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Determination of Organophosphorus Insecticides (Malathion and Diazinon) Residue in the Drinking Water

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Abstract: In this study, the amount and origin of the pollution of Karaj river and Amir-Kabir dam, which is the most important supply of Tehran's (capital of Iran) drinking water with malathion and diazinon, have been assessed by High Performance Thin Layer Chromatography (HPTLC) technique. This is a descriptive and cross-sectional study. After initial assessments, four stations were selected for water sampling. In all stations, sampling was done in one day, one week, two weeks, one month, two months and three months after insecticide spraying. Extraction and separating of organophosphorus insecticides from water samples was done by acetone and methylen chloride solvents, using decanter. Then, the residue of malathion and diazinon insecticides was determined using HPTLC technique. The residue of malathion and diazinon in water decreases with increasing the distance and time of spraying. The residues of malathion and diazinon insecticides at station 1 and 2, which were close to spraying places, 1-2 months after spraying was more than allowed limits. But at the ending stations (3 and 4), malathion and diazinon were detectable just in the 1 day and 1 month after spraying, respectively. It is unlikely that Tehran's drinking water be polluted with these two insecticides and the most adverse effect can be seen in the area of spraying. Therefore not only the environment, but also the people in area of Karaj river are at risk of chronic toxicity with malathion and diazinon through consuming polluted water and agriculture products.

Key words: Drinking water, insecticides, organophosphorus, thin layer chromatography

INTRODUCTION

Today, insecticides are widely used for control of medically important insects and also agriculture pests. Organophosphorus are used in agricultural pest control, more than other pesticides (Hela *et al*., 2005; Rawn *et al*., 2006). The most important side effect of using organic insecticides, especially organochlorine and organophosphorus ones is the pollution of human environment including water, soil, food and living organisms (Dogheim *et al*., 1996).

Since the organophosphorus insecticides are used more than carbamate and pyrethroide and they are also more permanent in environment, the main pollution of surface and drinking waters are of organophosphorus insecticides like chloropyrifus, chloropyrifus methyl, malathion, diazinon, prymfus methyl and so on (Castilho and Fenz, 1999; Puglise *et al*., 2004; Na *et al*., 2006).

Studied have shown that washing fruits and vegetables with tap water can not remove some insecticides like chloropryfus (Krol *et al*., 2000).

Insecticides can enter the food chain and also directly to drinking waters. In this way, they can affect the life of human directly or indirectly.

Karaj river is one of the biggest rivers of Iran and its water has an appropriate physico-chemical characteristics for drinking purposes. On the other hands, according to the geographical classification, Iran is an arid country. Therefore, sanitation of this river is important. The water of this river passes through many villages and agricultural areas and is finally stored behind Amir-Kabir dam. Insecticides, especially organophosphorus ones like malathion and diazinon, spray the gardens in the route of this river, for pests control of fruit trees. Therefore, malathion and diazinon enter the river directly or indirectly through rain, wind and other ways. Finally the pollution of side's stream causes the pollution of the main Karaj river. In this study pollution of Karaj river and dam with malathion and diazinon and their origin, have been studied and the residue of those insecticides in the water of sides' streams, the main Karaj river, Amir-Kabir dam and also Aderan river which is branched from Amir-Kabir

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dam, have been determined by High Performance Thin Layer Chromatography (HPTLC) technique.

MATERIALS AND METHODS

This is a descriptive and cross-sectional study, which has been done in two phases:

- Water sampling from streams and the main Karaj river, Amir-Kabir dam and Aderan river.
- Extraction of malathion and diazinon insecticides from water samples and determining their amount by HPTLC technique.

Water sampling phase: Firstly, four stations were selected for water sampling, based on geographical study and preparation the map of streams, which end in Amir-Kabir dam and some scientific consultations. Since organophosphorus insecticides, especially malathion and diazinon, are the mostly used insecticides for pests control of fruit gardens in that area, they were selected for the study.

The mostly used phosphate insecticides by farmers and gardeners of that area were Emulsifiable Concentrates (EC) of malathion (57%) and diazinon (60%).

The selected stations of water sampling were as follows:

Station 1: Chahar-bagh location of Sira village, which its streams end in the main Karaj river.

Station 2: The main Karaj river, 15 km before Amir-kabir dam.

Station 3: Amir-kabir dam.

Station 4: Aderan river, 5 km after Amir-kabir dam (Fig. 1).

An initial sampling was done before spraying, in order to assuring of not pollution of sample waters with phosphate insecticides. The main sampling was done after spraying of fruits gardens, in following method:

Twenty one litre samples were collected from different parts of each station. In station 1 which contained the streams of gardens sampling was done at different parts, including the beginning middle and the end of streams. The collected samples of each station were mixed and a final

Two litre sample was prepared for each station. In order to preventing the hydrolysis insecticides in water sampling during carrying them to laboratories, 50 cc methylene chloride solution was added to each sample.

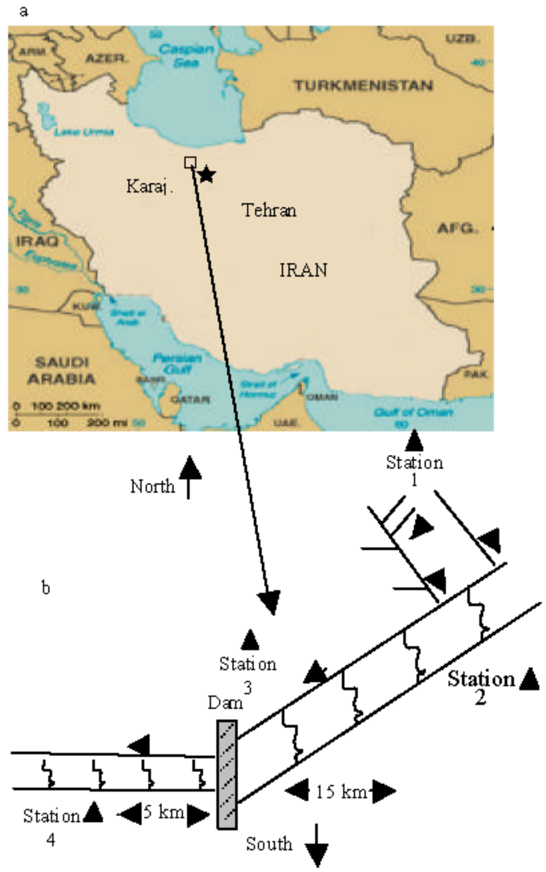

Fig. 1: Schematic map of water sampling stations. (station 1, 2, 3 and 4: is villages, Karaj river, dam and Aderan river, respectively)

Sample contain were sealed by parafilm, tagged and transferred to laboratory for insecticide extracting. It should be note sampling was done in six times (one day, one week, two week, one month, two months and three months after spraying) for each station.

Extraction and determining phase: The solvent was used in this study, such as acetone, methylene chloride, hexane and also silica gel (SiO_4) $60F_{254}$ plate (20*20 cm), purchased from Merck Company. The malathion and diazinon standards were purchased from the representative of Accustandard Company of Switzerland.

Extraction and separating of phosphate insecticide was done by acetone and methylene chloride solutions using decanter, which is current method (Butz and Stan, 1995). After separating and extracting malathion and diazinon insecticides from water samples, additional materials were deleted using separator funnel and the insecticides were extracted by acetone-methylene chloride solvent. The resulted solution was cleaned up in silica gel (SiO_4) columns. Then, the prepared solution was concentrated by evaporation rotary device. The resulted solution was tagged as unknown samples and their insecticide contents were determined. Allowed limit of malathion and diazinon in drinking water is less than 0.5 ppm (Butz and Stan, 1995).

Recovery rate of (extraction efficiency) malathion and diazinon was determined by blind technique.

The residue of malathion and diazinon insecticides was measured by HPTLC technique, which is common in recent years (Futagami *et al.*, 1997; Butz and Stan, 1995). Spotting was done by applicator and capillary 5, on an Aluminium plate containing silica gel (SiO_4) $60F_{254}$, as stationary phase, using standard solution and the unknown solutions of water samples.

Multiple level method was used for spotting of standard sample. In this method, some different concentrations, or different volume of a standard concentration, are used for spotting of standard sample. These spots were allowed to dry before being placed to run in respective solvent systems. Then plates were put in solvent tank (chamber tank) for development.

The organic solvent (as mobile phase) for developing malathion and diazinon spot, was 80:20 acetone-hexane mixture.

This solvent was poured in chamber thank and the prepared plates were put in it after saturation of tank space (about 30 min).

In this test, development of spots and running of solvent was done in 20-25 min. Then the plates were exited from the tank and the spots were seen by fluorescent light in UV cabinet at 254 nm. After development, R_f value was calculated for each insecticide. The chromatographic zones corresponding to spots of malathion and diazinon were scanned at 257 nm by using of TLC scanner 3 (V. 1.14 S/N: 080320) (CAMAG company, Switzerland) and CATS4 software (version 4.06, S/N: 0805A007), in Reflection /Absorption measurement mode (Denistrop, 2000; Ahmad, 2001). The source of radiation utilized was the deuterium lamp. At the end, the amounts of malathion and diazinon of each spot and their R_f (retardation factor) values were determined. The position of a substance zone (spot) in a thin layer chromatogram can be described by R_f. This is defining as the quotient obtained by dividing the distance between the substance zone and the starting line by the distance between the solvent front and the starting line (Denistrop, 2000).

Data analysis: To compare organophosphorus insecticide residue among four stations in different times after spraying, analysis of variance (ANOVA) and non-parametric Mann-Whitney test were used.

RESULTS

Results of this study showed that there is a significant difference between the malathion and diazinon residue of the water samples from the four stations ($p<0.05$). The highest amount of organophosphorus insecticide residue was seen in station 1, where the water was directly exposed to insecticides spraying and the lowest amount was seen in station 3 (Table 1). The amount of malathion and diazinon insecticides in water samples of station 4 (the river after dam), was significantly more than of the water samples of station 3 (water behind the dam) ($p<0.05$).

Statistical analysis of the data shows that the amount of detectable organophosphorus insecticides in the water samples of stations significantly decreases with time ($p<0.05$).

The amount of malathion residue of the water samples of stations 1 and 2 after on month of spraying was 4.1 and 3 ppm, respectively, which are more than allowed limits. The malathion residue of the water samples of stations 3 and 4 was more than allowed limits just one day after spraying and decreased to the allowed limits (0.5 ppm<) after it (Table 1).

The R_f values of malathion and diazinon were calculated 0.47 (±0.01) and 0.58 (±0.02), respectively.

The amount of diazinon residue in water samples of stations 1 and 2, were more than allowed limits, respectively in one and two months after spraying. Diazinon residue of the samples of stations 2, were in allowed limits three months after spraying (Table 2). Diazinon residue of the samples of stations 3 was more than allowed limits (12 ppm), even one week after spraying. diazinon residue of the samples of stations 4 was more than allowed limits in one month after spraying.

Table 1: Residue of malathion in water of selected stations (ppm). (Amount of more than allowed limit (0.5 ppm<) showed bold)

Water sampling station	1 day	1 week	2 week	1 month	2 month	3 month
1	**341.8**	**75.6**	**18.5**	**4.1**	0.2	0
2	**318.0**	**110.0**	**25.0**	**3.0**	0.2	0
3	**2.0**	0.0	0.0	0.0	0.0	0
4	**30.0**	0.0	0.1	0.1	0.0	0

Table 2: Residue of diazinon in water of selected stations (ppm) (Amount of more than allowed limit (0.5 ppm<) showed bold)

Water sampling station	1 day	1 week	2 week	1 month	2 month	3 month
1	**491.6**	**125.3**	**43**	**5.3**	0.2	0.1
2	**454.0**	**101.2**	**43**	**11.0**	**2.0**	0.1
3	**10.0**	**1.0**	0	0.0	0.0	0.0
4	**76.0**	**12.0**	**4**	**0.8**	0.1	0.0

Comparing the trend of decreasing malathion and diazinon residue in different times of sampling shows a significant difference between the two insecticides ($p<0.05$), so that the decreasing rate of diazinon residue is slower than the decreasing rate of malathion one. Recovery rate of malathion and diazinon was 85-92%.

DISCUSSION

The health of Amir-Kabir dam water which is the main supply (60-70%) of Tehran's drinking's water is very important.

In stations 1 and 2 which were close to gardens and spraying places, malathion insecticide was detectable and more than allowed limits till one month after spraying, while it was detectable just one day after spraying in stations 3 and 4.

Diazinon was more than allowed limits, 2 months after spraying in station 1 and 2, but unlike malathion it was more than allowed limited till one month after spraying in station 4 which shows its higher lasting capacity in water compared to malathion regarding to the alkaline pH of Karaj river water (pH: 7.8) it can be thought that malathion and diazinon insecticide are hydrolyzed considerably in the route of streams and river to the dam, meanwhile concentration of organophosphorus insecticides may be decreased in the route of Karaj river, due to their mixing with the deep water of the river. The results of some similar studies in Nicaragua have shown such phenomenon too (Castilho and Fenz, 1999).

Therefore spraying the gardens of Karaj river sides can be the origin of this rivers pollution to malathion and diazinon insecticides.

According to the results of this study the amounts of malathion and diazinon insecticides were more than allowed limits in the water of streams and main Karaj river, especially in the region of Sira village which is the main site of spraying at least one and two months after spraying, respectively. Unfortunately the same water is used by local people for irrigating gardens, washing purposes and even sometimes for drinking of people and live stocks.

Based on the results of this study malathion content of dam water has been more than allowed limits one day after spraying but has been in acceptable limits after one week. The low contents of malathion insecticide in the first day after spraying (2 ppm) can be deleted during refining process and thus the drinking water of Tehran has no malathion pollution diazinon content of dam water has been more than allowed limits till one week after spraying but it has not been detectable in next weeks. Other studies also show that organochlorine and organophosphorus insecticide are the main causes of the pollution of drinking water supplies especially in rural areas close to fruits gardens the highest risk of environment (Pedersen *et al*., 2006). Water pollution with insecticides and their acute effects can be seen in inhabitants of spraying region but their long-term effects can even threaten inhabitants of distant regions too (Abdel-Halim *et al*., 2006). Organophosphorus pesticides can pollute surface water and also sometimes underground water supplies (Castilho and Fenz, 1999; Wilsont and Foos, 2006) the result of some studies Pakistan approve it too (Ahmad Karam and Tahir, 2000). Insecticides can even spread to the interior surfaces of houses. The results of a study in New York have shown that insecticides residue is detectable in interior surfaces of houses and the facilities like furniture, carpets and even dust. They are mainly transferred to houses by mechanical ways like air flow and wind (Obendore *et al*., 2006).

Although the amount of phosphate insecticides is not considerable in the water of Amir-kabir dam and there is a very low risk of the pollution of Tehran's drinking water supply with these insecticides inhabitants of spraying region including Sira village are highly at risk of the adverse effects of such pollutions. This may cause environment pollution and also chronic toxicity with malathion and diazinon insecticides.

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