derivative of  $L_{2}(\epsilon)$ .

TABLE 6. Observed and predicted mortality for various concentrations at fixed time t = 2 days using 2, 3 and 4 parameter probit models (A. millefolium)

Concentrations		1.56	3.13	6.25	12.5	25.0	37.5	50.0
Observed		0.000	0.068	0.098	0.194	0.269	0.341	0.417
2 Parameters	Probit	0.029	0.058	0.108	0.182	0.282	0.351	0.403
	Logit	0.037	0.064	0.108	0.178	0.278	0.351	0.407
3 Parameters	Probit	0.026	0.054	0.102	0.175	0.276	0.345	0.399
	Logit	0.037	0.064	0.107	0.175	0.272	0.342	0.397
4 Parameters	Probit	0.023	0.050	0.097	0.171	0.274	0.345	0.400
	Logit	0.032	0.057	0.099	0.168	0.270	0.345	0.404

TABLE 7.  $\epsilon$  and  $L_2$  for A. biebersteinii, A. santolina and A. millefolium with 2,3 and 4 parameter models

Parameter	Model	A. biebersteinii		A. santolina		A. millefolium	
		t = 8 days		t = 2	t = 2 days		days
		$\epsilon$	$L_2$	$\epsilon$	$L_2$	$\epsilon$	$L_2$
2 Parameters	Probit	0.0077	0.0037	0.0455	0.00087	0.0264	0.0008
	Logit	0.0218	0.0029	0.0568	0.00099	0.0362	0.0007
3 Parameters	Probit	0.0077	0.0601	0.0119	0.0211	0.0284	0.0084
	Logit	0.0218	0.0602	0.0269	0.0227	0.0491	0.0098
4 Parameters	Probit	0.0077	0.0563	0.0324	0.0127	0.0202	0.0085
	Logit	0.0218	0.0563	0.0432	0.0158	0.0317	0.0096

3. **Results.** In Table (1) at  $C = 50 \ \mu \ \text{L/L}$  and t = 8 days mortality reached 1 (*i.e.* all sample insects are killed) and in Tables (2 and 3) at  $C = 1.56 \ \mu \ \text{L/L}$  and t = 2 days, mortality is 0 (*i.e.* the tested oil did not effect on the target insects). In these cases, we need to use the perturbation technique to change these values by

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the nearest values which minimize  $L_2$ , all other cases do not need this technique. An overview of Tables (1-3), indicates that the first oil is more effective than the second and the second is more effective than the third. Tables (4-6) contain observed and predicted mortality data using the probit and logit models with 2, 3 and 4 parameter. Values of  $\epsilon$  and corresponding  $L_2$  are presented in Table (7), which shows that, in general, 2 parameter model is more efficient than 4 parameter and 4 parameter is more efficient than 3 parameter. It is well-documented that the 2 parameter model is not a degenerated model of the 4 parameter one. So, for a global and precise management strategy the 2 parameter model should be adapted. Therefore, an optimum control strategy could be reached, if we use individual time-based models. Estimator parameters  $a, b, b_1, b_2$  and  $b_3$  for 2, 3 and 4 Parameters probit and logit models are presented in Tables (8 and 9). Table (10) gives concentrations for 50% and 99% mortality. Fig. (1) displays the observed mortality, 2 parameter probit and logit predicted mortality at t = 8 days for A. biebersteinii. Fig. (2) displays the same parameters at t = 2 days for A. santolina. Fig. (3) shows the discrepancy between observed and predicted mortality at fixed time t = 2 days using 2 and 4 parameter probit models for A. millefolium. Fig. (4) explains the advantage of using  $\epsilon$  which minimizes  $L_2$  over its other values. Figs (5 and 6) show  $\epsilon$  which minimizes  $L_2$  for the two cases. Data show that the probit and logit procedures may be acceptable models for the observed mortality in terms of least square error, and the 2 parameter models usually better than the 3 or 4 ones, whereas, in case of 3 or 4 parameter the probit are better than the logit models.

Plant oil	Model	2 days		4 days		8 days	
		a	b	a	b	a	b
A. biebersteinii	Probit	0.8349	1.3515	1.0418	1.3032	-0.5765	1.9604
	Logit	-7.2366	2.3483	-6.5368	2.1515	-9.3502	3.2940
A. santolina	Probit	1.2941	1.0714	0.7954	1.2843	1.6080	0.0208
	Logit	-6.2801	1.8222	-7.1082	2.1681	-8.4719	2.7413
A. millefolium	Probit	1.2267	1.0375	0.9141	1.1592	1.2451	1.1292
	Logit	-6.8590	1.9177	-7.1090	2.0223	-6.1726	1.8553

TABLE 8. The parameters a and b for 2 Parameters probit and logit models.

TABLE 9. The parameters  $a, b, b_1, b_2$  and  $b_3$  for 3 and 4 Parameters probit and logit models.

Plant oil	Model	3 Parameters				4 Parameters			
		a	$b_1$	$b_2$	a	$b_1$	$b_2$	$b_3$	
A. biebersteinii	Probit	-1.143	2.364	1.538	0.935	1.316	-0.466	1.011	
	Logit	-10.50	4.052	2.598	-7.269	2.424	-0.516	1.571	
A. santolina	Probit	-0.801	2.050	1.405	1.072	1.147	-0.197	0.777	
	Logit	-9.708	3.434	2.358	-6.675	1.953	-0.325	1.317	
A. millefolium	Probit	0.144	1.599	1.124	-0.019	1.667	1.209	-0.032	
	Logit	-8.057	2.639	1.885	-8.781	2.958	2.355	-0.204	

TABLE 10. Concentrations corresponding to 50% and 99% mortality using 2 parameter probit model.



FIGURE 1. Observed and predicted Mortality for various concentrations at a fixed time t = 8 days using 2 parameter probit and logit models (A. biebersteinii -Adults



Plant oil	Mortality percentage	2 days	4 days	8 days
A. biebersteinii	50~%	25.154	11.353	3.6416
	99~%	1324.2	692.27	55.974
A. santolina	50~%	59.921	19.572	6.5039
	99~%	8888.1	1267.8	181.93
A. millefolium	50~%	83.671	34.885	11.016
	99~%	10958	3544.6	1265.3

4. **Discussion.** In this study, probit and logit models are presented for mortality data of *T. granarium* when fumigated with three plant essential oils. According to our results, it was better to log-transform concentration and time as explanatory variables in modeling the mortality of *T. granarium* fumigated with the plant essential oils, rather than use the untransformed variables, the same result was obtained by Shi and Renton [25]. Therefore, only results for the logarithmic function are presented here. Moreover, for all data sets, the probit model is usually better than, or at least, equals its alternative logistic model. Therefore, we conclude that the probit model based on log-transform concentrations and times. In related studies, it was an arbitrary decision about what value to use instead of 0 or 1 (e.g. changing 0 to 0.0001 or 1 to 0.9999) when using a Least-Squares approach to fit probit (two or four-parameter) models to mortality/survival data of *Rhyzopertha dominica* due to phosphine fumigation [24, 26, 27]. Nonetheless, mortality estimations were



strongly affected by this arbitrary decision. Therefore, the perturbation approach was used herein to make the fitted models much more accurate. Choosing  $\epsilon$ , which minimizes  $L_2$ , improves the models, for example, Fig. (4) shows that, at C = 12.5, using  $\epsilon$  with minimum least square error ( $\epsilon = 0.0077$ ) the mortality M = 0.8528 and  $L_2 = 0.0037$ , whereas when  $\epsilon = 0.0001$  the mortality becomes M = 0.8966 and  $L_2 = 0.0105$  with increasing percentage reached 183%. Also, for A. millefolium the  $L_2$  value at  $\epsilon = 0.0001$  reaches approximately 30 times of its least square value (Fig. 5).

The estimations using 3 parameter probit and logistic models result in bigger  $L_2$  errors than those using corresponding 4 parameter models (Table 7), because the latter considers the interactions between concentration and time, which already occur in reality. The advantages of these models will help us more confidently predict mortality in *T. granarium*, in order to weigh the merits of various management tools for delaying or avoiding evolution of resistance in this destructive primary pest of stored grains.

We have also compared how fumigation tactics based on extending the duration of fumigation or increasing the concentration of fumigation influence the control of the Khapra beetle. In related studies, it was reported that extending the fumigation period (while lowering concentration) will increase toxicity of phosphine as a fumigant against stored grain insect pests [5, 7, 8, 28]. High concentrations, therefore, may cause insects to go into a protective narcosis [25]. However, the toxic effects of fumigants may, therefore, be accumulated slowly in the target insects and the resistance mechanism can be overwhelmed during long time periods. Results of



for a fixed time t = 2days using 2 parameter probit models (A. santolina and A. millefolium

for a fixed time t = 2days using 4 parameter probit models (A. santolina and A. millefolium

the predicted mortality obtained from the two models in our study revealed that extending the fumigation period could be an effective control strategy, even at lower concentrations of the test oils. Our results could, therefore, help in continuing to use relatively safe and effective strategy for the control of insect pests this serious pest, and thus help safeguard world-wide grain crops.

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