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Determination of Carbendazim Fungicide and Oxymatrine Insecticide Residues in the Soils of Four Agriculture Stations in Basrah Governorate by HPLC

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Abstract
The current study was concentrated on the determination of carbendazim fungicide and oxymatrine insecticide residues in four agriculture stations at Basrah governorate ; Abu Al-Kaseeb,Al-Hartha, Al-Zubair and ShattAl-Arab. High pressure liquid chromatography (HPLC) was used to determinedthe carbendazim residues which range between (0-3.05µg/g) .The highest value 3.05µg/g was recorded in Shatt Al- Arab station while the lowest value was recorded in Al-Zubair station.oxymatrine residues range between (0-1.89 µg/g) and the highest value 1.89µg/g was recorded in Al-Hartha station while the lowest value was recorded in Shatt Al- Arab station.There is no study on carbendazim and oxymatrine residues in soil, so this study was the first of its kind in the region which could be used as a baseline study for incoming study.
Keywords: Oxymatrine, Carbendazim, Basrah soil, HPLC.

Introduction

Environmental pollution is one of the serious predicaments of the modern world, currently a large number of pollutants and waste are eliminated to the environment(Helal & Abo-Seoud, 2014).

Among these substances are chemical pesticides ,the widespread use of pesticides for agricultural and non-agricultural purposes has resulted in the presence of their residues in various environmental matrices, such as soil, water and air and cause harmful effect to both human and environment and their bioaccumulation takes place via food chain (Azmi *et al*., 2009 ; Diez, 2010).

fungicides are a vast groups that used to control a wide range of fungal diseases in agriculture and on of the Carbamates fungicides applied is carbendazim.Carbendazim is a systemic benzimidazole fungicide and used to control abroad range of diseases on arable crops (e.g., cereals), fruits, vegetables ,ornamentals and medicinal herbs and it is also a main metabolic product of some other systemic fungicides, such as benomyl and thiophanate-methyl. Table 1(Singh, 2016).

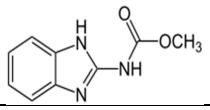
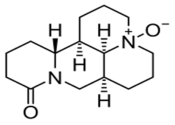
Due to slow degradation carbendazim persist in soil for along time (6-12 months). It classified as a hazardous category of chemicals by WHO carbendazim is a major pollutant detectable in food, soil and water, extensive and repeated use of it induce acute and delayed toxic effects on humans, invertebrates, aquatic life forms and soil microorganisms.(Lim *et al*., 2014;Singh, 2016).

Botanical pesticides are naturally occurring insecticides derived from plants that have been formulated specifically for their ability to control insects and used universally from commercial agriculture to institutions, homes and landscapes,plant derived materials such as from rotenone, pyrethrum, sabadilla, ryania, etc., (Sarwar, 2015).

Oxymatrine it is a new vegetable insecticide with mode of action contact and stomach. It is a tetracycloquinolizindine alkaloid derived from *Sophora flavescens* Aiton (Leguminosae) roots, oxymatrine, with trade name(Levo 2.4) are used to control dubas bug and other insect that threat vegetables. Table 1 (Gholami and Sadeghi, 2016).

Basrah was one of big city in Iraq and there is many use of pesticides in agriculture areas at it and there is many researchers dealing with the study of pesticides residues in the basrah governorate but there is no study on carbendazim and oxymatrine residues in soil ,so this study is afirst of its kind in the area which could be as a base line study for incoming study.

Table 1 : Chemical & physical properties of carbendazim and oxymatrine pesticides.

Pesticides	Chemical name	Trade name	Chemical formula	Mol.wt. (g/mol)	Mode of action	Chemical structure
carbendazim	Mercarzole, Carbendazole	Bavistin,	$C_6H_9N_3O_2$	191.18	Systemic Fungicide	
oxymatrine	Matrine oxide, MatrineN- oxide	Levo 2.4	$C_{15}H_{24}N_2O_2$	264.37	Contact & stomach poison	

Materials and methods

Collection of samples

Soil samples were collected from 4 agriculture areas in basrah city with farming activities and a history of pesticides contamination for several years(Fig 1) .These areas are Al-Hartha, Abi-Al-Kaseeb, Al-Zubair and Shatt-Al-Arab .Twenty four soil samples were collected from August 2016 to January 2017 . According to the method of Letcher & Powell(2001) six soil samples form each agriculture area were collected. The surface soil (5-15 cm) was removed by using sterile spatula , placed in polyethylene bags ,transported to the laboratory and stored at 4^0C until analysis.

Extraction and clean up of pesticides residues from soil samples

The extraction procedure employed in the present study was based upon the procedure described by (EPA,2007) and (Al-Ali,2012).

Soil samples, exactly 25 g of grind dry sample was placed in a pre-extracted thimble and soxhlet extracted for 24 hours with 200ml of dichloromethane then the extract was concentrated with rotary evaporator to 2ml .The extracts were cleaned by transferring to separating funnel containing 25 ml of the n- hexane and 25 ml acetonitrile at the ratio of 25:25 to remove the residual fat, the funnel was shaked well several times and left to settle down until two layer are formed.,the upper layer was taken and the lower layer was canceled. The concentrated hexane layer passed through a separating column (30*2.5 cm) was filled with glass wool (for installation of classes) 1-2 cm of anhydrous sodium sulfate(to remove water) then 3cm of silica gel 100-200 mesh was added then 5cm of florisil and 1cm of sodium sulfate then mixed solution of diethyl ether / hexane at the ratio of 6%,15% and 50% respectively was passed through the column separately then the passing solution was collected and concentrated to 2ml by rotary evaporator and used to measure the concentration of carbendazim and oxymatrine pesticides by HPLC.

Standard solution and analysis of pesticides by HPLC.

Carbendazim and oxymatrine pesticides were obtained from Toronto company, Canada. Stock solution of carbendazim fungicide of the concentration 2000 µg/ml $^{-1}$ was made by dissolving 2 mg of carbendazim in 1 ml DMSO while oxymatrine stock solution was prepared by dissolving 3mg of oxymatrine in 1 ml ethanol.(Fig 2&3).

HPLC device type Shimadzu LC solution equip was used for the identification and quantification of these pesticides .The condition that HPLC operated were : the column was C18(250mm, 25cm,4.6 mm) the mobile phase was acetonitrile /water (90:10 v/v) flow rate was 0.5 ml/min ,the injection volume was 20µl,the wavelength of the UV/ visible detector was 254 nm .

Fig 1: The study area

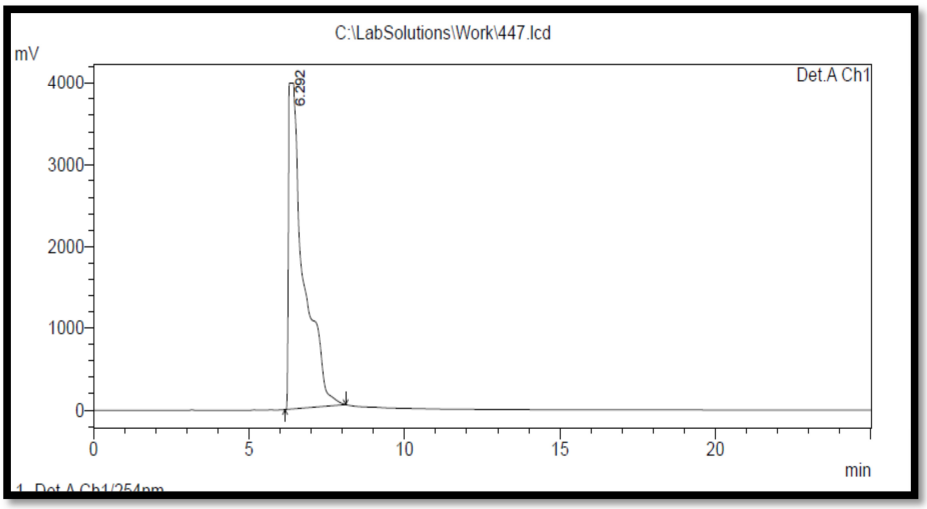


Fig 2: Carbendazim standard

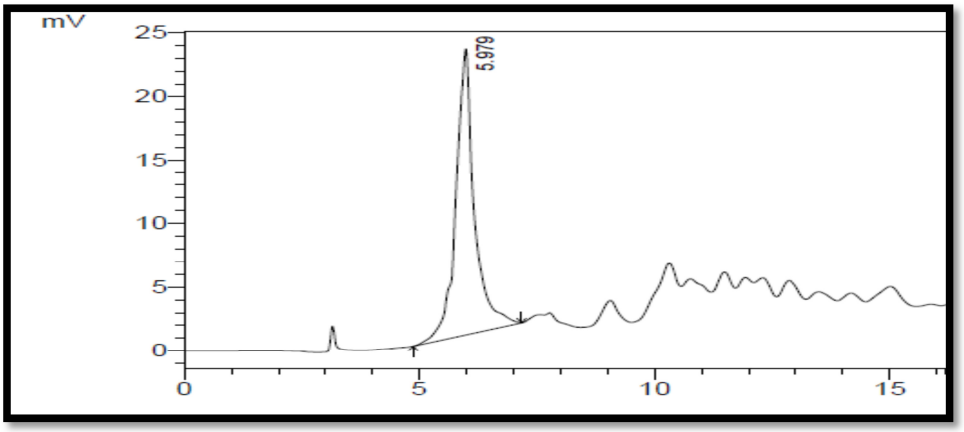


Fig3: oxymatrine standard

The Results

1. Determination of carbendazim fungicide residues in soil.

The concentration of carbendazim residues ranged from (0.466 µg/g)at station 3 to (3.051µg/g) at station 4 (Table 2,Fig 4) the highest concentration(3.051µg/g) was recorded in station 4 whereas the lowest concentration (0.466 µg/g) was recorded in station 3, the residues of carbendazim appeared in all stations samples .

ANOVA test- one way shows non- significant differences (p>0.05)between the soil of different stations in the content of carbendazim pesticide. The highest mean value (1.615 µg/g)was found at st.1 while the lowest mean value (0.931 µg/g/dry weigh) at st.4, the mean values of st.3 and st.2were(1.291, 1.200 µg/g/dry weigh) respectively.

2. Determination of oxymatrine insecticide residues in soil.

oxymatrine residues ranged from (0.0002 µg/g) at station 4 to (1.895µg/g) at station 2 (Table 3, Fig 5) the highest concentration(1.895 µg/g) was recorded in station 2 whereas the lowest concentration (0.0002µg/g) was recorded in station 4.

ANOVA test- one way shows non- significant differences (p>0.05) between the soil of different stations in the content of oxymatrine pesticide. The highest mean value (0.582, 0.510 µg/g)were found at st.1 and st.2 respectively while the lowest mean value (0.282, 0.209µg/g) at st.3 and st.4 respectively .

Table 2: The mean and the range of the fungicide carbendazim in the study stations .

No.	Name of station	Range µg/g	mean	±SD	±SE
St.1	Abu-Al-Kaseeb	0-2.67	1.615	0.961	0.392
St.2	Al-Hartha	0.58-1.92	1.200	0.480	0.196
St.3	Al-Zubair	0.46-2.09	1.291	0.555	0.226
St.4	Shatt-Al-Arab	0-3.05	0.931	1.215	0.496

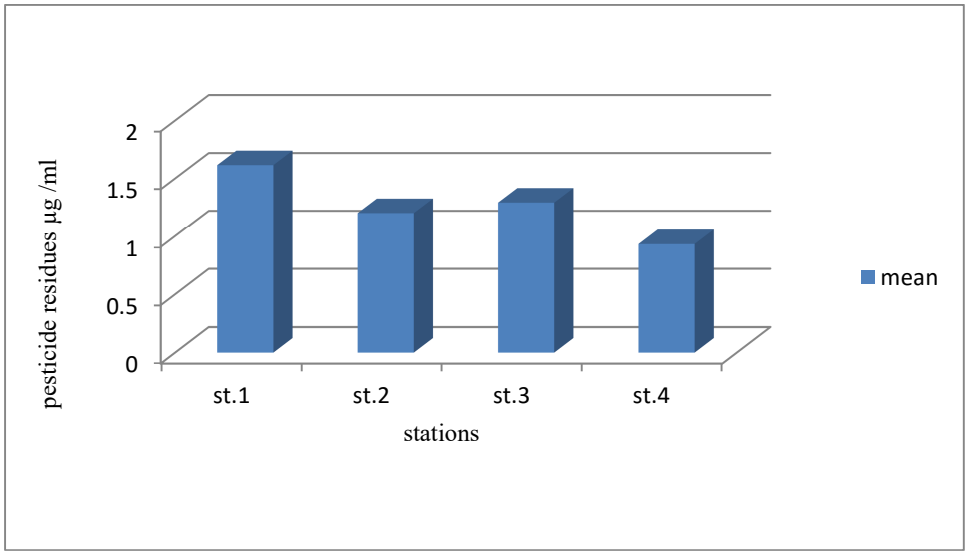


Fig 4: The mean value of the fungicide carbendazim in the study stations.

Table 3: The mean and the range of the insecticide oxymatrine in the study stations.

No.	Name of station	Range µg/g	mean	±SD	±SE
St.1	Abu-Al-Kaseeb	0-1.836	0.582	0.757	0.309
St.2	Al-Hartha	0-1.895	0.510	0.822	0.335
St.3	Al-Zubair	0-1.688	0.281	0.688	0.281
St.4	Shatt-Al-Arab	0-0.749	0.209	0.330	0.135

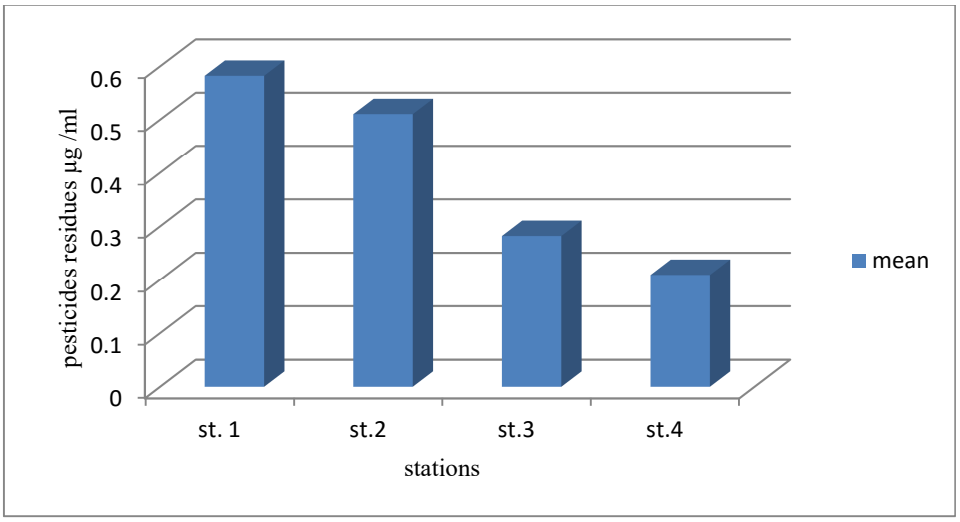


Fig 5: The mean value of the insecticide oxymatrine in the study stations

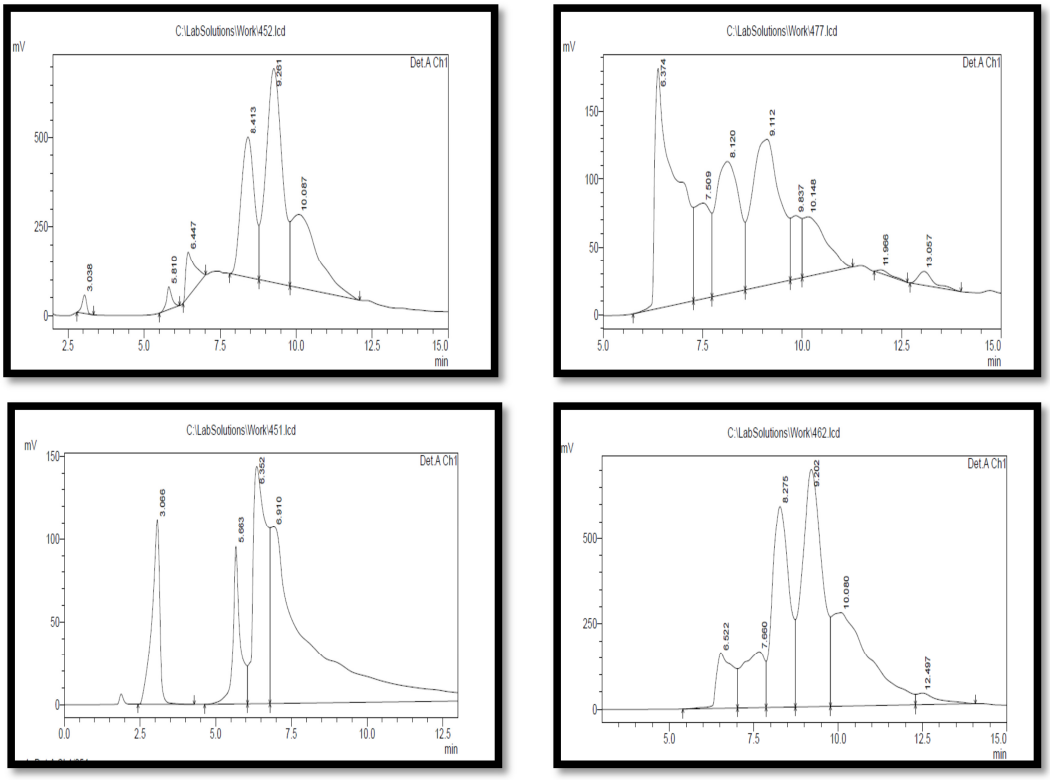


Fig 6 : HPLC chromatograph of some soil samples.

Discussion

Determination of pesticides residues in soil

The present study showed clear concentration of each pesticides in the soil which appeared very clearly in all soil samples.

The results showed that the mean of carbendazim residues ranged from 0.931 in Shatt-Al-Arab station to 1.615 in Abu-Al-Kaseeb while Al-Hartha and Al-Zubair recorded the mean 1.200 and 1.291respectively , Abu-Al-Kaseeb station recorded the highest mean value 1.615 and this attributed to the increase used of this pesticide to eliminate fungal diseases and led to accumulate of its residue in soil or may be due to the non-observance of instructions when using pesticides where each pesticide has a recommended safety period in order to remove

their effect from the soil in addition to the illegal entry of these pesticides to the country by traders and the lack of control of its entry, it reach easily to the farmers which used it each 2-5 days and led to their accumulation in the soil (Khammas and Ahmad 2016).

While the lowest mean value 0.931 was recorded in Shatt Al-Arab station and this may be because carbendazim pesticide may be was not used during the study period.

The same conclusion have been arrived by Wenjun *et al*.,(2004) and Qin *etal*.,(2016) in soil of china when they found the concentration 0.025mg/kg and 0.02mg/kg respectively.Vega *et al*.,(2005)were also recorded (0.1-5.5µg/l)in the river located in Spain which characterized by extensive agriculture farms around it, but Nemeth-Konda *et al*.,(2002)were found the same conclusion of our results ,they recorded the residual concentration(0.96-19.68mg/kg) of carbendazim on Hungarian brown forest soil.

By the other hand the residual concentration of oxymatrine insecticide was ranged from (0-1.89 µg/g) and the highest mean value was also recorded in Abu-Al-Kaseeb 0.582 and this may be because the station have a high agriculture activity with the increase use of pesticides which led to remain the residue in the soil , the pesticide may disrupt the activities of soil microorganisms that degraded it, the difficulty of its to the biodegradation process because it consist of five rings that gave it stability in the soil(Shao *et al*.,2014) .

These results were may be similar to that obtained by Yanget *et al*., (2010) in a china soil which ranged (0.045-0.187mg/kg) and also to Baofeny and Jinwang (1998).

Conclusion

These pesticides (oxymatrine and carbendazim) have been found in different concentration in the soil of Basrah governorate due the increase use of pesticides by farmers and this led to remain their residues in soil and cause harmful effect to human and environment , the carbendazim fungicide was the most occurrence in all soil samples than the oxymatrine insecticide .These study could be used for coming researchers in the future.

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