Research article

Drug Testing and Analysis

Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1351

Determination of hexaconazole in field samples of an oil palm plantation

Halimah Muhamad,[a]* Maznah Zainol,[b] Ismail Sahid[b] and Idris Abu Seman[a]

In oil palm plantations, the fungicide hexaconazole is used to control Ganoderma infection that threatens to destroy or compromisethe palm. The application of hexaconazole is usually through soil drenching, trunk injection, or a combination of these two methods. It is therefore important to have a method to determine the residual amount of hexaconazole in the field such as in samples of water, soil, and leaf to monitor the use and fate of the fungicide in oil palm plantations. This study on the behaviour of hexaconazole in oil palm agro-environment was carried out at the UKM-MPOB Research Station, Bangi Lama, Selangor. Three experimental plots in this estate with 7-year-old Dura x Pisifera (DxP) palms were selected for the field trial. One plot was sprayed with hexaconazole at the manufacturer's recommended dosage, one at double the recommended dosage, and the third plot was untreated control. Hexaconazole residues in the soil, leaf, and water were determined before and after fungicide treatment. Soil samples were randomly collected from three locations at different depths (0–50 cm) and soil collected fromthe same depth were bulked together. Soil, water, and palm leaf were collected at −1 (day before treatment), 0 (day of treatment), 1, 3, 7, 14, 21, 70, 90, and 120 days after treatment. Hexaconazole was detected in soil and oil palm leaf, but was not detected in water from the nearby stream. © 2012 John Wiley & Sons, Ltd.

Keywords: hexaconazole; water; soil and leaf

Introduction

Malaysia has become one of the most important sources of oils and fats in the world. In 2010, the export of crude palm oil (CPO) in Malaysia and Indonesia was 17.0 and 16.7 million tons, respectively. Therefore, Malaysia is the largest exporter of CPO in the world, followed by Indonesia.[1] Besides the development and quality control of palm oil and its products, pest and disease management are essential in ensuring the continued growth and expansion of the industry. Although integrated pest management strategies are being promoted and implemented in Malaysia, pesticides remain essential tools in pest management of oil palm plantations.

CPO is obtained from the mesocarp of the oil palm fruit, while crude palm kernel oil (CPKO) is derived from the kernel of the fruit. Palm oil possesses highly beneficial, nutritional, and culinary properties due to its unique composition of fatty acids and antioxidants.[2] CPO consists mainly of palmitic acid (45%), followed by oleic acid (40%), linoleic acid (10%), and stearic acid (5%), while CPKO contains mainly lauric acid (48%).[2] The benefits of palm oil for health, food production, and cosmetics have created a demand for this product worldwide. Therefore, palm oil is one of the 17 edible oils that has been accepted as meeting the requirements of the FAO/WHO food standards under the CODEX Alimentarius Commission Programme.[3]

The major diseases of oil palm include basal steam rot (BSR), vascular wilt, and bud and spear rot.[4] BSR rot caused by the fungus *Ganoderma*, is the most serious disease of oil palm in Malaysia. Latiffah and Ho recorded that disease incidence in Batu Anam, Johore, Gemenceh, Negeri Sembilan, and Malacca.[5] The disease also has been reported in Africa, Papua New Guinea, Indonesia, and Thailand.[6] The disease causes the death of more than 80% of oil palm plantings midway through their economic life.[7] Primary infection of palms by *Ganoderma* species has been considered to occur by contact of living palm roots with colonized debris within soil.[8] The best approach to control this disease consists of removal of infected palms, soil mounding, fungicide treatment, or a combination of these methods.[9] Chemical treatments are considered as the immediate short-term control measures. The use of systemic fungicides, together with a correct technique of application helps to reduce the progress of the BSR on the palm.

Fungicides that showed a good response against *Ganoderma*, were hexaconazole and bromoconazole.[8] These fungicides were classified under triazole group and found effective in prolonging the productive life of infected palms in comparison with four other fungicides such as thiram and benomyl, triadimefon, triadimenol, and tridemorph.[9] Previous field studies[9,10] evaluated the different methods of hexaconazole application of mature palms that include: soil drenching, trunk injection, pressure injection, or combination of the methods. It was reported that the application of hexaconazole with pressure-injection had limited the spread of *Ganoderma* infection within the infected standing palms.[6,8] The latest study[7] indicated that appropriate application of hexaconazole has the potential to reduce the risk of *Ganoderma* infection in healthy mature oil palm.

Hexaconazole is the common name for (RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol. It is available in a variety of formulations under the trade names: Proseed (Syngenta), Bullet 5 (Agro Chemicals), and Canvil (Vapco). Hexaconazole belongs

* *Correspondence to: Halimah Muhamad, Malaysian Palm Oil Board, No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. E-mail: halimah@mpob.gov.my*

a *Malaysian Palm Oil Board, Kajang, Selangor, Malaysia*

b *School of Environmental and Natural Resource Sciences, University Kebangsaan Malaysia, Selangor, Malaysia*

to the family of azole fungicides with a melting point range from 110 to 112 °C and molecular mass of 314.2. The structure of hexaconazole is shown in Figure 1.[14] It is a systemic fungicide with protective and curative properties used to treat and control *Ganoderma* in oil palm. The mode of action is through inhibition of ergosterol biosynthesis and thus, it is used for control of many fungi, especially ascomycetes and basidiocetes.

The application of pesticides on oil palm plantations is constantly used to safeguard the crop from deleterious insects and diseases. Because of their high biological activity, and in some cases of their persistence in the environment, the use of pesticides may cause undesired effects on human health and the environment. A number of studies have been conducted to determine the effects of pesticide used on oil palm plantations in Malaysia; studied pesticides include cypermethrin, deltamethrin, endosulfan, fluroxypyr-MHE and hexaconazole[11–13] but the use of hexaconazole on oil palm plantations has not been reported. Such information is needed to understand the fate of hexaconazole in oil palm plantation agro-environment. Therefore, the objective of this study is to investigate the presence and amount of residual hexaconazole in soil, water and leaf in oil palm agroecosystem.

Experimental site

The study was conducted at Research Station, Bangi Lama, Selangor owned by Malaysian Palm Oil Board (MPOB) and University Kebangsaan Malaysia (UKM). The oil palm trees used were 7-year-old Dura cross-breeding with Pisifera (DxP). The study was done at plot number 0.409 and covered 225 palm trees. The experiment was conducted by dividing into nine subplots. The nine subplots were subjected to three treatments, namely the manufacturer's recommended dosage, double the manufacturer's recommended dosage, and control (without pesticide treatment). Each treatment was replicated thrice.

Insecticide

Hexaconazole (Anvil®) used in this study contained 4.8% a.i. (active ingredient). It was applied in an aqueous solution to the palms by soil drenching. To prepare the recommended and double the recommended dosages, 90 and 180 ml of the chemical product were diluted in 10 l water, respectively. Ten litres of the diluted product were drenched around the palm base.

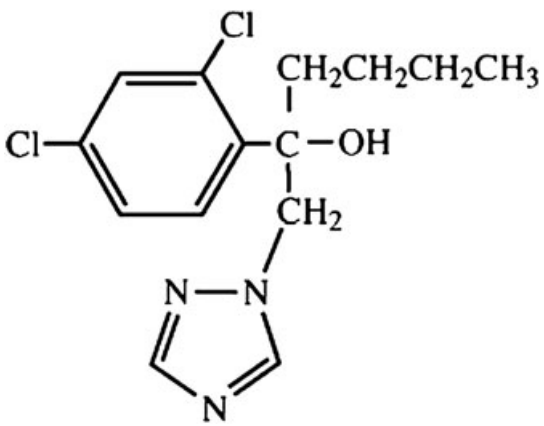

Figure 1. Structure of hexaconazole. [14]

Sampling

Soil samples were collected from each plot by using an auger set at different depths: 0–10, 10–20, 20-–30, 30–40, and 40–50 cm. One-litre water samples were collected from each plot from five points. Leaves were taken from the middle of plot from each seedling for analyses. Soils, were collected at day −1 (i.e. before treatment), 0 (6 h after spraying), 1, 3, 7, 21, 70, 90, and 120 days after treatment. Meanwhile, water and leaf were collected at day −1 (i.e. before treatment), 0 (6 h after spraying), 1, 3, 7, 14, 21, 30, and 70 days after treatment.

Methodology

Reagents and insecticide

All reagents and solvents used in this study were of analytical grade. Acetone, n-hexane, cyclohexane, methanol, dichloromethane, and ethyl acetate were obtained from Merck (LiChrosolv®). Standard hexaconazole (99.5% purity) was purchased from the Riedel-de Haen (Germany). Commercial solid-phase extraction (SPE) cartridges (AccuBond C_{18}, 200 mg/3 ml) were purchased from Agilent Technologies, (UK)). Sodium chloride and sodium sulfate was obtained from Merck (Darmstadt, Germany).

Standard solutions

Standard hexaconazole (0.01g) was dissolved in 50 ml acetone to make up a stock solution of 100 μg/ml. An intermediate stock solution of 50 μg/ml was prepared by dissolving 5 ml of the standard stock solution with 5 ml of acetone in a 10-ml volumetric flask. Working standard solutions containing 1.0 to 10 μg/L were prepared by appropriate dilution of the standard stock solution with acetone from the intermediate stock solution. All the standard solutions were stored at −20°C in glass bottles with Teflon-lined screw caps.

Apparatus

The instrument used was a gas chromatograph (GC) (HP 6890 series II) fitted with an auto-sampler injector and an electron capture detector (ECD). The sample preparation for leaf sample used Gel Permeation Chromatography (GPC) consisting of a Konik Tech pump with a 25 mm ID x 500 mm GPC column packed with Bio-Beads (SX-3, 200–400 mesh size) from LCTech GmbH. The evaporator, N-Evap Model 1111 used to evaporate the solvent was purchased from Organomation Associates Inc. (266, River Road West, Berlin MA 01503 USA). Vortex mixture Type 37600 was purchased from Thermolyne Co., New Hampshire, USA. Other instruments used were a solid phase extraction (SPE) manifold (Supelco), Sonicator (Bransonic, USA) and rotary vacuum evaporator (Heidolph, USA). Micro-pipette (100–1000 μL), vials (20 ml), volumetric flasks (10 ml) and round bottom flasks (100 ml and 50 ml) were used.

Conditions for GC-ECD

Sample extracts were analyzed using a GC (HP 5890 series II) with an electron capture detector (ECD) operated at 280 °C (Agilent Technologies, California, USA). The injection mode was splitless operated at 250 °C and the injection volume was 2.0 μL. A HP 5% MS column (30 m x 0.25 mm ID, 0.25 μm film thickness) was used to separate the analytes from matrix components. Nitrogen was used as carrier and makeup gas,

with flow rate of 1.3 and 1.5 ml/min, respectively. The initial temperature was 150 °C, with an initial hold time of 2 min. The oven was then heated to 220 °C at 15 °C/min and then held at that temperature for 2 min and then followed by a programmed increase of 5 °C/min to 250 °C and held at this final temperature for 2 min. Chemstation software was used for instrument control and data analysis.

Conditions for GC-MS

The GC-MS was used from Hewlett-Packard (Agilent Technologies, California, USA) Model 7890A Series fitted with 5975C Triple-Axis detector. An HP 5% MS (30m x 0.25 ID, 0.25 μm film thickness) column was used with nitrogen gas as carrier gas at flow rate of 1.5 ml/min. Splitless injection of 1.0 μL was carried out at 250 °C and solvent delay time 6.0 min. The following temperature program was applied: initial temperature 50 °C, held for 2 min, then a positive gradient of 15 °C/min was applied to 220 °C, held for 5 min and finally increasing at rate 15 °C/min to 280 °C and held for 15 min. The mass spectrometer was operated in electron impact ionization mode (ionization energy 70 eV), ion source and quad temperatures were set at 230 °C and 150 °C, respectively. Full scan chromatograms obtained in mass range 50–550 *m/z* were used.

Preparation of spiked soil samples

Standard laboratory methods[15] were used to determine the physico-chemical properties of the soil. Table 1 shows the physico-chemical properties of soil in the field trial. The soil was classified as sandy loam soil according to soil characteristic. Soil samples used for the spiking study were collected from an oil palm plantation at Research Station, Bangi Lama, Selangor owned by MPOB and UKM Bangi, Selangor, Malaysia. The samples were air dried and sieved through 2-mm mesh.

Preparation of control leaf samples

The leaf sample was taken from oil palm seedling in the control plot and cut to smaller sizes. The cut leaves were reduced to smaller sizes using the WARING blender and the ground leaves were stored at −20 °C prior to analysis.

Preparation of spiked water samples

One liter hexaconazole-free water was put into Schott bottles. Standard hexaconazole solutions, containing the dilutions from 0.05 to 0.8 μg/ml, were spiked into the 1-L Schott bottles. The final hexaconazole concentration in spiked water samples were 0.05, 0.1, 0.2, 0.5, 0.8 μg/L. The bottles were shaken for 5 min and then stirred for 10 min.

Determination of hexaconazole in soil

Twenty-five g of soil were added to a 250-ml conical flask. The soil was spiked with standard hexaconazole solution in acetone to obtain 2 to 80 μg/kg hexaconazole in soil, and the contents were mixed using a vortex mixer. One hundred ml of dichloromethane were then added to the conical flask and again mixed for 30 s on the vortex mixer. The conical flask was placed in an ultrasonic bath for 30 min. Fifty ml of the extract were then transferred into a 100-ml round bottomed flask using a 20-ml pipette. The solution was evaporated to about 5 ml using a rotary evaporator and transferred into a graduated micro-vial. The extract was further evaporated to 2 ml using an N- evaporator. The content was then mixed in a vortex mixer for 3 s and filtered with 0.45 μm prior to injection into the GC-ECD. A similar procedure was applied for the untreated and treated field trial samples before injecting into the GC/ECD.

Table 1. Physico-chemical properties of the soil.

Soil Type	Clay (%)	Sand (%)	Silt (%)	Total Carbon (%)	pH	CEC[a]
Sandy loam	27.29	62.62	10.09	0.86	5.28	6.55

[a]Capacity exchange cation (meq/100).

Determination of hexaconazole in leaf

Approximately 5 ± 0.002 g of ground leaf samples was placed in 250-ml conical flask. The ground leaf was spiked with hexaconazole standard solution at 1.0 to 5.0 μg/ml equivalent to 0.1 to 0.5 μg/g of hexaconazol in leaf. Forty-five ml of ethyl acetate, 45 ml of acetone and 10 ml water were added and the flask was shaken for 3 min. Three g of sodium chloride was added to the conical flask and placed in an ultrasonic bath for 30 min after which the solution was decanted into a second conical flask. Approximately 20 g of anhydrous sodium sulfate was added and left to stand for 20 min. Fifty ml of the solution was then transferred into a round bottomed flask and rotary evaporated to 3–5 ml. Then the extract was transferred to 25-ml volumetric flask and diluted with cyclohexane:ethyl acetate (1:1, *v:v*) to make up to 25 ml. Ten ml of each extract was injected in turn into the GPC Rheodyne injector (5-ml loop) using a syringe with PTFE filter. The eluting solvent was pumped through the column at a constant flow rate of 5.0 ml/min. The operating conditions were: dump cycle, 90 ml; collect cycle, 100 ml; wash cycle, 20 ml. The 100-ml eluate collected from GPC was concentrated by rotary-evaporator at 35 °C to about 5 ml and evaporated to dryness using an N- evaporator. The residue was redissolved with an accurately measured volume of 1.5 ml acetone and 1.0 μL was injected into the GC-ECD for quantification of hexaconazole. The determination of hexaconazole from leaf-spiked samples was carried out in triplicate.

Determination of hexaconazole in water

Method of extraction and concentration of hexaconazole in water by SPE

To determine hexaconazole in the spiked water samples, an SPE cartridge was attached to the manifold and pre-washed with 5 ml methanol and 5 ml distilled water. The washings were discarded. The water was spiked with hexaconazole standard solution into 1 L water samples (free of hexaconazole residue) in Schott bottles at 0.05 to 0.8 μg/ml equivalent to 0.05 to 0.8 μg/L of hexaconazole in water. The bottles were then stoppered and the contents mixed by shaking for a few seconds. The stopper was removed and the SPE tubing was immersed in the mixture and then the aspirator was switched on. With the aspirator on, the water was sucked through C_{18} cartridge. The flow of the water through the SPE cartridge was controlled by a pressure knob (20 mm Hg) so as to get the water to elute drop by drop. A preliminary study on the effect of loading the water sample through the SPE cartridge had shown that the suitable flow rate was 8–10 ml/s. After the water was completely drawn through the SPE cartridge, the bottle was rinsed with 10 ml water. The rinsing was also

passed through the SPE cartridge and the cartridge was then vacuumed dried. Hexaconazole absorbed in the cartridge was eluted with 15 ml methanol. The eluate was dried to 2–5 ml using a rotovap, transferred to a graduated micro-vial, and evaporated to dryness using an N- evaporator. The residue was then redissolved in 1.0 ml acetone and filtered using syringe filter (0.45 μm) prior to injection into GC-ECD. Quantification of the analyte was made by comparison with the hexaconazole standard solution curve.

Results and discussion

Figures 2 and 3 show the daily volume of rainfall and maximum and minimum air temperatures (°C) recorded at Research Station MPOB-UKM, Bangi Lama, Selangor during the study period. The monthly rainfall for May, June, July, August, and September 2009 was 115.1 mm, 78.90 mm, 66.20 mm, 237.70 mm, and 205.5 mm, respectively. The maximum air temperature for May, June, July, August, and September was 34.9 °C, 34.7 °C, 33.8 °C, 34.4 °C, and 34.0 °C, respectively, while the minimum air temperature was 22.1 °C, 23.1 °C, 22.3 °C, 21.5 °C, and 21.0 °C, respectively.

Figure 4 shows a calibration curve of standard hexaconazole against the GC peak area obtained using the ECD detector. The coefficient of determination (R^2) was 0.998, and the equation derived from the calibration curve was $y = 14417x + 9654$, where y is the area of hexaconazole and x is the concentration of hexaconazole in μg/L. Figure 5 shows a spectrum of hexaconazole from GC-MS analysis and 3 ion mass representing hexaconazole that is *m/z* 83, 214 and 174. The retention time of hexaconazole was 16.752 min.

The extraction efficiency of the methods was evaluated by conducting recovery studies. Samples of soil, palm leaf, and water were spiked with known concentrations of hexaconazole. Soil

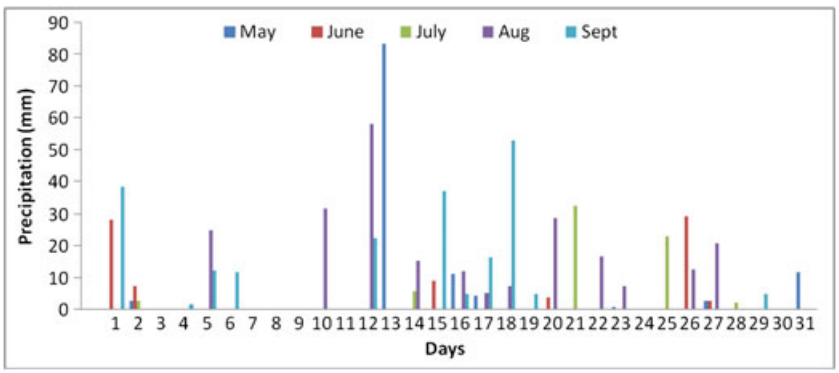

Figure 2. Daily rainfall from May to September 2009.

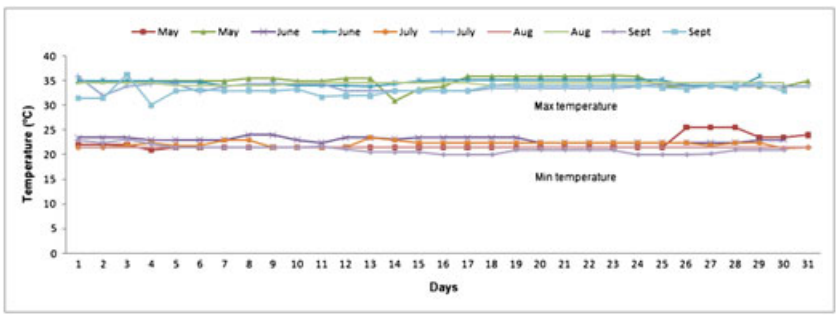

Figure 3. Variation of maximum and minimum air temperature from May to August 2009.

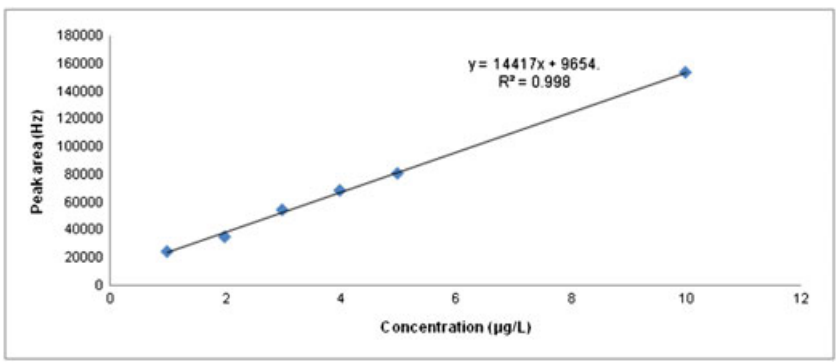

Figure 4. The calibration curve of standard hexaconazole by GC-ECD.

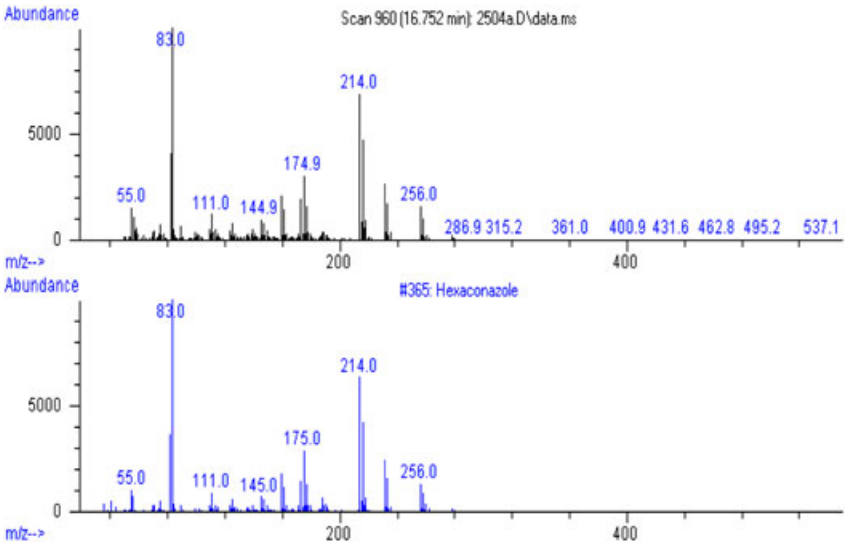

Figure 5. Spectrum of hexaconazole from GC-MS.

samples from the control plot were spiked with hexaconazole to provide concentrations from 0.01μg/g to 0.8 μg/g. Table 2 shows the recovery of hexaconazole from spiked soil ranging from 100% to 106% with relative standard deviations (RSDs) of 1.18% to 5.64%. The efficiency of the method for determination of hexaconazole in leaf was tested with various spiking concentrations and are presented in Table 3 with recoveries from 91.8% to 104% and RSDs from 0.73% to 8.11%. The recoveries and RSDs of hexaconazole spiked water samples were 95.2% to 106% and 2.85% to 6.03%, respectively, as shown in Table 4. Each spiked concentration of the hexaconazole was determined in triplicate.

Figure 6 shows the concentration of hexaconazole residue in soil. The hexaconazole residue was found at all depths (0–50 cm) at both application rates with decreasing concentrations of hexaconazole from day 0 to day 90. As expected, the amount of hexaconazole remaining in the soil at day 90 was higher in the double manufacturer's recommended dosage plot (1.659 mg/kg versus 0.776 mg/kg) than in the manufacturer's recommended dosage plot. At the manufacturer's recommended dosage, the amount of hexaconazole found on day 0 to day 90 ranged from 2.98 to 0.776 mg/kg at all soil depths. At double the manufacturer's recommended dosage the amount of hexaconazole deposited in the soil ranged from 7.12 to 1.66 mg/kg. On day 120 after treatment of hexaconazole, no residue was detected irrespective of the dosage rate. This demonstrates that hexaconazole, classified as a triazole fungicide, has a moderate persistence in soil. From this study the persistence of hexaconazole in soil could be due to low organic content in soil (0.86%). Persistence of hexaconazole in soils may be attributed to its higher sorption on soil particles due to the hydrophobic nature of the fungicide.[16,17] The fate of pesticides in the soil is also greatly influenced by their interaction with the soil environment. It is now well established that the persistence of most pesticides is influenced by the soil properties (e.g. organic matter content (OMC), CEC, soil moisture, content, and proportion of sand and clay[18,19]) and environmental factors, such as rainfall and temperature.[20] In laboratory studies by Singh, a lysimeter was used to study the mobility of hexaconazole in Indian soil.[17] The results indicated that penconazole, hexaconazole, and

Table 2. Recovery of hexaconazole from spiked soil samples.

Concentration (μg/g)	(%) Recovery	(%) RSD
0.8	100 ± 1.92	1.92
0.5	105 ± 3.74	3.56
0.2	100 ± 5.64	5.64
0.1	102 ± 1.20	1.18
0.01	106 ± 4.28	4.03

Table 3. Recovery of hexaconazole from spiked leaf samples.

Concentration (μg/g)	(%) Recovery	(%) RSD
0.1	97.6 ± 2.25	1.18
0.2	91.8 ± 5.50	5.99
0.3	97.5 ± 0.71	0.73
0.4	94.0 ± 7.62	8.11
0.5	104 ± 2.73	2.63

Table 4. Recovery of hexaconazole from spiked water samples.

Concentration (μg/L)	(%) Recovery	(%) RSD
0.8	96.4 ± 4.17	4.32
0.5	104 ± 6.29	6.03
0.2	95.2 ± 2.71	2.85
0.1	106 ± 3.16	2.99
0.05	102 ± 4.20	4.13

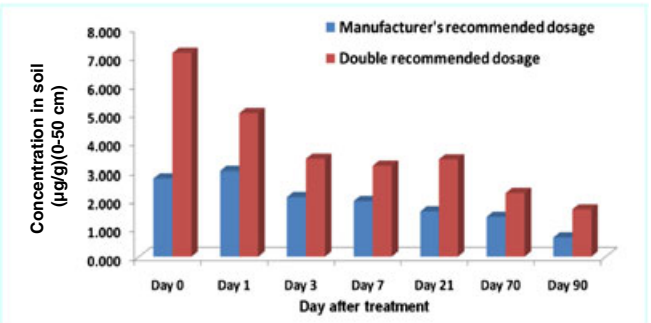

Figure 6. Concentration of hexaconazole residue in soil.

Table 5. Concentration of hexaconazole residue in leaf.

*DAT	dosage	Double manufacturer's recommended dosage
	Concentration (μg/g)	
0	0.128 ± 0.004	0.216 ± 0.005
1	0.097 ± 0.012	0.171 ± 0.007
3	0.052 ± 0.001	0.091 ± 0.004
7	0.059 ± 0.003	0.065 ± 0.003
14	0.044 ± 0.001	0.056 ± 0.006
21	0.028 ± 0.001	0.062 ± 0.002
30	ND	0.029 ± 0.003
70	ND	ND

* DAT = day after treatment.
ND = not detected.

propiconazole were moderately mobile in low OMC, sandy loams soils and leached down to lower soil profiles (0–15 cm depth). The observed mobility results in this study were different from those of the current study.[17] This could be due to the different experimental conditions; the current experiment was carried out under field (natural) conditions, while the previous study was conducted in the laboratory. Therefore, the current experiment should provide a more realistic assessment of compound mobility under natural condition. The differences also could be attributed to environmental and soil differences such as soil OMC, rainfall, temperature, pH, micro-organism, etc.[13,21–26]

Table 5 shows the amount of hexaconazole residue in leaf samples collected from both treatment plots. In the plot treated with the manufacturer's recommended dosage, the residue in leaf depleted from 0.128 μg/g (Day 0) to 0.028 μg/g (Day 21 post treatment). When the plot was treated with double the recommended dosage, 0.216 μg/g hexaconazole was detected on day 0 and 0.029 μg/g was still present on day 30 after treatment and after 70 days after treatment, no residue was found. Analysis of hexaconazole was also conducted on water samples taken from the nearby streams and drains in the plots during the study period. However, no hexaconazole residue was detected in the water samples during the experiment.

Conclusion

In conclusion, these results indicated that hexaconazole is moderately persistence in soil and detected in leaf samples after treatment. Hexaconazole was not detected in water samples. Repeated application may lead to accumulation, leaching, or affect the soil micro-organisms. Therefore, further study is required to determine its effect to the environment. Future study should also include spiked leaf samples over the range of hexaconazole analyzed concentrations in leaf samples from treated plots (0.028–0.128 or possibly higher if accumulation is predicted).

Acknowledgements

We thank the Director-General MPOB for permission to publish this article. We are extremely grateful to our technical staff of the Pesticides Laboratory, MPOB and MPOB-UKM Research Station for their invaluable technical assistance.

Conflicts of interest

The authors have no conflicts of interest to declare.

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