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Original Research Article

Pesticides residues in retail meat and offal

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ABSTRACT

Pesticides are used extensively especially in developing countries like Egypt to control pest either in animal or in agriculture, which may lead to harmful residues in foods of animal origin. The current study was conducted to estimate the residue level of OC and pyrethroid in 320 beef and sheep samples (160 each) collected from different shops at Beni-Suef governorate during summer and winter season. The collected samples were liver, muscle, kidney, and fat (80 each; 40 from each animal species). Among fourteen organochlorine compound examined, only Alpha HCH was detected in samples of cattle and sheep collected through winter season in a level below the MRL, while through summer season, only Alpha HCH and Delta HCH were detected in sheep samples in a level below the MRL. Pyrethroid pesticides residues represented by cypermethrin, deltamethrin, Es-fenvalerate, permethrin were not detected through winter season, while they were detected in muscles of cattle and fat of sheep through summer season, while Labdacyhalothrin, bifenthrin, cyfluthrin, Meothrin were detected in most of examined samples from different species through winter and summer seasons, most of these results revealed higher mean level than the maximum residue limits. From these results most of OC could not be detected may be due to these compounds not used since 1970, on other hand pyrethroid it still used nowadays in Egypt either in agriculture or as spray in animals to control ectoparasites spatially in summer season.

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Introduction :

Meat is an excellent source of high nutritional protein and fat contents as essential amino acids and high source of minerals as Heme iron. Therefore, the meat must be derived from food animals free from harmful residues. Pesticides residues may be used extensively in developing countries like Egypt to control pest either in animal or in agriculture in many African countries. OCPs are characterized by their bioaccumulation in the environment, especially in the food chain, where they find their way into the human body. Pesticides are used to control pests in plants which transmit to human through food chain. Moreover, pesticides are used as spray for animal to control ectoparasites, therefore bioaccumulation of pesticides residues in food of animal origin like meat, milk, and fish (Benbrook, 2002), (Lehotay, 2005) and (Qiu, 2005). Pesticides are classified into 4 major groups as organochlorines (OC), organophosphates (OP), carbamates and synthetic pyrethroids (SYP). Despite ban imposed by WHO on use of certain Organochlorine Pesticides (OCP), some of these pesticides are still used in limited quantity in many developing countries including India for agricultural and public health programmes. Nine OCP have been targeted for global elimination under the signed Stockholm Convention on persistent organic pollutants. OCP are highly persistent as OP and PYS are less persistent (Moye, 1981). Organochlorine pesticides are widely used by farmers due to their broad-spectrum activity and their effectiveness. Organochlorine pesticides such as lindane, DDT and endosulfan are also used to control ectoparasites of pets and farm animals (Ntow et al., 2006). The extensive presence of organochlorine

pesticide residues (OCPs) Organochlorine in food has ascended due to their extensive agricultural application and industrial production in the environment. A large number of pesticides may possibly be used in the production of agricultural crop, leading to indirect exposure of animals through feed and the possible for residues in animal products (MacLachlan and Bhula, 2008). High thermodynamic stability and lipid solubility of Organochlorine (OCPs) lead to bind them to lipid components in animal tissues, where becoming a major route of human exposure when consumed as food, causal to extra than 90% of the daily exposure to these compounds, Organochlorine have a high toxic effects and persistence in the environment, posing considerable hazards. The problem becomes more serious when bioaccumulation of these lipophilic compounds (such as lindane and heptachlor), (Voldner, 1995) and (Zumbado et al., 2005)

Pest control in intensive agriculture involves treatments with a variety of synthetic chemicals as pyrethroid pesticides. These chemicals can be transferred from plants to animals via the food chain. So that contamination routes lead to bio-accumulation of pesticides in food products of animal origin as fish, offal, meat, milk, fat and eggs, Also Pyrethroids were ranged from non-polar to low-polarity lipophilic compounds. Due to their metabolism in animals, they tend to bio-accumulate in lipid compartments, becoming a possible source of human exposure through foodstuffs that reported by (LeDoux M 2011).

Pyrethroids are synthetic insecticides derived from naturally occurring pyrethrin compounds, which combine

safety, effectiveness and low environmental hazard. Pyrethroids are preferred above organochlorine, organophosphates and carbamates as these have low toxicity, high efficiency and easy biodegradability. There are seven types of pyrethroids (bifenthrin, lambda-cyhalothrin, deltamethrin, cyfluthrin, fenvalerate, cypermethrin, and permethrin). The extensive use of pyrethroids in plant safety and farming animals can lead to the transfer of residues to animal milk, tissues, honey, and eggs. The veterinary applications for these types of pesticides include sprays, ear tags, dips and spray. As an importance of their chemical, physical properties and toxicological profile, pyrethroids are to be monitored in food of animal origin, in order to control food quality and prevent any possible risk for human health that reported by (Niewiadowska et al 2010, Sharaf et al., 2010).

Therefore, this study is planning to monitor and determine organochlorine and pyrethroid pesticide residues in retail meat and offal (muscle, liver, kidney and fat) of beef and sheep, collected from different butcher shops in Beni-suef Governorate.

Material and method: A total 320 Samples were collected from 80 cattle and sheep carcasses (40 each) from different butcher shops in BeniSuef governorate in the period between January and July. For each animal, 160 samples were collected; 40 each of meat, kidney, fat and liver for cattle and 40 each fat, muscle, liver and kidney for sheep. Each sample put in polyethylene bags. The samples were immediately transported to the laboratory in an insulated box and were stored at -20c° until analysis.

Reagents: Solvents: petroleum ether, n-Hexane, diethylether, Acetonitrile

Sodium sulfate anhydrous: pesticide residue mark (washed with n-hexane for numerous time then dried in an oven and kept in a glass Stoppard container) and were bought from El Nasr pharmaceuticals and chemical Company

Adsorbents: Florisil, (60-100) mech, pesticides reagent mark Sigma (USA), Florisil was stimulated in an oven at 130°C for (12 hours) .

Sodium chloride: Reagent grade purchased from El Nasr pharmaceuticals and chemicals Co .

Sample preparation: The samples were washed several times with deionized water to clean them from any sediment. The meat samples were identified and given identification codes .

***Extraction procedure of organochlorine was carried out according to the method of(AOAC Official method 970.52 (1996).**

-Extraction: fifty grams of the sample were blended with 100 g of anhydrous sulphate and 150 ml petroleum ether for 2 minutes then the extract was poured through filter funnel into a suction flask .

The flask was put in rotary evaporator till complete evaporation and formation of dry fat film .

-Clean up by acetone partitioning: the dry fat film mix with 15 ml petroleum ether and 30 ml acetonitril saturated with petroleum ether. Vigorously shake till formation of two layers then the lower one was drained into one liter separatory funnel containing 650 ml distilled water, 40 ml saturated NaCl solution and 100 ml petroleum ether well shake till separation of layers then drain the upper

one into 250 ml flask through filter funnel containing sodium sulphate anhydrous and conditioned with petroleum ether. Put the flask in rotary evaporator till complete dryness

Clean up by florisil column: Activated florisil was put in chromatography column till 10cm height, then topped with 1cm sodium sulphate anhydrous and condition them with 50 ml petroleum ether

-Two grams sodium sulphate anhydrous was added with 20 ml petroleum ether to obtained dry film then pass them via prepared florisil

-The column was eluted 3 times at the same rate with 20 ml of 6, 15 and 50% diethyl ether and petroleum ether, respectively and concentrate the elute in rotary evaporator till obtaining dry film .

-Two ml n-hexane was added to the dry film then transfers it into 2 ml auto sampler vial and transports it to GC for analysis.

****The extraction method for analysis of pyrethroid pesticides in animal meat and offal's by GC according to (Bordet et al 2002).**

standards solutions: cyfluthrin, 96% purity, bifenthrin, 95.5% purity, lambda-cyhalothrin, 98% purity, deltamethrin, 99% purity, cypermethrin 94% purity, permethrin, 94.5% purity and fenvalerate, 98% purity.

All of them were obtained from **Dr. Ehrenstorfer (Germany)**.

The solution was mixed at the level of 1 µg/mL prepared by dilution of specific standard in n-hexane and kept in the dark at a temperature under - 18°C for no extended than six months. The working standard solutions 0.01, 0.05, and

0.10µg/mL were prepared by serial dilutions of the mixed solution with (n-hexane).

Extraction: Ten grams sample of ground muscles was placed in centrifuge tube with 100 ml of acetone-petroleum ether mixture (1:1,v/v).

-The sample was homogenized and then centrifuged at 3,000 rpm, 2°C for 15 min . The clear solvent layer was poured through anhydrous sodium sulphate layer .The remaining matrix, another 100 ml of acetone-petroleum ether mixture (1:1, v/v) was added and the sample was homogenized and centrifuged blew the similar conditions.

-The flask was put in rotary evaporator till complete evaporation and formation of dry fat film .

-Cleanup: fat were dissolved with (2x2 ml) of acetonitrile dichloromethane mixture (3:1, v/v) and vortex mixed for 10 second

-Extracts were centrifuged (3,000 rpm) for 15 min at -12°C.The supernatant was moved to a new tube and the extraction from fat was repeated in the similar method.The residue was dissolved in (2x2 ml) of n-hexane and transferred to the chromatographic column packed with 10 g of Florisil. The pyrethroids were then mixed with 200 ml mixture of n-hexane diethyl ether (3:2 v/v) and the mixture was evaporated to (1 ml) on a rotary evaporator.The final extract was evaporated to dry blew a mild stream of nitrogen and then dissolved in 2–5 ml of n-hexane for Gas-Chromatography (GC) analysis.

Conditions and apparatus: Quantitative determination of organochlorine and pyrethroid: The extracts were injected into gas chromatography apparatus

(Agilent GC model 6890) equipped with an Ni⁶³ electron capture detector (ECD), capillary column of 30m length, 0.32mm internal diameter, and 0.25µm film thickness. The oven temperature was programmed from an initial temperature 160°C (2 min hold) to 280°C at a rate of 5°C/ min and maintained at 280 and 320°C, respectively. Nitrogen was used as carrier gas at flow rate of 4ml / min and injection volume of 1µl.

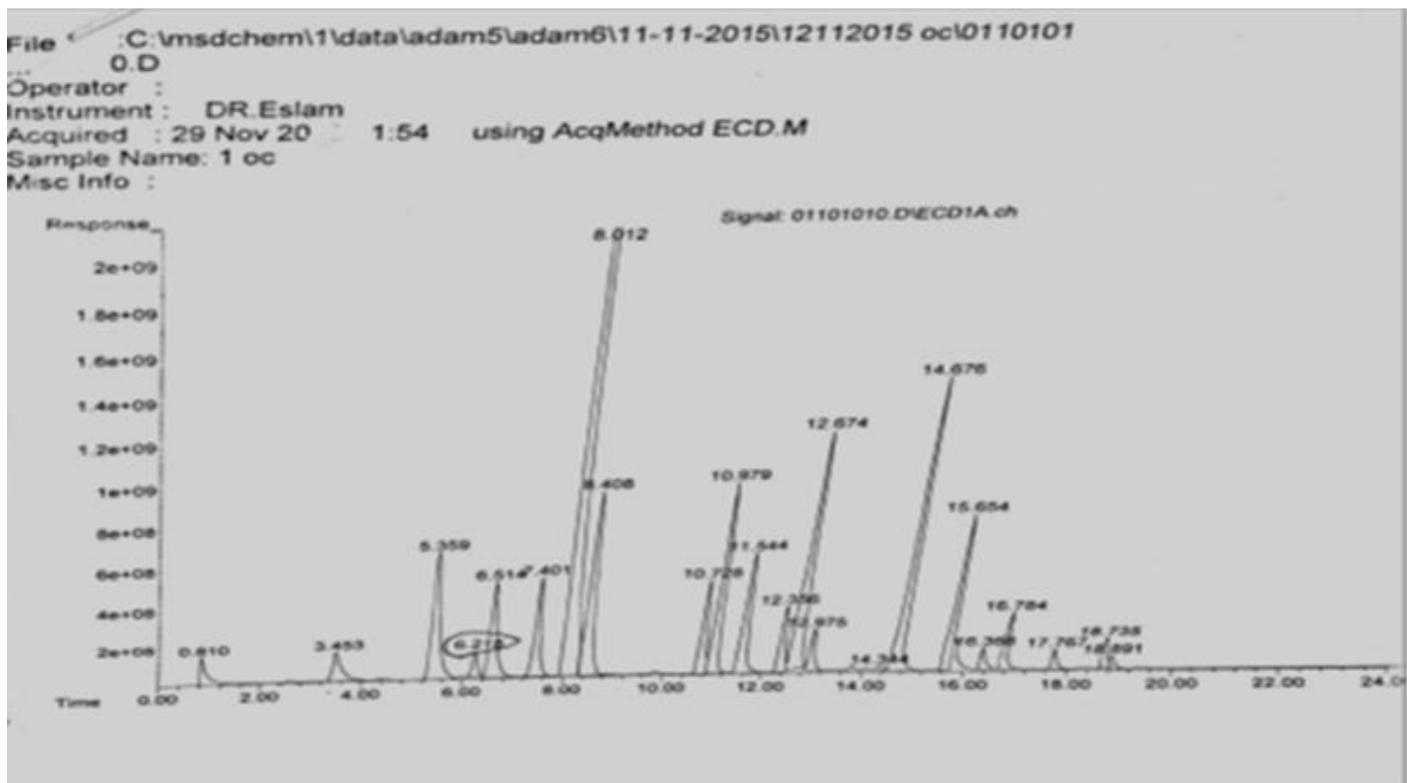
Results

Organochlorine and pyrethroid pesticides have been widely used and become a worldwide concern due to their persistence, bio-accumulative potential, chronic toxicity, and potential negative impacts on humans and wildlife (**UNEP 2001**). It is known that most of the total intake of pesticides residues by human beings is through the food chain (**Martinez et al., 1997**).

Levels of Organochlorine residues in beef and sheep tissues through winter and summer seasons:

	Winter								Summer							
	Cattle				Sheep				Cattle				Sheep			
	Fat	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat	Muscle	Liver	kidney	Fat	muscle	live r	Kidney
Methoxychlor	ND	ND	ND	ND	ND	ND	ND	ND	ND							
P,p'-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND							
P,p'-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND							
P,p'-DDE	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Gamma Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Heptechlor Epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Delta HCH	ND	ND	ND	ND	0.419 ±0.047	0.290 ±0.048	0.107± 0.038	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gamma HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND							
Alpha HCH	0.390 ±0.041	0.253 ±0.025	0.155 ±0.016	0.106 ±0.016	0.504 ±0.049	0.457 ±0.041	0.377± 0.044	0.348± 0.043	ND	ND	ND	ND	0.081±0. 044	0.359 ±0.079	0.145± 0.061	0.184±0.063

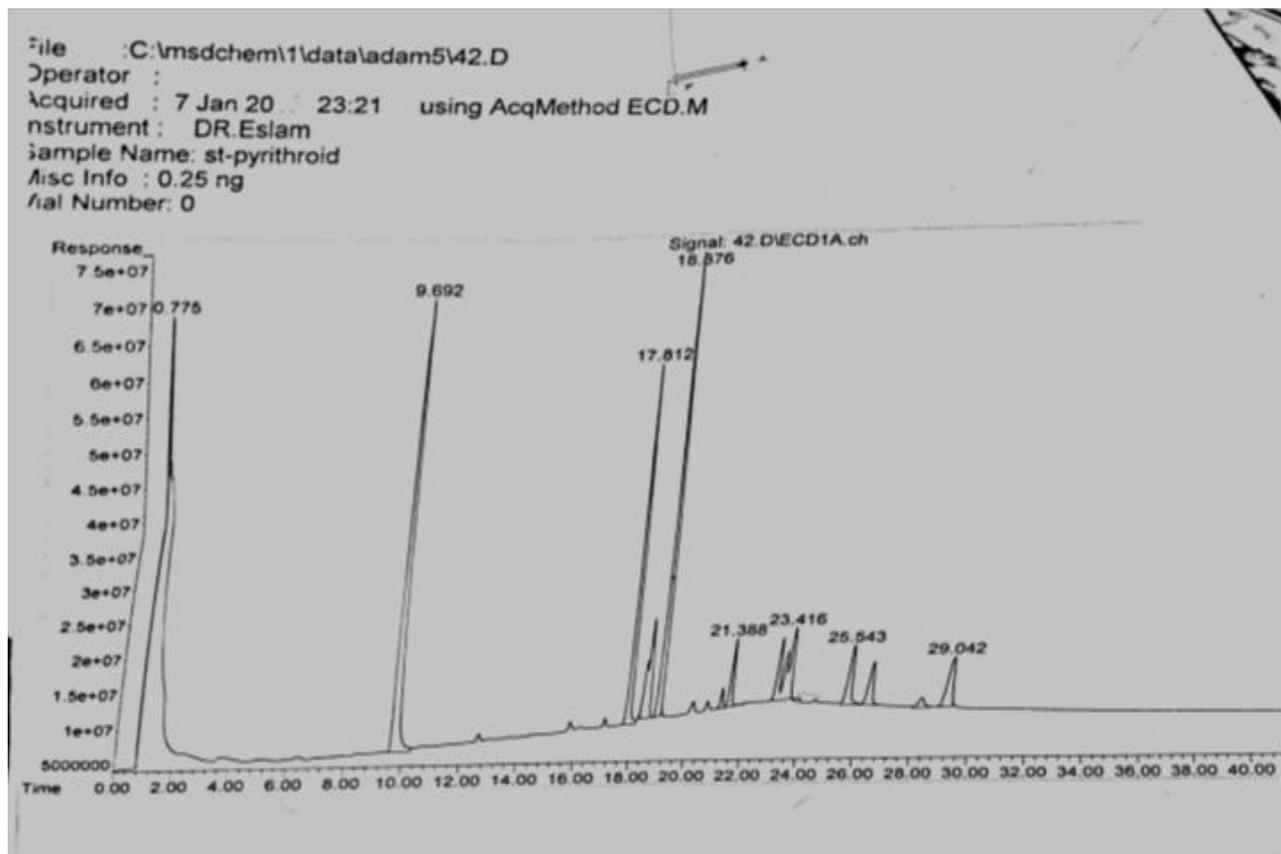
Chromatographic stranded of organochlorine



Levels of Pyrethroide residues in beef and sheep tissues through winter and summer season($\mu\text{g}/\text{kg}$ body weight):

Pyrethroid compound	Winter								Summer								
	Cattle				Sheep				Cattle				Sheep				
	Fat	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	Fat	Muscle	Liver	Kidney	
Deltamethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.066	ND	ND	ND	ND	ND	ND	ND
										± 0.046							
Es-fenvalerate	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.0002	ND	ND	0.159	ND	ND	ND	ND
										± 0.0001			± 0.110				
Cypermithrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.0003	ND	ND	39.872	ND	ND	ND	ND
										± 0.0002			± 28.028				
Permethrin	ND	ND		ND	ND	ND	ND	ND	ND	0.0004	ND	ND	ND	ND	ND	ND	ND
										± 0.0003							
Labdacyhalothrin	0.149	0.231	0.201	0.291	0.467	0.022	0.09	0.008	ND	0.0003	ND	ND	ND	ND	ND	ND	ND
	± 0.042	± 0.048	± 0.061	± 0.087	± 0.445	± 0.013	1 ± 0.052	± 0.004		± 0.0002							
Fenpropathrin	ND	ND	ND	ND	0.193	0.006	0.25	0.004	1.076	0.837	1.643	1.643	2.657	1.874	0.890	0.536	
					± 0.089	± 0.004	1 ± 0.250	± 0.004	± 0.313	± 0.274	± 0.332	± 0.349	± 0.910	± 0.504	± 0.251	± 0.149	
Meothrin	0.008	ND	ND	0.0004	ND	0.007	0.00	0.004	ND	0.0004	ND	0.0007	ND	ND	ND	ND	ND
	± 0.006			± 0.0003		± 0.003	1 ± 0.0003	± 0.002		± 0.0003		± 0.0005					

Chromatographic standard of pyrethroid



Discussion:

Organochlorine residues in different organs in cattle and sheep through winter and summer seasons:

The mean values of Alpha HCH in examined cattle sample during winter season were (0.390 ± 0.041 , 0.253 ± 0.025 , 0.155 ± 0.016 and 0.106 ± 0.016) for fat, muscle, liver and kidney, respectively, (Table 1). While the other thirteen compounds of OC were not be detected.

As for sheep, these figures in winter were (0.504 ± 0.049 , 0.457 ± 0.041 , 0.377 ± 0.044 and 0.348 ± 0.043) for fat, muscle, liver and kidney, respectively, (Table 1). On other hand, the mean residues of Delta HCH were (0.419 ± 0.047 , 0.290 ± 0.048 and 0.107 ± 0.038) for fat, muscle and liver, respectively, while it could not be detected in all kidney samples. While through summer season only alpha HCH metabolite was detected in examined samples of sheep with mean value of 0.081 ± 0.044 , 0.359 ± 0.079 , 0.145 ± 0.061 and 0.184 ± 0.063 , for fat, muscle, liver and kidney, respectively. All positive samples were within the permissible limits Codex Alimentarius Commission., (1999) set at (2000ppb for total HCH) who found These results agree with Hernandez et al. (1994) found that o-HCH, B-HCH, heptachlor epoxide and p,p DDE) in samples of cattle varied from 0.0006 to 0.0541 $\mu\text{g}/\text{kg}$. And also Kiranmayi et al (2016) mentioned that the contamination levels of Organochlorine (OC) pesticides in beef and mutton samples were 0.074 and 0.039 ppp for α HCH (hexachlorocyclohexane), 0.058 and 0.046 $\mu\text{g}/\text{kg}$ of β HCH, 0.081 and 0.058 $\mu\text{g}/\text{kg}$ of γ HCH, 0.051 and 0.022 $\mu\text{g}/\text{kg}$ of δ HCH, respectively. On other hand FAO/WHO (2008) reported that the mean levels of α HCH and Lindane during the rainy

season exceeded the MRL (100.00 $\mu\text{g}/\text{kg}$) for cattle fat. Moreover European Union (2009) set the residual level of α HCH in bovine liver and kidney tissues were 207.63 and 307.08 $\mu\text{g}/\text{kg}$, respectively, which exceed 1.0 and 1.5 times, maximum residue limit (MRL). Farther more Walker (2009) linden was in meat samples imported from India with limits that higher than the MRL. Most of OC could not be detected may be due to these compounds not used since 1970. The low level of detected HCH isomer may be attributed to its long persistence in the environment.

Pyrethroid pesticides residues in different organs (fat, muscle, liver and kidney) of cattle and sheep through winter and summer seasons:

Deltamethrin, Es-fenvalerate, Cypermethrin and Permethrin were not detected in cattle and sheep samples during winter season, while in summer they were detected in muscle of cattle with a mean value of 0.066 ± 0.046 , 0.0002 ± 0.0001 , 0.0003 ± 0.0002 and 0.0004 ± 0.0003 , respectively. As for sheep only Cypermethrin and Permethrin was detected in fats samples with a mean value of 0.159 ± 0.110 and 39.872 ± 28.028 , respectively (Table 2). Regarding Labdacyhalothrin, it was detected in all samples of cattle in winter season with a mean value were 0.149 ± 0.042 , 0.231 ± 0.048 , 0.201 ± 0.061 and 0.291 ± 0.087 for fat, muscle, liver and kidney, respectively. On other hand it was detected in muscle of cattle only through summer season with a mean value 0.0003 ± 0.0002 .

Considering value of Fenprothrin were detected in sheep samples through winter season with mean value 0.193 ± 0.089 ,

0.006±0.004, 0.251±0.250 and 0.004±0.003 for fat, muscle, liver and kidney, respectively, on other hand it not detected in cattle samples through winter season. Farther more its level in cattle and sheep tissues through summer season was (1.076±0.313, 0.837±0.274, 1.643±0.332 and 1.643±0.349) and (0.193±0.089, 0.006±0.004, 0.251±0.250 and 0.004±0.003) for fat, muscle, liver and kidney, respectively. Meothrin was detected in fat and kidney of cattle through winter season with mean value 0.0008±0.0006 and 0.0004±0.0003, respectively. And also in sheep it was detected in muscle, liver and kidney not detected in fat these represented with mean value 0.007±0.003, 0.003±0.001 and 0.004±0.002, respectively. Furthermore in summer season it was detected in muscle and kidney of sheep only with mean value 0.0004±0.0003 and 0.0007±0.0005, respectively. Not be detected in cattle samples. All positive examined samples were within MRL European Commission (EC),(2005) stated MRLs in meat of different species of farm animals for following pyrethroids have been established: bifenthrin (50–100 µg/kg), cyfluthrin (50 µg/kg), lambda-cyhalothrin (20–500 µg/kg), cypermethrin (50–200µg/kg), deltamethrin (30–500 µg/kg), fenvalerate (20–200 µg/kg), and permethrin (50 µg/kg).

While the examined sheep samples through winter season showed that 15% of fat and liver samples were higher than permissible limits for fenpropathrin. While in examined cattle samples through winter season labdacyalothrin was detected higher than permissible limits in 10%, 20%, 15% and 25% of examined samples for fat, muscle, liver and kidney, respectively. While through summer season fenpropathrin was

detected in examined cattle sample with limits higher than permissible limits in 50%, 55%, 70% and 60% of examined samples for fat, muscle, liver and kidney, respectively. Also deltamethrin was exceeded permissible limits in 5% of examined muscle samples. US EPA (2004) stated that Beta-cyfluthrin residue in cattle fat samples with mean value was 20µg/kg. EL-Shemi and Abou EL-Ella (2015) found that mean residual levels of cypermethrin in tissues of male sheep were 64.2 ±3.05, 12.2 ±0.20 and 62.4 ±3.03 for kidney, liver and muscle, respectively. Moreover Khashan(2016) reported that the levels of deltamethrine pesticide residues in cattle meat samples during October and March were (0.66±0.16µg/kg), and (0.28±0.08µg/kg), respectively. And also Abdu-rrahman (2016) mentioned that mean of DMT (deltamethrin) residues in sheep meat collected from different locations of Sulaimaniya province during June and July was (0.50±0.08µg/kg), (0.99±0.18µg/kg), respectively. Stefanelli et al. (2009) recording higher results for pyrethrin in sheep muscle (100 µg/kg). pyrethroid it still used nowadays in Egypt either in agriculture or as spray in animals to control ectoparasites spatially in summer season.

Conclusion: From the present data, it could be concluded that meat and tissues from native beef and sheep contained trace level of organochlorine represented by Alfa HCH and Delta HCH in most cattle and sheep tissues through winter season not through summer season, these results due to cattle feed on green plant through winter which have persistent organochlorine in soil, while through summer season cattle feed on dry ration. And also Pyrethroid pesticides were detected in most samples through summer and winter seasons with a value

below MRL according to European Commission (EC),(2005). pyrethroids were commonly used because it's safety for animal and human because it breaks rapidly; but excessive dose cause severe problems for human and animals .

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