Egypt. J. Plant Prot. Res. Inst. (2020), 3 (2): 673 - 680

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Effect of storage on the stability on some fungicides and determination of residues in fish

Kamal, N. Y.; Mesbah, A. and Abdel-Latif, O.

Formulation Research Dept., Central Agricultural Pesticide Lab., Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received: 12/4/2020

Accepted: 22/ 6/ 2020

Keywords

Propiconazole, dimethomorph , fludoxanil , storage and residues .

Abstract:

The present work was carried out to study the persistence of naserzol (Propiconazole 25 % EC W/V), nofostar (Dimethomorph 5%WDG W/W) and self (Fludioxonil 25 %WG W/W). The stability of active ingredient of the tested fungicide under storage 54 ºC for 14 days and influence direct sun light, monitoring of pesticide residues in fish (gills and muscles) samples in Egypt in three months : June , November and December 2018 from three Governorates (Behera , Gharbia , and Alexandria). The pesticide residues in the fish muscles, gills, studies fungicides were estimated by HPLC, and GC/Ms. The active ingredient were propiconazole, dimethomorph and fludioxonil 25.31, 5.03 and10.21 before storage become after storage 54 ºC, 18.65, 4.67 and 7.68 , respectively , at the end experiment the loose percentages were 26.31 , 7.15 and 24.77 % when the fungicides compound previously were stored at 54 ºC for 14 days. Also, the stability active ingredient before influence sun light 25.07 , 5.03 and 10.21 after influence sun light 22.01, 4.10 and 9.11, respectively . The highest fungicide in Alexandria Governorate was fludioxonil more than ADI according to FAO in November. The less than in Gharbia and Behera Governorates were propiconazole and dimethomorph ADI according to FAO in June and December.

Introduction

Fungicides are widely accustomed kill or inhibit fungi or fungal spores that threaten to ruin greenhouse crops through incorporation into the soil and foliar application. On the contrary single fungicide applications, multiple applications over a season often end within the fungicide's permanency in agricultural soil (Bulbul and Ozhan, 2012). The risk to the environment posed by the employment of fungicides in horticultural production systems has received relatively little attention compared to other kinds of agrochemicals, like insecticides and herbicides (Gillom *et al*., 2006).

The environmentally available proportion of fungicides in water, sediment and soil can cause toxic effects to organisms. However, not all the fungicide is absorbed by organisms and react with toxic sites of action within exposed organisms (be toxicologically

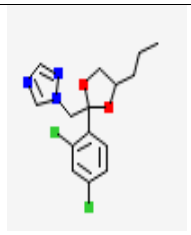
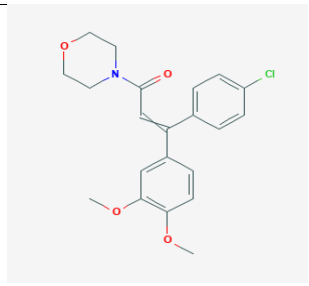
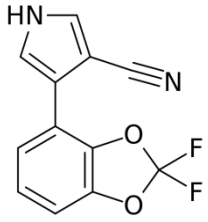
bioavailable or bio accessible) (Fairbrother *et al*., 2007 and Harmsen, 2007). In Egypt, as in many other agricultural countries, pesticides are widely accustomed control harmful pests, mainly, cotton, maize and rice. Nearly of these chemicals are readily soluble in plant oils and waxes; this common property places all under suspicion as food (Fatin *et al*. , 2016).

In this paper, we studied the effect of storage and influence sun light direct of fungicides (Propiconazole, dimethomorph and fludioxonil) during storage for 14 days at 54 ºC on also the degradation of tested fungicides was studied. Determination residues fungicides of the fish were collected from each of the three Governorates, Behera , Gharbia , and Alexandria.

Materials and methods

1. Source of sample pesticides: Central Agricultural Pesticide Lab. and Table (1) observed trade name, active ingredient and formulation types of fungicides:

Table (1): Showed the formulation of fungicides.

Trade name for fungicides	Active ingredient	M.W g/mol	Formulation types	Structure
Naserzol	Propiconazole 25 %	342	EC	
Nofostar	Dimethomorph5%	387.9	WDG	
Self	Fludioxonil 25 %	248	WG	

2. Sample preparation for tested pesticides:

Accurately weighed enough samples formulation equivalent to 10 mg of standard in a different 25 ml volumetric flask for each sample, and slowly mixed with methanol and the volume was completed with methanol.

3. Storage stability tests:

The Previously fungicides (Propiconazole , fludioxonil and dimethomorph) formulation were stored in oven at 54 ºC for 14 days according to the (FAO, 2018 and 2010), respectively, 14 days storage. During the storage period the

samples were taken at 0, 1, 3 ,7 and 14 days from storage to determine the active ingredient for formulation under testes if is present .

4. Influence of direct sunlight:

The fungicides (Propiconazole , fludioxonil and dimethomorph) formulation were influence of direct sunlight for 14 days according to the (Naser *et al*., 2003) .The samples were taken at 0, 1, 3 ,7 and 14 days from influence direct sun light to determine the active ingredient and impurities content for formulation under testes if is present .

5. Fish samples:

Thirty Tilapia (*Oreochromis niloticus*) growing in drainage water were collected from each of the three Governorates (Behera, Gharbia, and Alexandria) under study.

6. Extraction and cleanup of fish samples:

Fish muscles and gills were ground and blended in a warring blender just to pulplike consistency. 200 ml methanol / acetone (4/1) were added to 100 g blended both muscles and gills samples , then the mixture was shaken mechanically using electrical shaker for one hour and filtered through Buchner funnel. The collated solvent mixtures were evaporated under reduced pressure using rotary evaporator till dryness. The residue was quantitatively transferred into small vials with 5 ml methanol. The solvent was then evaporated till dryness and vials were stored at -15 ºC until the cleanup (Muir *et al*., 1981).

7. Identification of pesticides in gills and muscles fish samples:

A study was done to check the three pesticides residues (Table, 1), in different samples of both gills and muscle fish samples collected from three different Governorates (Behera , Gharbia , and Alexandria) in Egypt. The residues analysis was done using HPLC and identified the type of pesticides with gas chromatography-Mass spectrometer (GC-MS) (Iqbal *et al.*, 2009).

8. Determination of Active ingredient by high Performance Liquid Chromatography HPLC :

The type of chromatographic HPLC system model (Agilent Technologies 1260 Infinity) with Quaternary pump, UV-detector was employed. The chromatographic C18 stainless steel column (25 cm length, 4.6 mm inner diameter, and 4.0 µm particles) .

9. Determination of fungicides by GC/Ms:

Apparatus Agilent B, 5977 AMSD gas chromatography equipped with an Agilent mass spectrometric detector , with a direct capillary interface and fused silica capacity Colum (30 m x 0.025 mm HP -5 0.25 microm 60 to 325/325 ºC). Samples were injected under the following condition; helium was used as carrier gas at approximately 1 ml /min , pulsed split mode , split ratio (10:1) split flow 10 ml /min . The solvent delay was 4 min and the injection size were 1 UL . Oven temperature program , %0 ºC for O<5 min , the 10 /min ramp to 190 ºC followed by a 10 ºC /min ramp to 210 ºC for 1 min followed by a 10 ºC /min ramp to 300 ºC and held for 2 min (total run time followed by an injection temperature was set at 280 ºC. Wiley mass spectral data was used in the identification of the separated peaks .

Results and discussion

1. The effect of storage 54 ºC and influence sun light on propiconazole, dimethomorph and fludioxonil:

Date in Table (2) stability of active ingredient were propiconazole dimethomorph and fludioxonil 25.31, 5.03 and 10.21 before storage become after storage 54 ºC, 18.65 , 4.67 and 7.68 at the end experiment, respectively . The loose percentages were 26.31, 7.15 and 24.77 % when the fungicides compound previously were stored at 54 ºC for 14 days . The stability active ingredient before influence sun light 25.07, 5.03 and 10.21 after influence sun light 22.01, 4.10 and 9.11, respectively.

Table (2): The effect of storage 54 ºC and influence sunlight on propiconazole , dimethomorph and fludioxonil .

Storage period (days)	Storage 54 C						Sunlight					
	Propiconazole 25 %		Dimethomorph 5 %		Fludioxonil 10		Propiconazole 25 %		Dimethomorph 5 %		Fludioxonil 10%	
	(a.i)	Loss	(a.i)	Loss	(a.i)	Loss	(a.i)	Loss	(a.i)	Loss	(a.i)	Loss
0*	25.31	00	5.03	00	10.21	00	25.07	00	5.03	00	10.21	00
1	25.31	00	5.03	00	10.02	1.86	25.00	2.79	5.00	0.59	10.00	2.20
3	25.07	0.23	4.95	1.59	9.02	11.65	24.00	4.26	4.85	3.57	9.94	2.60
7	21.41	15.40	4.80	1.51	8.87	13.12	22.27	11.16	4.17	17.09	9.59	6.07
14	18.65	26.31	4.67	7.15	7.68	24.77	22.01	1.03	4.10	18.48	9.1 1	10.77

0* one hour before exposure to storage.

2. The degradation product and possible pathway of propiconazole, dimethomorph and fludoxanil by GC/Ms :

The data presented in Table (3) showed that propiconazole after storage 54 ºC for 14 days and influence direct sun light product m/z 344 1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4- triazole , M/Z 172 2,4-dichlorophenyl 1,2,4-triazole-1-ylmethyl ketone, m/Z 301 1-[2-(2,4-dichlorophenyl)-4-(hydroxypropyl)-1,3-dioxolan-2-ylmethyl]- The degradation fragment ion spectrum of m/z 344 was identified by its (M -16)+ which was due to loos of OH from 344 suggesting 1-[2-(2,4-dichlorophenyl)-4-(hydroxypropyl)-1,3-dioxolan-2-ylmethyl]. Propiconazole has propyl side-chin on the dioxolane ring. Therefore, this degradation product was suggested to be compound hydroxlated on different carbon position of propyl side chin (Hen and Trak, 2003). Dimethomorph the fragment ion m/z 388 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxy-phenyl)-1-oxo-2-propenyl]-and fludioxonil 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile (Figures 1-3) , influence sun light and storage 14 days on 54 ºC.

Table (3): The degradation product on propiconazole, fludioxonil , dimethomorph and by GC/Ms .

Pesticides	M/Z	Compound product
Propiconazole	344 172 301	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4- triazole 2,4-dichlorophenyl 1,2,4-triazole-1-ylmethyl ketone 1-[2-(2,4-dichlorophenyl)-4-(hydroxypropyl)-1,3-dioxolan-2-ylmethyl]-
Fludioxonil	248	4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
Dimethomorph	388	4-[3-(4-chlorophenyl)-3-(3,4-dimethoxy-phenyl)-1-oxo-2-propenyl]-

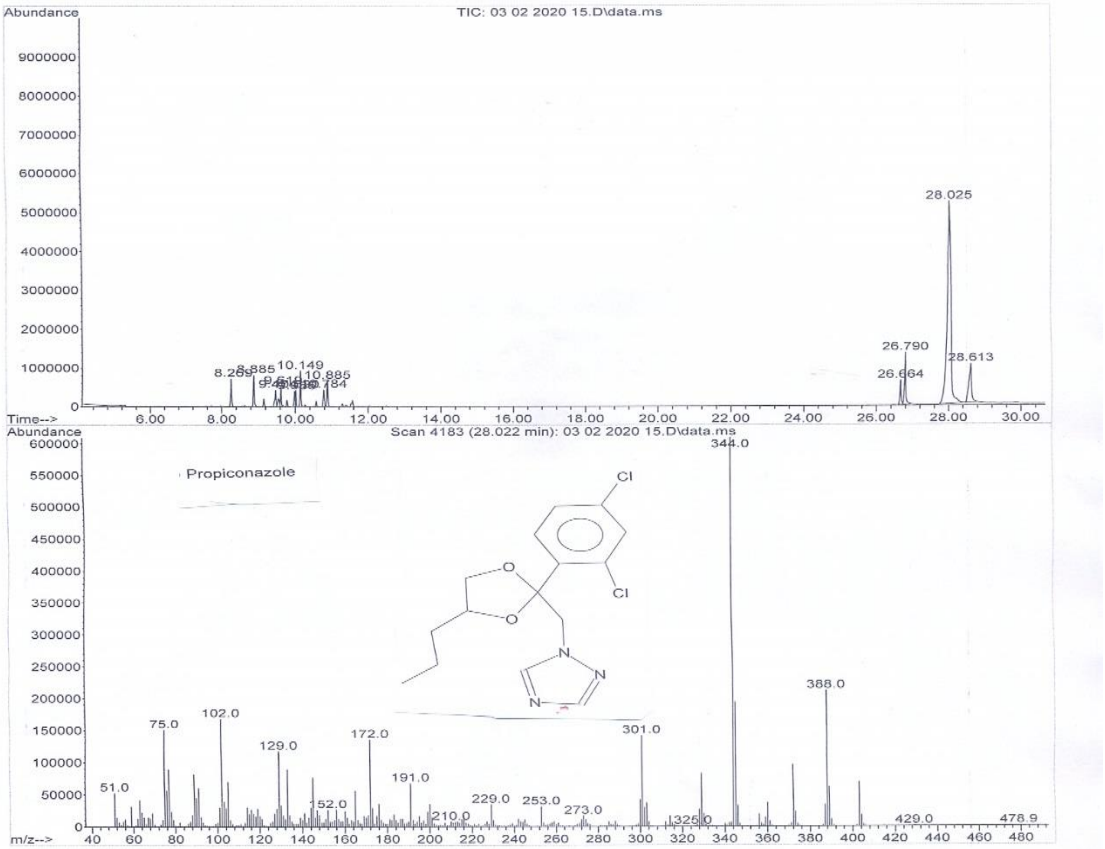


Figure (1): Fragment of propiconazole by GC/Ms.

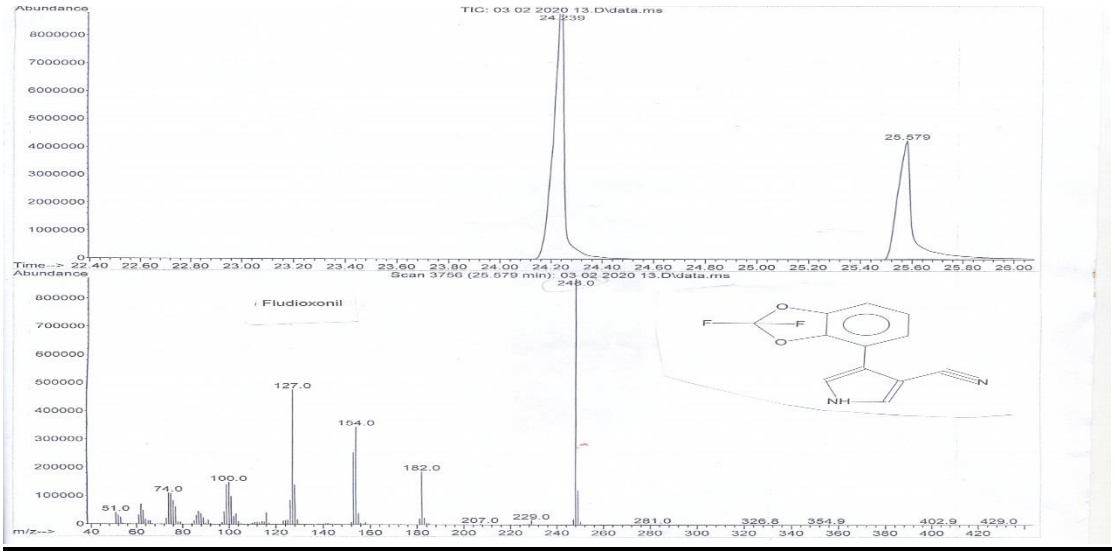


Figure (2): Fragment of fludioxonil by GC/Ms.

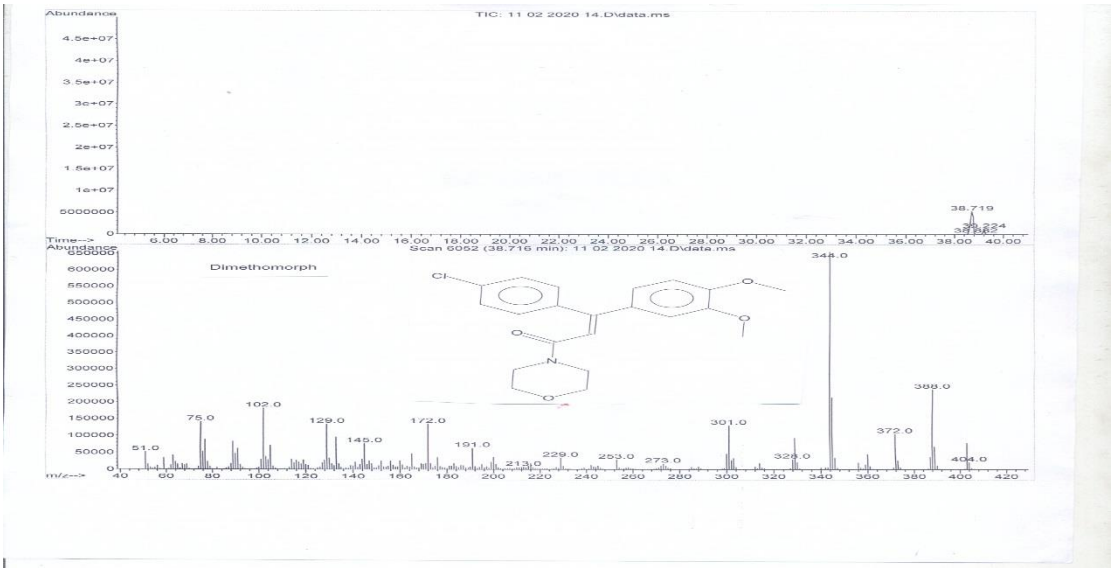


Figure (3): Fragment of dimethomorph by GC/Ms .

3. Fungicides residues detected in *Oreochromis niloticus* fish gills and muscles samples collected from three different Governorates during June 2018:

Data in Table (4) indicated the presence residues of pesticides in fish muscles samples from three different Governorates November in 2018. The average of the detected pesticide residues in these Governorates were as follows: gill of fish samples collected from Gharbia Governorate contained propiconazole UND, fludioxonil 0.001246 ppm dimethomorph 0.000404 ppm in gills. The muscles of fish samples collected from Gharbia Governorate propiconazole, fludioxonil, dimethomorph UND, respectively.

The pesticide residues in gills of fish samples collected from Behera , propiconazole 0.00323 ppm , fludioxonil 0.00052 ppm , dimethomorph UND and fish samples collected from Alexandria propiconazole 0.00558 ppm, fludioxonil UND, dimethomorph 0.00803 ppm less than ADI. The pesticide residues in muscles of fish samples collected from Behera Governorate were propiconazole 0.00644 ppm fludioxonil 0.0001986 ppm dimethomorph UND and samples collected from samples collected from Alexandria propiconazole 0.00102 ppm fludioxonil UND, dimethomorph 0.000508 ppm dimethomorph is less than the ADI in gills and fish muscles according to FAO.

Table (4) : Pesticide residues detected in *Oreochromis niloticus* fish gills and muscles samples collected from three different Governorates during June 2018 .

Name of pesticides	Gills µg/g (ppm)			Muscles µg/g (ppm)			
	Gharbia	Behera	Alex.	Gharbia	Behera	Alexandria	ADI mg/kg
Propiconazole	UND	0.00323	0.00558	UND	0.00644	0.00102	0.07
Fludioxonil	0.001246	0.00052	UND	UND	0.0001986	UND	0.01
Dimethomorph	0.000404	UND	0.00803	UND	UND	0.000508	0.008

UND: Undetectable
ADI : Acceptable daily intake

4. Residue of pesticides in fish muscles and gills from three different Governorates June in 2018:

Data in Table (5) indicate the presence residues of pesticides in fish muscles samples from three different Governorates November in 2018 .The average of the detected pesticide residues in these Governorates were as follows: gills of fish samples collected from Gharbia Governorate contained propiconazole 0.00303 ppm, fludioxonil 0.009042 ppm, dimethomorph UND. The pesticide residues in gills of fish samples collected from Behera propiconazole UND fludioxonil UND , dimethomorph UND and the pesticide residues in gills of fish samples collected from Alexandria propiconazole 0.0002191 ppm , fludoxanil 0.05632ppm , dimethomorph UND less than the ADI in gills and fish muscles According to FAO . The average of the detected pesticide residues in these Governorates were as follows: muscles of fish samples collected from Gharbia Governorate contained propiconazole 0.0023090 ppm fludioxonil 0. 001512 ppm dimethomorph UND. The muscles of fish samples collected from Behera and Alexandria Governorates propiconazole fludioxonil and dimethomorph UND .

Table (5) : Pesticide residues detected in *Oreochromis niloticus* fish gills and muscles samples collected from three different Governorates during November 2018 .

Name of pesticides	Gills µg/g (ppm)			Muscles µg/g (ppm)			
	Gharbia	Behera	Alexandria	Gharbia	Behera	Alexandria	ADI mg/kg
Propiconazole	0.00303	UND	0.0002191	0.002309	UND	UND	0.07
Fludioxonil	0.009042	UND	0.05632	0.001512	UND	UND	0.01
Dimethomorph	UND	UND	UND	UND	UND	UND	0.008

UND Undetectable

5. Residue of pesticides in fish muscles and gills from three different Governorates December in 2018:

Data in Table (6) indicate the presence residues of pesticides in fish muscles samples from three different Governorates November in 2018. The average of the detected pesticide residues in these Governorates were as follows: gills of fish samples collected from Gharbia Governorate contained propiconazole 0.012108 ppm, fludioxonil 0.0016594 ppm , dimethomorph UND**.** The pesticide residues in muscles of fish samples collected from Behera propiconazole UND fludioxonil 0.003131 ppm, dimethomorph UND and Alexandria propiconazole 0.0002815 ppm fludioxonil 0.0036963 ppm fludioxonil 0.0036963 ppm dimethomorph UND less than AID according to FAO . The muscles of fish samples collected from Gharbia , Behera and Alexandria Governorates propiconazole UND dimethomorph UND, but fludioxonil 0.000194 , 0.001933 and 0.000694 ppm, respectively.

Table (6) : Pesticide residues detected in *Oreochromis niloticus* fish gills and muscles samples collected from three different Governorates during December 2018 .

Name of pesticides	Gills µg/g (ppm)			Muscles µg/g (ppm)			
	Gharbia	Behera	Alexandria	Gharbia	Behera	Alexandria	ADI mg/kg
Propiconazole	0.012108	UND	0.0002815	UND	UND	UND	0.07
Fludioxonil	0.0016594	0.003131	0.0036963	0.000194	0.001933	0.000694	0.01
Dimethomorph	UND	UND	UND	UND	UND	UND	0.08

UND Undetectable

References

Bulbul , E.N. and Ozhan, G. (2012): cytotoxic effect of triazole fungicides. Journal Fac . Pharm. Istanbul, 42(1): 23- 31.

FAO (2010) : Manual on development and use of FAO and WHO Specifications for pesticides. November 2010 2nd rev . of the st Edd. Available only on the internet . prepared by the FAO/WHO. Joint metting on pesticides specification (JMPS) .

FAO (2018): Specifications and evaluations for Agriculture.

Fairbrother, A.; Wenstel, R.; Sappington, K. and Wood, W. (2007): Framework for Metals Risk Assessment. Ecotoxicology and Environmental Safety, 68 (2): 145-227. pesticides Propiconazole. p 1-29.

Fatin , S.; Hassanen , K. E.A ; Gaafar–Rehab, A.M. and Shheen , A.A. (2016): Effect of grilling on pesticides residues in O, Niloticsles Muscles . Benha Veterinary Medical Journal , 30 (1): 348- 357.

Gillom, R.J.; Barbash, J.E.; Crawford, C.G.; Hamilton, P.A.; Martin, J.D.; Nakagaki, N.; Nowell, L.H.; Scot, J.C.; Stackleberg, P.E.; Thelin, G.P. and Wolock, D.M. (2006): The quality of our nation's waters – pesticides in the nation's streams and groundwater, 1992 – 2001. U.S Geological Survey Circular 1291. United States Geological Survey,

Harmsen, J. (2007): Measuring bioavailability: from a scientific approach to standard methods. Journal of Environmental Quality, 36:1420 – 1428.

Hen, J.S. and Tark, Y.S. (2003): Laboratory studies formation of bound residues and degradation of propiconazole in soils. Pest Management Science, 59(3): 324-330.

Iqbal, M. F.; Maqbool, U.; Perveez, I.; Farooq, M. and Asi, M. R. (2009): Monitoring of insecticides residues in brinjal collected from market of Noshera Virkan, Pakistan. The Journal of Animal and Plant Sciences, 19(2): 90-93.

Muir, D.C. ; Grift , N.P. and Solomon , J. (1981) : extraction and cleanup of fish sediment , and water for determination of triaryl phosphates by gas liquid chromatography. J. Asoc. off Anal. Chem., 64 (1): 79- 84 .

Naser, I.N. ; Ahmed, N.S. and Al-Maz, M.M . (2003): Effect of boiling and some environmental factor on residues behavior of penconazole Fungicide vine leaves, Annals Agri. Sci. Ain Shams Univ., 48 (1): 365-372 .