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Presence of Chemical Nerve Agent in Agriculture Lands of India: Critical Information on Pesticide Degradation in the Environment?

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Abstract: ***Some intermediate products of environmental degradation of organophosphate (OP) are reported as potential neurotoxic chemicals. The fate and biogeochemical dynamics of such chemicals are little known, especially in Indian agricultural soils. This study analysed the presence of secondary metabolites and qualitatively developed a profile of organic compounds from the soils collected from mango plantations in Kerala, India. Samples were extracted with acetone and the chemical profile was developed with Gas Chromatography Mass Spectrometer. We identified the presence of ethyl-3methyl cyclohexyl methyl Phosphonate (Ethyl-3CyMP) which is listed as a degradation product of chemical warfare agent in the database of Organization for Prohibition of Chemical Weapon. The presence of this chemical compound poses ecological and human health risks due to its neurotoxic effects. Even though cyclohexyl methyl phosphonates groups were identified from various regions in the world, this may be the first report from India.***

Keywords: Organophosphates, Secondary metabolites, Mango plantation, Nerve agents, Ecological risk

1.Introduction

Organophosphates (OPs) are the major pesticide group applied in agricultural lands around the globe (Sharma et al., 2019) and most of these compounds are derivatives of phosphoric, phosphonic or phosphinic acids (Sevrain et al., 2017). During the environmental degradation of OPs, a few specific alkyl phosphonic acids are produced as intermediate compounds which were used as chemical nerve agents (CNAs) or chemical warfare agents (CWAs) (Kireev et al., 2002). The OP compounds include nerve agents such as G agents (tabun, sarin, soman and cyclosarin), V agents (VX methyl phosphonothioic acid) and fourth generation agents (Novichoks or A –Series nerve agents) (Franca et al., 2019). These compounds possess rapid toxicity resulting in respiratory failure, depression of the central nervous system, excessive secretions and can be lethal within minutes (https://www.opcw.org/).

Chemical transformation and properties of the OP nerve agent intermediate compound is regulated by specific ions called leaving group (Hamelin et al., 2014). The fluoride anion in the leaving group of G-series agents gives isopropyl methyl phosphonic acid (IMPA), pinacolyl methyl phosphonic acid (PMPA), cylcohexyl methyl phosphonate (CyMP), ethyl methyl methyl phosphonate (EMMP) and some alkyl phosphonate compounds during hydrolysis (Valdez et al., 2018; Valdez et al., 2019). These can irreversibly inhibit acetylcholine esterase enzyme (AChE) and disrupt neurotransmission mechanism after exposure to humans or animals, like organophosphates (Gupta, 2009; Richardson et al., 2007; Andreu and Pico, 2004). In many low and middle-income countries like India, monitoring pesticides and their effects on the environment are inexistent due to the scarcity of regulation capacity, leading to increased health risk (Brodesser et al., 2006; WHO 2018). It is crucial to determine the fate and degradation products of organophosphorus pesticides (OPPs) in agriculture fields. The present study assesses the presence of toxic degradation products of organophosphates from an agriculture plantation in South India. It mainly attempts to evaluate the presence of organophosphates degradation products as a chemical marker for the presence of nerve agents in agriculture plantations.

2.Materials and Methods

Study area and sampling

The study was conducted in the mango plantations of Muthalamada Panchayath, central Kerala (10° 38' 0" North, 76° 48' 0" East) (Figure 1) which is the largest mango producing region of Kerala. A survey conducted in the region indicated the long-term application of chemical fertilisers and pesticides. It is understood that malathion, chlorpyrifos thiamethoxam, matlaxyl, ethion, imidacloprid, and carbendazim are the major OPs used in these plantations. Soil samples (4 nos.) were collected from mango plantations for the screening of pesticides and secondary metabolites.

Accurately weighed soil (10 g) in an Erlenmeyer flask was suspended in 20 ml HPLC grade acetone and shaken for 2 h. The suspension was filtered through Whatman No 41 filter paper and centrifuged and passed through a chromatographic column (sintered) packed with high-purity anhydrous sodium sulphate, silica gel and activated alumina (Weissberg et al., 2018; Sandra et al., 1998; USEPA 3630C). Subsequently, the extracts were concentrated in a rotary vacuum evaporator at 50°C at 500 psi. The extracts were transferred to pre-cleaned glass vials and sealed using parafilm in order to avoid further contact with air. These vials were refrigerated until further analysis in GC-MS.

AI/AS1310-auto sampler ISQ Series-mass TRACE 1300 Series - gas chromatograph with helium carrier gas (99.9995) was used for the separation of the pesticide metabolites. TGS-MS with length - 30m, diameter - 0.25mm, film thickness - 0.25 µm capillary column was used for all separation experiments. A detailed description of the GC parameters is provided in Table 1.

3.Results and Discussion

The analysis of the soil samples collected from different sites in mango plantations shows the presence of ethyl-3 methyl cyclohexyl methyl phosphonate (Ethyl-3 Methyl CyMP) (Graph 1-4), which is listed with RI Index 1472 in the database of the Organisation for the Prohibition of Chemical Weapons (OPCW) (Mesilaakso, 2005; Kostiainen, 2016). Two degradation products are possessing high toxicity; cyclohexyl methyl ethyl phosphonate (CyMEP) and cyclohexyl methyl phosphonate (CyMP), derived from the hydrolysis and methylation of nerve agents like Sarin (GB), Soman (GD) and Cyclosarin (GF) (Valdez et al., 2018; Valdez et al., 2016; Gilly et al., 2009; Soderstorm and Ketola, 1994; Evans et al., 2008; Sidhu et al., 2018; Sindhu et al., 2019; Andreu and Pico, 2004). Di methyl methyl phosphonate (DMMP) groups are the parent chemicals of organophosphate insecticides (DaBell et al., 2004; Elsinghorst et al., 2015).

Valdez et al., (2016) demonstrated the effective methylation of phosphonic acids from chemical warfare agents (CWAs) with trimethyloxonium tetrafluoroborate (TMO. BF4). The method successfully methylates a mixture of nerve agents and produced pinacolyl methylphosphonate (PMPA), cyclohexyl methylphosphonate (CyMP) and ethyl methylphosphonate (EMPA). Similarly, sarin degradation products in soil indicated the presence of methyl phosphonic acid and isopropyl phosphonic acid (Williams et al. 1987; Minami et al., 1997). There is a high probability that organophosphorus pesticides might lead to the formation of cyclohexyl methyl phosphonate (CyMP) in the agricultural soil in the study area. Since carbon-phosphorus (C-P) bond is the backbone of the phosphonic acid group which is resistant to hydrolysis, thermal degradation and photolysis, their metabolites are highly persistent in the environment. Cyclohexyl methyl phosphonate also has a similar C-P bond indicating its persistence in the mango plantations of Muthalamada.

OP pesticides and some nerve agents are chemically related and pose the same mechanism of toxicity, and are thereby included in studies investigating the efficacy of countermeasures against their poisoning in animal models (Albuquerque et al., 2006). Even though, several technologies were suggested for the degradation and decontamination of these toxic compounds (Wagner et al., 2007; Yang et al., 1992) minimising the ecological and human health risk should be a prime concern in reported areas. Amitai et al., 2006 studied the toxicity of OP chiral isomers and nerve agents and observed that the inhibition of AChE by OPs chiral isomers was more effective than cyclosarin and soman, similar studies were done by Barakat et al., 2009. Cyclohexyl methyl phosphonate, dimethyl methyl phosphonates are the degradation or hydrolysis products of G series nerve agents (Kanu et al., 2005; Douglas et al., 2007). Hence CyMP also poses a similar mechanism of toxicity. Eman et al., 2007, used cyclohexyl methyl phosphonate as a degrading agent of cyclosarin for the toxicity assay of lycerophosphodiesterase and mentioned that organophosphate diesters are the most toxic and persistent degradation product of the nerve agent VX which includes Cyclohexyl methyl phosphonate. Analysis and detection of cyclohexyl methyl phosphonates were reported from environmental samples such as soil, water, air, clothing, polymers (Tornes et al., 1991; Black et al., 1994; D'Agostino et al., 2001; Naomi et al., 2002; Sega et al., 1997; Rohrbaugh et al., 1998; Kataoka et al., 2000; Tomkins et al., 2001; Hacock et al., 1991; Staan'kov et al., 2004; D'Agostino et al., 2006). From these reviews it is confirmed that CyMP is an organophosphonate monoester compound, also a degradation or hydrolysis product of cyclosarin nerve agent poses environmental toxicity and human health risks. Exposure of CyMP occurs through dermal contact because of its lower volatility (Yang et al., 1999; Samuel et al., 1983; Valdez et al., 2014; Evans et al., 2008; Albo et al., 2014). Studies on quantification or identification of nerve agent metabolites are scarce in the Indian agroecosystems (Chauhan et al., 2008; Ganesan et al., 2010).

There is a very high possibility of the formation of organophosphate metabolites from OP pesticides applied in the mango plantations of Muthalamada. It also demonstrates the requirement of further investigation about the OP pesticides and their environmental fate in similar agricultural ecosystems of Kerala and India. Several studies have been conducted for the quantification of organophosphorus and organochlorine pesticides in agricultural soils of India. However, report on highly toxic secondary metabolites in the agricultural environment is scarce in India. Proper identification of these compounds is necessary for the minimisation of environmental and community health risks. To date, this is the first report on cyclohexyl methyl phosphonate detection in agricultural soils of India.

4.Conclusions

This study is the first report on ethyl-3methyl cyclohexyl methyl phosphonate from the soils of mango plantations in India. The available reviews confirm that this metabolite is an organophopshonate monoester compound of Cyclosarin. Since many OPPs were used in the study area, the environmental degradation processes have influenced the formation of Ethyl-3CyMP, as reported in the OPCW's database. The presence of this compound in the agriculture field indicates the presence of highly toxic secondary metabolites, which may pose ecological and human health risks. We recommend further investigations for a better understanding of the biogeochemical dynamics and ecotoxicology of such compounds.

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Figures

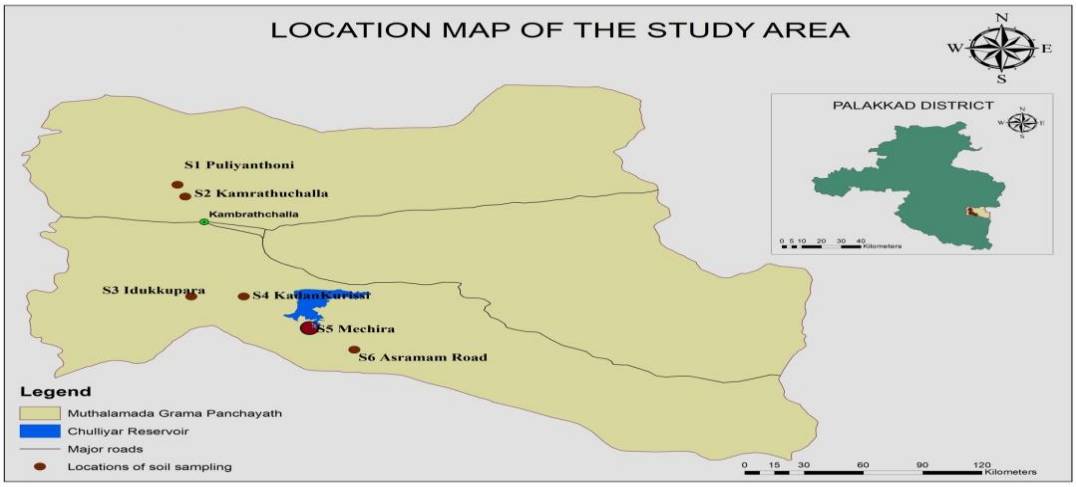
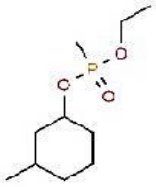
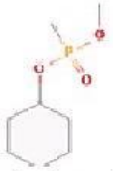


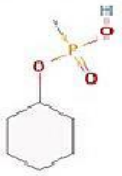
Figure 1: Google map image of Study area showing sampling locations



Ethyl-3 Methyl cyclohexyl methyl phosphonate
($C_{10}H_{21}O_3P$)



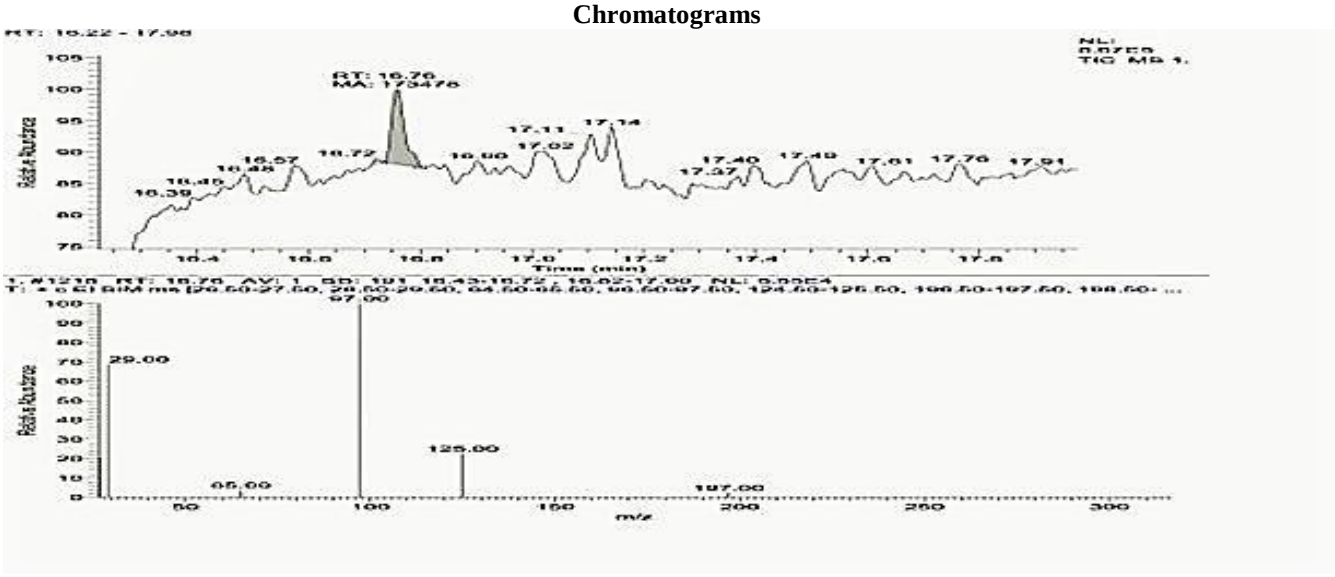
Cyclohexyl methyl methyl phosphonate($C_8H_{17}O_3P$)



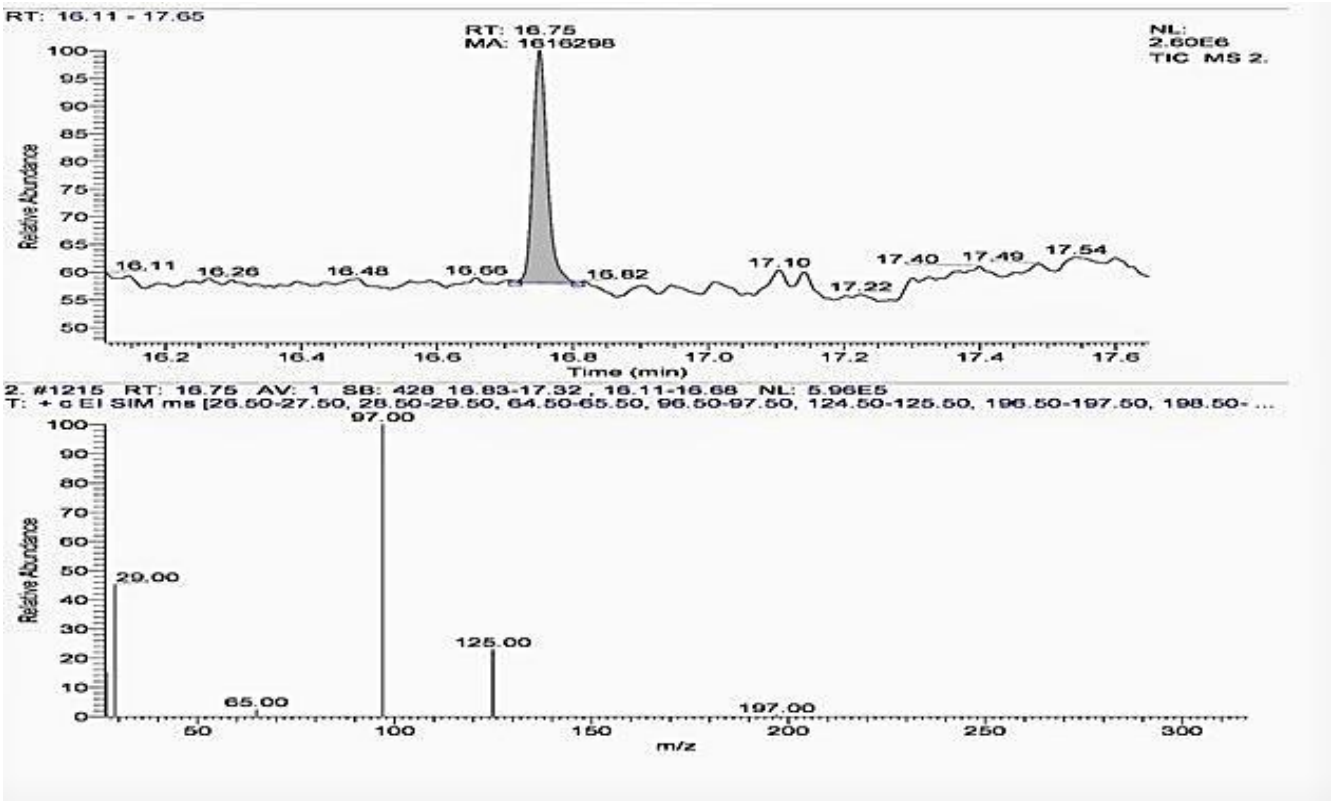
Cyclohexyl methyl phosphonate ($C_7H_{15}O_3P$)

Figure 2: Cyclohexyl phosphonate degradation products (Source: https://pubchem.ncbi.nlm.nih.gov)

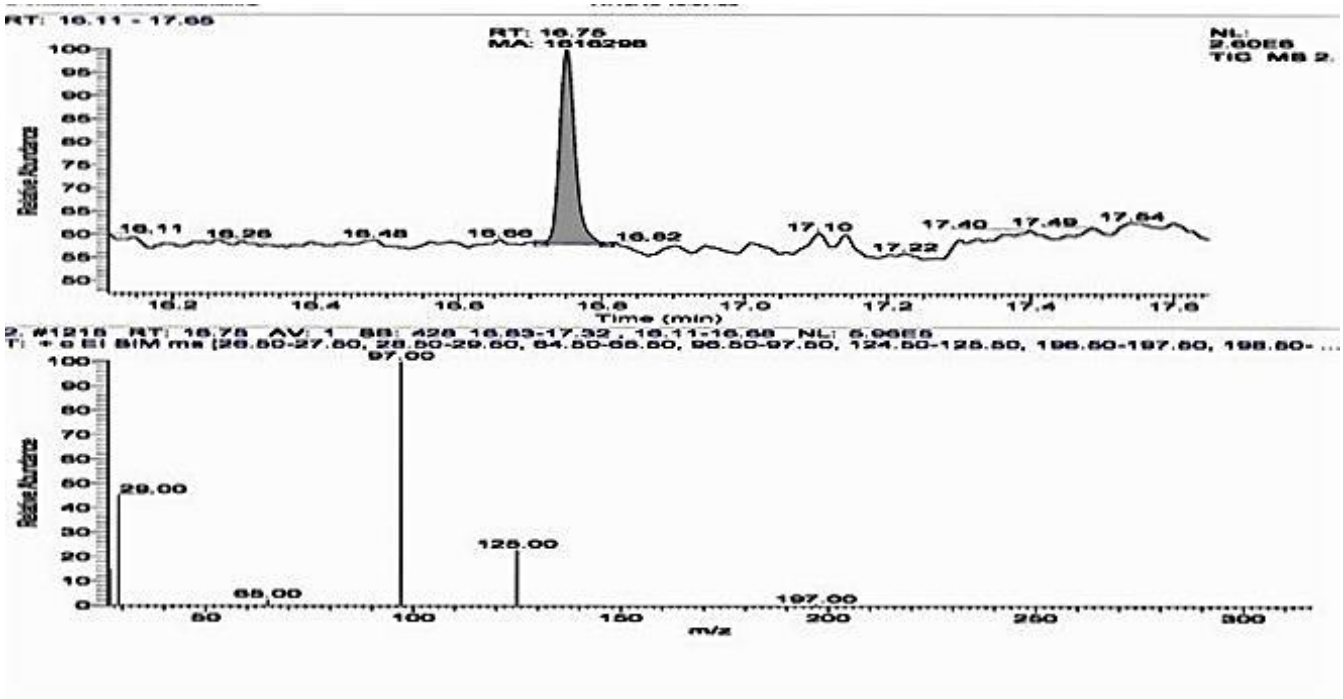
Chromatograms



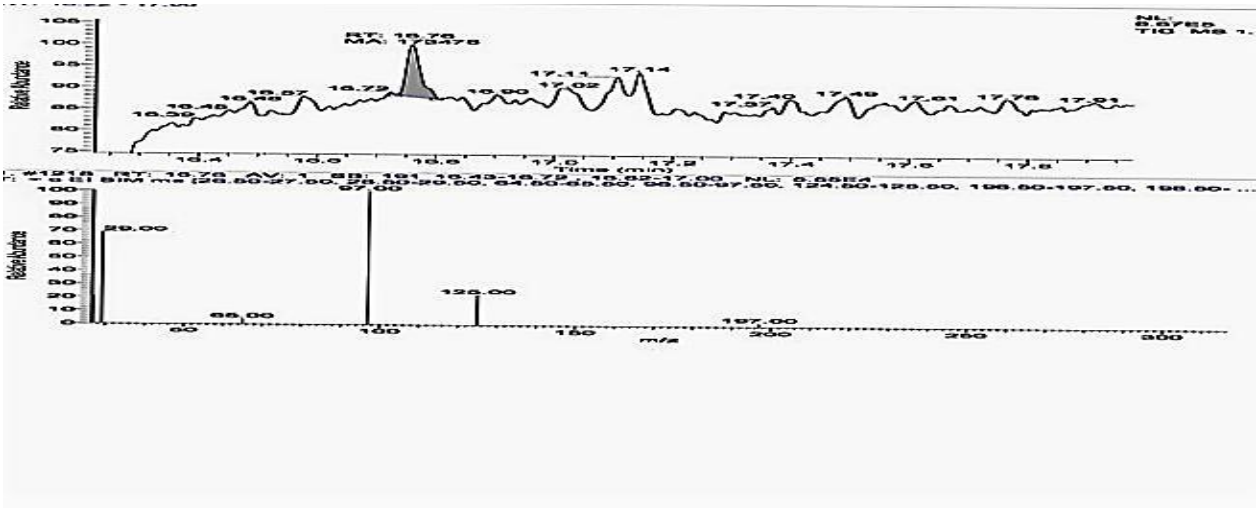
Graph 1: Chromatogram of ethyl-3 methyl CyMP from site1



Graph 2: Chromatogram of Ethyl-3 methyl CyMP from site 2



Graph 3: Chromatogram of Ethyl-3 methyl CyMP from site 3



Graph 4: Chromatogram of Ethyl-3 methyl CyMP from site 4

Table 1: GC Instrumental Parameters

Instrumental parameter	Specifications
Instrument	AI/AS1310-Auto Sampler, ISQ Series-mass, TRACE 1300 Series - GC
Carrier Gas	Helium(99.9995)
Injection	1.2ml/min
Purge Time	5ml/min
Injection Volume	1 µl
Oven Program	Start temp-80˚C, Hold Time-6min, Increasing temp-9˚/min Final temp-280℃, Final hold time-5 min
Column	TG5-MS, Length-30m,diameter- 0.25mm, film thickness-0.25 µm