Research Article

Dissipation and Residues of Mandipropamid in Grape Using QuEChERS Methodology and HPLC-DAD

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The HPLC method for determination of mandipropamid residues and its dissipation in grape was investigated. The mean recoveries of the analytical method were 98–102%. The samples were collected within 2 weeks after pesticide application, and the pesticide residues were extracted by an optimized QuEChERS method. Mandipropamid dissipated rapidly with half-life 2.20 days in grape. According to maximum residue limit (MRL) the preharvest interval (PHI) of mandipropamid on grape was 4 days, after the last treatment.

1. Introduction

Pesticides will continue to be used in the production of food and fiber especially in the developing countries. Drastic reductions of pesticide usage will increase the production cost and lower the quality of the agriculture productivity. It is well recognized that there are risks attached to the consumption of pesticide-treated crops because of the presence of residues on them [1, 2]. Therefore, the rational recommendation of a pesticide requires that it must not only provide an effective control of pests but at the same time its residues on the commodity must also be toxicologically acceptable. The dissipation of the pesticides after their application depends on several factors, such as the applied dose and formulation [3], application parameters, the number of applications, climatic conditions, the species cultivated, physical phenomena, and chemical degradation [4–6].

Of late several new molecules have been developed as pesticides, which can be degraded easily in the environment and are less harmful for human beings. Mandipropamid [4-chloro-N-[2-[3-methoxy-4-(2-propynyloxy)]phenyl]ethyl]-{a-(2-propynyloxy)-benzeneacetamide} (Figure 1) is a new fungicide in the mandelamide class developed by Syngenta Crop Protection, Inc. for the control of foliar oomycete pathogens in a range of crops including Plasmopara viticola in grapes, Phytophthora infestans in potatoes and tomatoes, and Pseudoperonospora cubensis in cucurbits. Mandipropamid is also proposed for uses on leafy vegetables to control downy mildew (Bremia lactucae) and blue mold (Peronospora effusa). At present, studies of mandipropamid focus on chemical synthesis, toxicology, mode of action, and efficacy. In contrast, papers on analytical methods for residues of mandipropamid in food stuffs seem to be rare. The QuEChERS method (named for quick, easy, cheep, e ffec tive, rugged, and safe) is well known for its applicability in simultaneous analysis of a large number of pesticides residues in variety of food matrices [7]. This method and several modified versions have been lately applied for the extraction of different types of pesticides from all fruits and vegetables. Recently, the QuEChERS method has received the distinction of an AOAC official method for multiple pesticides in fruit and vegetable [8].

This paper investigated the residual behavior and the dissipation of mandipropamid applied on grape for identifying the preharvest interval (PHI). For this purpose a high-performance liquid chromatographic (HPLC) method com-
2. Experimental

2.1. Chemicals. All organic solvents were of HPLC grade and supplied by Alliance Bio, USA. Primary secondary amine (PSA, 40 μm Bondesil) and graphite carbon black (GCB) sorbents were purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulfate was of analytical grade and purchased from Merck Ltd. Sodium chloride is of analytical grade and was purchased from El Naser Pharmaceutical (Egypt). Anhydrous magnesium sulfate was of analytical grade and was purchased from El Naser Pharmaceutical (Egypt).

All organic solvents were of HPLC grade and was purchased from El Naser Pharmaceutical (USA). Anhydrous magnesium sulfate was of analytical grade and was purchased from El Naser Pharmaceutical (Egypt). Anhydrous magnesium sulfate and Sodium chloride were activated by heating at 250°C for 4 h in the oven before use and kept in desiccators. Mandipropamid reference standard was of 99% purity and obtained from central agricultural pesticide laboratory (Egypt). Stock solution was prepared in acetonitrile and stored at −18°C. The standard solutions required for constructing a calibration graph (0.01, 0.25, 0.5, 1, 5, 10 mg/kg) were prepared from stock solution by serial dilution with acetonitrile and were stored at 4°C before use.

2.2. Field Experiment. The field experiment was conducted on grape cultivar at Aga district, El-Dakahlia Governorate, Egypt during June to July 2011. The experimental area had received routine horticultural practices. Trees were planted 15 years before the experiment, variety banaty with 3.5 m space between rows and 1.5 m between the individual trees in the row. Treatment was carried out by knapsack sprayer equipped with one nozzle. The commercial formulation Pergado 27% WG formulation (50 gai/kg) was used. Three randomized plots were treated on 20 June 2011 by mandipropamid at the maximum dose recommended by the manufacturers (400 g/100 L) and one untreated plot was left to serve as control. There was no rainfall at any time during the experimental period. The average daily temperature during the experiment was from 27 to 35°C.

2.3. Sampling. Sampling was performed by randomly collecting from various places of the experimental plots according to the FAO/WHO recommendations [9]. Three replicate samples about 2.5 kg of ripened grapes were collected from pesticide-treated plant. Samples were taken 2 h after the pesticide application. Subsequent samples were taken 1, 3, 7, 10, 15, and 21 days after treatment. During experiment, a control sample was taken in each sampling time. Immediately after collecting the samples, all the samples were packed in plastic bags and transported to the laboratory in an ice box. The samples were homogenized using a food processor (Thermomix, Vorwerk). The homogenate of each sample was done where three representative samples of 15 g were taken. Samples were then placed into polyethylene 50 ml centrifuge tube and frozen at −20°C until the time of analysis.

2.4. Sample Preparation. The samples were extracted according to the QuEChERS method described by Anastassiades et al., with slight modification [7]. 15 g of the homogenized samples were weighted in a 50 ml centrifuge tube and 15 ml of acetonitrile (1.0% acetic acid) were added, the screw cap was closed, and the tube was vigorously shaken for 1 min using a vortex mixer at a maximum speed. Afterwards, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride were added, then extracted by shaking vigorously by hand up to down for 10 min, and centrifuged for 10 min at 6000 rpm and 4°C. An aliquot of 4 ml was transferred from the supernatant to new clean 15 ml centrifuge tube and cleaned by dispersive solid-phase extraction with 100 mg PSA, 20 mg GCB, and 600 mg of magnesium sulfate. Afterwards, centrifugation was carried out as mentioned above. Then, aliquot of 3 ml of the supernatant was taken and evaporated to dryness using test tube evaporator. The residue was then reconstituted in 2 ml of acetonitrile (i.e. 1.5 g matrix per ml extract) and it was then filtered through 0.45 μm PTFE filter (Millipore, USA). The sample was then ready for the final analysis in LC system.

2.5. Liquid Chromatographic Analysis. HPLC analysis was performed with an Agilent 1100 HPLC system (USA), with quaternary pump, manual injector (Rheodyne), thermostat compartment for the column, and photodiode array detector. The chromatographic column was C18 Zorbax XDE (250 mm × 4.6 mm, 5 μm film thickness). The column was kept at room temperature. Flow rate of mobile phase (acetonitrile/methanol/water = 40/20/40 v/v/v) was 0.8 ml/min. and injection volume was 20 μL. Detection wavelength for detection of mandipropamid was set at 205 nm. The retention time of mandipropamid was about 12.9 min.

2.6. Recovery Assays. Control grape samples were fortified with a standard solution of mandipropamid at three levels. Final concentrations of mandipropamid in control samples were 0.01, 0.1, and 0.25 mg/kg. Each fortification level was replicated five times. Extraction of control samples was performed as mentioned earlier. Results of recovery study are shown in Table 1. Results were corrected according to recovery rate. Blank analyses were performed in order to check interference from the matrix.

2.7. Statistical Analysis. Data were statistically evaluated by one-way analysis of variance (ANOVA). All statistical analysis was done using the statistical package for social sciences (SPSS 16.0) program.
3. Results and Discussion

3.1. The Limit of Detection (LOD) and Limit of Quantification (LOQ). The LOD and LOQ were determined as the sample concentration of mandipropamid at signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ were estimated to be 0.003 mg/kg and 0.01 mg/kg, respectively. The LOQ was in all cases lower than MRLs established by different legislation for mandipropamid, which is indicative that the used method is valid for the determination of mandipropamid residues in grape. The matrix effect of the present method was investigated by comparing standards in solvent with matrix-matched standards for 5 replicates at 1 mg/kg. The relative response (response matrix/response solvent) was 0.98 for grape. It may be concluded that the matrix does not significantly suppress or enhance the response of the instrument.

3.2. Linearity. A standard calibration curve of mandipropamid was constructed by plotting analyte concentration against peak areas. At 205 nm, for mandipropamid, the calibration range was linear from 0.01 to 20 mg/kg. The standard curve equation was $Y = 189.39x - 1.198$ ($R^2 = 0.9999$), where $y$ = peak area and $x$ = concentration (mg/kg). Linearity correlation is shown in Figure 2.

3.3. Recovery. To evaluate the accuracy and precision, a fortified recovery experiment was done. The mean recoveries of mandipropamid ($n = 5$) at the spiking levels (0.01, 0.1, and 0.25 mg/kg) appear in Table 1. Satisfactory results were found in the 3 instances, with recoveries between 95 and 102%, and relative standard deviation (RSD) ranged from 2.3 to 5.6%. Figure 3 shows the chromatograms of the mandipropamid standard, grape blank and grape spiked at 0.25 mg/kg.

3.4. Pesticide Residual Behavior. Data in Table 2 and Figure 4 demonstrated the initial deposits, dissipation rate, and half-life time periods of mandipropamid in/on grape. The average initial deposit of mandipropamid (2 h after application) was 9.09 ± 0.02 mg/kg. The mandipropamid residues were decreased with the time. The residues amount decreased to 5.43 ± 0.13 mg/kg, in grape within the first 24 hours after application. Following that period, mandipropamid residues in/on grape decreased to 2.01 ± 0.37, 0.3 ± 0.07, 0.1 ± 0.25, and 0.07 ± 0.64 mg/kg, at 3, 7, 10, and 15 days after spraying, respectively. The residues of mandipropamid were dissipated in grape to undetectable limits twenty-one days after plant treatment.

The dissipation of the pesticide residues in/on crops depends on environmental condition [10], type of application, plant species, dosage [11], and interval between application, the relation between the treated surface and its weight, and living state of the plant surface, in addition to harvest time [12, 13].

First-order kinetics has been extensively used to describe degradation process of many chemicals [14–16]. The degradation kinetics of the mandipropamid in grape was determined by plotting logarithm residue concentration against time (Figure 4). The rate of degradation ($K$) and half-life ($t_{1/2}$) values were obtained from the following equation of Gomaa and Belal [17]:

$$\text{Rate of degradation} \ (K) = 2.303 \times \text{slope} \quad (1)$$

$$\frac{\text{Half-life} (t_{1/2})}{K} = 0.693$$ \quad (2)

The results showed half-life ($t_{1/2}$) values of 2.20 days for mandipropamid in grape. In practice, the PHI for residues in different vegetables and fruits is the time required before the residue reaches a level that is lower than the maximum residue limits (MRLs) established. While the FAO/WHO

### Table 1: Recoveries and relative standard deviations for mandipropamid in grape at various fortification level.

<table>
<thead>
<tr>
<th>Fortified level (mg/kg) ($n^* = 5$)</th>
<th>Recovery</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>102</td>
<td>2.3</td>
</tr>
<tr>
<td>0.1</td>
<td>98</td>
<td>4.0</td>
</tr>
<tr>
<td>0.25</td>
<td>95</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Number of replicates.

### Table 2: Dissipation of mandipropamid residues ($\text{mg kg}^{-1} \pm \text{SD}^a$) in/on grape.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Residue level mean (mg kg$^{-1}$) $\pm$ SD</th>
<th>Dissipation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>$9.09 \pm 0.02$</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>$5.43 \pm 0.13$</td>
<td>40.26</td>
</tr>
<tr>
<td>3</td>
<td>$2.01 \pm 0.37$</td>
<td>77.88</td>
</tr>
<tr>
<td>7</td>
<td>$0.30 \pm 0.07$</td>
<td>96.69</td>
</tr>
<tr>
<td>10</td>
<td>$0.10 \pm 0.02$</td>
<td>98.89</td>
</tr>
<tr>
<td>15</td>
<td>$0.07 \pm 0.01$</td>
<td>99.22</td>
</tr>
<tr>
<td>21</td>
<td>ND$^b$</td>
<td>—</td>
</tr>
<tr>
<td>MRL</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>$K$ (days$^{-1}$)</td>
<td>0.314</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$ (days)</td>
<td>2.20</td>
<td></td>
</tr>
</tbody>
</table>

$^a n = 3$.

$^b$Not detectable.
Figure 3: Chromatograms of mandipropamid (a) standard 0.4 mg/kg, (b) grape blank, (c) grape spiked at 0.25 mg/kg.

Figure 4: Decline in mandipropamid residues over time following its application to grape.

4. Conclusion

In this study, the dissipation rates of mandipropamid after single application at recommended dose on grape was evaluated. The results of this study indicated that mandipropamid disappear rapidly in grape plant and field under natural conditions and exhibited first-order kinetics dissipation. Usually, the degradation of pesticides in the plant besides the effect of some physical and chemical factors like light, heat, pH, and moisture, and growth dilution factor might have played a significant role. Further studies are required to asess the residual behavior, exposure risk, and the environmental fate of mandipropamid, as a new fungicide.

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References


