Preliminary Study of Profenofos and Difenoconazole Pesticide Residue in Soil and Citrus Fruits from Citrus Farming in Serai Village Kintamani Bangli

I Wayan Muderawan*, Luh Mitha Priyanka, I Nengah Suparta*****
* Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Ganesha, Bali 81117
** Department of Physical Sciences and Natural Sciences Education, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Ganesha, Bali 81117
*** Department of Mathematics, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Ganesha, Bali 81117

Article Info

Article history:

Received Apr 30th, 2022
Revised Jun 9th, 2022
Accepted Jun 30th, 2022

Corresponding Author:

I Wayan Muderawan,
Department of Chemistry
Universitas Pendidikan Ganesha

Email:
wayan.muderawan@undiksha.ac.id

ABSTRACT

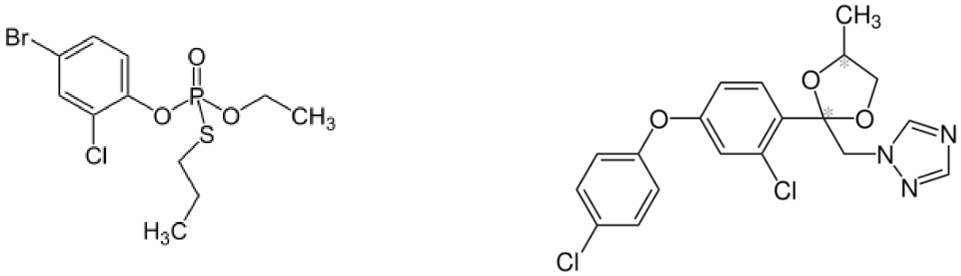
The intensively use of both profenofos and difenoconazole pesticides in Citrus farming in Serai Village Kintamani Bangli may remain as residue in the soil and citrus fruits. This study was performed in order to determine the level of pesticide residues in the soil and the citrus fruits from Citrus farming in Bangli. The pesticide residue levels in the soil and citrus fruit were performed by using solid-liquid extraction (SLE) and detected by high performance liquid chromatography (HPLC). The result shows that the pesticide residue levels in soil, citrus peel and citrus flesh are 0.057±0.002, 0.112±0.013 and 0 mg/L for profenofos and 0.069±0.002, 0.180±0.013 and 0 mg/L for difenoconazole. The results indicated that the soil and citrus peel from citrus farming area in Serai Village Bangli Regency contained both pesticide residues but not the citrus flesh.

Keyword: analysis, profenofos, difenoconazole, pesticide residue, soil, citrus fruit

1. INTRODUCTION

Pesticides or also known as plant protection products are substances or mixtures of substances which are extensively used in agriculture productions and public health programs, to reduce pests and vector borne diseases (Aktar et al. 2009, Dasika et al. 2012). The main reason of the use of pesticides in agriculture is to increase the yield, improve the quality, extend the storage life of food crops and help to secure food supplies. However, the use of plant protection products can leave pesticide residues in soil and agricultural products and then raises serious health and environmental problems (Nicolopoulou-Stamati et al. 2016).

Citrus belonging to family Rutaceae is one of the most popular fruit commodities because of its refreshing flavor and nutritional values. The fruit has a sweet taste and contain lots of vitamin C. Citrus production continues to increase from year to year. Citrus production in Bali was 225,584, 349,775, 490,393 tons in 2018, 2019, and 2020 (BPPS Provinsi Bali, 2021). The main citrus production and plantation in Bali is Gianyar and Bangli Regencies. The type of citrus widely grown in this area is *Citrus nobilis*. Like other plants, citrus plants are also attacked by various types of pests. Farmers usually use types of systemic pesticides and contact poisons as well as fungicides that are protective, curative and eradicative. Citrus farmers in Kintamani Bangli usually use pesticides with a frequency of twice a month or every 15 days since the citrus plant is one year old. This is very likely to cause the active ingredients in the pesticide to seep into the parts of the citrus plant and settle in the soil. Based on our observation and interview with the famer, they usually used a combination of Curacron® and Newcore pesticides to control pest in the citrus farming. Curacron® is one of the organophosphate pesticide products which has an active ingredient profenofos. Profenofos is included in the category of gastric contact poison and has a broad spectrum which is quickly able to control attacks of various pests (Alen et al. 2015). Meanwhile, Newcore with the active compound difenoconazole is a systemic fungicide that shows broad-spectrum bioefficacy against various vegetable and ornamental plant diseases (Allen et al. 1997). Figure 1 shows molecular structure of profenofos and difenoconazole.



1 **2**

Figure 1. Molecular structure of profenofos (**1**) and difenoconazole (**2**) pesticides.

Profenofos [*O*-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate] is an organophosphate pesticide that was developed for pest strains resistant to chlorpyrifos and other organophosphates (Gotoh et al. 2001). It is a liquid with a pale yellow to amber color and a garlic-like odor. It has been classified as a moderately hazardous (Toxicity Class II) pesticide by the World Health Organization (WHO) and it has a moderate level of acute toxicity following oral and dermal administration (Malghani et al. 2009, Abass et al. 2007). Based on a study of patients poisoned with profenofos and its close chemical relative prothiofos, the compound has been described as "of moderately severe toxicity", causing respiratory failure (Eddleston et al. 2009). In one study of a patient who died of profenofos poisoning, the major metabolites of profenofos were identified as des-*S*-propylated profenofos, two isomers of despropylated propenofos, and desethylated propenofos (Gotoh et al. 2001).

Difenoconazole [1-((2-(2-chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1,2,4-triazole is a broad spectrum fungicide belonging to triazole class pesticides, widely used for disease control in many fruits, vegetables, cereals and various other grain crops (Munkvold et al. 2001, Mauryaa et al. 2019). It acts by inhibition of demethylation during ergosterol synthesis which results in blocking of fungal cell wall biosynthesis (de Figueirêdo et al. 2019, Hamada et al. 2011, Mu et al. 2015). Because of its preventive and curative action, it is used to control various crop diseases caused by fungi (Munkvold et al. 2001, Wang and Zang, 2012). Triazole group pesticides have high photochemical stability and low biodegradability, which makes them persistent in soil and water (Mu et al. 2015).

The use of both pesticides in citrus farming in Bangli may remain as residue in the soil and the citrus fruits which becomes a concern for human exposure. Therefore, it is necessary to perform study in order to determine the level of pesticide residues in the soil and the citrus fruits from citrus farming in Bangli.

2. RESEARCH METHOD

2.1 Chemicals and Reagents

Acetone, dichloromethane, methanol (HPLC grade) and anhydrous sodium sulphate were obtained from Merck. The HPLC grade-water was obtained by purification of deionized water through a Milli-Q system. Profenofos and difenoconazole as standard were isolated from Curacron® and Newcore pesticides purchased from farm shop.

2.2 Sampling location

The soil and citrus fruits were directly taken from Citrus farming in Serai Village Kintamani Bangli Regency, Bali Province on August 2021. The citrus fruits were collected from three different *Citrus nobilis* trees and the soils were collected from around (0, 0.5, 1.0 and 1.5 m) the citrus trees.

2.3 Standard solution and sample preparation

Two different pesticides to be were investigated and structures are given in Figure 1, as follows: profenofos and defenoconazole. Stock solutions were prepared containing 100mg/L of each compound investigated. Working standards were prepared by serial dilution. Final concentrations (in methanol) ranged to 0, 1.0, 2.0, 3.0, and 4.0 mg/L for each analyte.

Soil sample (100 g) was macerated using acetone (100 ml) for 24 hours. The acetone layer was separated using filtration and concentrated using a rotary vacuum evaporator until around 25 mL of solution. The concentrated aqueous acetone was then extracted using dichloromethane (3x25 mL). The combined dichloromethane extracts were dried over anhydrous sodium sulfate and filtered off. After that, the filtrate was concentrated in a rotary vacuum evaporator until dryness and then 10 ml of methanol was added. The extract was transferred to a sample vial and kept before further analysis.

Fresh *Citus nobilis* peel and flesh (100 g each) were macerated using dichloromethane (100 mL) respectively for 24 hours. The dichloromethane layer was separated using filtration and dried over anhydrous sodium sulfate. After filtration, the dichloromethane filtrate was concentrated using a rotary vacuum evaporator until dryness and then 10 mL of methanol was added. The extract was transferred to a sample vial and kept before further analysis.

2.4 High Performance Liquid Chromatography Analysis

The HPLC analysis was performed on a Shimadzu HPLC system consisting of a degasser (DGU-20A5), a pump (LC-20AD, UFLC), a sample injector, a column oven (CTO-20A), and diode array UV/VIS detector (SPD-20A). Shimadzu LC Solution Software was used for instrument control and the chromatographic data acquisition. For chromatographic separation, VP-ODS column (150L × 4.6 mm ID, 4.6±0.3 μm) was used. The column temperature was set at 40°C. A system consisted of mobile phase A (deionized water, 30%) and mobile phase B (methanol, 70%). The flow rate of mobile phase was 1.0 ml/min, and sample volume injected was 2.0 μL. The DAD detector recorded UV spectra in 254 nm for peak characterization.

2.5 Calculation of Pesticide Content

The pesticide content in sample is calculated using following equation:

$$C_{cal} = \frac{C_{obc} x V x f}{w_s}$$

where, C_{cal} = pesticide residue calculated (ppm), C_{obc} = concentration obtained from calibration curve (ppm), V = final volume of sample solution (ml), f = dilution factor (f = 1, without dilution), and W_s = weight of sample (g).

2.6 Statistical analysis

All the experiments were performed in triplicates, and the results were expressed as the mean ± standard error (SE). Linear regression analyses were performed with Microsoft Excel 2010 to evaluate the relationship between the concentration of pesticide and the peak area.

3. RESULTS AND ANALYSIS

Profenofos is an organophosphate insecticide. It is a liquid with a pale yellow to amber color and a garlic-like odor. The compound has a specific gravity 1.455 g/ml, solubility of 28 mg/L at 25°C and miscible in most organic solvents (Pub Chem, 2021). While, difenoconazole is a member of the class of dioxolanes that is 1,3-dioxolane substituted at position 2 by 2-chloro-4-(4-chlorophenoxy)phenyl and 1,2,4-triazol-1-ylmethyl groups. The compound is a gray white solid which has a melting point of 78.6 ^{o}C and density of 1.37 g/ml. The solubility of difenoconazole is 15 mg/L in water at 25^{o}C and very soluble (>500 g/L) in most organic solvents (Pub Chem, 2021).

Because both compounds have very low solubility in water, in this study the extraction of these two types of pesticides from soil, citrus peel and citrus fruit flesh was carried out using solid-liquid extraction (SLE) with acetone and dichloromethane as solvents. The results of the qualitative analysis using High Performance Liquid Chromatography (HPLC) showed that profenofos and difenoconazole eluted well using water: methanol (30: 70 %V/V) as eluent at retention times of 5.83 and 6.60 minutes, Figure 2.

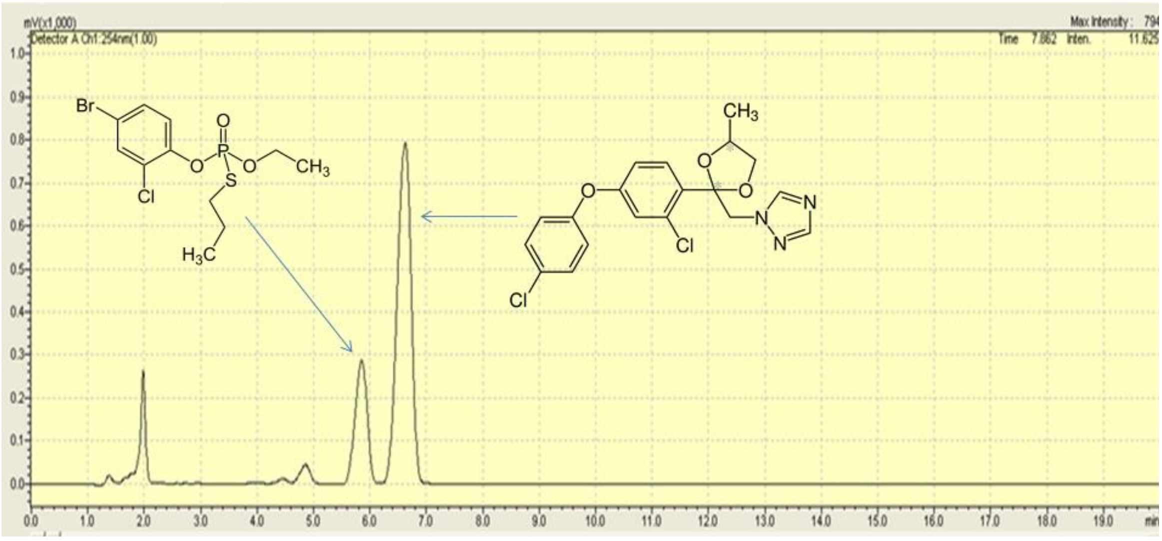


Figure 2. The chromatogram of profenofos and difenoconazole pesticides.

Quantitative analysis of pesticide residues in citrus fruit and soil samples was determined using HPLC. The measurement results of the standard solution in Figure 1 show a linear curve where the relationship between concentration and area is directly proportional to the concentration range of the standard pesticide solution. The correlation coefficient value obtained is 0.999, close to one and in accordance with the terms of acceptance which means that the performance of the method used for the measured concentration range is very good. Average pesticide residue contents in citrus peel were found 0.112±0.013 ppm and 0.180±0.013 ppm for profenofos and difenoconazole, respectively Table 1. However, these pesticides were not detected in citrus flesh. Duniaji and Puspawati also reported that profenofos pesticide was not detected in orange from Badung Regency, but there was no report for difenoconzole (Duniaji and Puspawati, 2016). While, Sumiati and Julianto reported that the profenofos residue in sweet citrus from farmer in Malang was 0.108 ppm (Supiyati and Julianto, 2018).

Pesticide residue analysis is tremendously an important process in determining the safety of using certain pesticides. Residue analysis provides a measure of the nature and level of any chemical contamination within the environment and of its persistence. The levels of pesticides residues are controlled by Maximum Residue Levels (MRLs), which are established by each country. In Indonesia are established by Agriculture Department through the Program for analysis of pesticide residues in food, started in 2011, which monitors the levels of pesticides in fruits, vegetables, and grains consumed by Indonesian. Since not all the Agriculture Department's data for these insecticide residues were available, it was compared with the MRLs established by the European Union (EU) and the Japan Food Chemical Research Foundation (JFCRF).

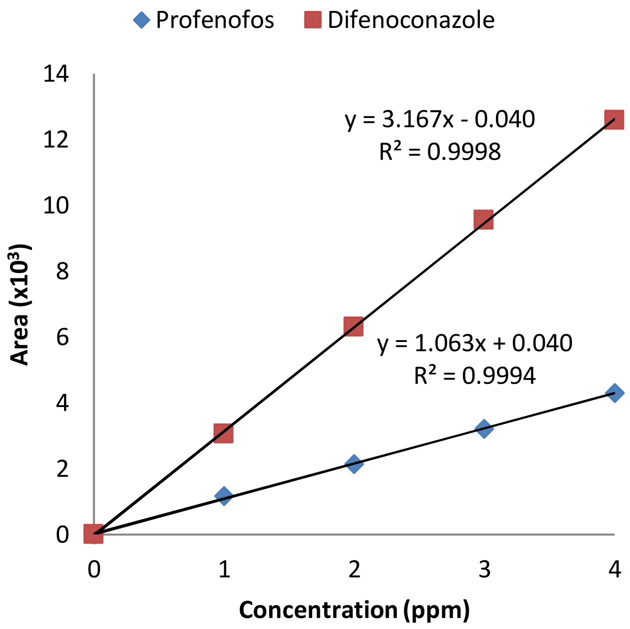


Figure 3. Calibration curve for standard sample.

Table 1. Pesticide residue contents in citrus fruits

Sample	Pesticide contents (ppm)			
	Peel		Flesh	
	P	D	P	D
1.1	0.095	0.157	ND	ND
1.2	0.097	0.159	ND	ND
1.3	0.097	0.192	ND	ND
2.1	0.123	0.187	ND	ND
2.2	0.126	0.188	ND	ND
2.3	0.129	0.188	ND	ND
3.1	0.114	0.181	ND	ND
3.2	0.117	0.185	ND	ND
3.3	0.109	0.181	ND	ND
Mean	0.112	0.180	-	-
SD	0.013	0.013	-	-

P: Profenofos, D: Difenoconazole, ND: not detected

Table 2. Pesticide residue contents in soil samples.

Sample	Pesticide Content (ppm)							
	0 m		0.5 m		1.0 m		1.5 m	
	P	D	P	D	P	D	P	D
1.1	0.055	0.067	0.059	0.070	0.055	0.067	0.054	0.066
1.2	0.055	0.067	0.059	0.070	0.055	0.067	0.054	0.066
1.3	0.055	0.067	0.059	0.070	0.055	0.067	0.054	0.066
2.1	0.054	0.070	0.064	0.072	0.060	0.069	0.059	0.067
2.2	0.054	0.070	0.063	0.072	0.060	0.069	0.059	0.067
2.3	0.054	0.070	0.064	0.072	0.060	0.069	0.059	0.067
3.1	0.055	0.068	0.060	0.069	0.055	0.069	0.054	0.067
3.2	0.055	0.068	0.060	0.069	0.055	0.069	0.054	0.067
3.3	0.055	0.068	0.060	0.069	0.055	0.069	0.054	0.067
Mean	0.055	0.068	0.061	0.071	0.057	0.068	0.056	0.067
SD	0.001	0.001	0.002	0.001	0.002	0.001	0.002	0.001

P : Profenofos, D : Difenoconazole
Profenofos content = 0.057 ± 0.002 ppm; Difenoconazole content = 0.069 ± 0.001 ppm

In European Union, the MRL for profenofos is set at 0.05 mg/kg for citrus and MRL for difenoconazole is set at 0.05 mg/kg (European Union, 2021, Hingmire et al. 2015). The toxicological profile of difenoconazole was assessed in the framework of the EU pesticides peer review under Directive 91/414/EEC where an acceptable daily intake (ADI) value of 0.01 mg/kg body weight (bw) per day and an acute reference dose (ARfD) of 0.16 mg/kg bw was derived (Brancato et al. 2018). The profenofos residue is over than the Maximum Residue Limits (MRL) that established by The Japan Food Chemical Research Foundation (0.05 ppm for profenofos) and lower than MRL of 0.6 for difenoconazole even though Indonesian Government and World Health Organization (WHO) have not established Maximum Residue Limits (MRL) profenofos and difenoconazole on types of citrus (JFCRF, 2021).

The presence of pesticides in soil samples was confirmed by comparing the standard chromatogram with the sample chromatogram, and the content was calculated based on the calibration curve. According to the results obtained, Table 2, pesticide residues were detected in samples of soil taken around (0 – 1.5 m) citrus three area. The pesticide residue contents in soil samples are 0.057 ± 0.002 ppm for profenofos and 0.069 ± 0.001 ppm for difenoconazole. Residues of difenoconazole in soil origin were not assessed. European Food Safety Authority (EFSA, 2021) has identified that difenoconazole is very potent to aquatic organisms and as of 2015 it was not approved in the European Union.

4. CONCLUSION

The use of pesticides has greatly enhanced agricultural production. But this is only one side of the coin; the other side showed that the presence of these pesticides has resulted in serious threats to human health and the environment. This preliminary study reveals the presence of pesticides in citrus fruit and soil taken from citrus farming in Serai Village Kintamani Bangli Regency. The contents of pesticide residues in citrus peels from the citrus farming are 0.112 ppm for profenofos and 0.180 ppm for difenoconazole. In addition, the pesticiede residue contents of soil samples from the citrus farming location are 0.057 and 0.069 ppm for profenofos and difenoconazole, respectively. In Bali, some legal measures should be taken in order to monitor the pesticide residues in fruits and vegetables because of potential health risks and to accomplish the standards and to make the products safely consumable by the consumers. It is also recommended that in future studies the health risk for human can be calculated.

ACKNOWLEDGEMENTS

We would like to thank EFForTS ABS Fund Project for financial support and our partner, Götingen University, Germany.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

REFERENCES

Abass, K., Reponen, P., Jalonen, J., Pelkonen, O. (2007). *In vitro metabolism and interaction of profenofos by human, mouse and rat liver preparations. Pestic. Biochem. Physiol., 87, 238–247.*

Aktar, W., Sengupta, D., Chowdhury, A. (2009). *Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol., 2(1), 1–12.*

Alen, Y., Zulhidayati, Suharti, N. (2015). *Pemeriksaan Residu Pestisida Profenofos pada Selada (Lactuca sativa L.) dengan Metode Kromatografi Gas, Jurnal Sains Farmasi & Klinis, 1(2),140-149.*

BPPS Provinsi Bali. (2021). *Produksi Buah Jeruk Provinsi Bali 2018-2020, Statistik Pertanian Hortikultura SPH-BST, BPS Provinsi Bali. https://bali.bps.go.id/indicator/55/200/1/produksi-buah-jeruk-provinsi-bali-menurut-kabupaten-kota.html. Retrived 19 December 2021.*

Brancato, A., Brocca, D., de Lentdecker, C., Erdos, Z., Ferreira, L., Greco, L., Janossy, J., Jarrah, S., Kardassi, D., Leuschner, R., Lythgo, C., Medina, P., Miron, I., Molnar, T., Nougadere, A., Pedersen, R., Reich, H., Sacchi. A., Santos, M., Stanek, A., … (2018). *Reasoned Opinion on the modificationof the existing maximum residue levels for difenoconazole in various crops. EFSA Journal, 16(1): 5143, 28 pp. doi: 10.2903/j.efsa.2018.5143.*

Dasika, R., Tangirala, S., Naishadham, P. (2012). *Pesticide residue analysis of fruits and vegetables, J. Environ. Chem. Ecotox., 4(2), 19-28. DOI: 10.5897/JECE11.072.*

Duniaji, A.S., Puspawati, N.N. (2016). *Contamination of Pesticides Residues of Fruits in Badung Regency. Research Report, Department of Food Science and Technology, Faculty of Agricutural Technology, Udayana University.*

de Figueirêdo, L.P., Daam, M.A., Mainardi, G., Mariën, J., Espíndola, E.L., van Gestel, C.A., Roelofs, D. (2019). *The use of gene expression to unravel the single and mixture toxicity of abamectin and difenoconazole on survival and reproduction of the springtail Folsomia candida. Environ. Poll., 244, 342–350.*

Eddleston, M., Worek, F., Eyer, P., Thiermann, H., Von Meyer, L., Jeganathan, K., Sheriff, M.H., Dawson, A.H; Buckley, N.A. (2009). *Poisoning with the S-Alkyl organophosphorus insecticides profenofos and prothiofos*. *QJM.* ***102*** *(11): 785–92.* doi:10.1093/qjmed/hcp119

Ellen, T., Ottw, J. C. G., & Gero, B. (1997). *Degradation of the fungicide difenoconazole in a silt loam soil as affected by pre-treatment and organic amendment. Environ. Poll., 96, 409–414.*

European Food Safety Authority (EFSA). (2021). *https://www.efsa.europa.eu/en. Retrieved 20 December 2021.*

European Union. Maximum Residue Levels (MRLs). (2021). *(http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=commodity.resultat. Retrieved 20 December 2021.*

Gotoh, M., Sakata, M., Endo, T., Hayashi, H., Seno, H., Suzuki, O. (2001). Profenofos metabolites in human poisoning. *Forensic Sci. Int*., *116, 221–226.*

Hamada, M.S., Yin, Y., Ma, Z. (2011). *Sensitivity to iprodione, difenoconazole and fludioxonil of Rhizoctonia cerealis isolates collected from wheat in China. Crop Protect., 30 (8), 1028–1033.*

Hingmire, S., Oulkar, D.P., Utture, S.C., Ahammed Shabeer, T.P., Banerjee, K. (2015). *Residue analysis of fipronil and difenoconazole in okra by liquid chromatography tandem mass spectrometry and their food safety evaluation, Food Chem., 176, 145–151.*

Malghani, S., Chatterjee, N., Yu, H.X., Luo, Z.J. (2009). *Isolation and identification of profenofos degrading bacteria. Braz. J. Microbiol., 40, 893–900.*

Mauryaa, R., Dubeya, K., Singha, D., Jaina, A.K., Pandey, A.K. (2019). *Effect of difenoconazole fungicide on physiological responses and ultrastructural modifications in model organism Tetrahymena pyriformis, Ecotox. Environ. Safety, 2019, 182, 109375.*

Mu, X., Wang, K., Chai, T., Zhu, L., Yang, Y., Zhang, J., Li, X. (2015). *Sex specific response in cholesterol level in zebrafish (Danio rerio) after long-term exposure of difenoconazole. Environ. Poll., 197, 278–286.*

Munkvold, G.P., Martinson, C.A., Shriver, J.M., Dixon, P.M. (2001). *Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. Phytopathology, 91 (5), 477–484.*

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., Hens, L. (2016). *Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front. Public Health, 4:148. doi: 10.3389/fpubh.2016.00148.*

Pub Chem. Difenoconazole. (2021). *https://pubchem.ncbi.nlm.nih.gov/compound/Difenocona-zole. Retrieved 19 December 2021.*

Pub Chem. Profenofos. (2021). *https://pubchem.ncbi.nlm.nih.gov/compound/Profenofos. Retrieved 19 December 2021.*

Sumiati, A., Julianto, R.P.D. (2018). *Analisis Residu Pestisida Wilayah Malang dan Penanggulangannya Untuk Keamanan Pangan Buah Jeruk, Buana Sains, 18(2), 125-130.*

The Japan Food Chemical Research Foundation (JFCRF). (2021). *Maximum Residue Limits (MRLs) List of Agricultural Chemicals in Foods, https://db.ffcr.or.jp/front/. Retrieved 20 December 2021.*

Wang, X., Zhang, R., (2012). *Control efficiency and increase yield of two difenoconazole and propiconazole 30% EC to rice false smut. Agric. Technol. Service, 29, 300–304.*