Monitoring Pesticide Residues in Forage Crops and Buffalo Meat in Egypt

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Abstract

This research aimed to quantify the pesticide residues on both maize and faba bean forage crops during the seasons of 2021 and 2022 at the Sides station in Beni Suef Governorate. In addition, the quality of the sampled meat from the buffaloes fed on these forage crops was assessed to determine its content of pesticide residues. After consuming maize and faba beans as fodder, pesticide residues that were transmitted to buffalo meat were observed for 3 consecutive months. Acetone was used for the extraction process. Clean-up of the extracts was accomplished by dividing saturated hexane with acetonitrile. Both Gas Chromatography (GC) with a flame ionization detector and High Performance Liquid Chromatography (HPLC) with an ultra violet detector were used to examine all samples for pesticide residues. Except for methomyl, which was higher than the ADI in all samples of maize and in samples of faba beans taken exclusively during the winter, other pesticides were below the values of the Acceptable Daily Intake (ADI) and the Maximum Residue Limits (MRL). Additionally, between 56% and 67% of the pesticides applied on faba beans and maize during the seasons of 2021 and 2022, respectively, were transmitted to buffalo flesh.

Keywords: *Residue ,Pesticide, Forage , HPLC, GC.*

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1. Introduction

It is vital to expand livestock production and quality to supply the additional food needed to keep up with the world population's constant growth.

Agricultural pests must be managed in order to produce crops used as fodder for livestock. Pesticides are chemical substances used to eradicate pests. Although pesticides used in agriculture not only prevent crop damage but also boost crop output, their residues have shown to pose a serious risk to human, animal, and environmental health **Mahdavi, *et al.*, 2022** and **Dai , *et al.*, 2023 .** The research of pyrethroid residues **Emert, *et al., 2023*** , demonstrated that the biodiversity of agricultural environments depends on the prudent use of pesticides. As residues may transfer to, collect in, or deposit on fruit tissues, pesticide residues in agricultural products can be detrimental to human health **KUMAR, *et al.*, 2011**. Meat is considered one of the most important sources of protein for humans as it contains high levels of essential amino acids and many minerals. Given the importance of meat, it is important to ensure that it is free of various contaminants, especially harmful residues of pesticides widely used in agriculture and animal pest control in developing and African countries. It is an essential protein source for humans because it is rich in the most important essential amino acids and many minerals. Given the importance of meat, ensuring that meat is free of harmful residues from various contaminants, especially the widely used pesticides in agriculture in developing and African countries is an important task. It can cause bio magnification in plants and animals as well as the environment, reaching humans through ingestion and causing health hazards **Lehotay** , ***et al.*, 2005** ; **Qiu**, ***et al.*, 2005** ; and **Maclachlan & Bhula 2008 .** A study in Cameroon was done using QuEChERS method where samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), indicated that 81pesticides residues

were present in 160 samples collected from 11 dry agricultural commodities in three major cities **Galani, *et al.*, 2020 .**

The objectives of this research were to monitor and quantitatively estimate the nine most commonly used pesticides on two forage crops. In addition, the migration of pesticide residues into the meat of buffalo fed three consecutive months of contaminated crops during the 2021 and 2022 growing season will be tracked. The daily intake (ADI) and maximum residue limit (MRL) set by the WHO/FAO Food Commission **Codex 2015**, were compared to provide the correct intake recommendations.

2. Material & Methods

Plant materials:

a) Maize:

Single Cross 128 maize was planted in 2021 for the first season and in 2022 for the second season. Crops were harvested at full maturity 120 days after sowing. Maize cobs were air dried. Five representative samples weighing 500 grams each and one untreated sample (control) was used for the analysis and determination of pesticide residues in each season.

b) Faba bean :

The Giza 111 beans were planted in 2021 for the first season and in 2022 for the second season. Crops were harvested 120 days after sowing, when fully ripe. Broad beans were allowed to air dry, then, five random representative samples (500 grams each) were collected from each seasonal crop in addition to a number of untreated samples (control) for the analysis and the determination of pesticide residue levels.

c) Pesticides used:

Pesticide formulations included in the various pest control program during the cultivation of faba bean and maize at Sides Station, Beni Suef Governorate which were **methomyl** (Lannet SP 90%), **emamectin benzoate** (Spedoo WG 5.7%), **glyphosate** (herbazed SL 48%), **profenofose** (selectron EC 72%), **chloropyrifos** (pestban EC 48%), **nicosulfuron** (active SC 6%) , **pendimethalen** (omega EC 33%), **clethodim** (select super EC 12.5%), and **prodiamine** (barricade WG 65%).

Sampling:

A total of 38 samples were taken, 14 from corn and 14 from kidney beans, 5 of which were in 2021, 2 were pesticide-free (control samples), 5 were in 2022, and 2 were controls. In 2021, 10 buffalo meat samples were collected: 3 from corn-fed buffalo, 3 from fava-fed buffalo, and 2 control samples. The same number of samples was collected in 2022.

1) Plant material samples:

Ten random samples of approximately 500 g each of maize and kidney beans were collected from the field after full maturity. The broad bean and corn cobs were subsequently sampled, washed, and air dried.

2) Buffalo meat samples:

Two samples of Longissimus buffalo meat were used. The longissimus dorsi muscle (latissimus dorsi muscle; LD) was harvested from a meat-producing buffalo after it was fed on the two forage plants for 3 months. Bodies were dismembered, stripped, circumcised and bagged. Three 250 grams samples of the tissue of the LD. Samples were randomly excised into bags and placed in sterile, colorless, self-sealable polyethylene (PE) bags. The bags were labeled, frozen, and shipped frozen in foam boxes with ice packs to the lab. Samples were stored at -20 °C before analysis **Biswas, *et al.*, 2010 .**

Extraction and preparation of plant samples.

To extract residues, 50 g each of corn and fava bean homogeneous samples were taken after fine grinding in a mechanical mill and placed in a 250 ml Erlenmeyer flask containing 100 ml of acetone. Flasks were shaken for 15 minutes. Then, the extract was centrifuged for 5 minutes using 2000 rpm for better separation of the liquid phase. A Buchner funnel is then used to separate the solids from the organic solvent containing pesticide residues, and the separation process is repeated three times to ensure that all pesticide residues are quantitatively transferred. A 50 mL polypropylene tube and extractor were transferred. All sample extracts were concentrated by evaporation in a water bath vacuum evaporator at 45 °C. The analytical method was adapted from **Ohlin 1998.**

Partitioning and clean-up of the extract.

This washing method was developed by **SGARBIERO *et al.*, 2003.** Purification of extracts was performed by liquid/liquid partitioning between acetonitrile (8 mL) in saturated hexanes and acetonitrile-saturated hexanes (25 mL) in polypropylene tubes. After manual shaking and centrifugation for 5 minutes at 2500 rpm, the bottom layer (acetonitrile) was transferred to another polypropylene tube. The extract was then reconcentrated by evaporation in a 60° C water bath and resuspended in 5 mL of hexane. Purification was completed by column cartridge C18 transferring the hexane extract to these columns. Elution was performed with a mixture of hexane and acetone (15 ml, 1/9 v/v) and the clean extract was collected in another polypropylene tube. After further concentration, the remaining insecticide was dissolved in 10 ml of acetone at 45°C. It was dissolved in a water bath using a rotary evaporator and transferred to a 10 mL volumetric flask.

Extraction and clean-up of meat samples:

According to **Biswas *et al.*, 2010**, frozen tissue samples were thawed, trimmed of exogenous fat and fascia, and then minced with scissors. The minced samples were blended in a high speed (5000 rpm) tissue blender for 2 minutes. A representative portion (10 g) of this sample was weighed into a polypropylene tube and homogenized in 10 mL of Milli-Q water for 5 minutes using a tissue homogenizer. Aliquots (0.5 g) of the homogenized samples were then transferred to glass test tubes, supplemented with 50 µL of working standard solutions of various concentrations, and the specimens were contacted with the meat samples for 30 minutes. After adding 3 ml of McIIvaine buffer, the mixture was vortexed at high speed, incubated at room temperature for 5 minutes, and centrifuged at 3500 rpm for 15 minutes in a refrigerated centrifuge. Extraction was repeated by adding 2 ml of McIIvaine buffer and supernatants were pooled. The supernatant was filtered and loaded onto cartridge C18 with 3 mL of methanol and 2 mL of water. Cartridges containing samples were washed with 5 ml water, filtered for pesticide residues through a 0.22 µm nylon filter, vortexed and centrifuged, and 20 µl aliquots were injected onto the HPLC system.

Preparation of blank solution.

Equal volumes of solvent and anhydrous sodium sulfate (pesticide extraction solution) were subjected to the same procedure as the tested samples to detect possible trace levels of the monitored pesticides, and the results were adjusted accordingly. Method sensitivity and recovery were determined using samples spiked with the test compounds. Prior to analysis, relevant standards were run to check column performance, peak height, resolution, and detection limits. Peaks were identified by comparing the retention time values of the samples with those of the corresponding pure standard compounds. Solvent blanks, standard mixtures, and method blanks were run sequentially on each set of samples analyzed to check for contamination, identify peaks, and quantify. For all test compounds in each plant and meat sample, the mean pesticide recovery of the enriched samples at various concentrations was determined and calculated. ADI and MRL'S values for buffalo, corn and kidney beans are listed in **Table 1.**

Chemicals:

All solvents (acetone, methanol, acetonitrile and Dichloromethane) and reagents (Anhydrous sodium sulphate, florisil and sodium chloride solution 10%) used in this study were high analytical and HPLC grade.

Table 1: values of ADI and MRL'S for buffalo meat, maize and faba bean crops.

Pesticide	MRL mg/kg			ADI
	maize	bean	meat	
Profenofos	0.001	0.01	0.05	0.03
Glyphosate	5	20	0.05	0.3
Emamectin benzoate	0.03	0.005	0.004	0.0025
Nicosulfuron	0.01	0.01	----	0.02
Chloropyrifos	0.05	0.01	----	0.01
Clethodim	0.1	0.5	0.2	0.01
Pendimethalin	0.05	0.05	0.2	0.02
Prodiamine	0.4	0.7	0.15	0.07
Methomyl	0.02	0.1	0.02	0.0025

Chromatography analysis:

a) High Performance Liquid Chromatography:

Pesticide residues were determined using High-Performance Liquid Chromatography (HPLC) analysis, using standards of glyphosate, emamectin benzoate, nicosulfuron, clethodim, methomyl, prodiamine, and

methomyl. The HPLC system used was the Agilent series 1200, equipped with a solvent delivery system and a UV spectrophotometer detector. A C18 stainless column (4.6 x 250 mm) was employed for the analysis. The elution solvent grade from the Merk company. The detection limits for the mentioned standards were found to be 0.05 ng, 0.03 ng, 0.04 ng, 0.03 ng, 0.05 ng, 0.04 ng, and 0.05 ng, respectively.

Conditions of analysis:

Liquid chromatography with UV detector at a wavelength of 197 nm for each of glyphosate and clethodim, a wavelength of 254 nm for methomyl and 235 nm for pendimethalin. Solvents used were methanol 10% + acetonitrile 90%, with a flow rate of 1 ml / min, wavelength 210 nm for each of emamectin, benzoate, nicosulferon, and prodiamine were in the same system as the previous solvents. Only emamectin, benzoate had a 5% acidic water + 95% acetonitrile as a solvent with a flow rate of 1 ml/min.

(b) Gas Liquid Chromatography:

The separation and detection of the analyzed pesticides were performed by high resolution Gas chromatography (GC). The analysis was performed using an Agilent 7890A Gas chromatography equipped with Flame Ionization Detector. Determination of pesticide residues by GLC analysis compared with the external standard used chloropyrifos and profenofose were carried using out GLC model Agilent Technologies, column HP50 + (30m X 0.53 mm X1 micron) with flame ionization

detector (FID). The detection limits were (0.02 and 0.04) ng for chloropyrifos and profenofose insecticides, respectively. Injection temperature was 225 ºC, detector temperature was 300 ºC and oven temperature was 130 ºC for 5min.

Rate of Recovery

The reliability of analytical method was examined by fortifying the tested samples with known quantities of tested pesticides followed by the same procedure of extraction, clean up and analysis. The percentage rates of recoveries of each one of the nine pesticides were 83%, 86%, 84% for glyphosate 88%, 86% , 89% for profenofose, 85%, 83%, 86% for emamectin benzoate, 86%, 88%, 82% for nicosulfuron, 87%, 89%, 92% for chlorpyrifos 92%, 91%, 94% for methomyl, and 91%, 98%, 95% for pendimethalin and 93%, 89%, 96% for Clethodim and 89%,87%,93% for prodiamine in maize , faba bean and buffalo meat, respectively.

Statistical analysis

Descriptive statistics such as mean values for each group and its corresponding standard deviation (SD). Statistical analysis of differences among means was carried out using one way analysis of variance (ANOVA). When significant difference was found, Least Significant Difference (LSD) test was used for multiple comparisons was used to evaluate between groups at P >0.05 level of significance **.**

3. Results & Discussions

All samples of maize, faba bean and buffaloes meat collected during the seasons of 2021 and 2022 from sides station in Beni seuif Governorate were analyzed for nine different pesticides residues (**Table 1**). The residue analysis revealed that about 62.96% of samples were contaminated with all montored pesticides. The occurrence of the methomyl and prodiamine residues were 100%, followed by each of glyphosate (83.3%), clethodim, and chlorpyrifo (66.6%), emamectin benzoate, and pendimethalin (50%). followed by profenofos (33.3%). On contrast, nicosufuron was not detected in maize or faba bean samples, as well as buffalo meat, fed on maize and faba bean as fodder for 4 consecutive months in 2021 and 2022 seasons. Average pesticide concentration for the monitored pesticides in maize, faba bean, and buffaloes' meat was calculated. Sstatistical analysis indicated that there were no significant differences between the replicates of the same pesticide for all tested materials (maize, faba bean, Buffaloe meat) in both seasons of 2021 and 2022, as well as among replications. because samples of maize and faba bean were collected by methods reference sampling in terms of randomness and representativeness for each treatment, in addition used official methods of extraction and clean-up for pesticides from samples were also evaluated and the recovery rate for each pesticide was evaluated.

Pesticide residues in forage crops samples:

a) Maize crop samples

The results of the screening of ten samples of maize crop indicated that glyphosate, emamectin benzoate, clethodim, prodiamine and methomyl were detected in maize crop samples which cultivated in two seasons in 2021 and 2022, while pendimethalin and profenofose were detected in maize crop samples of second season only. On the other hand, chlorpyrifose and nicosulfuron were not detected in the two seasons. Also, it was observed that there was a significant increase of the residue values of each of the pesticides emamectin benzoate, clethodim and methomyl in the samples collected from the maize crop in first season compared with that of second season (**Table 2**). Our results were consistent with those of **Shaban *et al.,* 2015] and Shaban *et al.,* 2016 ,** who carried out two field experiments during 2013/2014 and 2014/2015 seasons at Sides Agricultural Research Station, Agricultural Research Centre (A.R.C) and found that the pendimethalin herbicide studied active ingredient was under acceptable daily intake (ADI**). Nyarko,** *et al.,* 2021 ,determined grain quality and pesticide residue concentrations in maize stored in polypropylene and hermetic bags. They found that, out of 35 pesticides screened; only lambda-cyhalothrin was detected in polypropylene bags and deltamethrin was detected in hermetic bags. The presence of these pesticide residues may be due to their long-lasting abilities. Levels of lambda-cyhalothrin residues were above the maximum residue limit (MRL) of 0.02 mg/kg, but have no significant effect on health **Codex 2015 .** Deltamethrin residue concentrations in hermetically stored maize samples were below the MRL.

b) Faba bean crop samples

The results of the screening of ten samples of faba bean crop indicated that chloropyrifose ,clethodim , prodiamine, and methomyl were found in faba bean crop samples which cultivated in 2021 and 2022, while emamectin benzoate was detected in faba bean crop samples which cultivated in second season only. Glyphosate was detected in the faba bean samples that was cultivated in 2021 only. On contrast, profenofos, chlorpyrifose and nicosulfuron were not detected in the two seasons' samples. Also, a significant increase in the residue values of each of the pesticides chlorpyrifos and clethodim in the samples collected from the faba bean crop in 2021 season was observed when compared with those of 2022 season **(Table 2).** Meanwhile, a significant increase of the residue values of both prodiamine and methomyl in the samples collected from faba bean crop in 2022season was evident when compared with those of 2021 season **(Table 2).** Our results were in agreement with **El-Saeed *et al.*, 2020.** They conducted two laboratory tests to compare the efficacy of pendimethalin at a rate of 773.5 g a.i/fed (Stomp extra 45.5% CS) and clethodium at a rate of 62.5 g a.i/fed (Select super 12.5% EC) in the 2017–18 and 2018–19 growing seasons at Moshtohor, Benha University, Qalyubia Governorate, Egypt. In the second season, faba bean seeds were examined in the Central Agri. Pesticides Lab. A.R.C., Dokki, Giza, Egypt, for the presence of the herbicide residues pendimethalin (Stomp extra) and clethodium (Select super) using the Gas Liquid Chromatography method as described in **Nguyen *et al.*, 2008**. In the field, sunlight and a variety of microorganisms quickly broke down herbicide residues. Therefore, So, the residues remained on faba bean seeds were less than the allowable level according to European Food Safety Authority **EFSA 2012 .**

c) Buffaloes meat samples

Glyphosate, chloropyrifose, pendimethalin, prodiamine, and methomyl were found in six samples of buffalo meat that had been fed maize and faba beans as fodder for four consecutive months during the seasons of 2021–2022, while profenofose was only found in buffalo meat samples from the 2022 season that had been fed the same type of fodder for four consecutive months. In contrast, clethodim, emamectin benzoate, and nicosulfuron were not found in buffalo meat samples from the 2021 and 2022 seasons that were fed faba bean and maize as fodder. The findings also revealed a marked increase in the residue levels of the pesticides glyphosate, chloropyrifos, pendimethylene, prodamine, and methomyl in buffalo meat fed on beans and maize grown in the 2021 season as fodder as opposed to residues of the same pesticides in buffalo meat fed on beans and maize grown in the 2022 season as fodder. Our findings concurred with those of **Koli and Bhardwaj 2018** , who investigated the presence of pesticide residue in food, livestock, and livestock products, drinking water, and other sources. Many types of dry and green fodder were shown to contain pesticide residues, including Fluazifop-P-butyl, Imazethapyr, Pendimethalin, Pinoxaden, Saflufenacil, and Penthiopyrad. By using high performance liquid chromatography to check and quantify liver sample data, **Kumar *et al*,**. 2019 , discovered Chlorpyrifos residues in 9.05% of the samples, of these, only 0.78% of the liver samples were found to exceed the Codex maximum residue limit (MRL) for chlorpyrifos content. The contamination in the flesh and organs of cattle raised in pesticide-spraying districts of Faisalabad, Pakistan, was identified by **Muhammad *et al.*, 2010** .About 13 muscle samples were found to be chloropyrifos-contaminated by the residue analysis. These pesticide residues may still be present because of their longevity. Despite being above the maximum residue limit (MRL) of 0.02 mg/kg, lambda-cyhalothrin residue levels had no discernible negative effects on health **Codex** 2015 **.** According to the study's findings, glyphosate, chlorpyrifos, pendimethalin, prodiamine, and methomyl residues were significantly higher in buffalo meat from animals fed maize and faba bean grown in 2021 than they were in buffaloes meat from animals fed maize and faba bean grown in season in 2022 **(Table 2).**

4. CONCLUSION

In conclusion, our study evaluated the presence of pesticide residues in several agricultural components and discovered that samples of maize and faba beans from the 2021–2022 seasons included a variety ofpesticides, including glyphosate, clethodim, prodiamine, and methomyl. Pesticides were identified in both crops, while some were present only in the maize samples. In samples of beans, chlorpyrifos was found; it was not present in maize. Profenfos was not discovered in beans but did show up in tests of winter-grown maize, which is how it ended up in buffalo meat. With the exception of methomyl, which during the winter months surpassed the ADI in samples of maize and faba beans, pesticide levels in maize, faba beans, and buffalo meat were generally below permissible limits.

5. Summary of findings:

The residue analysis revealed that about 62.96% of samples were contaminated with all montored pesticides. The occurrence of the methomyl and prodiamine residues were 100%, followed by each of glyphosate (83.3%), clethodim, and chlorpyrifo (66.6%), emamectin benzoate, and pendimethalin (50%). followed by profenofos (33.3%). On contrast, nicosufuron was not detected in maize or faba bean samples, as well as buffalo meat, fed on maize and faba bean as fodder for 4 consecutive months in 2021 and 2022 seasons.

6. Recommendations :

Even in low quantities, the presence of pesticide residues in meat is a risk to human health because they build up inside the body, so even if the level is beyond the allowable limit, it is still dangerous. Therefore, it is essential to educate dairy producers about the need to prevent pesticide residues in meat.

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Table (2) Residue of pesticides in buffalo meat samples and plant materials in two seasons 2021-2022

Pesticide names	Maize samples mg/kg		Faba bean samples mg/kg		Buffalo meat mg/kg	
	Season 2021	Season 2022	Season 2021	Season 2022	Season 2021	Season 2022
Profenofos	Und	0.0021 ±0.00011	Und	Und	und	0.00039 ±0.00009
Glyphosate	0.00071(bc) ±0.00050	0.00069(ac) ±0.00031	0.001486(ab) ±0.00037	Und	0.000046(f) ±0.00046	0.000031(e) ±0.00017
Emamectin benzoate	0.00099(bcd) ±0.00041	0.000132(acd) ±0.00082	Und	0.00054(ab) ±0.00025	Und	und
Nicosulfuron	Und	Und	Und	Und	und	und
Chloropyrifos	Und	Und	0.00193(cd) ±0.00010	0.00148(cd) ±0.00027	0.000014(f) ±0.00017	0.0000091(e) ±0.00011
Clethodim	0.00102(bcd) ±0.00012	0.000865(acd) ±0.00028	0.00529(abd) ±0.00014	0.00096(abc) ±0.00017	Und	und
Pendimethalin	Und	0.000574 ±0.00022	Und	Und	0.000067(f) ±0.00017	0.0000013(e) ±0.00014
Prodiamine	0.00043(bcd) ±0.000020	0.000633(acd) ±0.000024	0.00015(abd) ±0.00023	0.00279(abc) ±0.000017	0.000037(f) ±0.00032	0.000027(e) ±0.00034
Methomyl	0.00342(ab) ±0.00017	0.006991(ab) ±0.00008	0.00177(abd) ±0.00024	0.00321(abc) ±0.00025	0.000152(f) ±0.00012	0.000078(e) ±0.00043

Data are represented mean ± SD, different letters indicate significance at ($p<0.05$).

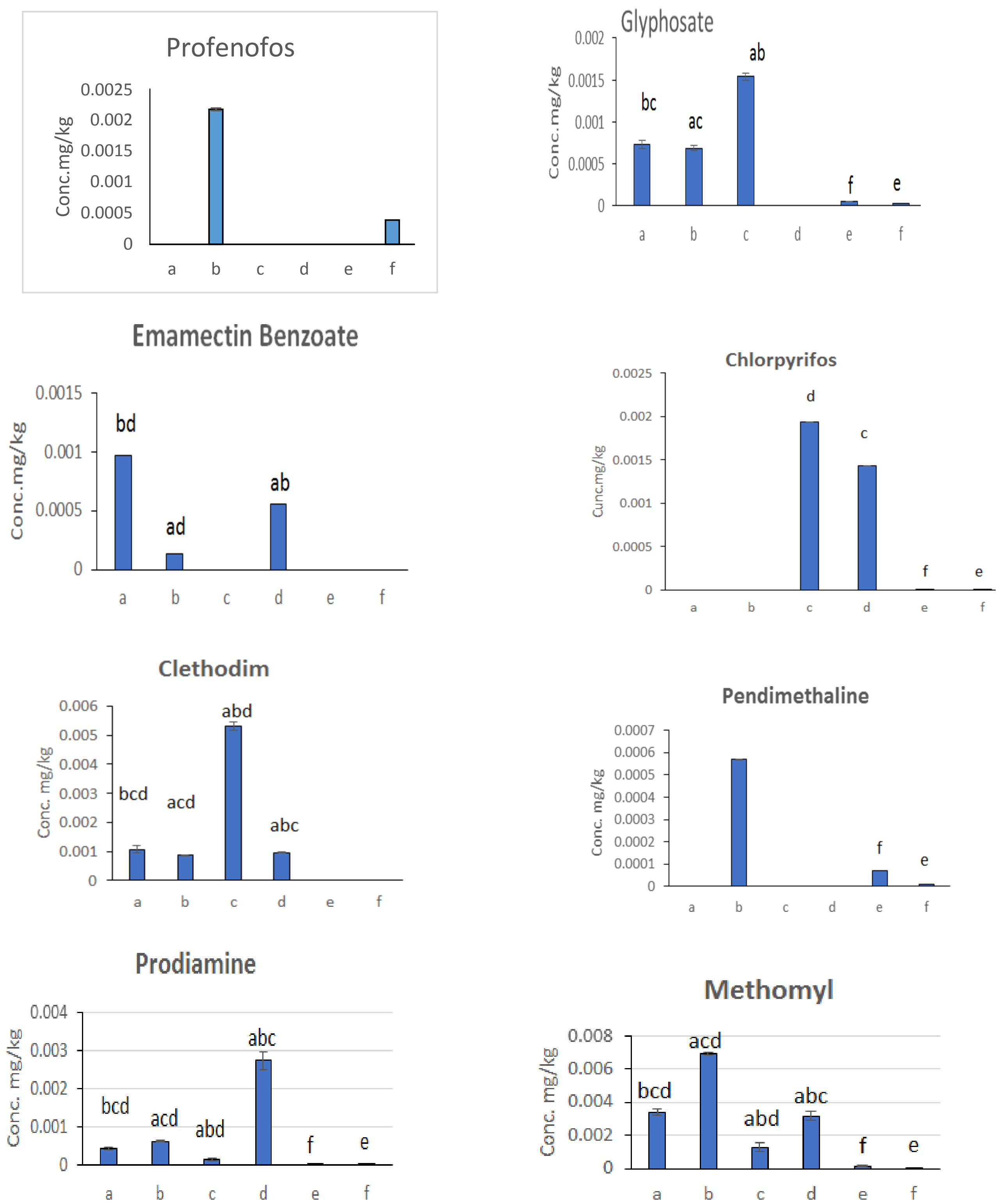

Fig. (1) Concentration of each pesticide in maize , faba bean and buffalo meat in seasons 2021-2022 , data are represented in column each column represent mean ± SD , different letters on the column indicate significance at ($p<0.05$). **(a)** maize in first season 2021 , **(b)** maize in second season 2022 , **(c)** faba bean in first season 2021 , **(d)** faba beans in season 2022 , **(e)** buffaloes flesh meat fed on fodder crops cultivated in 2021 , and **(f)** buffaloes flesh fed on fodder crops cultivated in 2022.