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Determination of Diazinon and Sevin insecticides residue in Saymareh and Konjanchem Rivers, West of Iran in 2011

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Abstract
In order to study the residues of Diazinon and Sevin insecticides in Saymareh and Konjanchem Rivers, Ilam province, Iran, the rivers water samples were collected in April 2010 to July 2011, throughout the Ilam province. Thin layer chromatography was used, since it was the best applicable method in area with reasonably high level of sensitivity up to 0-01 ppm and acceptable recovery of 80%. On 3 samples was conducted to assess two insecticide residues in various phases of the Saymareh and Konjanchem Rivers from the day after the spraying of the Diazinon and Sevin insecticides until when no trace of the insecticide was detectible in the studied Rivers of the study area. The highest level of Diazinon was detected the day after the spraying of station 3 of Saymareh River in amount of 1.97 ppm. Also the highest level of Sevin was detected the day after the spraying of station 1 of Saymareh River in amount of 2.33 ppm.

Keywords: Residue, Diazinon, Sevin, river, Ilam Province, Iran

1. Introduction

Insecticides are widely used for protecting products. Environmental pollution is one of the serious predicaments of the modern world (Hosseinabadi 2014) [6]. Due to the significant increase in the environmental pollutants and lack of precaution measures of observance of the environmental regulations, it has become a global problem (Abdel-Halim, Salama *et al.* 2006, Wilson and Foos 2006, Arjmandi, Tavakol *et al.* 2010) [1, 12, 2]. Organophosphorus and Carbamate insecticides are used more than other pesticides in agriculture. These compounds have been used in large quantities (Abdel-Halim, Salama *et al.* 2006) [1]; Therefore the residue of these insecticides in environment such as water may be hazardous to human health. Pollution of the water in the river and depleting its resources can put the lives of many people in danger (Chimwanza, Mumba *et al.* 2006) [3]. Due to wide spread use of insecticides in the world and our country which has led to substantial portion of the water resources in long term, effect on human health and different organisms including mammals and aquatic organisms, it is important to control such insecticides use. Organophosphorus and Carbamate (OPS, OC) compounds consist of a great group of chemical insecticides are generally among the most acutely toxic insecticides in environment. These insecticides are used to control broad spectrum pests while they have close relationship with rivers.

Ilam province is located in south-west and west of Iran. This province is bordered with Iraq country. Saymareh River resources from northern mountains of Lorestan province and southern slopes of mountains of province. Konjanchem River in which is located in Chovar city resources from Ilam province. These rivers are in close contact with agricultural lands in the province.

This study has been conducted to determine the concentration of Diazinon and Sevin insecticides in Saymareh and Konjanchem Rivers. Sampling was done along Saymareh and Konjanchem Rivers from different sites during April 2010 to July 2011. Samples were extracted by methylene chloride and determined quantitatively for insecticides by TLC method.

2. Materials and Methods

The sample size was calculated by the $n = (\delta/\delta/3)^2$ formula with a certainty of 95 % and the accuracy of 1.6 standard deviation. The extraction of pesticides was carried out using solvents, including methylene chloride and acetone (Merck, Germany). After removing excessive solvents by the rotary evaporation device, the samples were prepared for spotting. The produced solutions were labeled as the unknown samples and in the next phase, their level of insecticide contents were determined (Futagami, Narazaki *et al*. 1997) [4]. The residual levels of the pesticides in samples were detected using HPTLC (TLC Scanner, AMAG Company, Switzerland). For this purpose automatic spotting after extraction was done by silica gel plates (silica gel plate sio4 60F254) 20*20 cm Merck, Germany. The spots on plate had 2cm distance from another. Some spots belonged to the pesticides sample standards (Accustandard Company, Switzerland). After the spotting, plates were placed in chromatography tank having a ratio of 20cc acetone + 80cc Hexane and then after reaching to Rowing Flow appropriate (RF), plates were put in to UV cabinet having a wave length of 254nm in order to observe the spots. Then the plates were removed to scanner for quantitative analysis. This survey is a cross-sectional study. The study targets the assessment of organophosphate and Carbamate residues in water samples along Saymareh and Konjanchem Rivers located in Ilam province (Fig 1).

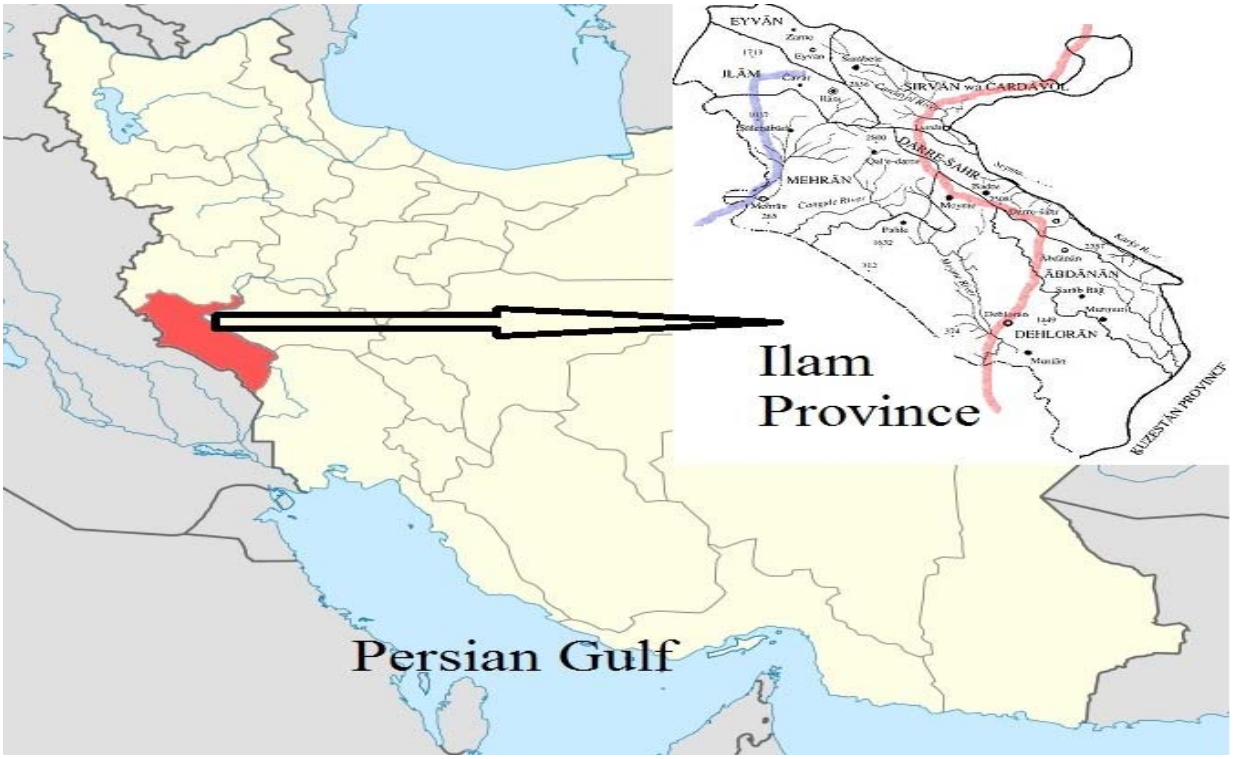

Fig 1: Ilam province is located in west of Iran. Saymareh (Blue) and Konjanchem (Red) Rivers are located in Ilam province

Sampling was carried out during April 2010 to July 2011. This study conducted in three phases: sampling, extraction and determination. Before use the glass wares they were washed with hot water, detergent and then rinsed with distilled water and acetone (Merck, Germany).

1-Sampling: Water samples were taken from April 2010 to June 2011 from six stations in two rivers. The water samples were collected in glass bottles (1 Liter) from surface down to depth of 20cm below water.

In order to water sampling, 20 samples of one-L Volume were collected. The collected samples were mixed together in a 20 L container. Eventually, from each station, 5 one L samples were collected (Tavakol 2007) [11]; Then, the samples were kept in refrigerators and sent to the Pesticides Chemical Laboratory (PCL) of the School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Water samples were filtered with whatman No 1. Shacked samples were studied in PCL. The solution was concentrated by the rotary evaporation apparatus. The produced solutions were labeled as the unknown samples and in the next phase, their level of insecticide content was determined (Tavakol 2007) [11]. A period of 20 min was adequate for the growth of spots and the rise of the solvent. Afterwards, the plate was taken out of the tank and dried. Then, the spots were examined inside a UV cabinet with florescent light at the wavelength of 254 nm.

Eventually, the TLC Scanner 3 and the CATS 4 software were utilized to scan the insecticides spots (Hahn-Deinstrop 2007) [5]. For scanning the plates, the deuterium light at the wavelength of 257 nm was selected. At the conclusion, the amounts of two insecticides present in each spot were directly determined by the CATS 4 software. The level of insecticides in unit volume of water was calculated from the amounts contained in each spot. At the end, the ANOVA test was used in the data analysis (Tavakol 2007) [11].

2-extraction: It was thoroughly mixed by inverting the flask three times. The samples were extracted with 200cc methylene chloride (40, 100, 40) shaken for 3 minute each time. The organic phase was concentrated to 2cc by the rotary evaporator. The samples were prepared for spotting.

3-Determination: For this purpose automatic spotting after extraction derived by silica gel plates (Silica gel plate si04 60F254, 20*20, Merck, Germany). The spots on plates had 2cm distance from another. Some spots belonged to the insecticides samples standards (Accustandard Company, Switzerland). After spotting plates were placed in chromatography tank having a ratio of 20cc acetone + 80cc Hexane, and then after reaching to rowing Flow Appropriate

(RF) plates were exit, and put in to UV cabinet having a wave length of 254nm in order to observe the spots. Then plates were removed to scanner for quantitative analysis. The residual levels of the pesticides in samples were detected using HPTLC (TLC Scanner, CAMAG Company, Switzerland).

To each sample 100cc methylene chloride (Merck, Germany) was added to preserve from degradation due to the long distance between sampling site station to the laboratory for analysis. During the transportation process the samples were kept in standard conditions. For control sample the water from network was also taken.

3. Results

Three samples were prepared to assess insecticide residues in various phases of each river, the Saymareh and Konjanchem Rivers from the day after the spraying of the Diazinon and Sevin insecticides until no trace of the insecticides were detectible in the studied rivers of the study area. As it is shown in Table 1, the highest level of Diazinon was detected the day after the spraying of station 3 of Saymareh River in amount of 1.97 ppm. The mean level of Diazinon residue a day after the spraying in 3 studied stations of Saymareh River was 1.746 ppm. The mean levels in a week, Fifteen days and 45 days after the spraying this river were 1.10 ppm, 1.456 and 0.756 ppm respectively. Also the mean level of Diazinon residue a day after spraying in 3 studied stations of Konjanchem River was 1.173 ppm. The mean levels in a week, fifteen days and 45 days after the spraying this river were 0.93, 1.063 and 0.763 ppm respectively (Table 1).

Table 1: Station numbers, Diazinon and Sevin insecticides residual in Saymareh and Konjanchem Rivers before spraying and various times after spraying (PPM)

Station No.	Before Spraying		after 1 day		after one week		after 15 days		after 45 days	
	Diazinon	Sevin	Diazinon	Sevin	Diazinon	Sevin	Diazinon	Sevin	Diazinon	Sevin
Saymareh 1	0	0	1.44	2.33	0.9	2.1	1.51	1.9	1.28	0.3
Saymareh 2	0	0	1.83	2.11	1.1	1.98	1.27	0.50	0.55	0.27
Saymareh 3	0	0	1.97	1.9	1.31	1.4	1.59	0.8	0.44	0
Konjanchem 1	0	0	1.26	0.27	1.49	1.46	0.37	0.50	0.85	0.1
Konjanchem 2	0	0	1.77	1.23	0.78	0.84	1.36	0.51	0.87	0.07
Konjanchem 3	0	0	0.49	1.36	0.52	0.54	1.46	0.3	0.57	0.02

The highest level of Sevin was detected the day after the spraying of station 1 of Saymareh River in amount of 2.33 ppm. The mean level of Sevin residue a day after the spraying in 3 studied stations of Saymareh River was calculated as 2.113 ppm. Also the mean level of Sevin residue a day after the spraying in 3 studied stations of Konjanchem River was 0.953 ppm. Respectively, the mean levels in a week, fifteen days and 45 days after the spraying Saymareh River were 1.826, 1.066 and 0.19 ppm. Also the mean level of Sevin residue in Konjanchem River after a week, fifteen days and 45 days were 0.946, 0.436 and 0.063 respectively (Table 1).

4. Discussion

Due to the extensive application of insecticides in the farmlands of Ilam province and other wide farmlands, this investigation was carried out to obtain the necessary data and information on the residues of Diazinon and Sevin insecticides in Saymareh and Konjanchem Rivers. It is obvious that Diazinon and Sevin insecticides residues are significantly more in summer than other seasons in rivers containing Saymareh and Konjanchem Rivers, due to this fact that the most sprayings are in summer there. Similar studies (Shayeghi, Khoobdel *et al*. 2008) [10] have shown significant difference of Diazinon and Azinphosmetyl concentration during summer and other seasons in Qarahso and Gorganroud rivers ($P<0.05$).

The fate of Diazinon in river depends on water outflow and degradation. The water flow has a main role on outflow of Diazinon in Tajan River (Hosseinabadi 2014) [5]. Iran is one of the countries in which has located in semiarid district. This country has been always in drought; so it is important and vital to make the drinking water safe and usable. The investigations have revealed that Sevin and Diazinon insecticides have been widely used in agriculture fields in Ilam province for pest control. Our study revealed that, the concentration of Diazinon and Sevin in station 1, which is near to farmlands and spraying places was higher than WHO maximum residue limits even 45 days after spraying. It is obvious that the reason of these high residues of Diazinon and Sevin is due to this fact that the water samples have been taken from sprayed farmlands which are close to station 1. The amount of Sevin and Diazinon insecticides decreased when our sampling were in middle of rivers (station 2) and end of the river (station 3).

In an investigation, a similar monitoring performed on Organochlorine and Organophosphorus pesticides in the water of the Reconquista River in Argentina (Rovedatti, Castañé *et al*. 2001) [9]. This study provided first systematic data for measurement of pesticides in surface water of Reconquista River, based on a monthly monitoring program over two-year span and the results revealed that at all locations, pesticides levels were found to be between 40 and 400 times higher than the legal limits established for protection of aquatic life. (Rovedatti, Castañé *et al*. 2001) [9].

The results of this investigation by Shayeghi *et al*., revealed that the residue of Diazinon and Azinphosmetyl in summer is significantly different from others seasons ($P<0.05$). The most of Azinphosmetyl and Diazinon residue was determined in summer. Three other seasons (spring, autumn and winter) were not significantly different in Azinphosmetyl and Diazinon residue ($P>0.05$). The means of Azinphosmetyl residue in Qarahso and Gorganroud in summer was 14.56ppm (±SD= 10.59) and 14.9 ppm (±11.67), respectively. The means of Diazinon in Qarahso and Gorganroud in summer was 22.4ppm (±18.62) and 6.74 ppm (±6.89), respectively (Shayeghi, Khoobdel *et al*. 2008) [10]. In another study, residues of Diazinon insecticide Samples analyzing revealed that Diazinon toxin is observed frequently in aquatic ecosystems in Ab-bandans adjacent of three rivers of Mazandaran province, throughout the year especially in summer (Hosseinabadi 2014) [6].

Another investigation showed that Organophosphorus insecticides are the contamination resources of drinking waters in villages and cities which are near to gardens and farmlands (Pedersen, Yeager *et al*. 2006) [8]. Obendorf *et al.* carried out a study on residues for 17 pesticides in 41 households in central New York State that represented farm, rural, and urban houses. The results revealed the presence of some pesticides in family room carpet; furniture and even settled dust (Obendorf, Lemley *et al*. 2006) [7].

The results of this study show that by the use of T-Test, a significant difference could be seen in the residue of Sevin and Diazinon in water samples. The most insecticides residue was observed in station 1, which is near the farmlands and the lowest insecticides residue was observed in station 3, which is in the end part of rivers (Table 1). Also we revealed that the insecticides residue decreases from the first day to 45th day (Table 1); although in some cases we saw contraries in some samples. The reason of these contraries may be the spraying of the farmlands near station 2 or station 3 before sampling.

5. Acknowledgments

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