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ENVIRONMENTAL PESTICIDE RESIDUES AND HEALTH BIOMARKERS AMONG FARMERS FROM GREENHOUSES OF ERBIL CUCUMBER CROPS

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Abstract

This study intended to determine the residual concentrations of pesticides used in greenhouse cucumber fields and their effects on the farmers that spray it. Sixteen active cucumber greenhouse fields with 96 people (75 sprayers and 21 volunteers as control) randomly had been selected. In all selected greenhouses, abamectin, thiamethoxam, pyridabin, and spirodiclofen pesticides used, their cucumber with leaves and groundwater collected in December 2016, March, June, and September 2017 with workers blood samples, were immediately brought to the laboratory. Results showed the occurrence of residual pesticides in the plant, and groundwater samples with a higher level in plant samples decreased in water samples. In comparison with control, sprayer blood results showed that cholinesterase activity decreased significantly meanwhile, liver function enzymes: serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, direct and total serum bilirubin increased significantly. The kidney function test revealed that the mean serum urea concentration in the sprayers was considerably increased compared to the controls, while there was no significant change between the sprayers and the control serum creatinine. Slight variations in the declining number of red blood cells; however, white blood cells have significantly risen to their upper limit within normal ranges.

Key words : Erbil cucumber greenhouse, environmental pesticide residuals, serum cholinesterase activity, liver and kidney functions test, and blood cell count.

Introduction

Greenhouse environments provide a range of advantages for plant production; however, for this reason, many greenhouses also favor the growth of pests, hot, humid conditions and abundant food are perfect for the growth of pest, for these reasons, pest problems often grow faster and are more severe in enclosed systems. In the management of greenhouse pests, pesticides are essential tools. However, applications in enclosed and poorly ventilated areas increase the risk of exposure of workers and increase the risk of exposure, especially by inhalation (Bessin *et al.,* 1997).

Greenhouse cucumber plantations are considered to be still important vegetable crop grown on a large scale in Erbil City, but many insects attack the plant and then use insecticides commonly. Abamectin, Thiamethoxam, Pyridabin, and Spirodiclofen were used in most greenhouse plants to control the various insect of greenhouse cucumbers (Hassanzadeh *et al.,* 2012). Excessive use of pesticides has multiple adverse effects on the environment, and the health of individuals who were directly or indirectly exposed to it, which attracted attention in scientific disciplines, such as medicine, environmental studies, development studies, although the literature on the issue is going to grow. One of Erbil's main problems is the lack of an analysis based on empirical data on the attitudes of local farmers. Landowners are typically not well informed about the methods of chemical selection and application.

Also, information on the exact doses of pesticides

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used by companies in terms of region and product type is severely lacking. In some cases and some places, there is even a lack of willingness to apply the necessary dosage (Isin and Yildirim, 2007). Finally, the damages caused by excessive and incorrect chemical use to the environment with its health impacts on human and animal health was not recorded by authorized institutions such as the Government departments of Environment and Agriculture (Gun and Kan, 2009). Pesticides have been and continue to be useful against agricultural pests, since their use has led to numerous health risk effects on non - target species, especially humans. In farm-related projects, occupational exposure to pesticides has regularly associated with increased risk of developing some chronic diseases. Biomarkers such as hematological parameters, liver, kidney function tests, and BChE activities could be evaluated to detect the hazardous impacts of pesticides. As far as we understand, there were no investigations into the health impacts on farm employees in Erbil.

The objective of our study was to evaluate the bioaccumulation of pesticides in the plant and groundwater as well as assessing their exposure and toxicity to blood cell variables, liver, kidney functions test and monitor the activity of BChE in Erbil greenhouse cucumber farmer sprayers.

Materials and Methods

In a commercial greenhouse in the agricultural land of Erbil (plain), cucumber crops were grown. The cucumber with its leaf and groundwater samples (within a depth of 48 - 165 m from the same farm used for irrigation) within sixteen active cucumber greenhouse fields in the north of Erbil have collected after 2 months of growing cucumber, the spacing of the row was 60 cm, and the spacing of the plant was 25 cm with 28 - 45 relative humidity. Samples were collected in polyethylene bags and groundwater bottles in December 2016, March, June and September 2017, properly stored at (4^{o}C) and transported to the laboratory for further analysis.

Collection and analysis of blood samples

Blood samples collected and quickly brought to the research laboratory for cholinesterase activity (BChE), direct bilirubin, total bilirubin, serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), Serum Urea and Creatinine, red blood cell (RBC) and white blood cell (WBC) analysis. Blood samples were analyzed using complete blood count (CBC), and the serum was analyzed by Biolyzer 300 to determine ALP, GOT, GPT, Total and Direct Bilirubin, Urea and Creatinine after 5 min centrifugation at 5000 rpm of the remaining blood and BChE was established by the fluorescent activity kit Butyrylcholinesterase.

HPLC Instrumentation and

Extraction of environmental samples

100 μl of water and cucumber plant juice (cucumber with leaves was already cut and chipped with a juicer) and 300 μl acetonitrile also added to a 2 ml Eppendorf tube, and the sample was centrifuged at 12000 rpm for 10 minutes. The solvent phase was then collected and diluted 1:1 in a 200 μl HPLC vial (90 μl solvent phase, 90 μl water).

Through using Agilent 1260 infinity HPLC system w/1290 infinity thermostat, pyridabin, abamectin, thiamethoxam, and spirodiclofen insecticides were determined using stainless steel column C18 (300 mm long, 4.6 mm) packed with silica gel for chromatography (5μm particle size) at column temperature 30°C. Acetonitrile – water was selected using a UV wavelength of 254 nm as a mobile phase at a flow rate of 1.0 ml/min. Retention time (tR) for Pyridabin, Abamectin, thiamethoxam and Spirodiclofen pesticide detection were 2,455, 8,187, 11,893 and 42,793 minutes respectively and compared to a known standard (Fig. 1) under the same conditions. Based on the peak area, the quantities were calculated. 20 μl injection volumes were used in all experiments, and LC solution software controlled the chromatographic device. The recorded data during the entire study period were evaluated statistically using the accessible software programs (GraphPad Prism 8.1.2 and Microsoft Excel Professional Edition 2013).

Results and Discussion

The HPLC technique used to identify and quantify residues of pyridabine, abamectin, thiamethoxam, and spirodiclofen. Thirty-one samples had higher residue concentrations from 128 environmental samples, most frequently noticed thiamethoxam in plant and groundwater samples, followed by pyridabine, spirodiclofen, and abamectin. Surprisingly, abamectin concentration was identified in some plant samples and undetected in any water samples (Figs. 2 and 3), with statistically significant variations between plant and water samples ($P<0.001$). The concentration and amount of pesticide residue in the studied samples might have altered based on the types of pesticides, half-life, bioaccumulation trend, and biodegradation properties.

Overall, Pyridabin pesticide residue concentration was measured in 75.0% of the plant and groundwater samples, the higher level was ranged from 18.918 and 1.086 ppm to N.D as a lower concentration with mean of 7.053± 0.164 and 0. 38±0.01 for plant and groundwater samples respectively. It was revealed that 90.6% of the plant and water samples determined the concentration of thiamethoxam residues and its higher range was established in plant samples within the studied period, higher concentration ranged from 33.647 and 1.633 ppm to N.D, with a mean of 11.25±2.74 and 1.63±0.13 respectively for the plant and groundwater samples.

The elevated levels of spirodiclofen ranged from 12.83 and 0.85 ppm to N.D and with a mean concentration of 3.985±1.13 and 0.205±0.01 in the plant and groundwater samples, respectively. Residues of spirodiclofen pesticide were detected in 65.6 % of the plant and water samples and their higher range determined in plant samples within the period of study. While residues of abamectin pesticides were detected in 15.6 % of plant samples, there were no residues of abamectin pesticides detected in groundwater samples were ranged from 5.913 ppm to ND with a mean of 0.1± 0.005 in plant samples, and non- detected in groundwater samples during the studied period (Fig. 3).

Table1: Liver and kidney function variables with a blood cell count of sprayer and control subjects (mean± SE).

Parameters	Sprayers	Control	P value
BChE (ng/ml)	6.705 ± 0.325	12.08 ± 0.575	0.0001
D. Bilirubin (mg/dl)	0.483 ± 0.025	0.214 ± 0.0217	0.0001
T. Bilirubin (mg/dl)	1.526 ± 0.0495	0.6795 ± 0.0484	0.0001
ALP (U/l)	144.9 ± 3.393	46.46 ± 7.825	0.0001
GOT (U/l)	56.54 ± 1.817	24.17 ± 1.579	0.0001
GPT (U/l)	56.87 ± 2.019	20.89 ± 2.41	0.0001
RBC (1012/l)	4.255 ± 0.0728	4.67 ± 0.11	0.01
WBC (109/l)	8.26 ± 0.239	6.208 ± 0.186	0.0001
Urea	37.07 ± 1.406	28.98 ± 1.794	0.005
Creatinine	0.9619 ± 0.0433	0.9124 ± 0.043	0.5

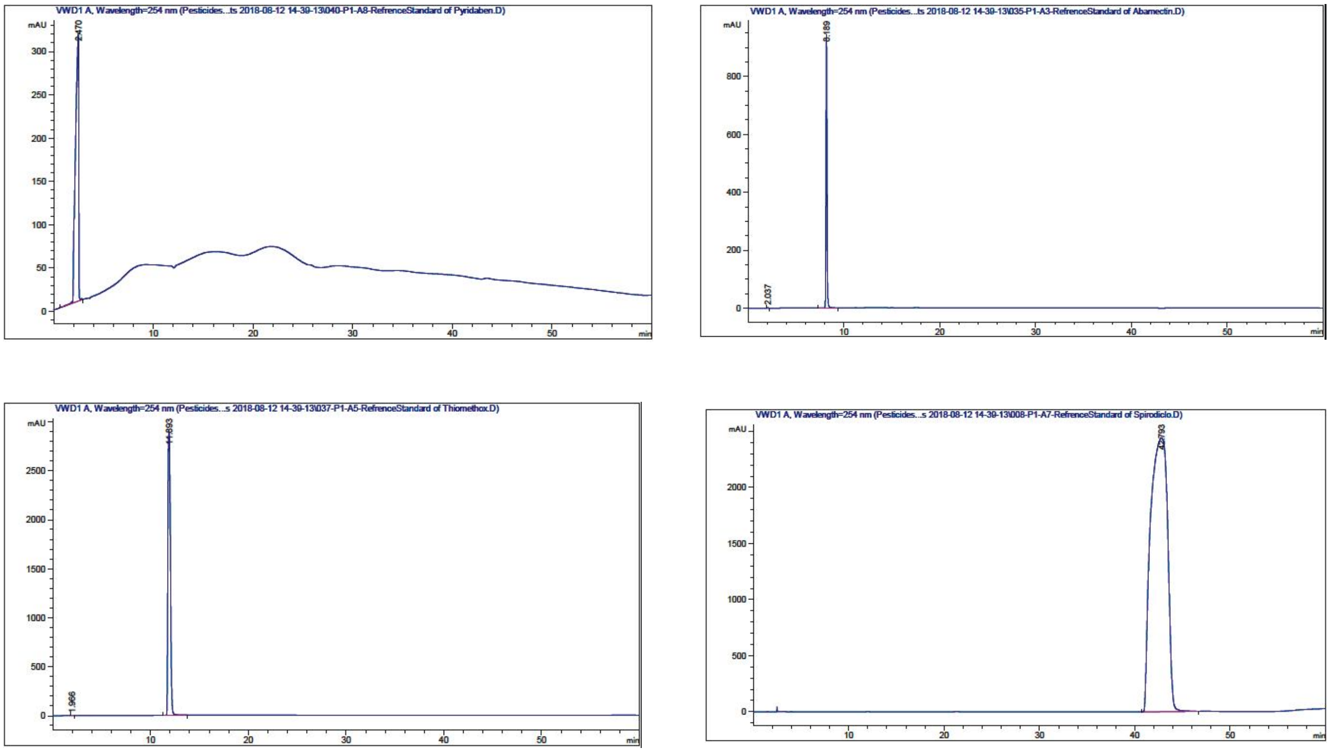
The remaining and detection of pesticides in selected samples during the study period were consistent with the structure and formation of pesticides that abamectin pesticide was rapidly reduced due to photo-degradation and the action of abamectin-degrading enzymes that groundwater was not reached, while other three pesticides reached groundwater. The same results had been obtained by (Jodeh *et al.*, 2016, Fantke *et al.*, 2014, Vineyard and Stewart, 2017, Jiandani and ashraf, 2015, EFSA, 2018).

According to questioner lists, most sprayers complained about fatigue, headache, and loss of appetite for nausea, dizziness, excessive sweating and salivation, blurred vision with excessive watering, muscle weakness, all signs and symptoms of pesticide exposure. While spraying the pesticides, no precautions were taken by workers when spraying pesticides.

BChE activity is a reliable biomarker of pesticide

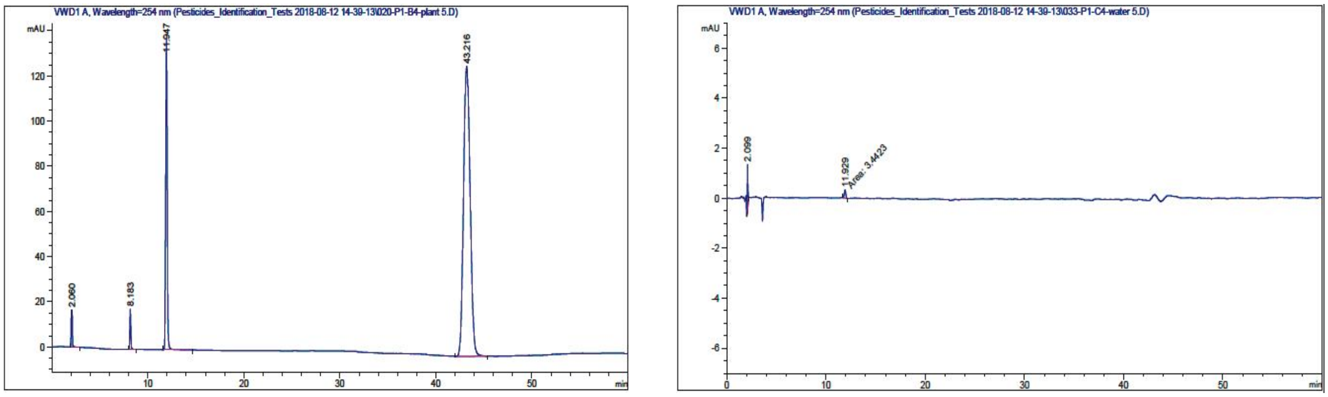
Table 2: Illustrate statistical correlation among liver, kidney function test, and blood cell count variables of sprayer and control subjects.

Parameters		BChE	D. Bilirubin	T. Bilirubin	ALP	GOT	GPT	Creatinine	Urea	RBC	WBC
BChE	Pearson (r)	1.000									
	P value	-									
D. Bilirubin	Pearson (r)	-0.641	1.000								
	P value	0.01	-								
T. Bilirubin	Pearson (r)	-0.797	0.756	1.000							
	P value	0.001	0.001	-							
ALP	Pearson (r)	-0.721	0.428	0.578	1.000						
	P value	0.01	0.05	0.01	-						
GOT	Pearson (r)	-0.718	0.416	0.683	0.834	1.000					
	P value	0.01	NS	0.001	0.001	-					
GPT	Pearson (r)	-0.798	0.416	0.692	0.807	0.873	1.000				
	P value	0.001	0.01	0.001	0.001	0.001	-				
Creatinine	Pearson (r)	-0.100	-0.143	-0.142	-0.091	-0.186	0.202	1.000			
	P value	NS	NS	NS	NS	NS	0.01	-			
Urea	Pearson (r)	-0.433	0.417	0.367	0.279	0.164	0.452	0.373	1.000		
	P value	NS	0.05	0.05	NS	NS	0.01	0.01	-		
RBC	Pearson (r)	0.485	-0.684	-0.617	-0.604	-0.587	-0.654	0.106	-0.677	1.000	
	P value	NS	0.01	0.05	0.05	0.05	0.01	NS	0.001	-	
WBC	Pearson (r)	-0.459	0.405	0.446	0.542	0.588	0.321	-0.718	0.055	-0.532	1.000
	P value	0.05	0.05	0.05	0.01	0.001	0.001	0.001	NS	0.05	-



(a) Pyridabin (b) Abamectin (c) Spirodiclofen (d) Thiamethoxam

Fig. 1: The Chromatograms of a. Pyridabin, b. Abamectin, c. Thiamethoxam, and d. Spirodiclofen standards.



(a) Plant (b) Water

Fig. 2: HPLC Chromatogram sample of Pesticides residues in (a) plant and (b) water sample.

exposure and is generally used to estimate pesticide toxicity in occupational and clinical toxicology, and experts recommended that BChE activity be less than 60% of the reference value as a critical value of pesticide infection (Aroonvilairat *et al.,* 2015). According to our results (table 1 and Fig. 4) BChE activity significantly decreased at ($p<0.001$) in sprayers blood samples in comparison to control groups with a mean concentration of (6.705±0.325) to (12.08±0.575), which was highly correlated with the intensity and duration of exposure during spray and its lower recovery rates of BChE (Al-Ghais and Saif, 2013, Araoud *et al.*, 2011). In the present study, the decrease in serum BChE in pesticide sprayers was mainly due to infections with pesticides, confirming reports that nicotine pesticide compounds inhibit BChE activity (El- Nahhal, 2016, Jamal *et al.*, 2016). The main effects of pesticides are on channels of sodium and chloride as they modify the gating characteristics of voltage-sensitive sodium channels to delay their closure, these agents increase the influx of Na^+ into synaptic terminals and create a hyperpolarized and hyperirritable synaptic membrane that in turn increases the release of the acetylcholine neurotransmitters. The results were utterly consistent with (Dalvie *et al.*, 2006, Aroonvilairat

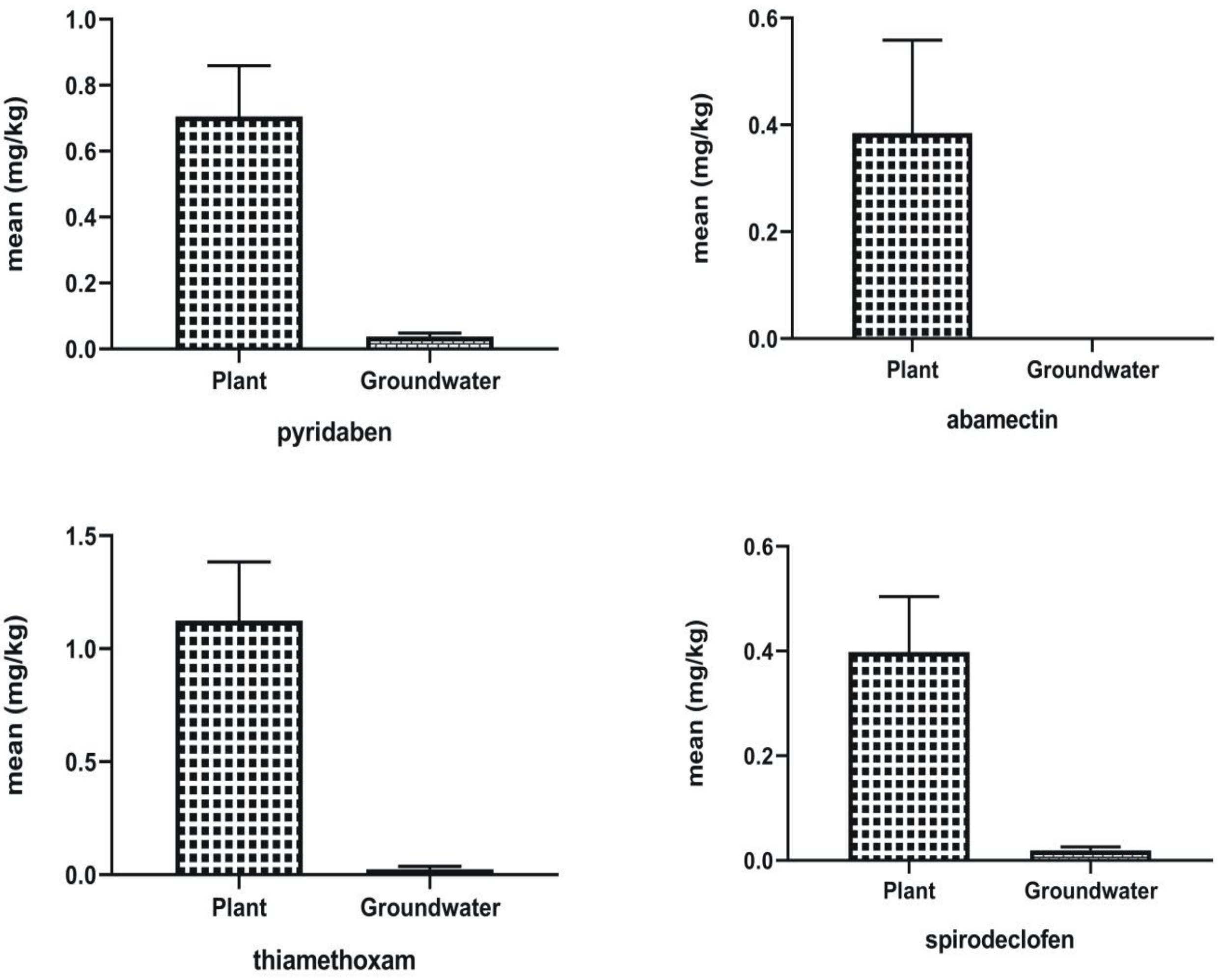
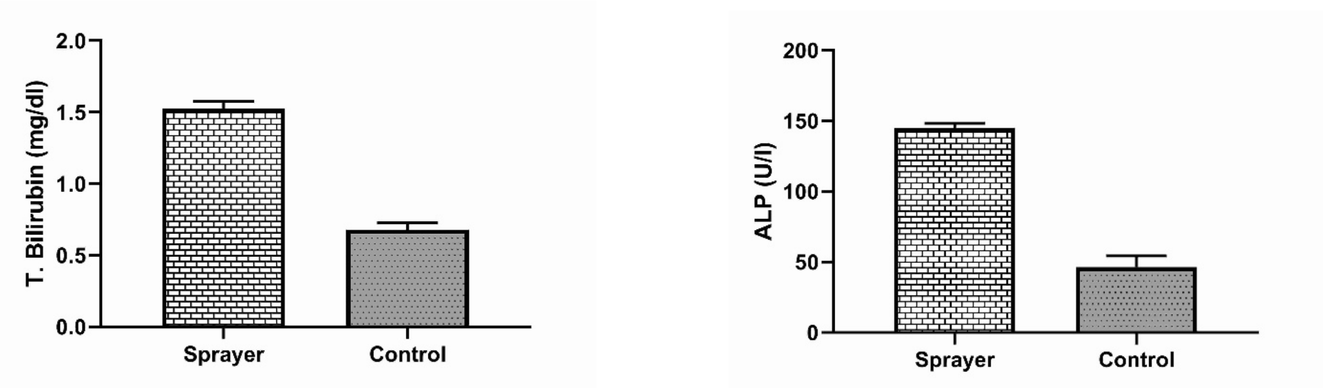


Fig. 3: The mean concentration of a. pyridabin, b. Abamectin, c. Thiamethoxam, and d. spirodiclofen pesticide residues within the plant and water during the studied period.

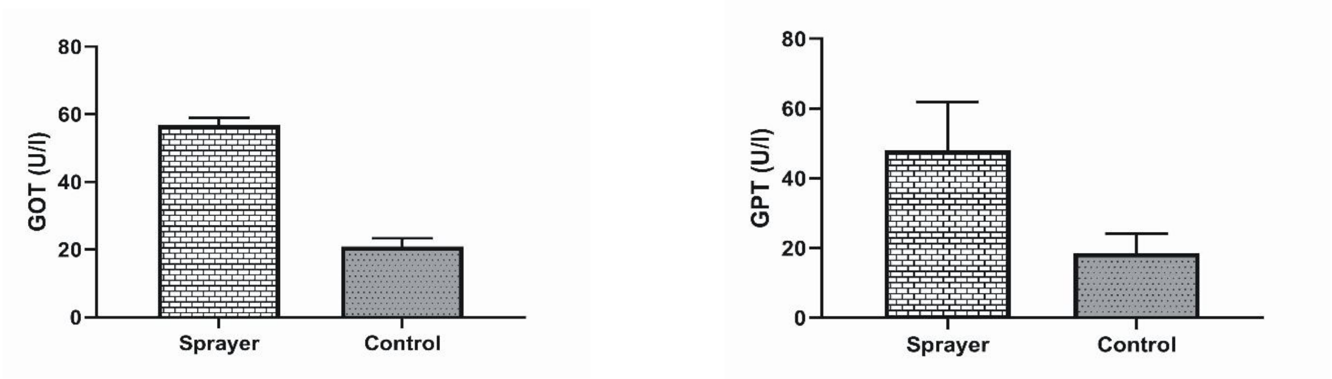
et al., 2015). According to investigated results there were significantly relation between BChE activities in exposed sprayers and other variables at (r = -0.65, p < 0.01; -0.797, p< 0.001; -0.721, p < 0.01; -0.718, p < 0.01; -0.798, p<0.001; -0.459, p<0.05) for D. bilirubin, T. bilirubin, ALP, GOT, GPT and WBC respectively. While non-significantly with creatinine, urea, and RBC (Table 2).

Exposure to pesticides and liver functions among sprayers hepatotoxicity were monitored through quantitative analyses of serum GPT, GOT, ALP, direct, and total bilirubin used as biochemical markers of liver damage among farm workers. According to our investigated outcomes, the liver function enzymes were significantly increased at statistically different levels (P<0.001) in exposed sprayers compared to the control group during the studied period (Table 1 and Fig. 4). Pesticides are hepatotoxic, mostly based on animal studies, increased liver weight, and documented enzyme induction in animals that have been exposed to pesticides for a long time, especially those with higher chlorine due to their long half-life and bioaccumulation properties (Serdar *et al.*, 2014). The higher levels of liver enzymes (GOT, GPT, ALT, direct, and total bilirubin) at the most upper exposure sprayers were our most consistent findings for blood biochemistry datasets. On the other hand, there were significantly correlation between D. bilirubin at (r= 0.756, p< 0.001; 0.428, p< 0.05; 0.416, p< 0.01; 0.417, p< 0.05, -0.684, p< 0.01; 0.405, p< 0.05) with T. bilirubin, ALP, GPT, urea, RBC and WBC respectively. While non-significantly with creatinine and GOT. ALP have significantly correlation (r= 0.834, p <0.001; 0.807, p< 0.001; -0.604, p<0.05; 0.542, p<0.01) with GOT, GPT, RBC and WBC respectively. While non-significantly with creatinine and urea. Also, GOT have significantly correlation (r= 0.873, p <0.001; -0.587, p<0.05; 0.588, p<0.001) with GPT, RBC and WBC respectively. While non-significantly with creatinine and urea. GPT have significantly correlation (r= 0.834, p <0.001; 0.807, p<



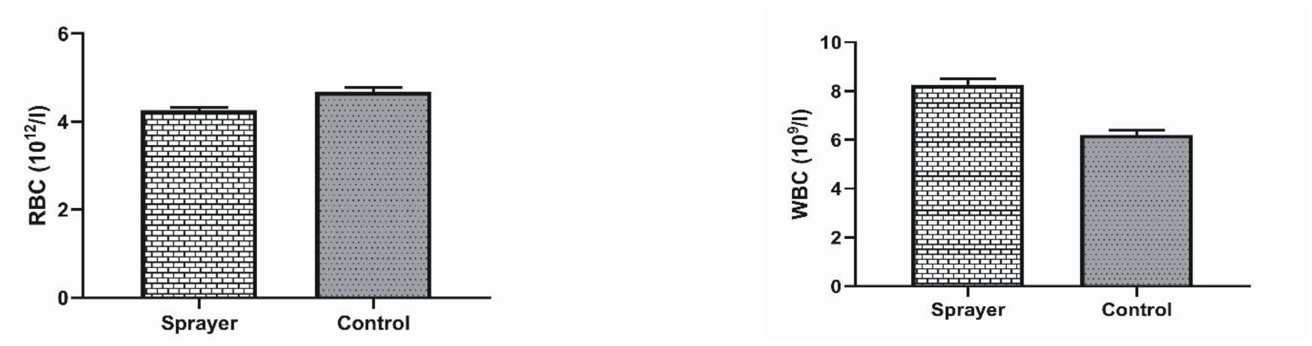
BChE Concentration

D.Biluribin Concentration



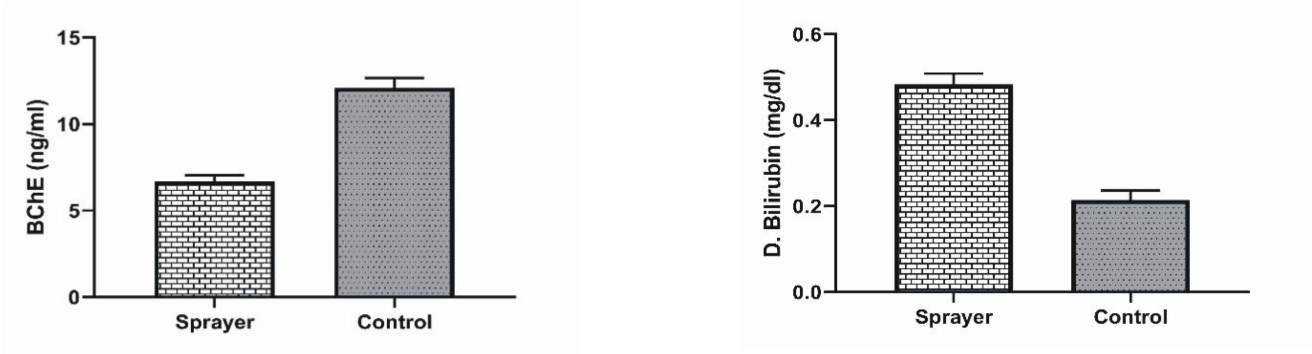
T.Biluribin Concentration

ALP Concentration



GOT Concentration

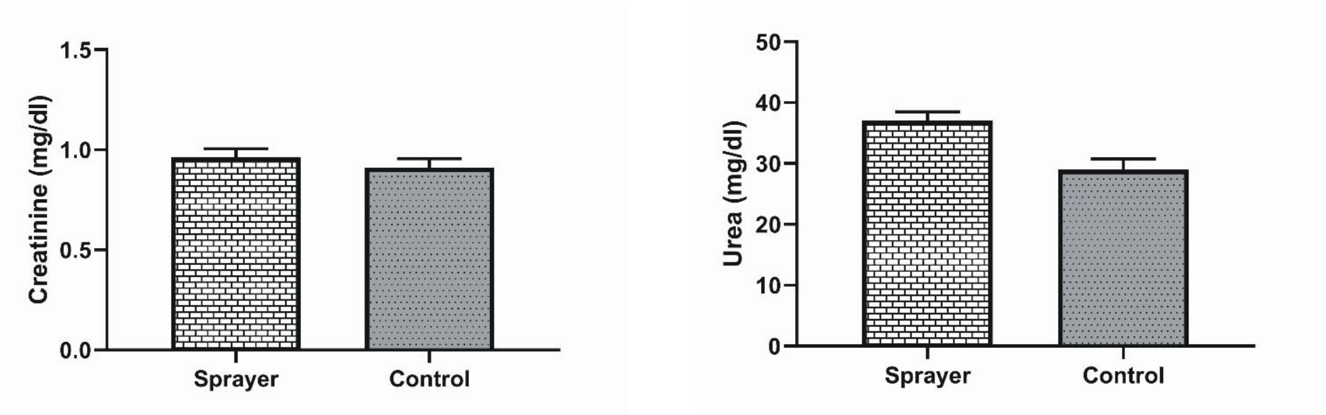
GPT Concentration



RBC Count

WBC Count

Fig. 4: Mean concentrations of 1. BChE, 2. D. bilirubin, 3. T. bilirubin, 4. ALP, 5. GOT, 6. GPT, 7. RBC, and 8. WBC within the serum of sprayers and control groups during the studied period.



Creatinine Concentration

Urea Concentration

Fig. 5: Mean concentration of a. creatinine and b. urea within the serum of sprayer and control groups within the studied.

0.001; -0.604, $p<0.05$; 0.542, $p<0.01$) with GOT, GPT, RBC and WBC respectively. While non-significantly with creatinine and urea (Table 2).

A positive association between OC pesticides and liver enzymes was already previously reported, indicating that these enzymes are an early and sensitive biomarker of exposure to pesticides, although levels were observed within normal clinical parameters, same observations have been reported by (Podprasart *et al.*, 2007, Dewan *et al.*, 2004, Ibrahim *et al.*, 2011). Increasing levels of bilirubin may be attributed to prolonged exposure to pesticides that have disturbed the healthy red blood cell metabolism, affected hepatic dysfunction and increased blood bilirubin levels, resulting in hyperbilirubinemia that may result from the production of more bilirubin than healthy liver, as indicated by other researchers (Awad *et al.*, 2014, Khawja., 2013, Aroonvilairat *et al.*, 2015).

Our observations showed that there were significantly decreasing at ($p<0.01$) in RBC counts between sprayers in comparison to control group blood samples. However, results showed an increase in the WBC count to the upper limit with a statistically significant difference in sprayer blood samples ($P<0.001$) compared to control blood samples (table 1 and Fig. 4) with significantly correlation (table 2) between RBC and WBC at ($r= -0.532$, $p<0.05$). Our outcome, contrary to the previous finding, that there were no significant differences between hematological parameters in exposed pesticides and control groups (Sudjaroen, 2017, Gaikwad *et al.*, 2015, Serdar *et al.*, 2014, Aroonvilairat *et al.*, 2015).

As shown in Fig. 5 during the studied period, the mean serum urea concentrations of the sprayer were significantly increased at ($P<0.01$) compared to controls (Table 1). The longer the duration of the work, the higher percentage of urea concentration increases were observed and in fact, mean concentrations of serum sprayers creatinine slightly fluctuated non-significantly compared to controls group. Previous studies have shown significant variations in the concentration of serum urea and creatinine in farmers exposed to pesticides, which are inconsistent with our investigation results (Ritu *et al.*, 2013, Haghighizadeh *et al.*, 2015). Statistical correlation results revealed that creatinine have significantly correlation ($r= 0.373$, $p <0.01$; -0.718, $p<0.001$) with urea and WBC respectively and non-significantly with RBC. While, Urea have significant correlation with ($r= -0.677$, $p <0.001$) with RBC and non-significantly with WBC (Table 2). Serum levels of urea and creatinine in pesticide-exposed farmers are of clinical value; urea is formed by amino acid deamination in the liver and then transported through the blood to the kidneys where it is excreted with urine. According to our research findings, excessive exposure to pesticides probably resulted in cytotoxic changes in hepatic and renal biochemical markers which were positively correlated with exposure to pesticides as indicated by (Reddy and Jagdish, 2012, Khan *et al.*, 2012, Khan *et al.*, 2012 Yassin *et al.*, 2016, Aroonvilairat *et al.*, 2015).

Conclusions

The present study revealed that both pyridabine, abamectin, thiamethoxam and spirodiclofen pesticides found in groundwater and sprayed greenhouse cucumber. Studied pesticide residues had an impact on both the BChE activity and certain enzymes (GOT, GPT, ALP, complete and direct bilirubin). Significant changes in serum urea observed, with small fluctuations in creatinine. WBC and RBC had already altered significantly. According to the questioner lists, most farmers in the studied fields were unaware of the hazards induced by inappropriate handling of pesticides. We suggest increasing awareness among farmers about the use of proper protective measures while handling and managing pesticide. Farmers must be encouraged to reduce, if not eliminate, the use of pesticides by offering incentives to assist farmers.

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