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Dose-response relationship between abamectin and mortality of *Panonychus ulmi* (Acari: Tetranychidae)

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In this study, the dose-response relationship between abamectin and mortality of larval and adult forms of *Panonychus ulmi* (Koch) was established. Abamectin was investigated at a dilution series of 1/1, 1/1.15, 1/1.35, 1/1.6, 1/2, 1/2.55, 1/4, 1/8 and 1/16 of its field recommended concentration, in order to calculate the EC_i – values. Results showed that the lowest EC*i* – values were observed on larvae, which showed high sensitivity to abamectin than adults. Both statistical models led to satisfactory findings and the linear model was ranked as the best model for describing the dose-response relationship. This research highlighted the importance of toxicity risk assessments to obtain a more accurate estimation of the compatibility of abamectin in the integrated pest management (IPM) programs.

Keywords: Abamectin, Effect concentration, Modeling, REGTOX Macro, Toxicity.
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Introduction

The effect of pesticides in plant protection is currently taken into account when establishing control strategies in accordance with the principles of integrated management. However, the control of such a population can be easily conducted especially in the presence of natural enemies. Although there is sufficient information available regarding the harmful effects of pesticides on these useful mites, concerns have been expressed about the risks related to their massive use in intensive agriculture, as well as the threats incurred by the aquatic environment[1-4].

Management of phytophagous organisms is an integral part of IPM programs for many crops. In principle, the pesticides used must be chosen so that they cause minimal disruption to naturally occurring biological control agents. Also, control agents applied against the same pest must also be chosen carefully so that they do not disrupt each other[5,6].

The laboratory investigations reported here were conducted to assess the effects induced by abamectin, on the most dominant phytophagous mite on apple tree in the world, *Panonychus ulmi* (Koch)[7,8]. In the experimental orchard, this species of the family Tetranychidae (Donnadieu), develops and reproduces differently on the planted varieties during the growing season[9] and causes considerable physiological and economical damages in the absence of a reasoned management strategy.

Concurrently with the descriptive analysis of toxicological data, mathematical modelling has been advocated to provide assistance in developing a dose-response relationship, in particular when extrapolation to low doses is necessary. Mathematical models have been used for several decades in the field of toxicology[10,11]. The dose-response relationship is an association between the dose and the incidence of a defined biological effect in an exposed population, usually expressed as a percentage of mortality among individuals[12].

The objectives of this study were to determine the efficacy range of activity and effective concentrations EC*i* (i= 5, 10, 15, 20, 25 and 50) of abamectin. The previously published results on abamectin showed toxicity related to this active ingredient to terrestrial and aquatic invertebrates[13–16]. Calculations of ECi were done using the numerous statistical models and the software packages[11,17] and their values slightly

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stable can change whatever the statistical method used[18]. In ecotoxicology, different calculations have been developed for modeling the dose-response relationship. The values presented in this document were calculated for the Hills model by running Reg Tox algorithm, whereas the results of linear regression were generated using R software (R Foundation for Statistics Computing, North America).

Material and Methods

Mites rearing

Field-collected adults of *Panonychus ulmi* (Koch) were taken to the Health and Environment Laboratory, Department of Biology, Moulay Ismail University (UMI), Morocco. All developmental stages were reared for at least three generations on green bean plants *Phaseolus vulgaris* (L.) at 25 ± 1 °C, 65±5 % relative humidity (RH) and 16L: 8D, for use in a series of toxicological experiments.

Abamectin properties

Abamectin was purchased from Syngenta (Morocco) and commercialized under a commercial name of Vertimec 018 EC. The recommended dose of abamectin against mite pests on apple tree was determined at 200 $mL.hL^{-1}$, this acaricide is known for its effectiveness against phytophagous mites, it acts on all mobile forms by contact and by ingestion. Avermectins represent a novel class of macrocyclic lactones that have demonstrated nematicidal, acaricidal, and insecticidal activity. They are a mixture of natural products produced by a soil actinomycete, *Streptomyces avermitilis* MA-4680 (NRRL 8165). The discovery of the avermectins from this organism in 1976 has greatly influenced the arsenal of chemicals available for control of household and agricultural arthropod pests as well as parasites of mammals[19]. Avermectins are designated as A_1, A_2, B_1, and B_2, referring to mixtures of the homologous pairs containing at least 80% of the component and no more than 20% of the b component. The difference between the A and B series is a methoxy group at the C5 position of the cyclohexene moiety in the A-series and a hydroxyl group in the B-series. A double bond links carbons 22 and 23 (C22, C23) in the 1 series; this bond is reduced in the 2 series and is a hydroxyl group at C23. The series has a secondary butyl substitution at C25, whereas the b series has an isopropyl group in that position.

Larva and adult bioassays

Laboratory tests were carried out according to the method described by Knight *et al*[20]. Ten *P. ulmi* adults were transferred to bean leaf disk (diameter of 4 cm), wholly placed into a petri dish for evaluation. At least 50 adults were evaluated for each sprayed concentration of abamectin. the tests on larvae are carried out using the same method as the adults.

Abamectin was investigated at a dilution series of 1/1, 1/1.15, 1/1.35, 1/1.6, 1/2, 1/2.55, 1/4, 1/8 and 1/16.of its field recommended concentration, corresponding to eight final nominal concentrations of 200, 174, 148, 125, 100, 78, 50, 25, 12.5 and 0 mg/hL (control). The tests on larvae and adults were replicated five times and followed regularly for a period of 72 hours, live and dead individuals were counted every 24 hours at the same time as the newly formed individuals (post-treatment spawn) that were removed from each petri dish each day. The mixtures obtained were sprayed on leaf discs containing mites by a hand-held sprayer as described by Butt and Goettel (2000)[21], the volume of each concentration as previously measured by the same authors was 9.6 ± 0.83 $\mu L/cm^2$.

Data analysis

The mortality rates were corrected according to Abbott's formula[22] and were calculated after log transformation using first the linear regression model. The effective concentrations (EC*i*) were obtained applying the Excel Macro REGTOX, according to Vindimian *et al.*[23]. In this case, the REGTOX tool was based on Hill's model, which is usually formulated for a function that increases from zero to a maximum and has proved extremely relevant for many applications, receptor-ligand interactions, Michaelian enzymatic kinetics or not, dose-response curve or concentration-response in toxicology[24], while the logistic model is often presented in different formulae identical to Hill's model. The experiment data were analysed by plotting the logarithmic mortality on logarithm dose of abamrctin using the R Software program v.i 386 3.4.3

Assessment of models

The maximum likelihood method[25] was used to fit both models to the data. To assess the goodness of fit of a single model to the data, it is necessary to compare two or more different models fit with the same data. The goodness of fit was determined using the deviance Δ_{LL}. One useful formal criterion for

doing that is Akaike's information criterion (AIC) for model selection. In general, the model with the smaller AIC is preferred[26].

$$\Delta_{LL} = -2(LL_c - LL_f)$$

The parameter LL_c is the natural logarithm of the likelihood of the actual fitted (current) model and LL_f is the natural logarithm of the likelihood of the experimental data. The Δ_{LL}, however, cannot be used to determine significant differences between models or to test whether a model describes the data properly. Therefore, a Monte Carlo hypothesis test was applied. The probability of finding a worse fit was determined from 1000 artificial datasets. To rank the accepted models, the number of model parameters was taken into account using the second-order Akaike's information criterion (AIC)[27]:

$$AIC = -2LL_c + 2k + \frac{2k(k+1)}{n-k-1}$$

The parameter k is the number of parameters and n is the number of individuals exposed to abamectin. According to the AIC-values, both models were evaluated based on the probability that each model minimizes significantly the loss of information. The percentage of fits to the artificial datasets with a higher Δ_{LL} than the fit to the original dataset is interpreted as the goodness of fit, where the criterion for model acceptance was 5%.

Results and Discussion

Computation of EC*i*-Values

A dose-response relationship was modelled and results of both models showed that the EC*i*-values were lower on *P. ulmi* larvae. The available trend suggests that mortality of mites increased with increasing concentration. Linear and Hill's model's approximate EC_{50} values of abamectin on *P. ulmi* adults to 128.7 and 123.4 mL/hL, respectively (Table 1), and for larvae to 118.4 and 116.1 mL/hL (Table 2). The EC*i*-values calculated using both models were not significantly different. Hill's model allows integrating the effects on the different life cycle parameters of *P. ulmi*. The instars appear more sensitive to abamectin than adults. In general, the differences between the means values of EC*i* calculated using both models were insignificant either on adults or on larvae. Both models lead to establishing the dose-mortality relationship. All results were graphically summarized and the EC_i curves are given in Fig. 1.

The bootstrap method was used to calculate the mortality rates at 5 and 10% of confidence. In contrast to conventional methods; the bootstrap method is

Table 1 — Bootstrap calculation of abamectin toxicity parameters corresponding to Hill's model on *P. ulmi* adults

Calc. Parameters	parameter values			Confidence intervals		500 Set of simulated Yes. weighted residues	
HILL	Optimal	Average	Median	< alpha = 5	> alpha = 5	< alpha = 1	> alpha = 1
Control	9.883	9.966	9.862	9.365	9.998	9.276	10
Hill number	1.78	1.78	1.78	1.68	1.88	1.46	2.08
EC50	123.7	123.4	123.3	122.3	124.7	111.4	139.8
Maximum effect	0	No object: non-adjusted parameter					
EC5	28.6	28.6	28.2	28.1	29.4	27.3	29.8
EC10	38.1	38.1	38.1	37.4	39.5	37.01	39.8
EC15	55.3	55.3	55.1	54.3	56.8	54.1	57.6
EC20	62.3	62.3	62.3	60.4	63.6	60.1	63.8
EC25	74.5	74.4	74.5	74.1	75.9	73.4	76.5

Table 2 — Bootstrap calculation of abamectin toxicity parameters corresponding to Hill's model on *P. ulmi* larvae

Calc. Parameters	Parameter values			Confidence intervals		500 Set of simulated Yes. weighted residues	
HILL	Optimal	Average	Median	< alpha=5	> alpha=5	< alpha=1	> alpha=1
Control	9.883	9.966	9.862	9.365	9.998	9.276	10
Hill number	2.35	2.35	2.35	2.25	2.45	2.06	2.94
EC50	116.1	118.1	118.3	115.4	117.6	102.4	127.5
Maximum effect	0	No object: non-adjusted parameter					
EC5	22.6	22.6	22.2	22.1	23.4	20.3	25.2
EC10	33.1	33.1	33.1	32.4	34.5	31.4	35.5
EC15	48.3	48.3	48.1	47.3	49.8	45.6	51.6
EC20	57.3	57.3	57.3	56.4	58.6	54.1	60.2
EC25	68.5	68.4	68.5	68.1	69.6	63.4	70.7

Table 3 — Dose-response modelling using linear and Hill models and probability values to limit the loss of information for each one.

Modeling	Life stage	EC_{50} value	95% CI	b	Δ_{LL}	AIC	p
Linear model	*P. ulmi* adult	128.7	116.3-143.1	0.43	234.62	238.73	0.672
	P. ulmi larva	118.4	105.4-133.2				
Hill's models	*P. ulmi* adult	123.4	111.4-139.8	0.40	235.12	239.15	0.328
	P. ulmi larva	116.1	102.4-127.5				

EC_{50}: application rate killing 50% for the exposed mites; CI = confidence interval; m = slope of the curve; Δ_{LL} = deviance; AIC = Akaike's information criterion. The Δ_{LL} and AIC were calculated from the larvae and adults together. p = probability to limit the loss of information

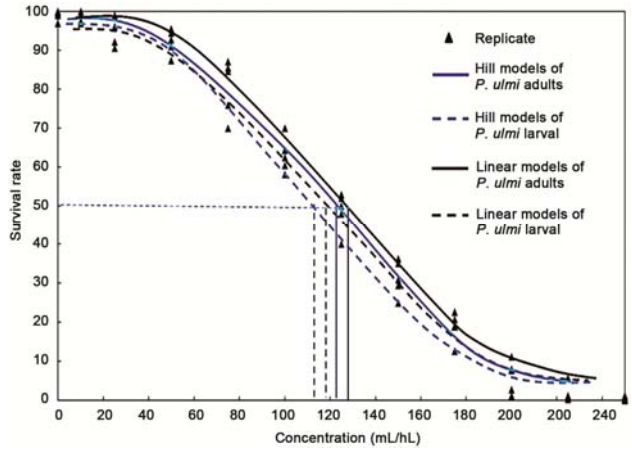

Fig. 1 — Bootstrap calculation of toxicity parameters related to abamectin using linear and Hill models.

based on Monte-Carlo simulation type[28] and provides an efficient methodology for building confidence intervals using the initial sampling data to calculate densities function and estimate its distribution without having assumptions about the basic distribution of each population[29].

Comparison of models

The goodness of fit, indicated by the Δ_{LL} and the AIC, did not show a significant difference between the linear model and Hill's model (Table 3). Akaike's information criterion of the Hill and linear models were AIC = 239.15 and 238.73, respectively. The probability to limit the loss of information or the relative likelihood for each model was calculated, however, the results showed that Hill's model evaluates the dose-response relationship with a probability value of 0.328 compared to 0.672 that corresponding to the linear regression model. Although the linear model seems to be more elected for establishing the dose-response relationship, both models have a similar quality for fit and led to satisfactory results. This similarity observed could be checked using a maximum of replicated data and wide weighted averages using more models.

The evolution of phytosanitary chemistry has made it possible to limit the potential losses related to crop pests; pesticides exert direct and indirect effects on the targets but present a real concern by their non-intentional effects. Abamectin is a potent miticide and is a mortality factor that can reach up to 100% of the target populations. This result is beneficial to the health of the plant but the pest mites could eventually manifest multiform resistances to this active product as a transmitting strategy to future individuals.

Regression modelling of toxicity data is becoming increasingly required in the ecotoxicology community. The advantages of regression over hypothesis testing have been promoted by numerous authors. As previously emphasised, the use of the linear model approach to predict the mixture toxicity of a chemical is well established in toxicology[30]. The classic toxicological approach aims to establish a proportional relationship between doses and effects[31]. The dose-response relationship is considered monotonous to determine quantitative variables that characterize the toxicity of abamectin to *P. ulmi* adults and larvae. The linear regression model, for which the probability to limit the loss of information was significantly higher, was found to be the best fitting model based on the AIC.

These results related to abamectin constitute not a source of concern for practitioners of commercial orchards but can offer useful indications to manage the biological control of phytophagous mites using the predatory mites. The family Phytoseiidae and other predators have proved to be a successful alternative to conventional chemical controls[32]. In general, the action of acaricides has already been proven and ranked in several toxicological studies often coupled to resistance tests of phytoseiids[33]. The biological control includes significant limitations as well, which makes the use of acaricides still indispensable. In crop protection, abamectin should be rational compounds: highly effective against mite pests and relatively saving to their predators[34]. In addition, the results of a toxicological test of abamectin on *P.ulmi* were similar to those given in many previous studies on species of Tetranychidae[35], or mite pests with the presence of a potential phytoseiids[36].

Abamectin can induce the emergence of resistance in predators and pests when it is frequently used against pest mites. The beneficial or undesirable effects of exposure to abamectin are not only related to the external dose received but depend on the amount of active material reaching the target cells. Thus, two phases are distinguished: the path of the substance in the body to the target tissues (pharmacokinetics) and the action of the substance in the target tissues (pharmacodynamics)[37].

Conclusion

To optimize the use of abamectin to control the *P. ulmi*, effective concentrations of abamectin causing hierarchized mortality levels were calculated by simulation. The violation of the principles of integrated pest management (IPM) is an inevitable failure of good chemical control. In ecotoxicology, modelling approaches provide relevant tools in pest management and understanding the effects of inputs on surviving organisms and agrosystem biodiversity.

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