Determination of Environmental Pollution by Pesticides on Raw Buffalo Milk in Assiut Governorate, Egypt

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Abstract
From September 2022 to October 2023, 30 random samples (1 kg each) of raw buffalo milk were collected from the same location (a village known for producing large amounts of milk near the city of Assiut, Egypt) it was done. Each sample was divided into five subsamples. The first subsample sample was kept as a control, and the second subsample was pasteurized at (72 EC/15 s) and cooled immediately. The third subsample was autoclaved (121 EC/15 min) and rapidly cooled. The fifth subsample was sterilized (121 °C/15 min), and then the fourth subsample was fermented with a yogurt starter culture (0.05 g LG1) at 43 °C/2.5 h and cooled immediately. The fifth subsample was cured with animal rennet (2 ml LG1). Heat at 40 °C until complete solidification, separating the quarks; the curd was cutting, and the whey was depleting. All milk, yogurt, and cheese samples were extracted and purified. Detection and identification of pesticide residues by gas chromatography-mass spectrometry (GC/MS) was studied. As a result, the concentrations of all organ phosphorus and organ chlorine pesticides in raw buffalo milk exceed the maximum permissible MRL limits for these pesticides set by the European Commission (EC) and are subject to heat treatment, fermentation, and coagulation. The process was shown to degrade pesticide residues from raw buffalo milk. Processing raw milk involves heat treatments such as pasteurization and sterilization and exposure to microorganisms during production and storage. In most cases, lactic acid bacteria growth leads to reduced pesticide levels.

1. Introduction

Milk and dairy products are considered one of the most important balanced and healthy foods for all stages of human life, as they are high in important minerals such as calcium and sodium, as well as amino acids and free fatty acids (Ayoub et al., 2012). It also contains important nutrients such as carbohydrates, fats, proteins, vitamins, and minerals (Abou-Donia et al. 2010). They are essential for growth, body structure, and strengthening immunity against diseases and are considered indicators of environmental pollution by many pollutants, such as pesticides (Raheem S.W. & Niama A., 2021). Pesticides are compounds used on dairy farms to control agricultural pests, and these pesticides can contaminate the surrounding environment, including animal feed and drinking water (Akhtar and Ahad, 2017). These pesticides appear in milk when dairy cows feed or water contaminated with pesticides. This is because pesticides are lipophilic and accumulate in fatty tissues such as meat, milk, and eggs (Jayaraj et al., 2017). Pesticides are easily hydrolyzed, so they do not persist long in the environment and accumulate in large quantities in milk fat. Organophosphate and organochlorine pesticides are used on a large scale in dairy farms and on a small scale in Egypt.

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Where there is no official regulation, and they are used in animal feed and the animal environment, it is an opportunity for contamination (Ismail and Elkassas, 2016). It is rich in water and is found in raw milk. The most common (OP) insecticides were Chlorpyrifos, Malathion and Parathion-methyl Silica (C18), derivatized with octadecylsilyl, acetonitrile, petroleum, OC pesticides were Alachlor, dieldrin, hexachlorobenzene (HCB), lindane and methoxychlor already used (Abshara M.M., 2023). Some pesticide residues were lost during the milk production process by heat treatment (pasteurization and sterilization), fermentation (yogurt production), and coagulation (cheese production). (Ahad, K., 2017). Consumption of milk and dairy products contaminated with pesticide residues is harmful and dangerous to human health (Lozowicka, 2015). It is considered to be a major source of toxic and carcinogenic compounds for animals and humans.

Several previous studies have reported that pesticide residues were found in dairy products (Aguilera L. et al., 2011- Fagnani, R. et al., 2011 - Liu S. et al., 2013 – Bijeljina, 2021 - Ramezani S. et el., 2022). Therefore, it is important to develop rapid, sensitive, and selective analytical methods that allow the measurement and quantification of multiple pesticide residues of multiple classes in milk in a single analytical process. An improved, rapid, simple, low-cost, effective, robust, and safe extraction method combined with ultrasound and ultra-high performance liquid chromatography-orbitrap-mass spectrometry (UHPLC-orbitrap-MS) was developed as a highly sensitive and reliable extraction method. It was a new method for quantification of multiple classes of pesticides in whole milk (Ourania Koloka et al., 2023). The European Union (EU) has established maximum residue limits (MRLs) for pesticides that are allowed in products of plant or animal origin for human or animal consumption to reduce the risks associated with the consumption of foods containing pesticide residues mas (European Union Commission Regulation, 2010). Therefore, this study measured organophosphorus and organochlorine pesticide residues in raw milk samples from Asyut Governorate, Egypt, and investigated the effects of laboratory heat treatments (pasteurization and sterilization), fermentation (yogurt production), and coagulation (cheese production) during processing, organophosphorus and organochlorine pesticides remain in them. Concentrations of all organophosphate and organochlorine pesticides in raw buffalo milk exceeded the maximum permissible MRL limits for these pesticides set by the European Commission (EC).

2. Material and Methods

2.1 **Collection of samples:** 30 random samples (1 kg each) of raw buffalo milk were collected from the same location (a village known for producing large amounts of milk near the city of Assiut, Egypt) from September 2022 to October 2023, it was done. The samples were identified, placed in polypropylene bags, and transferred there for immediate testing.

2.2 **Preparation of Samples:** Each sample is divided into five sub-samples. The first sub-sample was stored as control, the second sub-sample was pasteurized at (72EC/15 sec) and cooled immediately, the third sub-sample was sterilized (121EC/15 min) in an autoclave and then cooled rapidly, the fourth and the fifth sub-sample were sterilized (121EC/15 min) then the fourth was fermented by yogurt starter culture (0.05 g LG1) at 43EC/2.5 h) and immediately cooled. The fifth was cured by animal rennet (2 mL LG1) at 40EC till complete coagulation; then, the curd was cut, and the whey was drained.

3. Results

Each sample of raw milk or yogurt was thoroughly mixed, and 10 ml was measured in glass containers covered by Teflon capped stoppers and stored at 20 ºC until examination. 25 gm. of each cheese sample was thoroughly mixed with 50 ml of distilled water in the blender, and then 10 ml from each prepared sample was stored at 20 ºC until examination. All milk, yogurt, and cheese samples were extracted and cleaned up according to Suzuki et al., 1979. The pesticide residues were detected and identified by gas chromatography-mass spectrometry (GC/MS). GC was performed on 7890 Agilent instruments and equipped with an insertion source mass of 5975 detection systems (Agilent Technologies). DB-1701 (J&W Scientific, USA) was the analytical capillary column at 40°C for 1min., then programmed at 30°C min-1 up to130°C, then 5°Cmin-1 up to 250°C, and finally 10°Cmin-1 up to 300°C, which was held for 5min., used Helium (purity, >99.999%) at a flow rate of 1.2ml min-1. At 260°C injection port temperature 1µL samples were injected split less with the purge on after 1.5min., The MS ionization energy was 70eV with an ion-source temperature of 230°C and a GC-MS interface temperature of 280°C. Used selected ion monitoring (SIM) with a dwell time of each ion 100ms. Retention time windows and base peak ions were designed for OC and OP pesticides. External standard methods were used to identify pesticide retention time and specific ions and quantified using. Statistical analyses were conducted using SPSS 16.0 for Windows (SPSS, Chicago, USA). Results were considered significant at the 5% level ($p<0.05$). The data obtained are shown in the tables below.

Table (1): Incidence of organophosphorus and organochlorine pesticide residues (ppm) in raw buffalo milk examined samples (n/30)

Type of Pesticid	Frequency		Min.	Max.	Mean ± SE	European Commission (ppm)
	No.	%				
Chlorpyrifos	12	40	0.294	0.342	0.318 ± 0.034	0.01**
Malathion	14	46	0.132	0.198	0.168 ± 0.073	0.02*
Parathion methyl	7	23.3	0.108	0.128	0.119 ± 0.046	0.01
Methoxychlor	15	50	0.124	0.168	0.142 ± 0.64	0.01
HCB	11	36.7	0.102	0.134	0.116 ± 0.024	0.01
Lindane	16	53.3	0.094	0.153	0.0.136 ± 0.037	0.01**
Dieldrin	9	30	0.073	0.104	0.094 ± 0.081	0.006*
Alachlor	13	43.3	0.0000	0.0004	0.0002± 0.0001	0.01

Table (2): Incidence of organophosphorus and Organochlorine pesticide residues (ppm) after laboratory pasteurization of positive examined samples

Type of Pesticide	Frequency		Min.	Max.	Mean ± SE	Degradation (%)
	No.	%				
Chlorpyrifos	8	26.7	0.126	0.187	0.167 ± 0.86	47.5
Malathion	9	30	0.076	0.093	0.082 ± 0.07	51.2
Parathion methyl	4	13.4	0.038	0.042	0.040± 0.47	66.3
Methoxychlor	10	33.4	0.057	0.089	0.064 ± 0.098	51.6
HCB	8	26.7	0.065	0.074	0.068 ± 0.024	58.6
Lindane	7	23.3	0.042	0.075	0.064 ± 0.084	47.1
Dieldrin	4	13.3	0.033	0.058	0.040 ± 0.075	42.5

Alachlor	6	20	0.0000	0.000	0.000± 0.00001	…

Table (3): Incidence of organophosphorus and Organochlorine pesticide residues (ppm) after laboratory sterilization of positive examined samples

Type of Pesticide	Frequency		Min.	Max.	Mean ± SE	Degradation (%)
	No.	%				
Chlorpyrifos	5	16.7	0. 095	0.116	0.106 ± 0.046	66.7
Malathion	7	23.3	0.058	0.075	0.068 ± 0.064	59.5
Parathion methyl	2	6.66	0.024	0.037	0.031± 0.053	73.9
Methoxychlor	7	23.3	0.031	0.053	0.043 ± 0. 87	69.7
HCB	5	16.7	0.048	0.057	0.052 ± 0.086	55.1
Lindane	4	13.3	0.029	0.057	0.034 ± 0.046	75.0
Dieldrin	2	6.66	0.018	0.033	0.025 ± 0.073	73.0
Alachlor	2	6.66	0.0000	0.000	0.000± 0.0000	…

Table (4): Incidence of organophosphorus and Organochlorine pesticide residues (ppm) after laboratory fermentation of sterilized positive examined samples

Type of Pesticides	Frequency		Min.	Max.	Mean ± SE	Degradation (%)
	No.	%				
Chlorpyrifos	5	16.7	0. 073	0.093	0.084 ± 0.074	73.6
Malathion	7	23.3	0.034	0.052	0.047 ± 0.094	72.0
Parathion methyl	2	6.66	0.016	0.024	0.021± 0.086	82.4
Methoxychlor	7	23.3	0.024	0.045	0.034 ± 0. 060	76.0
HCB	5	16.7	0.033	0.048	0.042± 0.039	63.8
Lindane	4	13.3	0.015	0.025	0.021 ± 0.085	84.6
Dieldrin	2	6.66	0.010	0.014	0.017 ± 0.036	81.9
Alachlor	2	6.66	0.0000	0.000	0.000± 0.0000	000

Table (5): Incidence of organophosphorus and Organochlorine pesticide residues (ppm) after laboratory curdling of sterilized positive examined samples

Type of Pesticide	Frequency		Min.	Max.	Mean ± SE	Degradation (%)
	No.	%				
Chlorpyrifos	5	16.7	0.069	0.091	0.083 ± 0.036	73.9
Malathion	7	23.3	0.031	0.048	0.036 ± 0.024	78.6
Parathion methyl	2	6.66	0.014	0.018	0.016± 0.061	86.6
Methoxychlor	7	23.3	0.025	0.045	0.037 ± 0. 025	73.9
HCB	5	16.7	0.038	0.047	0.044± 0.042	62.1
Lindane	4	13.3	0.018	0.032	0.028 ± 0.056	79.4
Dieldrin	2	6.66	0.017	0.022	0.019 ± 0.084	97.8
Alachlor	2	6.66	0.0000	0.000	0.000± 0.0000	000

4. Discussion

Organophosphate and organochlorine pesticides are widely used in many agricultural and industrial areas of Egypt, as they are considered economically important for high-yield production. Although pesticides provide effective and practical control of disease vectors and destructive insects, the use of these substances is considered toxic to humans and poses many public health risks. It is believed that the samples were measured in the upper Egyptian city of Assiut. Raw buffalo milk samples were collected from the same location (a village known for producing large amounts of milk). Organophosphorus and organochlorine pesticides were detected in 100% (30 samples) of raw buffalo milk samples investigated, and pesticide residues (Chlorpyrifos, Malathion, parathion methyl, methoxychlor, HCB, Lindane, and dieldrin are the frequency distribution of crawls) was found as follows {No. (%)}: 12 (40), 14 (46), 7 (23.3), 15 (50), 11 (36.7), 16 (53.3), 9 (30) and 13 (43.3); while the average concentration (ppm) /L) were measured: 0.318 ± 0.034, 0.168 ± 0.073, 0.119 ± 0.046, 0.142 ± 0.64, 0.116 ± 0.024, 0.0.136 ± 0.037, 0.094 ± 0.081, 0.0002 ± and 0.0001 respectively. Levels of all organophosphorus and organochlorine pests exceeded the maximum permissible limits for these pesticides set by the European Commission (EC) for residual amounts of toxic substances in raw buffalo milk. Comparing the obtained data of our study with the other previous studies, it is noted that almost similar results were achieved by (Ahmed et al., 2009 - Abou-Donia et al., 2010 - Shaker et al., 2015 and Abou El-Makarem et al., 2023). While, Higher results were obtained by (Waliszewski et al., 1997 - El-Asuoty et al., 2017 and Nasef et al., 2018). However, the results obtained from (Abd-Rabo et al., 2016 - and Raheem et al., 2021) are lower than obtained data. The frequency distribution (ppm) of pesticide residues after pasteurization of laboratory samples determined to be positive is shown in Table 2. The average concentrations (ppm/l) were {No. (%)}: 8 (26.7), 9 (30), 4 (13.4), 10 (33.4), 8 (26.7), 7 (23.3), 4 (13.3), and 6 (20) respectively. The average concentrations were: 0.167 ± 0.86, 0.082 ± 0.07, 0.040 ± 0.47, 0.064 ± 0.098, 0.068 ± 0.024, 0.064 ± 0.040 ± 0.075 or 0.000 ± 0.00001 and raw buffalo pasteurization process. All pesticide residues are degraded in milk samples (%) (47.5), (51.2), (66.3), (51.6), (58.6), (47.1), and (42.5), or disappear completely from Alachlor.

On the other hand, Table (3) shows the frequency distribution (ppm) of pesticide residues of different types of pesticides after positive samples were sterilized in the laboratories, which were {No. (%)}: 5 (16.7), 7 (23.3), 2 (6.66), 7 (23.3), 5 (16.7), 4 (13.3), 2 (6.66) and 2 (6.66). The average concentrations (ppm/L) were: 0.106 ± 0.046, 0.068 ± 0.064, 0.031 ± 0.053, 0.043 ± 0.87, 0.052 ± 0.086, 0. 034±0.046, 0.025±0.073, 0.000±0.0000 respectively. The sterilization process of raw buffalo milk samples completely degrades all pesticide residues (%) as 66.7, 59.5, 73.9, 69.7, 55.1, 75, 0, and 73.0. Loss of Alachlor or Thermal treatment of the investigated raw milk samples reduced the frequency distribution and average concentration of pesticide residues of different types of pesticides (Abd-Rabo et al., 2016), approaching the maximum permissible MRL. These limits are set by the legislator, the European Pesticides Committee (European Commission, EC). Also, the distribution frequencies (ppm) of pesticide residues of different types of pesticides are shown in Table 4. After laboratory fermentation of sterilization positive samples, organophosphorus and organochlorine pesticide residues were studied with the same average concentration (ppm/kg). The incidence rate (ppm) was shown as 0.084±0.074, 0.047±0.094, 0.021±0.086, 0.034±0.060, 0.042 ± 0.039, 0.021 ± 0.085, and 0.017 ± 0.036, respectively. The fermentation process of sterilized raw buffalo milk samples increases the degradation of all pesticide residues in the range of (%): 73.6, 72.0, 82.4, 76.0, 63.8, 84.6, and 81.9.

Almost similar results were obtained by (Zhao et al., 2010- Abd-Rabo et al. 2016 and Raheem et al., 2021), and lower-order results were obtained by (Bo et al., 2011 and Zhang et al., 2006). Yogurt production first requires heat treatment such as pasteurization or sterilization, and the action of microorganisms during production and storage, as well as the growth of lactic acid bacteria in milk, leads to a reduction in the pesticide content in yogurt. From Table 5, it can be seen that the occurrence rate (ppm) of pesticide residues after coagulation in the laboratory of sterilized positive samples with the same distribution frequency (ppm) of pesticide residues of different types of pesticides had average concentration (ppm). Also, it can be seen that the average concentrations (ppm/kg) are 0.083 ±, 0.036, 0.036 ± 0.024, 0.016 ± 0.061, 0.037 ± 0, 025, 0.044 ± 0.042, 0.028 ± 0.056, and 0.019 ± 0.084, respectively. The process of laboratory curdling sterilized raw buffalo milk samples caused an increase in the degradation of all pesticide residues reaching all pesticide residues (%): 73.9, 78.6, 86.6, 73.9, 62.1, 79.4, and 97.8 respectively. Almost similar results were achieved by (Pagliuca et al., 2006 - Zhang et al., 2006 - Kaushik et al., 2009 and Abd-Rabo et al., 2016). The curd process reduces pesticides in food by heat treatment and drying.

5. Conclusion

In our study, all milk, yogurt, and cheese samples were extracted and cleaned up to Detect and identify pesticide residues using gas chromatography-mass spectrometry (GC/MS). The results show that the levels of all Organophosphorus and organochlorines pesticide residue in raw buffalo milk exceeded the maximum permissible limit MRL values for these pesticides set by the European Commission (EC), and the heat treatment, fermentation, and curdling processes cause degradation of Pesticides residue of raw buffalo Milk. We can say that the processing of raw milk includes thermal processes such as pasteurization or sterilization, as well as the role of bacteria during production and storage. The proliferation of bacteria and acute lactic acid generally causes a decrease in the amount of pesticides. Therefore, in order to prevent the formation of pesticides in raw milk, farms, and dairy products must use the HACCP system from the first stage of production until they reach the consumer. Consumers should carefully warm raw milk before consumption to reduce pesticide exposure. Consumption of UHT milk is higher than that of raw milk and other processed dairy products, reducing the percentage with the opportunity to reduce pesticides through effective heat treatment (pasteurization or sterilization), fermentation, and coagulation.

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