Thermal Storage Effect on Metalaxyl and Detection of Its Residues on Tomato and Their Relevant Impurities

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Abstract: Metalaxyl fungicide under trade name vacomil 35% wettable powder (WP) was applied on tomato at the recommended dosage 262.5 g a.i./ha to study the residues and dissipation rate of tested fungicide under field condition by LCMS/MS. Also, the tested fungicide was stored according to Food and Agriculture Organization (FAO) for 14 days at 54 ± 2°C to study the effect of thermal storage on degradation of metalaxyl active ingredient and stability of their relevant impurities (2,6-dimethylaniline) beside its physical properties. Data illustrated that residues of metalaxyl reached below the maximum residue limit (MRL) of metalaxyl after 7 days from application at the recommended dosage. The half-life (t_{1/2}) for metalaxyl degradation was 1.84 days. Metalaxyl active ingredient was slightly affected with thermal storage and become conformity with FAO specifications and half-life (t_{1/2}) for metalaxyl active ingredient was 8.99 days. 2,6-dimethylaniline as relevant impurities of metalaxyl was increased by extending storage time. It became non conformity with FAO specification under thermal storage where, it was 0.024% before one day of storage and after 14 days of storage reached 0.042% recording increasing rate 75%. All physical properties tests passed successfully and became conformity with FAO specifications.

Keywords: Metalaxyl, fungicide, degradation and storage.

INTRODUCTION

Metalaxyl is the active ingredient of the fungicide Ridomil. Since its introduction in 1977, it has been widely used for the control of plant diseases caused by oomycetous fungi of the order Peronosporales House worth (1987). Fungicides are biocidal chemical compounds or biological organisms which used to kill parasitic fungi or their spores. A fungistatic inhibits their growth. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Fungicides are used both in agriculture and to fight fungal infections in animals. Chemicals used to control oomycetes, which are not fungi, are also referred to as fungicides, as oomycetes use the same mechanisms as fungi to infect plants Latijnhouwers et al. (2003). Fungicides can either be contact, translaminar or systemic. Contact fungicides are not taken up into the plant tissue and protect only the plant where the spray is deposited. Translaminar fungicides redistribute the fungicide from the upper, sprayed leaf surface to the lower, unsprayed surface. Systemic fungicides are taken up and redistributed through the xylem vessels. Few fungicides move to all parts of a plant. Some are locally systemic, and some move upwardly Mueller and Daren (2013). Fungicide residues have been found on food for human consumption, mostly from post-harvest treatments Brooks and Roberts (1999). Some fungicides are dangerous to human health, such as vinclozolin, which has now been removed from use Hrelia (1996). Sanjay and Dureja (1992) reported the identification of the impurities, which are commonly present or may be developed upon storage in technical metalaxyl. Residue decline may be attributed to volatilization that occur during the first days following application, removal by weathering, heat decomposition, sunlight, UV radiation, or the complex condition Spynu (1989). Temperature is known to be one of the most important factors influencing the stability, persistence and degradation of pesticides. It may affect other factors which are mainly responsible for the decomposition of pesticides Harris (1971) and Suett (1975). Pesticide formulation during storage depends on the stability of its active ingredient, the formulation itself and the protective function of the packaging material Franklin et al. (1991).

MATERIALS AND METHODS

Chemicals

All organic solvents used in this study were HPLC grade were purchased from thermo fisher USA. An anhydrous calcium chloride, magnesium chloride, magnesium sulphate, Sodium chloride and buffer pH 4, pH 7 and pH 9 solutions purchased from El Naser pharmaceutical chemical Egypt. Disodium hydrogen citrate sesquihydrate, trisodium citrate dehydrate, primary secondary amine (PSA) and C18 sorbents were purchased from Supelco USA. Deionized water prepared from elga water purification system. Metalaxyl and 2,6-dimethylaniline reference standards with a minimum of 98% purity were obtained from sigma Aldrich. The tested fungicide with trade name vacomil 35% wettable powder containing {metalaxyl 35% (C15H21NO4)} obtained from factories of veterinary and agricultural medicines VAPCO Jordan.

Determination of active ingredient

Metalaxyl percentage before and after storage were determined according to (365/WP/M/3, CIPAC E, p.128) the metalaxyl content shall be declared (g/kg) and when determined the content obtained shall not differ from that declared by more than ± 5% of the declared content in initial period. While 2,6-dimethylaniline determined according to (365/WP/M/4, CIPAC E, p.128).

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Storage stability at 54°C

Vacomil 35% wettable powder fungicide was stored for 14 days at 54 ± 2°C according to (MT 46.1.1, CIPAC F, p.149) and samples were taken during storage in periods at 0 (1 day before storage), 2, 4, 6, 8, 10, 12 and 14 days to determine active ingredients, relevant impurities and physical properties for tested fungicides formulations.

Physical properties

Physical properties determined before and after storage as the following suspensibility (365/WP/M/5, CIPAC E, p.128), pH range (MT 75.2, CIPAC F, p.206), Persistent foam (MT 47, CIPAC F, p.152), Wet sieve test (MT 59.3, CIPAC F, p.179) and Wettability (MT 53.3.1, CIPAC F, p.165).

Field experimental

Tomato (Lycopersicon esculentum) was planted in February 2018 in plots in Donshway village, El- Monofiya Governorate, Egypt. Treatment was carried out by using Knapsack sprayer equipped with one Nozzle. The fungicide vacomil 35% WP was applied at the recommended dosage 262.5 g a.i / ha. Three randomized plots were treated on 5 April 2018 and one untreated plot was left to serve as control. No rain fall at any time during the experimental period was recorded.

Sampling

Samples were collected randomly from each plot after 0 (2 hours after treatment), 1, 3, 7, 10, 13 and 17 days after spraying with tested fungicide. About ten marketable tomatoes size were collected randomly from each plot and labeled then transfer to the laboratory. The samples were homogenized and sub sampling was done and taken in three representation samples and placed into polyethylene bags and frozen at - 20 °C until analysis time.

Standard preparation

Metalaxyl stock solution was prepared at a concentration of 5 mg/L. Calibration solutions with concentrations of 0.1-0.5 μg/L (ppb) prepared in acetonitrile/water 50:50 (v/v) by serial dilution of the stock solution.

Extraction and Clean up

Samples were prepared by using QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method where is10 g of homogenous sample and 10 ml acetonitrile (1% acetic acid) were added into 50 mL polyethylene centrifuge tube and shaken vigorously by vortex mixer at maximum speed. Then add 6 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate then shaken vigorously by vortex mixer for 1 min and centrifuge at 4000 rpm for 5 min. An aliquot of 6 ml from supernatant transferred into dispersive SPE15-mL centrifuge tube (part no 5982-5158) containing 400 mg PSA, 1200 mg anhydrous magnesium sulphate and 400 mg C18 and shaken vigorously for 30 sec and centrifuged at 4000 rpm for 5 min. Then filtrated and make dilution in ratio 1:2 with mobile phase and transferred to LC system for analysis.

Apparatus

LCMS/MS from Thermo Scientific company USA. The autosampler was an HTC-PAL Autosampler (CTC Analytics, Zwingen, Switzerland). The chromatographic conditions were as follows: Hypersil GOLD aQcolumn (100 x 2.1 mm, 1.9 μm particle size) controlled at 40°C. Mobile phase A (water with 0.1% formic acid and 4 mM ammonium formate) with mobile phase B (methanol with 0.1% formic acid and 4 mM ammonium formate) with flow rate 300 μL/min, gradient for mobile phase was controlled described in Table (1). LC connected with TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with a heated electrospray ionization (HESI) source. And all samples were analyzed according this condition sheath gas flow rate was 55 units, aux gas flow rate 15 units, spray voltage: 3500 V, capillary temp: 280°C, heater Temp 295°C and cycle Time: 0.2 s. Metalaxyl precursor 280.11fragmented to quantitation ion 220.10 by collision energy 16 and confirming ion 192.1 by collision energy 15. While, 2,6-dimethylaniline precursor 122.1gived quantitation ion 107.1 by collision energy 15 and confirming ion 92.2 by collision energy 11.

| Table (1): LCMS/MS mobile phase gradient |
|---------------------------------|-------|-----|
| Gradient Time (min) | %A | %B |
| 0.00 | 98 | 2 |
| 0.25 | 70 | 30 |
| 35.00 | 0 | 100 |
| 40.00 | 0 | 100 |
| 40.01 | 98 | 2 |
| 45.00 | 98 | 2 |

Recovery Assays

Metalaxyl standard solution was spiked on a homogenized untreated tomato samples in levels 0.1, 0.5 and 0.7 mg/kg each level carried out with five replicates. These samples were processed according to the above procedure. Results of recovery are presented in Table (2).

| Table (2): Recovery and relative standard deviation for metalaxyl on tomato at various levels |
|---------------------------------|-------|-----|
| Spiked levels (mg/kg) | Recovery % (mean of 5 replicate) | RSD |
| 0.1 | 92 | 11 |
| 0.5 | 96 | 9.4 |
| 0.7 | 98 | 4.7 |
Kinetic study
Degradation rate of the tested active ingredient and half lives period (t_{0.5}) for the tested pesticides were calculated according to equation (Moye et al., 1987).
\[ T_{0.5} = \frac{\ln 2}{K} = 0.6932 / K \] Where, \( K \) = rate of decomposition.
a = initial residue.
\( t_x \) = time in days or hours
\( b_x \) = residue at \( t_x \) time

Statistical analysis
All statistic analysis was done using the statistical package for social sciences (spss 16.0) program.

RESULTS AND DISCUSSION
1. Dissipation of Metalaxyl in/on tomato under field conditions
Data in Table (3) illustrated the metalaxyl residues amount (mg/kg) in/on tomato. The initial level of metalaxyl residue on tomato was 2.53 mg/kg after two hour from treatment at recommended dosage. After one day of application the degradation of metalaxyl was increased and the residue reached 1.32 mg/kg recording loss 47.83% from the initial residue level. Milgroom and Fry (1988) observed that metalaxyl residues decreased rapidly in the first two days after application and thereafter the decrease was quite slow. Dissipation/degradation rates of metalaxyl increased with extending days after treatment to reached 0.678, 0.201, 0.056 and 0.021 mg/kg recording loss 73.20%, 92.06%, 97.79% and 99.17 after 3, 7, 10 and 13 days, respectively. While metalaxyl residue not detected in tomato after 17 days of application. The half-lives (t_{0.5}) of metalaxyl degradation of tomato at recommended dosage calculated according to Moye et al. (1987) and it was 1.84 days. According to FAO which reported in CODEX (2011) that MRL of metalaxyl in tomato was 0.50 mg/kg the residues of metalaxyl on tomato after 7 days form treatment at the recommended dosage were less than its maximum residue limits (MRL) values. So, results suggested that period 7 days is safe to harvest after applying with recommended dose of metalaxyl on tomato and will be safe for the consumer's health. These results in line with Malhat (2012) who reported that half-life of metalaxyl in tomato was 1.81 days and the initial deposit of metalaxyl in tomato fruit was 2.39 mg/kg and was decreased to 0.105 mg/kg after 7 days of treatment.

Table (3): Dissipation of metalaxyl on tomato fruits under field conditions

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Residue level (mg/kg) ± SD</th>
<th>Dissipation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (^{(1)})</td>
<td>2.53± 0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>1.32± 0.09</td>
<td>47.83</td>
</tr>
<tr>
<td>3</td>
<td>0.678± 0.06</td>
<td>73.20</td>
</tr>
<tr>
<td>7</td>
<td>0.201± 0.03</td>
<td>92.06</td>
</tr>
<tr>
<td>10</td>
<td>0.056± 0.01</td>
<td>97.79</td>
</tr>
<tr>
<td>13</td>
<td>0.021± 0.01</td>
<td>99.17</td>
</tr>
<tr>
<td>17</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

\(t_{1/2}\) (days) = 1.84
\(^{(1)}\) Initial = 2 hours after treatment
ND = not detectable
Each value is a mean of three samples (replicates)

2. Effect of thermal storage at 54 ± 2°C on metalaxyl active ingredient in vacomil 35% WP
Data in table (4) describe storage temperature effect on active ingredient of metalaxyl fungicide vacomil 35% WP formulation through 14 days of storage at 54±2°C according to FAO specification (1992). Results showed that active ingredient percentage of metalaxyl one day before storage was 34.83% recording loss 0.49%. Dissipation/degradation rate of metalaxyl active ingredient increased with extending time of storage periods and there is slightly decrease in metalaxyl active ingredient percentage to reached 33.32% recording loss 4.80% after 14 days of storage at 54±2°C. Photo degradation is one of the major transformation processes affecting the fate of pesticides in environment Dejonckheere and Kipa (1974). Data showed that metalaxyl half-life (t_{1/2}) after storage for 14 days at 54°C was 8.99 days. According to FAO specifications (1992) which reported that permitted tolerance (± 5%) for matalaxyl the fungicide vacomil 35% WP formulation become conformity with FAO specification when stored at 54°C for 14 days. Ramadan (2009) founded that metalaxyl in milor-Cu WP fungicide passed successfully under thermal storage for 14 days at 54°C where metalaxyl was 14.97% and reached 14.11% after storage. Results are in line with Ola and Shereen (2007) and Kamal El-Din (2007).
3. Effect of thermal storage at 54±2°C on metalaxyl impurities 2,6-dimethylaniline vacomil 35% WP

Data in Table (4) illustrated the percentage of 2,6-dimethylaniline as relevant impurities of metalaxyl content in fungicide vacomil 35% WP formulation under thermal storage at 54±2°C. The percentage of 2,6-dimethylaniline before one day of storage was 0.024%. Extending time of thermal storage increased percentage of 2,6-dimethylaniline to reached 0.042% recording increasing rate 75% after 14 days of storage at 54°C. 2,6-dimethylaniline is a nasal carcinogen in rats and humans may be exposed to this compound via several routes. 2,6-dimethylaniline is a pharmacologically inactive metabolite of some drugs like the local anesthetic lidocaine and pesticides as metalaxyl and it is an impurity in technical grade metalaxyl Puente and Josephy (2001). According to FAO specifications (1992) which reported that maximum limit of 2,6-dimethylaniline as relevant impurities is 0.1% of the metalaxyl content the tested fungicide percentage became higher than FAO specification limit after 10 days of storage and tested fungicide became non-conformity with FAO specifications when stored for 14 days at 54±2°C. Hala et al. (2016) indicated that ethylenethiourea as relevant impurities of mancozeb fungicide affected under thermal storage and become not conformity with FAO specifications.

4. Effect of thermal storage at 54°C on pH value for vacomil 35% WP

Data presented in Table (5) indicated the influence of thermal storage on changed of pH value for fungicide vacomil 35% WP formulation through 14 days of storage at 54°C. Data showed that pH values were slightly decreased with extending storage time for 14 days at 54°C where, pH value was 6.85 one day before storage and reached to 6.53 after 14 days from storage. These results are agreed with FAO specifications (1992) which reported that pH range for metalaxyl fungicide from 5 to 10. Ramadan (2009) reported that pH value for milor-Cu fungicide WP was 6.25 and decreased to 6.05 after 14 days of storage. Data are agree with El-badry and Mohsin (2007) and Kamal El-Din and Ola (2007).
5. Effect of thermal storage at 54°C on suspensibility percentage for vacomil 35% WP

Data in Table (5) showed the effect of storage on percentage of suspensibility for vacomil 35% WP during storage at 54°C for 14 days. The suspensibility percentage of vacomil 35% WP formulation was 97% one day before storage while this percentage was gradually decreased by time lapse period of storage and became 78% after storage for 14 days at 54°C. The results indicated that the suspensibility percentage of metalaxyl through the experiment passed successfully during storage for 14 days at 54°C according to FAO specifications (1992) which reported that a minimum of 60% of metalaxyl content shall be in suspension before 30 min. Ramadan (2009) reported that suspensibility test for milor-Cu fungicide passed successfully during thermal storage.

6. Effect of thermal storage at 54°C on wet sieve test for vacomil 35% WP

Data in Table (5) illustrated the effect of thermal storage on retained percentage of vacomil 35% WP on a 75 μm test sieve at 54°C. The results revealed that the retained percentage of fungicide vacomil 35% WP before one day of storage was none and after 14 days of storage reached to 0.39%. According to FAO specification (1992) for metalaxyl which reported that a maximum 2% of retained on a 75 μm test sieve the tested fungicide vacomil 35% WP become conformity with this specification. Khozymy et al. (2017) illustrated that fungicide uthane 80% WP and volar mz 69% WP become conformity with FAO specification under thermal storage at 54°C.

7. Effect of thermal storage at 54°C on persistent foam for vacomil 35% WP

Data in Table (5) and showed the amount of foaming as volume (ml) after 1 min for vacomil 35% WP fungicide formulation. Results indicated that foaming volume for vacomil 35% WP before one day of storage was 18 ml and it’s slightly decreased by increasing long time of storage to reached 10 ml after 14 days of storage 54°C. The product ought not to make foams when it is mixed with water for use and the suspension should show less that 2% foaming under different field dilution rates El-Attal (1979). According to FAO specification (1992) for metalaxyl which reported that a maximum 25 ml of foam after 1 min the tested fungicide vacomil 35% WP become conformity with FAO specifications when stored for 14 days at 54°C. Kamal El-Din and Ramadan (2011) founded that persistent foam test for milor-Cu fungicide WP passed successfully when stored for 14 days at 54°C.

8. Effect of thermal storage at 54°C on wettability for vacomil 35% WP

Data presented in Table (5) showed the required time as minfor fungicide vacomil 35% WP to completely wetting without swirling during storage for 14 days 54°C. The results showed that the tested fungicide was completely wetted in 1 min without swirling before one day of storage until 14 days of storage 54°C. The tested fungicide vacomil 35% WP become conformity with FAO specification (1992) which reported that the product shall be completely wetted in 1 min without swirling. Results in line with Ramadan (2009) and Khozymy et al. (2017).

CONCLUSION

Residues of metalaxyl was less than its MRL values at period 7 days and results suggested that period of 7 days is safe for the consumer's health. 2,6-dimethylaniline as relevant impurities of metalaxyl affected under thermal storage and become non conformity with FAO specification after 10 days of storage at 54°C.

REFERENCES


