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HPLC-PDA Determination of Chlorpyrifos and Glyphosate Residues in Mango Orchard Soil and their Subsequent Uptake to Mango Fruit

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Chlorpyrifos and glyphosate are used to control mango mealybug and weeds respectively in mango ecosystem. Chlorpyrifos (2.5 and 5.0 mL L^{-1} of water) and glyphosate (8.0 and 16.0 mL L^{-1} of water) were applied to mango (cv. Dashehari) orchard soil to study their dissipation in soil and subsequent uptake in mango fruit using HPLC-PDA. Chlorpyrifos and glyphosate applied at standard and double doses, respectively dissipated from 5.05 and 8.30 mg kg^{-1}, and 3.37 and 6.12 mg kg^{-1} after 2 h of soil application to 0.75 and 1.07 mg kg^{-1}, and 0.61 and 1.04 mg kg^{-1} after 50 and 30 d of application. Residue of glyphosate was below detectable limit in soil beyond 30 d of application. The residual half-lives of chlorpyrifos and glyphosate in soil were 17-18 and 12-13 d from the standard and double doses, respectively. The rate of dissipation followed first-order exponential kinetics in soil for both the concentrations. Residues of either pesticide were below detectable limit in mature fruits at harvest. Hence, both the pesticides can be considered safe for their respective uses in mango ecosystem.

Key words: Chlorpyrifos, glyphosate, dissipation, soil, uptake, mango fruit

India ranks first in the world and Uttar Pradesh first among Indian states in mango production with Dashehari, Chausa and Langra being the most popular varieties. Dashehari is the choicest table purpose variety for its taste, aroma and flavour and grown mostly in Lucknow and Malihabad region of Uttar Pradesh. For controlling various insect-pests, Dashehari requires frequent application of different pesticides throughout its growth and development, either in soil or on tree. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-3-pyridyl phosphorothioate), a broad-spectrum organophosphate insecticide with contact mode of action, is used widely in mango ecosystem for the management of various insects mainly mealybug (*Drosicha mangiferae*). It is generally used as soil applied insecticide during December – January[1] to control mealybug nymphs. Due to its moderate to high persistence, it is used for effectively controlling the soil insects. Persistence of chlorpyrifos in various kinds of soils under different agricultural systems has already been studied worldwide e.g. citrus orchard soil in Spain[2], apple orchard soil in China[3], soil under tomato cultivation in Spain[4], soil of oil palm plantation in Malaysia[5], Turkish soil[6], rice soil in China and India[7,8], andosol upland soil in Japan[9], sandy loam, loamy sand and acid soils in India[10, 11], etc. Its residues can be analysed either by gas chromatography[5, 8, 10] or by high performance liquid chromatography[12–14]. A simple HPLC method has been developed and validated in the laboratory for residue analysis of chlorpyrifos in soil and mango.

Glyphosate (N-phosphonomethyl glycine) has been one of the world's most widely used herbicides since 1974. It is a systemic herbicide with broad spectrum of activity, very effective even on plant roots with little harmful effect on mammals. It translocates throughout the plant via the phloem after absorption through the foliage. It is non-selectively used in mango ecosystem to control many weed flora. Residues of glyphosate have been analysed in various agricultural soils worldwide[15–22]. The analysis required derivatization with 9-fluoronylmethylchloroformate in borate buffer[15–16, 23], which is a cumbersome and time consuming process. A simple HPLC method, therefore, has been standardized for residue analysis of glyphosate in soil and mango without a need for derivatization.

Though both the pesticides are frequently used in mango ecosystem, so far no study has been conducted on residue analysis of chlorpyrifos and glyphosate in mango orchard soil and their subsequent uptake in mango fruits. Therefore, the present investigation was carried out to study the dissipation kinetics of chlorpyrifos and glyphosate in

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mango orchard soil and their subsequent uptake to mango fruit.

MATERIALS AND METHODS

Materials

Technical grade standards of chlorpyrifos and glyphosate were purchased from Lupin Agrochemicals (India) Ltd., Bharuch, India and Accustandard, USA, respectively. Chlorpyrifos (Dursban 20 EC, Dow AgroSciences India Pvt. Ltd) and glyphosate (Glycol 41 SL, Excel Industries Ltd.) were procured from the local market. Analytical and HPLC grade solvents along with other reagents were procured locally. Soils under mango cv. Dashehari cultivation and its fruits were chosen for this study because Dashehari is the most popular variety in this region of India.

Soil application and sampling

The soil under mango ecosystem in the Institute farm (26.50° N, 80.50° E) at Rehmankhera, Lucknow is of loam to fine sandy loam type with pH 7.3 and organic carbon content 0.68 per cent. Soils were treated with chlorpyrifos (Dursban® 20 EC) at 2.5 and 5.0 mL L^{-1} of water and glyphosate (Glycel® 41 SL) at 8.0 and 16.0 mL L^{-1} of water separately during first week of May to study their dissipation pattern in soil and subsequent uptake to fruits. Soil samples were collected at 0 (2 h after treatment), 3, 7, 10, 15, 20, 30, 40 and 50 d after treatment from 0-15 cm upper layer and fruit samples were collected at 15 d interval from second week of May to last week of June (up to harvest) for both the pesticides.

Extraction and clean-up of residues

Chlorpyrifos. Soil (50 g each, in triplicate) samples were extracted twice with hexane + acetone mixture (1:1, v/v, 100 + 100 mL) by mechanical agitation on a shaker at 150 rpm speed for 30 min. After filtration, the pooled extract was evaporated to near dryness in a flash vacuum evaporator. The residue was then passed through glass column (50 × 1.8 cm i.d.) using 100 mL of hexane:acetone mixture (1:1, v/v) as eluant and neutral alumina (5 g) as adsorbent. The eluate was completely evaporated in a flash vacuum evaporator and the residue dissolved in 5 mL of HPLC grade acetonitrile for final analysis.

For the extraction of chlorpyrifos from mango fruit (20 g, n = 3), acetonitrile was used as extraction solvent (50 + 50 mL). Further extraction was done by liquid-liquid partitioning with chloroform (50 + 50 mL) and clean-up performed by passing the chloroform layer through a glass column (same size as in soil samples) using magnesia-celite-charcoal (1:2:2, w/w) as adsorbent mixture. After complete evaporation of chloroform, the residue was dissolved in 5 mL of HPLC grade acetonitrile for final analysis.

Glyphosate. Soil samples (5 g each, in triplicate) were extracted twice with double distilled water (20 + 20 mL) by centrifuging at 1500 rpm (201.24 x g) for 15 min. The supernatant was filtered through a nylon membrane filter (Axiva, 0.45 µm thickness and 13 mm diameter) held in a filter holder attached to a glass syringe and directly used for HPLC analysis.

For the extraction of glyphosate from mango fruit samples, 20 g fruit each in triplicate was extracted with 50 + 50 mL of water:dichloromethane mixture (4:1, v/v) for 2 h by shaking at 150 rpm (20 x g) in a mechanical shaker. After filtration, the pooled extract was liquid-liquid partitioned with 50 + 50 mL dichloromethane and the organic fraction was cleaned up by passing through glass column (50 × 1.8 cm i.d.) containing 5 g of magnesia-celite-charcoal (1:2:2, w/w) mixture as adsorbent. Dichloromethane was completely evaporated in flash vacuum evaporator and the residue dissolved in 5 mL of mobile phase of HPLC for analysis.

HPLC-PDA determination of residues

A Shimadzu make HPLC (model LC10 ATVP) coupled with a photo diode array (PDA) detector and reverse-phase Waters µBondapak™ C-18 column (300 x 3.9 mm i.d., 125 Å porosity, 10 µ film thickness) was used for residue analysis of both the pesticides. For chlorpyrifos, the mobile phase, flow-rate and detection wavelength were acetonitrile-water-acetic acid (82.5:17:0.5, v/v), 0.8 mL min^{-1} and 254 nm, respectively. The same parameters for glyphosate were 75 per cent mixture of 0.05 M KH_2PO_4 (pH 6.0) in acetonitrile, 1.0 mL min^{-1} and 315 nm, respectively. The HPLC methods for both the pesticides were standardized at the Institute laboratory and validated through single laboratory validation procedure.

Accuracy and precision

Stock solutions of 1000 mg L^{-1} each of chlorpyrifos and glyphosate were prepared in HPLC grade acetonitrile.

Working solutions of 1, 2, 5 and 10 mg L^{-1} were prepared by subsequent dilution in the same solvent. The calibration curves for chlorpyrifos and glyphosate were found linear in the range of 0.5 to 20 mg L^{-1} concentrations. The precision of the method was checked through the calculation of limit of detection (LOD) and limit of quantification (LOQ) by considering signal to background noise ratio of 3:1 and 10:1, respectively. Recovery study was conducted to know the accuracy and efficiency of the analytical techniques. Soil samples were fortified with 1, 5 and 10 mg L^{-1} concentrations for recovery of chlorpyrifos and with 2, 5 and 10 mg L^{-1} concentrations for recovery of glyphosate. Similarly, fruit samples were fortified with 1 and 5 mg L^{-1} concentrations for recovery of chlorpyrifos and 2 and 5 mg L^{-1} concentrations for recovery of glyphosate.

Statistical analysis

The residue data were subjected to statistical analysis for the calculation of residual half-life values (DT_{50} in days)[24].

RESULTS AND DISCUSSION

Dissipation of chlorpyrifos in soil and its uptake into mango fruit

The recovery of chlorpyrifos from soil and mango fruit ranged between 85.73 to 90.72 per cent and 83.02 to 88.12 per cent, respectively (Table 1). LOD and LOQ for chlorpyrifos were calculated to be 0.1 and 1.0 mg kg^{-1}, respectively.

From its initial (0 d) residue of 5.05 and 8.30 mg kg^{-1} in mango orchard soil, chlorpyrifos dissipated to 0.75 and 1.07 mg kg^{-1} after 50 d of treatment at single and double doses, respectively (Table 2). The residues persisted in soil up to 50 d with 85.15 and 87.11 per cent dissipation from single and double doses, respectively. The rate of dissipation followed first-order exponential kinetics in soil. The residual half-life values were calculated as 18 and 17 d for single and double doses, respectively (Table 2). Residue of this insecticide was below detectable limit in unripe as well as mature mango fruits, which confirmed that chlorpyrifos might not be translocated to fruit.

Table 1. Recoveries of chlorpyrifos and glyphosate from soil and mango fruit at different fortification levels

Fortification level (mg kg^{-1})	Average residues recovered* (%) ± SD			
	Chlorpyrifos		Glyphosate	
	Soil	Mango fruit	Soil	Mango fruit
1	85.73 ± 1.28	83.02 ± 2.23	–	–
2	–	–	83.58 ± 2.01	81.92 ± 1.70
5	96.41 ± 1.92	88.17 ± 1.85	84.96 ± 1.49	85.81 ± 2.41
10	90.72 ± 1.56	–	92.08 ± 1.83	–

*Average of three replicates

Half-life values of 14 to 17 d for chlorpyrifos in soil under tomato cultivation with residues ranging between 0.008 to 0.15 µg g^{-1} were reported in Spain[4]. Dissipation half-life values of 20 and 18 d from surface (0-15 cm) and sub surface (40-60 cm) of silty clay loam soil were also reported in Turkey[6]. The dissipation of chlorpyrifos in soil of a Malaysian golf course[25] followed first-order kinetics with half-life values ranging from 3.4 to 15.3 d. In citrus orchard soil it dissipated with a half-life of 10 d and its concentration was always found highest in upper layer (0-50 cm) of soil during a two-month period[2]. It was found moderately stable in both loamy sand and sandy loam soils with half-life of 12.3 and 16.4 d, respectively[10]. The current results differ from those reported in Malaysia, where it has been mentioned that chlorpyrifos persists in oil palm plantation soil only up to 5 and 7 d after application at recommended (3.8 g a.i.) and double the recommended (7.6 g a.i.) doses, respectively[5]. The difference might be due to the different environmental factors like rainfall, temperature, soil pH and organic matter content, etc. The high persistence of chlorpyrifos in sandy loam, laterite and red loam soils in Kerala[11], India in two moisture conditions has also been highlighted with half-life values ranging from 39-115 d. The authors opined that its persistence in soil depended on pH, clay type, organic matter and its concentration. However, no literature has reported its translocation from soil to any fruit. When sprayed after fruit set[26], residues of chlorpyrifos in whole mango fruit were reported to be higher in non-IPM samples (1.33 µg g^{-1}) than the IPM samples (0.45 µg g^{-1}), the former being above its MRL in mango (0.5 µg g^{-1}).

Dissipation of glyphosate in soil and its uptake to mango fruit

The recovery of glyphosate varied from 83.58 to 92.08 per cent from soil and 81.92 to 85.81 per cent from mango fruit (Table 1). LOD and LOQ values for glyphosate were calculated to be 1.0 and 2.0 mg kg^{-1}, respectively.

Table 2. Dissipation of chlorpyrifos and glyphosate in mango orchard soil, their uptake in mango fruit

Days after application	Average residues in soil (mg kg^{-1})* ± SD			
	Chlorpyrifos		Glyphosate	
	T_1 (2.5 mL L^{-1} of water)	T_2 (5.0 mL L^{-1} of water)	T_1 (8.0 mL L^{-1} of water)	T_2 (16.0 mL L^{-1} of water)
0 (2 h)	5.05 ± 0.14	8.30 ± 0.13	3.37 ± 0.06	6.12 ± 0.08
3	4.11 ± 0.13	7.28 ± 0.21	2.83 ± 0.07	5.35 ± 0.09
7	3.62 ± 0.12	6.21 ± 0.16	2.51 ± 0.05	4.67 ± 0.09
10	3.10 ± 0.06	5.34 ± 0.09	2.18 ± 0.08	3.84 ± 0.07
15	2.55 ± 0.10	4.36 ± 0.07	1.77 ± 0.07	3.23 ± 0.10
20	2.12 ± 0.06	3.40 ± 0.09	1.33 ± 0.05	2.31 ± 0.05
30	1.76 ± 0.12	2.52 ± 0.05	0.61 ± 0.05	1.04 ± 0.08
40	0.92 ± 0.04	1.43 ± 0.04	BDL	BDL
50	0.75 ± 0.03	1.07 ± 0.09	BDL	BDL
15 (fruit)	BDL	BDL	BDL	BDL
30 (fruit)	BDL	BDL	BDL	BDL
45 (fruit)	BDL	BDL	BDL	BDL
60 (harvest)	BDL	BDL	BDL	BDL
Regression equation	$y = 4.699e^{-0.03x}$	$y = 8.193e^{-0.04x}$	$y = 3.601e^{-0.05x}$	$y = 6.708e^{-0.05x}$
Half-life (days)	18	17	13	12

*Average of three replicates; BDL – Below detectable limit (0.1 mg kg^{-1} for chlorpyrifos and 1.0 mg kg^{-1} for glyphosate)

The rate of dissipation of glyphosate followed first-order kinetics in mango orchard soil, though it was less persistent as compared to chlorpyrifos. Glyphosate persisted in soil up to 30 d after application beyond which its residue was not detectable. It dissipated in soil from 3.37 and 6.12 mg kg^{-1} after 2 h of application (0 d) to 0.61 and 1.04 mg kg^{-1} after 30 d of application at single and double doses, respectively (Table 2). The per cent reduction of glyphosate residues from single and double doses after 30 d of application was 81.90 and 83.00 per cent, respectively. The residual half-life values for glyphosate in soil were calculated as 13 and 12 d from single and double doses, respectively. Similar to chlorpyrifos, residues of glyphosate were below detectable limit in unripe as well as mature mango fruits (Table 2), meaning that glyphosate might not be translocated to fruit.

Generally glyphosate adsorbs strongly to soil making it practically immobile and then degrades to aminomethylphosphonic acid (AMPA). It has been reported that glyphosate residues persisted in sandy loam soil up to 86 d after application at 4.25 kg a.i. ha^{-1} but was not detected in field-grown barley, oat, wheat, sweet corn, beans, peas, red beet or carrots[15]. Glyphosate remained consistently in boreal forest soil of Ontario, Canada up to level below 50 per cent of its highest residual amount observed beyond 24 d, of which more than 95 per cent was found in upper organic layer[17]. Half-life of 9 d has been reported for this herbicide in agricultural soil, but 0.006 and 0.005 per cent of radioactivity was measured in rape and barley, respectively, after 41 d of application of ^{14}C-labeled herbicide[21]. Complete disappearance of glyphosate in soil by 20th day has been reported where it has been suggested that glycine pathway of degradation of glyphosate in soil mediated by fungi could be the major reason[19].

CONCLUSION

As residues of either chlorpyrifos or glyphosate were below detectable limit in unripe as well as mature mango fruit, it can be concluded that both the pesticides are safe for their respective use in mango orchard soil.

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REFERENCES

1. Anonymous (2011) http://www.cishlko.org/Technologies/MangoMealybug_advisory. pdf. Accessed 10.08.2013.

2. Redondo MJ, Ruiz MJ, Font G and Boluda R (1997) Dissipation and distribution of atrazine, simazine, chlorpyrifos, and tetradifon residues in citrus orchard soil. *Arch Environ Contam Toxicol* **32**: 346-352.

3. Zhang Q, Zhu L, Han C, Wang J, Xie H, Wang J and Sun S (2011) Analysis of chlorpyrifos and TCP residues in agricultural soil and apples by HPLC. *J Food Agric Environ* **9**: 659-662.

4. Castro J, Sanchez-Brunete C, Rodriguez JA and Tadeo JL (2002) Persistence of chlorpyrifos and endosulfan in soil. *Fres Environ Bull* **11**: 578-582.

5. Muhamad H, Ai TY, Sahid I and Mat N (2010) Downward movement of chlorpyrifos in the soil of an oil palm plantation in Sepang, Selangor, Malaysia. *J Oil Palm Res* **22**: 721-728.

6. Yücel Ü, Ýlim M, Gözek K, Helling CS and Sarýkaya Y (1999) Chlorpyrifos degradation in turkish soil. *J Environ Sci Health Part* **B34**: 75-95.

7. Zhang X, Shen Y, Yu XY and Liu XJ (2012) Dissipation of chlorpyrifos and residue analysis in rice, soil and water under paddy field conditions. *Ecotoxicol Environ Saf* **78**: 276-280.

8. Arora S, Mukherjee I and Trivedi TP (2008) Determination of pesticide residue in soil, water and grain from IPM and non-IPM field trials of rice. *Bull Environ Contam Toxicol* **81**: 373-376.

9. Rahman GKMM and Motoyama N (2000) Determination of chlorpyrifos residue in andosol upland soils using methanol-phosphoric acid extraction. *J Pestic Sci* **25**: 387-391.

10. Menon P, Gopal M and Prasad R (2004) Dissipation of chlorpyrifos in two soil environments of semi-arid India. *J Environ Sci Health* **B39**: 517-531.

11. George T, Beevi NS and Gowri Priya (2007) Persistence of chlorpyrifos in acid soils. *Pestic Res J* **19**: 113-115.

12. Islam A, Farrukh MA, Rahman A, Qureshi FA and Ahmed S (2010) Residue analysis of an organophosphate pesticide in wild plants in Lahore area. *American-Eurasian J Agric Environ Sci* **9**: 514-518.

13. Zhang C, Wu YJ, Jin SF and Yang H (2013) Analysis of chlorpyrifos and chlorpyrifos-methyl residues in multi-environmental media by cloud-point extraction and HPLC. *Anal Methods* **5**: 3089-3095.

14. Mukhtar M, Sherwani A, Wani AA, Ahmed SB, Sofi JA and Bano P (2015) Degradation of chlorpyrifos residues in apple under temperate conditions of Kashmir valley. *Environ Monit Assess* **187**: 482.

15. Ragab MTH, Abdel-Kader MKH and Stiles DA (1985) Fate of glyphosate in a sandy loam soil and analysis for residues in field-grown crops. *Proc Nova Scotian Institute of Science* **35**: 67-70.

16. Miles CJ and Moye HA (1988) Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils. *J Agric Food Chem* **36**: 486-491.

17. Roy DN, Konar SK, Banerjee S, Charles DA, Thompson DG and Prasad RP (1989) Persistence, movement and degradation of glyphosate in selected Canadian boreal forest soils. *J Agric Food Chem* **37**: 437-440.

18. Laitinen P, Siimes K, Eronen L, Rämö S, Welling L, Oinonen S, Mattsoff L and Ruohonen-Lehto M (2006) Fate of herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils. *Pest Manage Sci* **62**: 473-491.

19. Sailaja KK and Satyaprasad K (2006) Degradation of glyphosate in soil and its effect on fungal population. *J Environ Sci Engg* **48**: 189-190.

20. Hu JY, Chen CL and Li LZ (2008) A simple method for the determination of glyphosate residues in soil by capillary gas chromatography with nitrogen phosphorus detector. *J Analyt Chem* **63**: 371-375.

21. Simonsen L, Fomsgaard IS, Svensmark B and Spliid NH (2008) Fate and availability of glyphosate and AMPA in agricultural soil. *J Environ Sci Health* **B43**: 365-375.

22. Al-Rajab AJ and Schiavon M (2010) Degradation of ^{14}C-glyphosate and aminomethyl-phosphonic acid (AMPA) in three agricultural soils. *J Environ Sci* **22**: 1374-1380.

23. Ibáñez M, Pozo OJ, Sancho JV, López FJ and Hernández F (2005) Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry. *J Chromatogr A* **1081**: 145-155.

24. Hoskins ML (1961) Mathematical treatment of loss of pesticide residues. *FAO Plant Protect Bull* **9**: 163-169.

25. Tahir NM, Soon KH, Ariffin MM and Suratman S (2010) Chlorpyrifos and malathion residues in soils of a Terengganu golf course: a case study. *The Malaysian J Anal Sci* **14**: 82-87.

26. Arora S, Singh A, Trivedi TP, Shukla RP and Singh J (2006) Residues of chlorpyriphos and monocrotophos in IPM and non-IPM mango orchards. *Pestic Res J* **18**: 76-78.

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