New Study Concerning the Biodegradation of Dimethoate, Chlorpyrifos and Chlorfenvinphos in some Medicinal Plants

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Herbal drugs are widely used in pharmaceutical industry due to their beneficial effects on human health. The current research was carried out to evaluate the organic pollutant degradation in the flower part of the plant after a foliar application of pesticides. Also, based on the fact that selected pesticides are highly toxic to bees, it is important to monitor the removal rate of the pollutant. Under laboratory conditions, the marigold and French flowers (Calendula Officinalis and Tagetes Patula) were spiked with a mix of organophosphorus insecticide, dimethoate, chlorpyrifos and chlorfenvinphos, where the first two substances are still used on Romanian territory. The samples were extracted at different time moments over 300 min after sprayed treatment in order to evaluate the concentration in flower. The analytes were quantified by using gas chromatography with flame photometric detection (GC-FPD) technique. The developed method ensures the quality and comparability of analytical results, with a good sensitivity in determining the analytes over the concentration range 5-250 µg/L.

Keywords: GC-FPD, medicinal plants, organophosphorus pesticides

Instead of highly toxic and banned organochlorine pesticides, organophosphorus pesticides (OPP) are used as a good alternative in agriculture due to their low persistency in environment and higher killing efficiency of pests. Though, some pesticides present negative effects for the environment [1], like being a source of honey bee population decline [2]. Although, OPP and their metabolites residues have been found in various environmental compartments (water [3], air, soil, biota [4]), also in crude medicinal plant materials. Metabolism and degradation half-lives of pesticides in plants are required data for assessing the plant protection products and ecosystem impact. In order to fulfill the lack of information, the data is estimated by extrapolation from one medium to another [5]. Based on the assumption that in plants pesticides degradation is more efficient than in soil, but slower than in air, developed studies correlate the pesticides half-life on the herb with pesticides half-life on the soil, using the formula to approximate the value: \[ \text{half-life}_{\text{plant}} = \text{half-life}_{\text{soil}} \times \frac{1}{16} \] [5]. In response to this problem, several methods have been developed for the quantification and monitoring of multi pesticide residues in biota [6], water [7] and soil [8].

Medicinal plants are commonly used in treatments of health disease as a rich source of polyphenolcarboxilic acids, flavonoids and carotenoids, antioxidant compounds to capture free radicals [9]. The content of contaminants, not only from sprayed amount but also from soil and water used for irrigation, enforce safe measures in developing natural products with protective role [10].

The impact of the chosen organophosphorus insecticides, chlorpyrifos (0,0-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate), chlorfenvinphos ([(Z)-2-chloro-1-(2,4-dichlorophenyl)ethenyl] diethyl phosphorothioate) and dimethoate (2-dimethoxyphosphinothiylsulfanyl-N-methylacetamide) on honey bee population life is realized usually through pollen and nectar contamination, by uptaking from soil to the roots, or by foliar application, then translocated within the plant (for systemic pesticide). The removal rate depends on the biodegradation route of pesticides. Function of contaminants solubility and metabolism phases, pesticides are transformed through oxidation, hydrolysis and reduction processes to conjugation, resulting hydrophilic and nontoxic compounds. Aside from plant metabolism, the removal of pesticides in/on plant over time it is under the effects given by environmental factors as temperature, relative humidity and UV radiation, or by the pesticide formulation and its application technique [11]. Thereby, reported half-life values for dimethoate vary in different plants, for example from 2-3 days in tea leaves to 5-7 days in chrysanthemum flowers or cherries and grapes [12]. In Romania, dimethoate (Novadim Progress) and chlorfenvinphos (Pyrinex Quick) are intensively used for vegetable cultures and fruit trees.

The aim of the current study was to determine the fate by measuring biodegradation rate of dimethoate, chlorpyrifos and chlorfenvinphos in Calendula Officinalis and Tagetes Patula. Quantitation of these compounds was done by using a fast, reliable and precise GC-FPD method.

Experimental part

Reagents and chemicals

Hexane 99% and acetone RESI were acquired from J.T. Baker, and dichloromethane and toluene from Fluka, Sigma Aldrich. High purity of certified pesticides analytical standards: dimethoate 99.5%, chlorpyrifos 100 ng/µL in acetonitrile and chlorfenvinphos 100 ng/µL in ciclohexane were purchased from Fluka, Sigma Aldrich. It was prepared in acetone solvent 5 calibration solutions over the

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The GC-FPD method was used to obtain high efficient analytes peaks and a good separation between them, with less interference from the rich organic compound plant matrix. The relatively low boiling point of chosen compounds (dimethoate, chlorpyrifos, chlorfenvinphos) makes them suitable for GC analysis without signs of thermodegradation in the injection port, while FPD selectivity for sulphur or phosphorous containing matrices avoids some the interferences resulted from volatilization, photodegradation or contaminant absorption through cuticular waxes, function of chemical proprieties of the selected analytes in different solvents like toluene, n-hexane, acetone and acetonitrile [13], acetone was chosen but for the enhanced analytical response on lower concentration, 5 µg/L (15% relative standard deviation, RSD - lower than in case of n-hexane), where at higher concentrations the precision increases at 2% RSD at 100 µg/L in case of dimethoate (fig. 1). Smaller values of RSD indicate better repeatability of the analysis. The calibration curves for all three compounds were found to be linear in the range of 5 to 250 µg/L, with a very good correlation coefficient (> 0.9979, table 1), and a LOQ (limit of quantification) value of 5 µg/L by considering a satisfactory signal-to-noise starting from 3:1. The results demonstrate the method potential for routine quantitative analysis of organophosphorus pesticides in herbs.

This method is also useful in quantifying the limit restriction for pesticides residues in herbal drugs established in European Pharmacopoeia (2.8.13. Pesticide residue), as follow: chlorpyrifos - 0.2 mg/kg, chlorfenvinphos - 0.5 mg/kg and dimethoate - 0.1 mg/kg [14].

Optimization of the extraction procedure. In order to ensure the efficacy and the precision of the extraction procedure, parameters like extraction solvents and ultrasonication time had been optimized by adapting several methods based on MSPD (matrix solid phase dispersion) procedure [4, 13, 15] to the experimental needs and available laboratory resources. Among the most frequently used extraction solvents (acetone, toluene, dichloromethane, n-hexane, acetone:toluene 1:1) which provide high recoveries of pesticides over a wide range of polarity [4, 15], only acetone has showed good results for all three pollutants.

In order to evaluate the efficiency of the analytical procedure, a recovery assay was conducted at 0.2 mg/kg and 0.5 mg/kg, and processed according to the extraction procedure. The mean recovery of spiked samples of Tagetes Patula flowers ranged between 85 to 96% for dimethoate, from 71 to 78% for chlorpyrifos and from 138 to 143% for chlorfenvinphos, with a RSD variation of 3.2 - 9.0%. In case of the analysis of pesticide recovery from Calendula Officinalis at 0.2 mg/kg, the values are slightly different: dimethoate 65%, chlorpyrifos 128% and chlorfenvinphos 153%.

The results are comparable to EU method validation criteria (SANCO/12495/2011 [16]) which state an average recovery between 70 and 120%, and an RSD value lower or equal to 20%. The highest recovery of chlorfenvinphos and chlorpyrifos is a false positive result known as ‘matrix-induced chromatographic response enhancement’ which impact negatively the quantitation accuracy [17].

Pesticide persistence on flower

By using foliar spray application, the main ways to minimize the pesticide concentration consist in volatilization, photodegradation or contaminant absorption through cuticular waxes, function of chemical proprieties presented in table 2. Frequently, the analytes are conjugated to water-soluble compounds with modified structure and with less or non-toxic effects.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>tr</th>
<th>R²</th>
<th>Precision intra-day (%)</th>
<th>LOQ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Marigold</td>
<td>French marigold</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>16.38</td>
<td>0.9994</td>
<td>15.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>19.54</td>
<td>0.9997</td>
<td>13.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>20.44</td>
<td>0.9979</td>
<td>4.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Table 1

PERFORMANCE PARAMETERS FOR METHOD VALIDATION
In this study, the results were percentage reported at the first concentration which was extracted at the zero moment after application. For all the analytes there was a decrease of concentration on flower extracts on first 30 min for 10-25%, followed by a very slow degradation with results that vary in a range limited by a coefficient of variation of extraction, lower than 8.2%. For both flowers the contaminants have a decreasing linear trend as it is figured out in figure 2 and 3, almost constant in case of chlorpyrifos: 20% reduction for Tagetes Patula and 10% for Calendula Officinalis after 300 min of experiment.

The maximum measured removed amounts of dimethoate and chlorfenvinphos in 300 min was 37% and 26% on marigold flower respectively 34 and 33% on french marigold flowers. Low vapour pressure of dimethoate indicates that the pesticide will be present in air for a short period of time, during the spray application, which means the volatility have a low influence or none on the removal value.

The uncontrollable variation of the environmental factors complicate the monitored analysis by unpredicted influence on results uncertainty [5] for which reason

<table>
<thead>
<tr>
<th>Solubility in acetone</th>
<th>Half-life time</th>
</tr>
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</table>
| **Dimethoate** | acetone: > 300 g/kg | Air - none  
Water - pH > 9, 12 days  
Soil - 4-16 days [12] |
| water: 25000 mg/L | **Chlorpyrifos** | acetone: 6500 g/kg  
water: 1.4 mg/L  
Air - 4 hours  
Water - 30 days  
Soil - 103 days [18] |
| **Chlorfenvinphos** | acetone: miscible  
water: 124 mg/L  
Air - 7-92 hours  
Water - 13-51 days  
Soil - 14-159 days [19] |
dispersion of the results ranges from 1.4-20%, few exceptions above 20%, especially for the french marigold flower. Another factor that should be taken into account is the variety of the absorption routes within plant tissue of the high water solubility of dimethoate comparative to more lipophilic analytes chlorpyrifos and chlorfenvinphos (table 2) [15].

According to scientific data, pesticides half-life on fruits can be comparable to the whole vegetation due to small differences between them [5], which means that results obtained from flowers spiked samples studies could be similar with eventually data for leaves.

Conclusions
In this study it has been evaluated the temporal evolution of the dimethoate, chlorpyrifos and chlorfenvinphos concentration on some medicinal plants with the observation that 10-30% of pesticide concentration had been removed from Calendula Officinalis and Tagetes Patula flowers, within 300 min, under laboratory conditions. The analytes were quantified by GC-FPD technique. All the investigated compounds present a decreasing linear tendency. Due to pesticides toxicity to honey bees, an optimal result would be a higher degradation of pollutants on flower surface, which can improve the quality of the related products (honey and medicinal plant products).

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