Significance of Temperature and Ultraviolet Light in the Breakdown of Folpet and Penconazole

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Abstract: Temperature and UV light on folpet and penconazole were studied to examine the degradation as well as identification of some degradation products by GC/MS after exposure to 30, 45, and 54 °C for 96 h, and exposure of folpet to UV light for 96 h and 28 days for penconazole. Results showed that the degradation rate of folpet increased at 45 and 54 °C reaching 60.64% and 73.59% after 96 h, respectively, also there not a significant effect of stability of folpet at 30 °C. There was a slight effect in the degradation of penconazole after exposure to tested temperatures and loss percentage was increased to reach 55.80% at 54 °C for 96 h, while there was no effect on stability of penconazole at 30 and 45 °C. Folpet was more affected than penconazole to photodegradation by UV light. However, the loss percentage of penconazole reached to 52.8% after 28 d of exposure, while folpet degradation reached to 79.73% after 96 h. Analysis of folpet samples after exposure to UV light using GC/MS identified three degradation products phthalimide, phthalic acid and (N-(dichloromethylthio)phthalimide and no degradation products detected for penconazole.

Keywords: Folpet; Penconazole; Degradation; Photodegradation; UV

1. Introduction

Folpet (*N*-(trichloromethylthio)phthalimide) is a foliar fungicide with protective action follow the phthalimide family with a mode of action centered around its reaction with thiol groups **(MacBean, 2011 and Liu and Fishbein, 1967)**. Used more than fifty years ago, it is still widely employed on grapevines, fruit trees and vegetables as a preventive treatment against downy mildews, powdery mildews, leaf spot diseases, scab and rots in fruit, ornamentals and vegetables. Folpet is classified as a harmful substance with possible risks of irreversible effects and to be harmful by inhalation **(Couteux and Lejeune, 2006)**. Folpet is very sensitive to exposure to light and the bioactivity decrease in a study on photodegradation of folpet by **(Crank and Mursyidi, 1992)**. Folpet is decomposed rapidly in alkaline and acidic solutions. The half-life of folpet is 1.1 h at pH 7, while it was 12 h in a formulation sample of folpet at pH 7.35; also the half-life of folpet was 10.5 h at pH 3. Folpet can be degraded into many intermediates such as phthalimide, phthalamic acid, and phthalic acid **(Cabras *et al.*, 1997)**. The main metabolite of folpet is phthalimide in crops, while folpet decomposed into phthalimide under different conditions from high temperature or pHs (European Food Safety Agency). Triazoles are closely applied over the world to eliminate fungus and promote agricultural production **(Lv *et al.*, 2017)**. Penconazole (1-(2,4-dichloro-β-propylphenethyl)-1*H*-1,2,4-triazole) is systemic fungicide with protective and curative action used in control powdery mildew, pome fruit scab, and other pathogenic ascomycete **(Mercadante *et al.*, 2016 and Husak *et al.*, 2017)**.

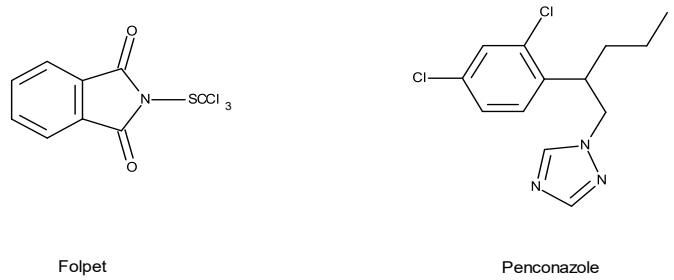
The stability of penconazole is very high in both acidic and alkaline conditions, also at high temperatures, when it exposure to direct sunlight decomposed with a half-life 4 days, however all photochemical reactions were investigated in this study. Exposure of penconazole to light of wavelengths greater than 280 nm, the main reaction was a cyclised and dechlorinated product formation **(Roberts and Hutson, 1999)**.

The aim objective of this applied study was to examine the degradation of folpet and penconazole after exposure to UV light and temperature of different intervals and time, furthermore recognition of some degradation products after exposure to UV light by GC/MS.

2. Materials and Methods

2.1. The tested pesticides

Active ingredients of the tested pesticides (folpet and penconazole) were obtained from Sigma-Aldrich; also solvents used in this investigation were of HPLC-grade. The chemical structure of folpet and penconazole are shown in Fig. (1).



Folpet Penconazole

Fig. (1) Chemical structure of folpet and penconazole

2.2. Procedure

A stock solution (200 µg ml^{-1}) of both folpet and penconazole analytical standard were prepared in methanol. One milliliter methanol containing 200 µg ml^{-1} active ingredient. 1 ml from stock solution was put in petri dishes and it was left to dry at room temperature then petri dishes were exposed to:

- Different temperature degrees at 30, 45, 54 °C for 1, 2, 4, 6, 12, 24, 48, 72, 96 hours using digital thermal oven.
- A short wave length of an ultra violet lamp (254 nm) for 1, 2, 4, 6, 12, 24, 48, 72, 144 hours for folpet, while the period of exposure for penconazole increased to reach 28 days.

All exposed tested folpet and penconazole were transferred quantitatively with methanol and the content of the residue of folpet and penconazole were determined by HPLC and GC, respectively.

2.3. Measurements

2.3.1. HPLC determination of folpet

This procedure according to **CIPAC 75/TC/M/-(1983).** The HPLC analyses were carried out with Agilent Technologies 1260 infinity II auto sampling system, consisting of quaternary pump, column and UV detector. The chromatographic separation was performed with Agilent Zorbax Eclipse plus C_{18} (4.6 mm ID x 250

mm x 5 µm) chromatographic column. The mobile phase was methanol – acetonitrile (70:30), at a flow rate of 1.5 ml/min. Folpet was detected by UV detector at wavelength of 210 nm. The injection volume was 5 µl. under
these conditions the typical retention time of folpet was 1.888 min. A good linearity was obtained in the range 5 -200 ng μl^{-1} of active ingredient with correlation coefficient of 0.99998 as shown in Fig. (2).

2.3.2. Gas Chromatography determination of penconazole

This procedure according to **(MacBean, 2011)**. Agilent 7890B gas chromatograph with autosampler 7693 equipped with Flame Ionization Detector (FID) at 250 °C, capillary column HP-50+ (30 m x 0.25 mm I.D., 0.25 µm film thickness). Nitrogen was used as a carrier gas with constant flow 4 ml/min. The oven temperature program was held at 200 °C for 1 min, then ramp 20 °C /min to 280 °C. Injector temperature was 250 °C with splitless mode. The injection volume was 1 µl. under these conditions the typical retention time of penconazole was 3.803 min. A good linearity was obtained in the range 5-200 ng μl^{-1} of active ingredient with correlation coefficient of 0.99998 as shown in Fig. (3).

2.3.3. Gas chromatography-mass spectrometry analysis (GC/MS)

The GC/MS analysis was performed with an Aglient 7890B gas chromatograph equipped with 5977 A MSD Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5MS(30 m x 0.25 mm x 0.25 µm film thickness). Helium was used as carrier gas at approximately 1.0 ml/min pulsed split mode, split ratio (10:1). The solvent delay was 4 min, and the injection volume was 1 µl. The GC temperature program was held at 50 °C for 0.5 min, then ramp 10 °C /min to 190 °C for 1 min. followed by ramp 10 °C /min to 300 and held for 2 min °C (total run time 29.5 min, the injector temperature was set at 280 °C. EI mass spectra were identified using Wiley mass spectral data base Library.

2.3.4. Kinetic study

The half-life time ($t_{0.5}$ (of folpet and penconazole was calculated according to (**Moye *et al.*, 1987)** and **(Anderson and Scott, 1991)**.

$$t_{0.5} = \ln 2/K$$

$$K = (1/t_x) \text{ Ln } (a/b_x)$$

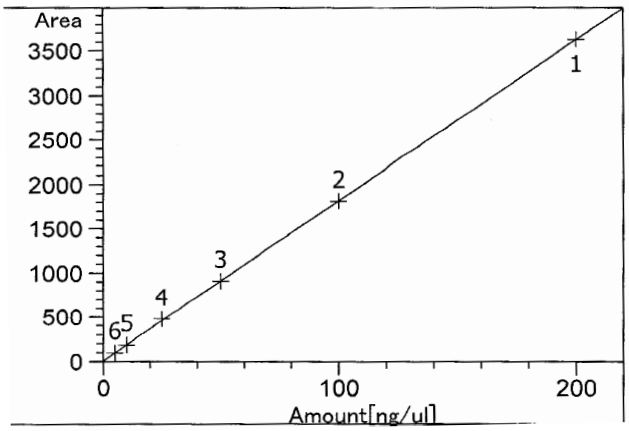
Where, $t_{0.5}$ = the time required to reach 50% of the initial concentration.

K = rate of degradation

a = initial concentration

t_x = time in hours

b_x = concentration at x time

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Folpet at exp. RT: 1.888
VWD1 A, Wavelength=210 nm
Correlation:              0.99998
Residual Std. Dev.:      10.07797
Formula: y = mx + b
      m:       18.13138
      b:        7.32577
      x: Amount
      y: Area
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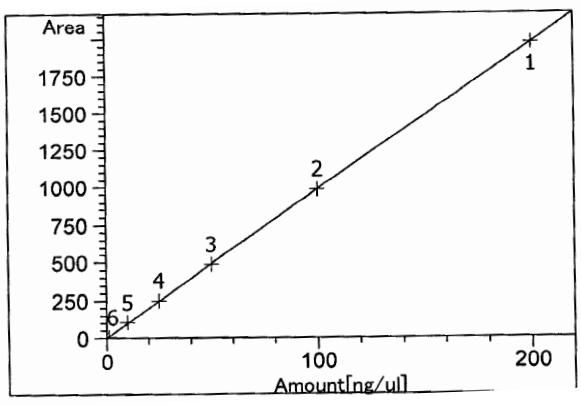
Fig. (2). Standard calibration curve of folpet using HPLC

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Penconazole at exp. RT: 3.803
FID1 A, Front Signal
Correlation:              0.99998
Residual Std. Dev.:       4.60080
Formula: y = mx + b
      m:        9.85687
      b:        7.96072e-1
      x: Amount
      y: Area
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Fig. (3). Standard calibration curve of penconazole using GC

3. Results and Discussion

3.1. Effect of different temperatures on the degradation of Folpet

Table (1) and Fig. (4) illustrated the effect of different temperatures on the degradation of folpet, the results indicated that there was a significant effect on folpet when exposed to high temperature and the loss of percentage increased to reach 60.64 and 73.59% after 96 h and the half-life ($t_{0.5}$) was 27.50 and 2.10 h at 45 and 54 °C, respectively, while folpet was more stable after exposure to 30 °C and the loss of percentage was 49.95% after 96 h and the half- life ($t_{0.5}$) was 97.20 h. the previously mentioned results clearly showed that the rate of degradation of folpet increased with increasing in temperature that folpet was sensitive to high temperature degrees and the rate of degradation increased with exposure to high temperature. The results are compatible with **(Raina-Fulton, 2014)** as indicated that the degradation of folpet occurs when exposed to heating even during the preparation of samples.

Table (1) Effect of different temperatures on the degradation of folpet

Exposure time (h)	30 °C		45 °C		54 °C	
	µg	Loss %	µg	Loss %	µg	Loss %
0	200	0	200	0	200	0
1	157.33	21.34	126.30	36.85	113.24	43.38
2	150.99	24.51	124.48	37.76	100.23	49.89
4	139.69	30.16	121.51	39.25	95.10	52.45
6	132.71	33.65	111.59	44.21	86.16	56.92
12	124.87	37.57	107.25	46.38	80.76	59.62
24	121.09	39.46	102.26	48.87	75.33	62.34
48	112.90	43.55	98.29	50.86	62.54	68.73
72	106.77	46.62	86.16	56.92	57.39	71.31
96	100.11	49.95	78.73	60.64	52.82	73.59
$t_{0.5}$ (h)	**97.20**		**27.50**		**2.10**	

3.2. Effect of different temperatures on the degradation of penconazole

Data from Table (2) and Fig. (5) showed the effect of different temperatures on the degradation of penconazole and indicated that there was a slight effect in the degradation of penconazole after exposure to different temperatures and the percentage loss reached to 50.23 and 55.80% after 96 h and the half-life ($t_{0.5}$) was 93.50 and 57.50 h at 45 and 54 °C, respectively. Penconazole has not been changed by exposure to 30 °C and it was more stable than exposure to other tested temperatures, as the percentage loss was 41.29% after 96 h and the half- life ($t_{0.5}$) was 126.50 h. The results showed that penconazole was more stable after exposure to different temperature degrees than folpet, and the rate of degradation was fairly stable, however our findings results are in harmony with **(Roberts and Hutson, 1999)**.

3.3. The photodegradation of folpet and penconazole after exposure to UV light

Data presented in Table (3) and Fig. (6,7) explained the effect of exposure to UV light on the degradation of folpet and penconazole, the results clarified that folpet is highly affected after exposure to UV light and loss of percentage increased to reach 79.73% after 96 h, further the half-life ($t_{0.5}$) was 1.3 h. Penconazole was more stable after exposure to UV light and the percentage loss reached 52.80% after 28 d where the half-life ($t_{0.5}$) was 17 d. The previously mentioned results clearly showed that the rate of degradation of folpet increased with exposure to UV light and long period of exposure, and this results are in agreement with **(Crank and Mursyidi, 1992),** while the degradation rate of penconazole did not affect even with increasing in the period of exposure and it was more stable than folpet, and our results in agreement with **(Roberts and Hutson, 1999)**.

3.4. Matching of the degradation products from the photodegradation of folpet and penconazole by GC/MS

The samples of folpet were analyzed after exposure to UV light for 96 hours using GC/MS to recognize some degradation products, and found that the characteristic ions at m/z 297, 147, 169 [M^++3] and 265 [M^++3] were molecular ions of folpet, phthalimide, phthalic acid and (N-(dichloromethylthio)phthalimide. The possible degradation pathways of folpet can be shown in Fig. (8).

Folpet is unstable to UV light, however it has unstable sulfenyl moiety as it cause the rapid degradation of folpet to pthalimide m/z, 147. The mass fragmentation of phthalimide can be shown in Fig. (9). Folpet can be degraded by UV light through decomposition of C-Cl bonds and loss a chlorine atom to give another by product (N-(dichloromethylthio)phthalimide m/z, 265 [M^++3]. The mass fragmentation of (N-(dichloromethylthio)phthalimide

Table (2) Effect of different temperatures on the degradation of pExposure time

Exposure time (h)	30 °C		45 °C		54 °C	
	µg	Loss %	µg	Loss %	µg	Loss %
0	200	0	200	0	200	0
1	168.73	15.64	145.47	27.27	134.81	32.60
2	160.34	19.83	141.70	29.15	118.18	40.91
4	157.78	21.11	138.79	30.61	114.53	42.74
6	150.39	24.81	116.29	41.86	112.08	43.96
12	145.47	27.27	112.95	43.53	109.51	45.25
24	138.79	30.61	107.58	46.21	106.95	46.53
48	129.49	35.26	106.95	46.53	105.26	47.37
72	123.66	38.17	103.30	48.35	91.61	54.20
96	117.43	41.29	99.55	50.23	88.41	55.80
$t_{0.5}$ (h)	**126.50**		**93.50**		**57.50**	

Table (3) Effect of exposure to UV light on the degradation of folpet and penconazole

Exposure time (h)	Folpet		Penconazole	
	µg	Loss %	µg	Loss %
0	200	0	200	0
1	107.70	46.15	165.36	17.32
2	94.11	52.95	158.63	20.69
4	82.72	58.65	149.54	25.23
6	68.32	65.84	140.76	29.62
12	50.56	74.72	133.79	33.11
24	47.25	76.38	124.10	37.95
48	45.47	77.27	120.10	39.95
72	44.36	77.82	117.23	41.39
96	40.55	79.73	111.42	44.29
168 (7 d)			106.02	46.99
336 (14 d)			102.19	48.91
672 (28 d)			94.40	52.8
$t_{0.5}$ (h)	**1.3**		**407.65 (17 d)**	

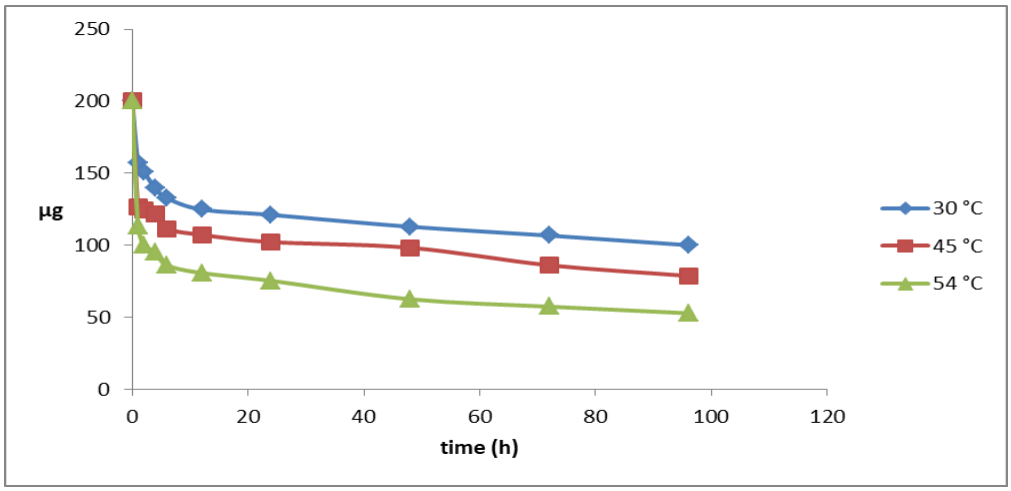

Fig. (4). Effect of different temperatures on the degradation of folpet

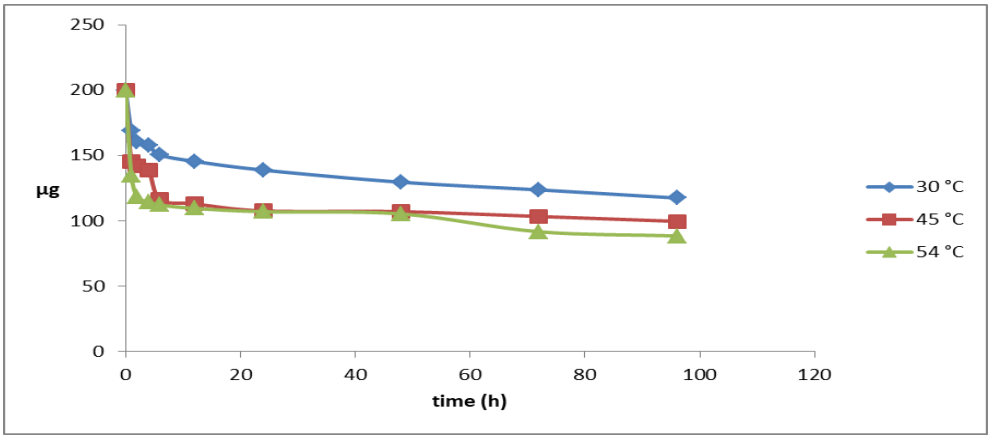

Fig. (5) Effect of different temperatures on the degradation of penconazole

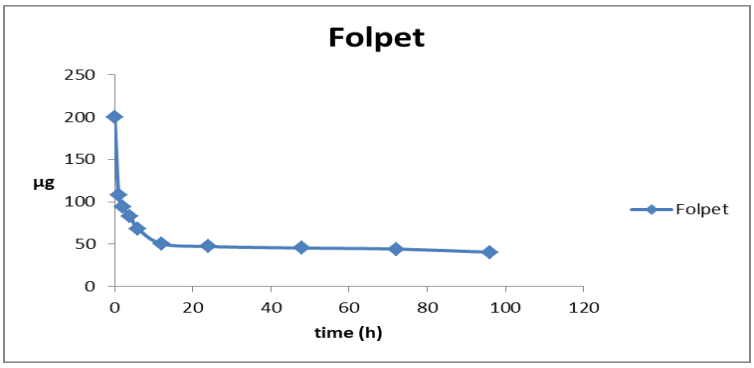

Fig. (6) Effect of exposure to UV light on the degradation of folpet

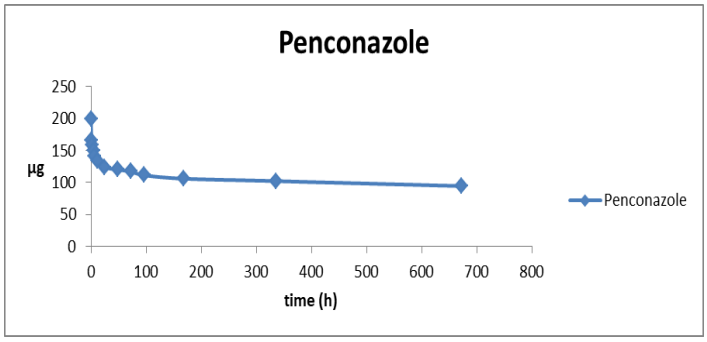

Fig. (7) Effect of exposure to UV light on the degradation of penconazole

can be shown in Fig. (10), however quickly dissociated to give phthalimide. Phthalimide can be broke down by photochemical reaction to phthalamic acid, where it can be transformed to phthalic acid m/z, 169 [M^++3]. The mass fragmentation of phthalic acid can be shown in Fig. (11). our finding results are in agreement with **(Crank and Mursyidi, 1992; Cabras *et al.*, 1997; Raina-Fulton, 2014 and Relana, 2017)**.

The samples of penconazole were analyzed after exposure to UV light for 28 days using GC/MS to recognize degradation products, and found that penconazole was comparatively stable and no photo degradation products were detected after 28 days of UV exposure**.**

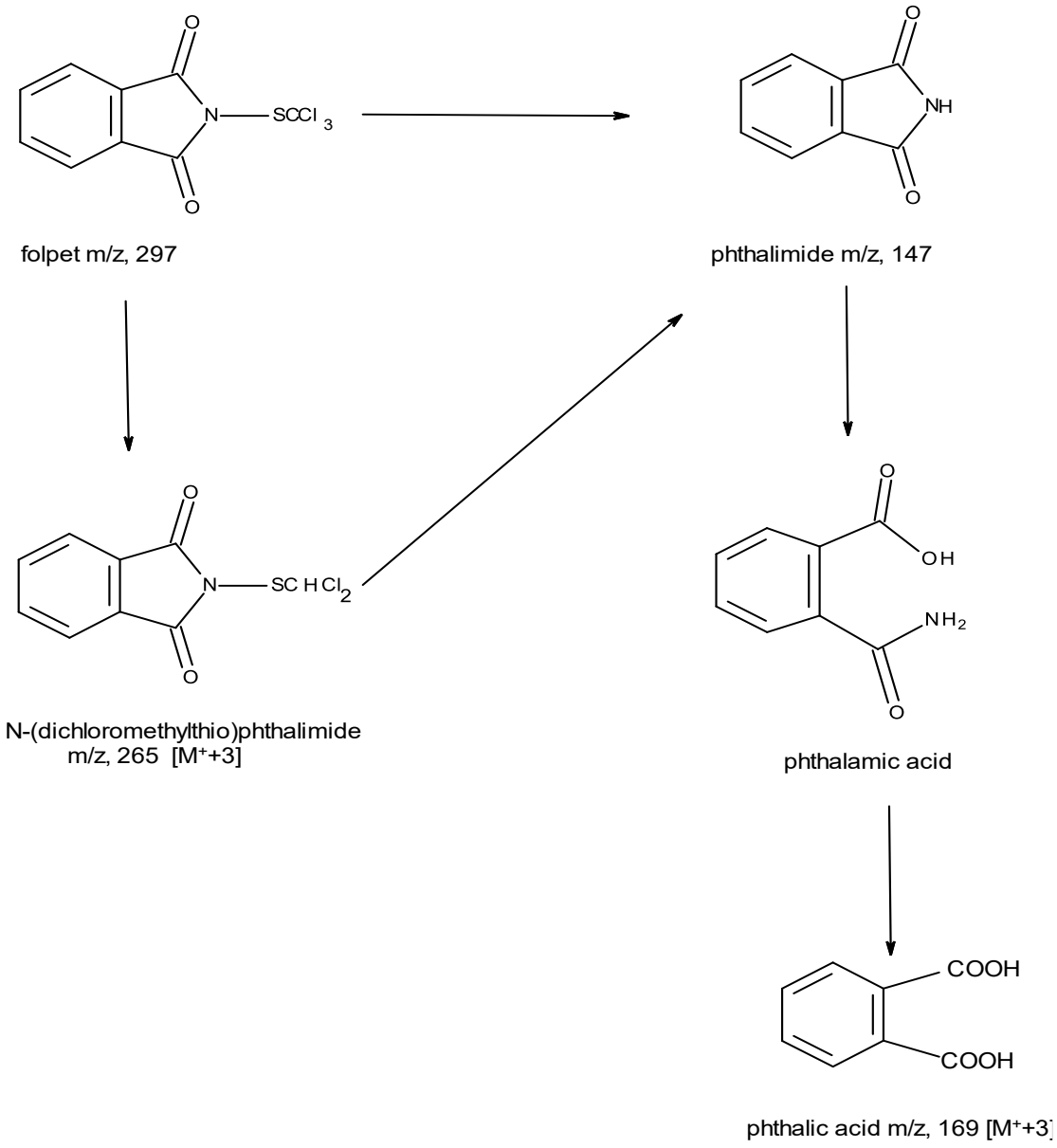

Fig. (8) Possible degradation pathways of folpet

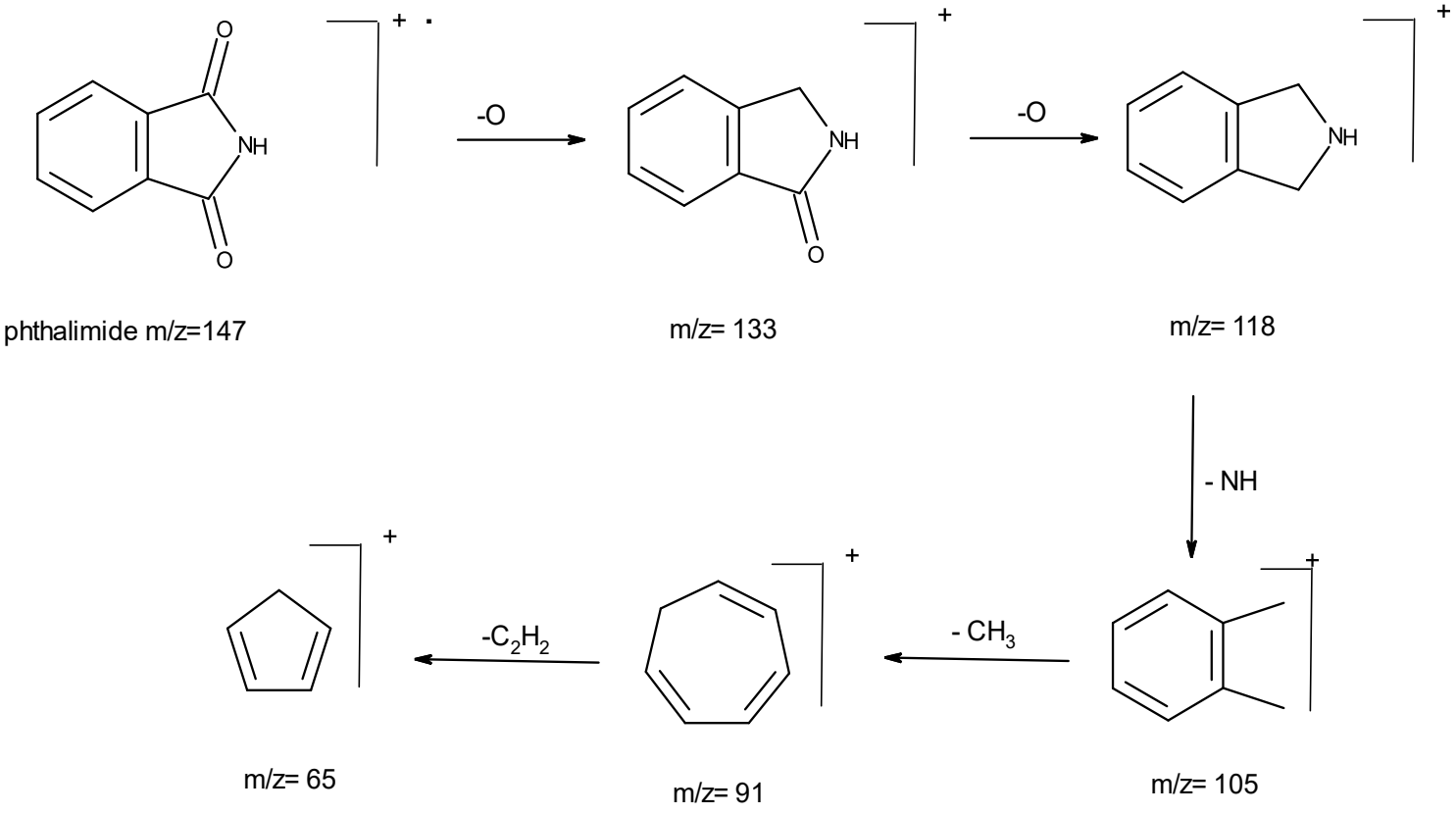

Fig. (9)Mass fragmentation of phthalimide

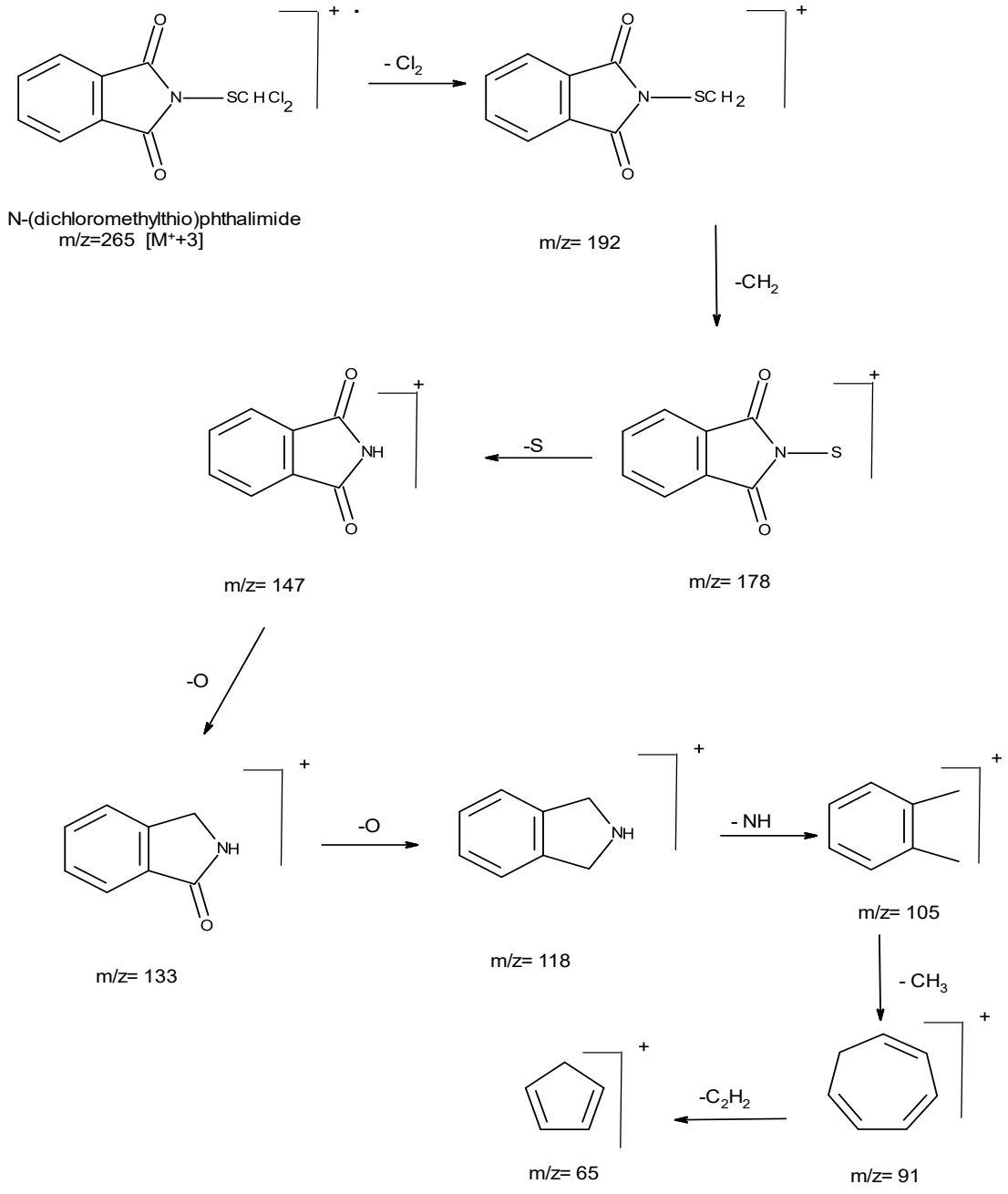

Fig. (10) Mass fragmentation of (N-(dichloromethylthio)phthalimide

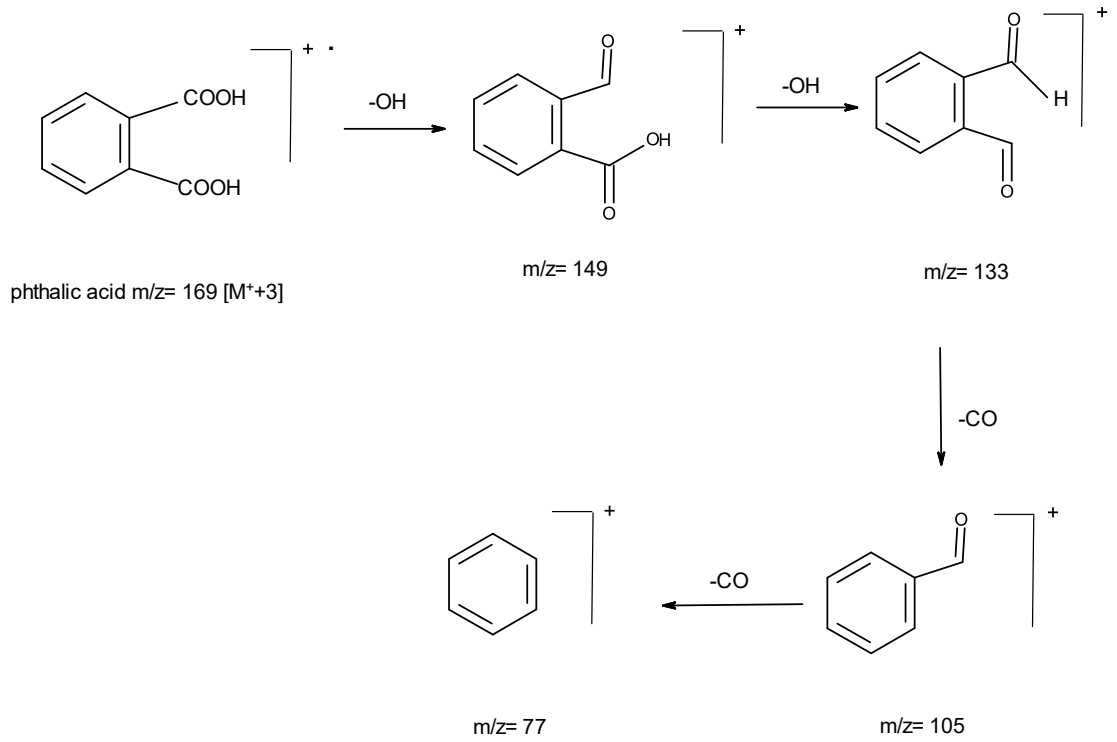

Fig. (11) Mass fragmentation of phthalic acid

Conclusions

There was a significant impact of folpet after exposure to different temperature degrees and UV light, where the rate of degradation increased by several factors such as, chemical structure, long period of exposure and high temperature, and this resulted in the formation of some by products. Penconazole was more stable after exposure to different temperature degrees and UV light.

References

Anderson G. and Scott M. (1991), Determination of product shelf life and activation energy for five drugs of abuse. *Clin. Chem*., .**37**(3), 398-402.

Cabras P., Angioni A., Garau V. L., Melis M., Pirisi F. M., Farris G. A., Sotgiu C. and Minelli E. V. (1997), Persistence and metabolism of folpet in grapes and wine. *J. Agric. Food Chem.,* **45**. 476-479.

CIPAC 75/TC/M (1983)/-, Folpet Technical; Vol., **1B**, p. 1845-1847 .

Couteux A. and Lejeune V.(2006), Index phytosanitaire ACTA, 42th ed., .

Crank G. and Mursyidi A. (1992), *J. Photochem. Photobiol. A*, 289-297, **68**.

Husak V. V., Mosiichuk N. M., Storey J. M., Storey K. B. and Lushchak V. I. (2017), Acute exposure to the penconazole-containing fungicide Topas partially augments antioxidant potential in goldfish tissues**.** *Comp. Biochem. Phys. C Toxicol. Pharmacol*., .) 1-8 ,**193**.

Liu M. K. and Fishbein L. (1967), Reactions of Captan and Folpet with thiols. *Experientia,* .81-82 ,**23**

Lv X., Pan L. M., Wang J. Y., Lu L. P., Yan W. L., Zhu Y. Y., Xu Y. W., Guo M. and Zhuang S. L. (2017), Effects of triazole fungicides on androgenic disruption and CYP3A4 enzyme activity. *Environ. Pollut.,* **222**, 504-512 .

MacBean, C. (2011), The pesticide manual (version 5.2). The British Crop Protection Council .

Mercadante R., Polledri E., Scurati S., Moretto A. and Fustinoni S.(2016), Identification of Metabolites of the fungicide penconazole in human urine. *Chem. Res. Toxicol*. **29**, 1179-1186 .

Moye H. A., Malagodi M. H., Yoh J., Leibee G. L., Ku C. C. and Wislocki P. G. (1987), Residues of avermectin B1a rotational crops and soils following soil treatment with (C14) avermectin B1a. *J. Agric. Food Chem*., **35**, 859-864.

Raina-Fulton R. .(2014), A Review of methods for the analysis of orphan and difficult pesticides: glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides. *Journal of AOAC International*, 965–977 ,**97**

Relana (2017), Position Paper No. 17-01; Phtahlimid part 2: Unavoidable Artefact! Version 2017/04/07. http://www.relana-online.de/wp-content/uploads/2017/04/

PP_1701_PI_Part2_vers20170407.pdf (Accessed 15 August 2017).

Roberts T. R. and Hutson D. H. (1999), "Metabolic Pathways of Agrochemicals Part 2 Insecticides and Fungicides". Azoles and analogues, Royal Society of Chemistry, Cambridge, p. 1011-1104 .

دور درجة الحرارة والاشعة فوق البنفسجية فى انهيار الفولبت والبينكونازول

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قسم بحوث تحليل المبيدات – المعمل المركزى للمبيدات- مركز البحوث الزراعية – الدقى - جيزه – مصر

الملخص العربى:

تمت هذه الدراسة لبحث تأثير درجة الحرارة والاشعة فوق البنفسجية على انهيار الفولبت والبينكونازول وكذلك التعرف على بعض نواتج التكسير باستخدام جهاز GC/MS بعد التعرض لدرجات حرارة 30 و 45 و 54م° لمدة 96 ساعة و ايضا التعرض للاشعة فوق البنفسجية لمدة 96 ساعة للفولبت و28 يوم للبينكونازول. أوضحت النتائج المتحصل عليها ان الفولبت معدل انهياره زاد عند 45 و 54م° ووصلت نسبة الفقد الى 60,64% و 73,59% بالتتابع بعد 96ساعة وكان اكثر ثباتا عند درجة حرارة 30م° . كان هناك تأثرا طفيفا فى انهيار البينكونازول بعد التعرض لدرجات الحرارة المختلفة و وصلت نسبة الفقد الى 55,80% عند 54م° لمدة 96 ساعة بينما كان اكثر ثباتا عند درجات حرارة 30 و45م° . التحلل الضوئى بالنسبة للفولبت كان اكثر تأثيرا من البينكونازول ومعدل انهيار الفولبت زاد بالمقارنة بمعدل انهيار البينكونازول حيث وصلت نسبة الفقد فيه الى 52,80% بعد 28 يوم بينما فى الفولبت وصلت نسبة الفقد الى 79,73% بعد 96 ساعة. تم تحليل العينات باستخدام جهاز GC/MS بعد التعرض للاشعة فوق البنفسجية وتم التعرف على ثلاث نواتج تكسير للفولبت وهم phthalimide و phthalic acid و(N-(dichloromethylthio)phthalimide) و لم يتم التعرف اى نواتج تكسير للبينكونازول.