Residues and Dissipation Kinetics of Two Imidacloprid Nanoformulations on Bean (Phaseolus vulgaris L.) under Field Conditions

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ABSTRACT: The current study investigates the dissipation kinetics of two imidacloprid (IMI) nanoformulations (entitled: Nano-IMI and Nano-IMI/TiO2) on common bean (Phaseolus vulgaris) seeds under field conditions and compares them with 35% Suspension Concentrate (SC) commercial formulation. To do so, it sprays P. vulgaris plants at 30 and 60 g/ha within green bean stage, sampling them during the 14-day period after the treatment. Following extraction and quantification of IMI residues, dissipation data have been fitted to simple-first order kinetic model (SFOK) and to first-order double-exponential decay (FODED) models, with 50% and 90% dissipation times (DT50 and DT90, respectively) assessed along the pre-harvest interval (PHI). With the exception of Nano-IMI at 60 g/ha, other decline curves are best fitted to the FODED model. In general, dissipation is faster for Nano-IMI (at 30 g/ha: DT50 = 1.09 days, DT90 = 4.30 days, PHI = 1.23 days; at 60 g/ha: DT50 = 1.29 days, DT90 = 4.29 days, PHI = 2.95 days) and Nano-IMI/TiO2 (at 30 g/ha: DT50 = 1.15 days, DT90 = 4.40 days, PHI = 1.08; at 60 g/ha: DT50 = 0.86 days, DT90 = 4.92 days, PHI = 3.02 days), compared to 35% SC (at 30 g/ha: DT50 = 1.58, DT90 = 6.45, PHI = 1.93; at 60 g/ha: DT50 = 1.58 days, DT90 = 14.50 days, PHI = 5.37 days). These results suggest the suitability of Nano-IMI and Nano-IMI/TiO2 application at both rates in terms of their residues on P. vulgaris seeds.

Keywords: Pesticide nanoformulation, imidacloprid, dissipation kinetics, Pre-harvest interval

INTRODUCTION

Application of chemical pesticides for promotion of crop production and quality is inevitable and is growing globally. However, the widespread and sometimes improper use of these agrichemicals has raised serious concerns regarding their residues on crops and in the environment which can ultimately result in adverse impacts on non-target organisms such as humans (Khan et al., 2018; Yu et al., 2018).

One of the most important approaches
towards improvement of chemical pesticides' efficiency in pest control and, thereby, reduction of pesticide consumption is to optimize their formulations. Nanoencapsulation, a kind of controlled-release formulation, is a new technology that has emerged to reduce the application rate of pesticides and their contaminations. It can minimize the residues of these agrochemicals on crops as well as their impacts on non-target organisms (Anjali et al., 2010; Bhattacharyya et al., 2010; Ghormade et al., 2011; Khot et al., 2012). In this type of formulation, the active ingredient of a pesticide is released in a controlled manner and the effective concentration remains on the target for a longer period of time. Previous researches successfully prepared nanoencapsulated formulations of IMI, testing their efficacies (Memarizadeh et al., 2014a; Memarizadeh et al., 2016). However, the residue decline of these new formulations compared to the commercial formulation of the insecticide is still a matter of question.

Imidacloprid (IMI) [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazoli-din-2-ylideneamine] is a neonicotinoid systemic contact insecticide, widely used for its high effectiveness against various pests (Kapoor et al., 2013). In Iran, the use of this insecticide is widespread against sucking pests of various crops, with its residues detected on several crops (Hassanzadeh et al., 2012; Leili et al., 2016). IMI has adverse effects on human health, with several liver and thyroid gland toxicities reported by several chronic and sub-chronic studies (Kunkel et al., 2001). Therefore, the residues of this pesticide on crops can have damaging impacts on consumers’ health (EFSA Scientific Committee, 2009; 2011; 2013; 2018; Geiser & Kreyling, 2010; Simon & Joner, 2008).

For the first time, this research evaluates the concentration and residue decline kinetics (i.e., dissipation rates and pre-harvest intervals) of two previously-synthesized IMI nanoformulations on P. vulgaris seeds under field conditions. Furthermore, it compares all factors to those of IMI’s commercial formulation (35% SC). Results from this study will be essential for risk assessment of such novel formulations under field conditions.

MATERIAL AND METHODS
IMI standard (99.9% purity) and technical (95% purity) were obtained from Pestanal Sigma - Aldrich (Aldrich, Germany) and Kavosh Kimia (Kerman, Iran), respectively. Also, the 35%-suspension concentrate (SC) formulation of IMI was purchased from Kavosh Kimia (Kerman, Iran) as a commercial one. Polyethylene glycol (PEG) (molecular weight [Mn] = 1000), citric acid, tetrahydrofuran, anhydrous magnesium sulfate, sodium chloride, methanol (HPLC grade), water (HPLC grade), and diethyl ether were purchased from Merck. Dialysis bags (Mn cutoff 2000) were obtained from Sigma-Aldrich (St Louis, Missouri) and primary secondary amine (PSA) and graphitized carbon black (GCB) were obtained from Agilent (United States).

Nano-IMI was prepared via direct encapsulation with ABA triblock linear-dendritic copolymers, composed of poly(citric acid) (PCA) as A block and poly(ethylene glycol) (PEG) as B block, according to the method described by Memarizadeh et al. (2014a), who synthesized PCA–PEG–PCA copolymers through three thermal stages. For the purpose of encapsulation, Imidacloprid dissolved in acetone (1g/100ml) and PCA–PEG–PCA copolymers dissolved in ethanol as a basic solvent (1g/20ml) were mixed at room temperature and stirred for 8h (Memarizadeh et al., 2014a). Also, Nano-IMI/TiO$_2$ was prepared by encapsulation of IMI with PCA–PEG–PCA copolymers, in addition to TiO$_2$ nanoparticles via supramolecular interactions according to the method described by Memarizadeh et al. (2014b) whose approach had synthesized TiO$_2$ nanoparticles dispersed in ethanol, followed by ultrasonication for 90
min in a bath sonicator at 35 °C. The PCA–PEG–PCA copolymers (typically 10 g/L) were immediately combined and mixed with the dispersed TiO₂, using ultrasound for 2 h. The indoxacarb dissolved in acetone (typically 1 g/L) was then added to the resulting suspension and the mixture was stirred for at least 10 h at room temperature (Memarizadeh et al., 2014b).

Preparation of both nanoformulations of IMI were confirmed, using spectroscopy and microscopy analyses. Transmission electron microscopy (TEM) images showed encapsulated particles with an average size of 10 nm for Nano-IMI and 12 nm for Nano-IMI/TiO₂. A United States patent and trademark office (USPTO) patent about this formulation also was published in 2016 (Memarizadeh et al., 2016).

Field experiments were conducted during 28 May 2015 to 15 July 2015 in a 300 m² field, located in agriculture research farms of the University of Guilan (Longitude: 49/6412’ E, Latitude: 37/2047’ N, Altitude: 21.00 m) in Rasht city. A local common bean (Phaseolus vulgaris) cultivar “Akhtar” was planted using the furrow method. Experiments were performed based on a completely randomized block design with three replications. At the green bean stage, plants were sprayed to the dripping point with three formulations of IMI including 35% SC, Nano-IMI, and Nano-IMI/TiO₂, at both the recommended and double the recommended rates (30 and 60 g/ha, respectively). Water-sprayed plots were also regarded as controls. Sampling took place 0, 0.5, 1, 2, 3, 4, 5, 7, and 14 days, following pesticide treatment. In order to achieve an accurate statistical analysis, each sample included 100 bean seeds, collected from the bean bushes in a randomized pattern. Following the sampling, a sum of 150 g of each sample was stored in dark polyethylene bags, transferred to the laboratory in a cold chamber, and kept at -40 °C until analysis.

Extraction and clean-up were carried out based on the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, described by Anastassiades et al. (2003). From each sample, ten grams of chopped and well-homogenized bean seeds were mixed with acetonitrile (10 mL) in 50-mL polypropylene centrifugation tubes and vortexed for 1 min, followed by addition of 4 g MgSO₄ and 1 g NaCl. The mixture was then shaken with hand for 1 min and was centrifuged (5 min, 4000 rpm). The clean-up happened by transferring 1 mL of the supernatant to another tube, containing 150 mg MgSO₄, 25 mg PSA, and 7.5 mg GCB, there to get mixed well by 1 min of shaking. The mixture was then centrifuged at 4000 rpm for 5 min. The final extracts got evaporated until becoming thoroughly dry under a gentle stream of nitrogen. They were diluted in 0.5 mL methanol.

IMI residues were analyzed by means of an HPLC apparatus (Shimadzu LC9A), equipped with an ultraviolet/visible (UV/VIS) detector. A C₁₈ column was used to separate IMI at 40 °C. The mobile phase was consisted of acetonitrile and water (70:30 v/v) with a flow rate of 0.8 mL/min, with each sample, injected three times (20 μL). The detection wavelength for IMI was 280 nm.

According to recovery tests, homogenized pesticide-free bean seeds (blanks) were fortified with IMI standard solution in methanol (2 and 4 μg mL⁻¹) in triplicate. Extraction and data analysis techniques adopted the same above-mentioned methods, the precision of which was evaluated by calculating relative standard deviations (RSDs) of the recoveries. Prior to fortification, blank samples, solvents, and IMI standard were tested for quality control, showing that all blanks were negative in terms of IMI presence.

The instrumental linearity was assessed by generating a calibration curve for IMI, using the pesticide’s standard solution in methanol at five points that ranged between 1 and 15 μg/mL.

The instrumental detection and
quantification limits (IDL and IQL, respectively) were measured, based on an approach, recommended by US Environmental Protection Agency (US EPA), using the Root Mean Square Error (RMSE) as well as the calibration curve's slope (Corley 2003, Torabi et al., 2017). The estimated method detection limit (EMDL) was calculated according to Torabi et al. (2017), based on the calculated IDL and IMI average recovery.

SFOK is a simple exponential model with two parameters (Torabi & Talebi, 2013) (Eq. 1):

\[ x = X_0 \exp(-kt) \]  

where \( X \) is the concentration of IMI at time \( t \) (day); \( X_0 \), the initial concentration of IMI dissipated through a first-order process; and \( k \), the dissipation rate constant.

FODED model involved two exponential equations with four parameters (Torabi & Talebi, 2013; Torabi et al., 2017) (Eq. 2):

\[ x = X_1 \exp(-k_1 t) + X_2 \exp(-k_2 t) \]  

In this model, the dissipation of the pesticide residue occurred in two phases: a solution phase (\( X_1 \)) which dissipated at a faster rate (\( k_1 \)) and an absorbed one (\( X_2 \)) which declined more slowly (\( k_2 \)).

For SFOK model, 50\% (\( DT_{50} \)) and 90\% (\( DT_{90} \)) dissipation times for IMI residue were calculated in each sample, using Equations. 3 and 4, respectively (Torabi et al., 2017):

\[ DT_{50} = \lim_{x \to n} \frac{2^{-\frac{1}{n}} - 1}{(x - 1)X_0 x^{n-1}} \]  

(3)

\[ DT_{90} = \lim_{x \to n} \frac{10^{-\frac{1}{n}} - 1}{(x - 1)X_0 x^{n-1}} \]  

(4)

where \( x \) (\( n \)) is the order of the kinetic model (1 for the first-order kinetic model).

In case of FODED model, an iterative procedure was adopted to estimate the degradation times (FOCUS 2006).

The PHI values for both models were estimated, using the maximum residue level (MRL) of 2 mg/kg, established by the Codex Alimentarius for IMI on beans (http://www.fao.org/fao-who-codexalimentarius/codextexts/dbs/pestres/pesticide-detail/en/?p_id=206).

The goodness of the exponential models' fit were evaluated by measuring the normalized root mean square error (NRMSE) (Eq. 5) as well as coefficient of the residual mean square (CRM) of each model (Eq. 6).

\[ NRMSE = \frac{1}{M_{av}} \sqrt{\frac{\sum_{i=1}^{n} (F_i - M_i)^2}{n}} \]  

(5)

\[ CRM = \frac{\sum_{i=1}^{n} (F_i - M_i)^2}{\sum_{i=1}^{n} (F_i)} \]  

(6)

where \( M_{av} \) is the average of the measured value; \( F_i \), the fitted value; \( M_i \), the measured value; and \( n \), the number of observations. NRMSE and CRM values close to zero indicate better agreement between the predicted values and the observed ones (Torabi et al., 2017). DataFit version 9.1.32 was used to calculate the equations and parameters.

RESULTS AND DISCUSSION

The method linearity for IMI standard analysis was evaluated by a calibration curve equation of \( Y = 102.76X + 32718 \) (\( r^2 = 0.99 \)). Table 1 lists recovery and detection limits for IMI. The recovery results fitted within the range of 70 - 110\% and with relative standard deviation (RSD) of <20\%, which confirmed the accuracy and precision of the extraction method, according to European Commission specifications (Yi & Lu, 2006). The detection and quantification limits of instrumental and extraction procedures proved the relevant sensitivity of these methods to IMI analysis in bean seeds. No interfering peaks were noticed at IMI retention time when the extracts from pesticide-free bean seeds were analyzed.
Results show that nearly 97% of the initially applied concentration of IMI commercial formulation (35% SC) at both application rates dissipated on bean seeds after 14 days (Table 2). Both SFOK and FODED models were significant (p < 0.01). However, the goodness of fit parameters revealed that at both application rates, the dissipation of IMI followed a bi-phasic pattern, which could be best described through the FODED model (Table 3), according to which, the initially applied concentration of pesticide dissipates within a faster rate while the remaining fraction, which may be absorbed by plant tissues, reaches equilibrium and dissipates slowly (Torabi et al., 2017). This model has been used to describe the dissipation of pesticides in cases where the decline deviates from the first-order trend (Torabi et al., 2017). Here, according to FODED model, DT_{50}, DT_{90}, and PHI of IMI (35% SC) were 1.58, 6.45, and 1.93 days at the recommended rate and 1.58, 14.50, and 5.37 days at double recommended rate, respectively.

In case of the Nano-IMI/TiO\textsubscript{2} formulation also, both models showed significant fits (p < 0.01). However, the goodness of fit parameters revealed a slightly better fit of the FODED model at both concentrations (Table 3). These results were consistent with the ones, reported by Torabi & Talebi (2013) and Rahimi et al. (2015) as well. Accordingly, DT_{50}, DT_{90}, and PHI for Nano-IMI/TiO\textsubscript{2} were estimated to be 1.15, 4.40, and 1.08 days at the recommended rate and 0.86, 4.92, and 3.02 at double recommended rate, respectively. The residues of IMI with the Nano-IMI/TiO\textsubscript{2} formulation were initially 6.36 and 11.34 µg/g at the recommended and double recommended rates, respectively, which declined by 96.40% and 97.39% at the recommended and double the recommended rates, respectively after 14 days (Table 2).

For the Nano-IMI, the residues of IMI declined to almost 0.28 µg/g after 14 days at both application rates, accounting for up to 95.78% and 96.93% dissipation at the recommended and double recommended rates, respectively (Table 2). Based on the goodness of fit parameters, the dissipation curve of the Nano-IMI at the recommended rate followed the FODED model, while at the double rate, the SFOK model could best describe the pesticide decline. According to the FODED model, the Nano-IMI dissipated at the recommended rate with DT_{50} and DT_{90} of 1.09 and 4.30 days, respectively and PHI was estimated 1.23 days. At double this rate, for the Nano-IMI, DT_{50}, DT_{90}, and PHI were 1.29, 4.29, and 2.95 days based on SFOK model, respectively. The effectiveness of SFOK model for describing the decline of pesticides has been dealt with in previous researches (Prieto, 2002; Talebi, 2006; Khay, 2006; Torabi & Talebi, 2013; Rahimi et al., 2015).

The fast dissipation of the initial IMI deposit on bean seeds under field conditions can be due to the impact of environmental factors such as sunlight and temperature. Previous studies have shown that since PCA–PEG–PCA copolymers have good absorbance in the UV region, more photons and energy can be absorbed within the UV spectrum (Memarizadeh et al., 2014b). Thus, including the nanometer PCA–PEG–PCA copolymers in the Nano-IMI and Nano-IMI/TiO\textsubscript{2} can speed up the photocatalytic degradation of these formulations. Therefore, at the recommended rate, the initial higher dissipation rate of the Nano-IMI (k_{1} =

<table>
<thead>
<tr>
<th>Spiked concentration (µg/g)</th>
<th>Recovery (%) (Mean* ± RSD)</th>
<th>IDL (µg/mL)</th>
<th>IQL (µg/mL)</th>
<th>EMDL (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>91.05 ± 4.21</td>
<td>0.07</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>4</td>
<td>89.41 ± 5.85</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* means of triplicates
RSD: relative standard deviation, IDL: instrumental detection limit, IQL: instrumental quantification limit, EMDL: estimated method detection limit

Table 1. Recovery and detection limits for IMI extraction and analysis
and Nano-IMI/TiO₂ (k₁ = 2.59/day), in comparison to the commercial formulation (k₁ = 2.50/day) (Table 4), can be related to the faster photodegradation of these nanoformulations.

**Table 2. Dissipation of IMI on common bean**

<table>
<thead>
<tr>
<th>Days</th>
<th>Remaining concentration² ± SD (µg/g)</th>
<th>% Dissipation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35% SC</td>
<td>Nano-IMI</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>0</td>
<td>11.73 ± 0.03</td>
<td>15.40 ± 1.22</td>
</tr>
<tr>
<td>0.5</td>
<td>8.61 ± 0.32</td>
<td>13.90 ± 0.99</td>
</tr>
<tr>
<td>1</td>
<td>7.65 ± 0.40</td>
<td>10.72 ± 0.71</td>
</tr>
<tr>
<td>2</td>
<td>5.29 ± 0.14</td>
<td>5.26 ± 0.34</td>
</tr>
<tr>
<td>3</td>
<td>3.22 ± 0.00</td>
<td>3.33 ± 0.24</td>
</tr>
<tr>
<td>4</td>
<td>1.77 ± 0.00</td>
<td>2.53 ± 0.09</td>
</tr>
<tr>
<td>7</td>
<td>1.37 ± 0.02</td>
<td>2.94 ± 1.00</td>
</tr>
<tr>
<td>14</td>
<td>0.29 ± 0.00</td>
<td>1.68 ± 0.11</td>
</tr>
</tbody>
</table>

² means of triplicates
SD: standard deviation, T1: recommended dosage, T2: twice the recommended dosage

**Table 3. DT₅₀, DT₉₀, PHI, and goodness of fit indices derived from exponential models fitted to the dissipation of IMI on common bean**

<table>
<thead>
<tr>
<th>Models</th>
<th>Indices</th>
<th>35% SC</th>
<th>Nano-IMI</th>
<th>Nano-IMI/TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>SFOK</td>
<td>DT₅₀</td>
<td>1.68</td>
<td>1.50</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>DT₉₀</td>
<td>5.59</td>
<td>5.00</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>PHI</td>
<td>4.25</td>
<td>4.52</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>RMSE</td>
<td>0.16</td>
<td>3.47</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>CRM</td>
<td>0.001</td>
<td>0.02</td>
<td>26.00 × 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.98</td>
<td>0.99</td>
<td>0.97</td>
</tr>
<tr>
<td>FODED</td>
<td>DT₅₀</td>
<td>1.58</td>
<td>1.58</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>DT₉₀</td>
<td>6.45</td>
<td>14.5</td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td>PHI</td>
<td>1.93</td>
<td>5.37</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>RMSE</td>
<td>4.03 × 10⁻⁵</td>
<td>4.16 × 10⁻¹⁰</td>
<td>1.59 × 10⁻⁹</td>
</tr>
<tr>
<td></td>
<td>CRM</td>
<td>3.35 × 10⁻⁷</td>
<td>2.48 × 10⁻¹²</td>
<td>2.67 × 10⁻¹¹</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.98</td>
<td>0.94</td>
<td>0.97</td>
</tr>
</tbody>
</table>

T1: recommended dosage, T2: twice the recommended dosage, RMSE: root mean square error, CRM: coefficient of residual mass

**Table 4. Parameters for exponential models fitted to the dissipation of IMI on common bean**

<table>
<thead>
<tr>
<th>Models</th>
<th>Parameter³</th>
<th>Unit</th>
<th>35% SC</th>
<th>Nano-IMI</th>
<th>Nano-IMI/TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>SFOK</td>
<td>X₀</td>
<td>day</td>
<td>11.37</td>
<td>16.09</td>
<td>6.68</td>
</tr>
<tr>
<td></td>
<td>k</td>
<td>per day</td>
<td>0.41</td>
<td>0.46</td>
<td>0.59</td>
</tr>
<tr>
<td>FODED</td>
<td>X₁</td>
<td>day</td>
<td>10.16</td>
<td>14.74</td>
<td>6.40</td>
</tr>
<tr>
<td></td>
<td>k₁</td>
<td>per day</td>
<td>0.50</td>
<td>0.60</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>X₂</td>
<td>day</td>
<td>1.36</td>
<td>1.70</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>k₂</td>
<td>per day</td>
<td>0.09</td>
<td>5.75 × 10⁻¹⁷</td>
<td>0.35</td>
</tr>
</tbody>
</table>

³ defined in Eqs. 1 and 2
T1: recommended dosage, T2: twice the recommended dosage
According to FODED model, the dissipation rate of IMI in the Nano-IMI/TiO₂ declined as the time passed (Table 4), hence demonstrating the systemic behavior of this pesticide, its absorption into the plant tissues, and --as a consequence-- its low availability to the environmental-degrading factors. For the Nano-IMI at twice the recommended rate, however, the dissipation rate was constant according to SFOK model, without any decrease noticed throughout the experiment (Table 4). This can be due to the effect of biodegradability of PCA-PEG-PCA copolymers in the formulation, without the need to being mixed with any photocatalysis materials (Memarizadeh et al., 2014b; Memarizadeh et al., 2016).

CONCLUSION
Assessing the dissipation behavior of Nano-IMI and Nano-IMI/TiO₂ by means of exponential decay models revealed that even at double recommended rate, DT₅₀₅ₐ₅ and PHIs of this insecticide were generally lower at both nanoformulations than those of the commercial formulation. This implies that Nano-IMI and Nano-IMI/TiO₂ residues dissipate quite fast in vegetables and can be recommended even at the double recommended rate. Furthermore, previous studies on pollution potential of TiO₂ nanoparticles on biochemical biomarkers showed the toxicity in bioassay experiments (Memarizadeh et al., 2014c; Memarizadeh et al., 2014d). As a result, Nano-IMI can be introduced into the pesticide market as a promising and eco-friendly pesticide system in order to improve the efficiency of IMI and reduce its adverse environmental impacts.

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