IRAQI Academic Scientific Journals
Samarra Journal of Pure and Applied Science
SJPAS
مجلة سامراء للعلوم الصرفة والتطبيقية
www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

Evaluation of the Effect of the Pesticides Glyphosate and Chlorpyrifos on the Activity of liver enzymes in serum of Group of Farmers in Samarra

Mareb A. Shawkat [1*], Wijdan I.A. Abd-alwahab [2]
1- Department of Biology, College of Education, University of Samarra, Iraq
2- Department of Biology, College of Education, University of Samarra, Iraq

https://doi.org/10.54153/sjpas.2025.v7i2.949

Article Information
Received: 08/06/2024
Revised: 29/07/2024
Accepted: 20/08/2024
Published: 30/06/2025

Keywords:
Pesticides, Glyphosate, Chlorpyrifos, Liver enzymes

Corresponding Author

E-mail:
Marib.Ahmed@uosamarra.edu.iq
Mobile

Abstract

The study aims to evaluate the effect of exposure to the pesticides glyphosate and chlorpyrifos on the health of farmers, whose ages are between (20 and 45) years, in rural areas. The experiment was conducted during the period from the beginning of March 2023 until the end of June. The sample was divided into three groups: the glyphosate pesticide group (15), the chlorpyrifos pesticide group (15), and the control group (15). Information was collected from farmers about the use of agricultural pesticides and their health conditions. After that, blood samples were collected from them and used to evaluate physiological and biochemical variables such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Gamma-Glutamyl Transpeptidase (GGT). The findings showed a significant increase in the activity of ALT, AST, ALP, and GGT enzymes in the two groups of chlorpyrifos and glyphosate pesticides compared with the healthy control group.

Introduction

Pesticides are chemical substances that have a positive effect in improving the production of crops, protecting them, and preventing diseases. Excessive use of pesticides that violate standards and controls will harm the crops and beneficial organisms and cause health disasters. Many of these pesticides are environmentally persistent [1].

Despite the role that pesticides play in combating pests of all kinds, we must not ignore the fact that they are dangerous toxic substances whose use without guidance leads to serious negative repercussions. It could affect humans and the environment. Its harm is not limited to the general health of humans but extends beyond it. Its residues pollute the air, soil, and water, and accumulate in the tissues of living organisms as a result of incorrect uses. So, it will negatively affect the complex ecosystem. The harmful effects of pesticides on human health include cases of poisoning resulting from exposure to large or low doses, and permanent

exposure to their residues in food crops increases the amount of pollution resulting from their use [2].

Toxic pesticides penetrate the skin upon contact with it or enter the digestive system through contaminated fruits and vegetables that carry the remaining toxins. Then, it reaches the blood and all organs of the body, settling there and causing many serious diseases, including (liver disease, kidney failure, and cancers [3]. The liver is considered one of the most important organs that controls basic activities in the body, such as digesting food (storing sugar - building protein - and maintaining the level of cholesterol in the body). Unfortunately, the accumulation of pesticides in the liver leads to its destruction. It reduces its ability to perform its functions and destroys the defense lines and production cells in the human body. Thus, this will lead to the spread of hepatitis, cirrhosis, and then liver failure). High liver enzymes may indicate inflammation or cell damage in the liver. Inflamed or damaged liver cells leak certain chemicals into the bloodstream in higher than normal amounts, which elevates liver enzymes in blood tests [4]. In this investigation , liver enzyme levels were measured upon exposure to glyphosate and chlorpyrifos pesticides and their effects on farmers' health

Materials and Methods

Samples Collection

The 45 blood samples were collected from farmers which were divided into three groups, including the glyphosate pesticide group (15), the chlorpyrifos pesticide group (15), and the control group (15). The questionnaire form was also filled out with special information about the farmers. Each group included (15) people whose ages ranged from (20-45) and were prepared within the objectives of the study.Samples of venous blood were gathered using disposable plastic medical syringes after taking some information regarding each of them. The blood samples were placed in gel tubes free of anticoagulants to separate the serum using a centrifuge. Then, they are placed in clean and sterile Eppendorf tubes with a tight cap, after writing the code and sample number on the tube. Finally, they are stored in a deep freezer at a temperature of (-20 degrees Celsius) until they are used to conduct the necessary tests (enzymatic and biochemical).

Determination of liver enzymes activity

The effectiveness of the enzymes ALT, AST, ALP, and GGT was measured based on natural enzymatic methods using a measuring kit supplied by (Agapp, Switzerland) and a measuring kit for the GGT enzyme supplied by Biolabo, France.

Statistical Analysis

The results of this study were analyzed using analysis of variance (ANOVA), and the arithmetic means of the coefficients were compared using the Duncun Multiple Range test with a significance level of $0.05 > (P)$ and using the statistical program SPSS [5].

Results and Discussion

The results shown in Figures (1, 2, 3, 4) and Table (1) show a significant increase (P≤0.05) in the activity of ALT, AST, ALP, and GGT enzymes in groups of farmers exposed to glyphosate and chlorpyrifos compared to the healthy control group. It is also noted that there is no significant difference (P>0.05) in the effectiveness of the previously mentioned enzymes between the two groups of glyphosate and chlorpyrifos, except for the effectiveness of the GGT enzyme. As it is observed, there is a significant decrease in the effectiveness of the GGT enzyme in the group exposed to glyphosate compared to the chlorpyrifos group.

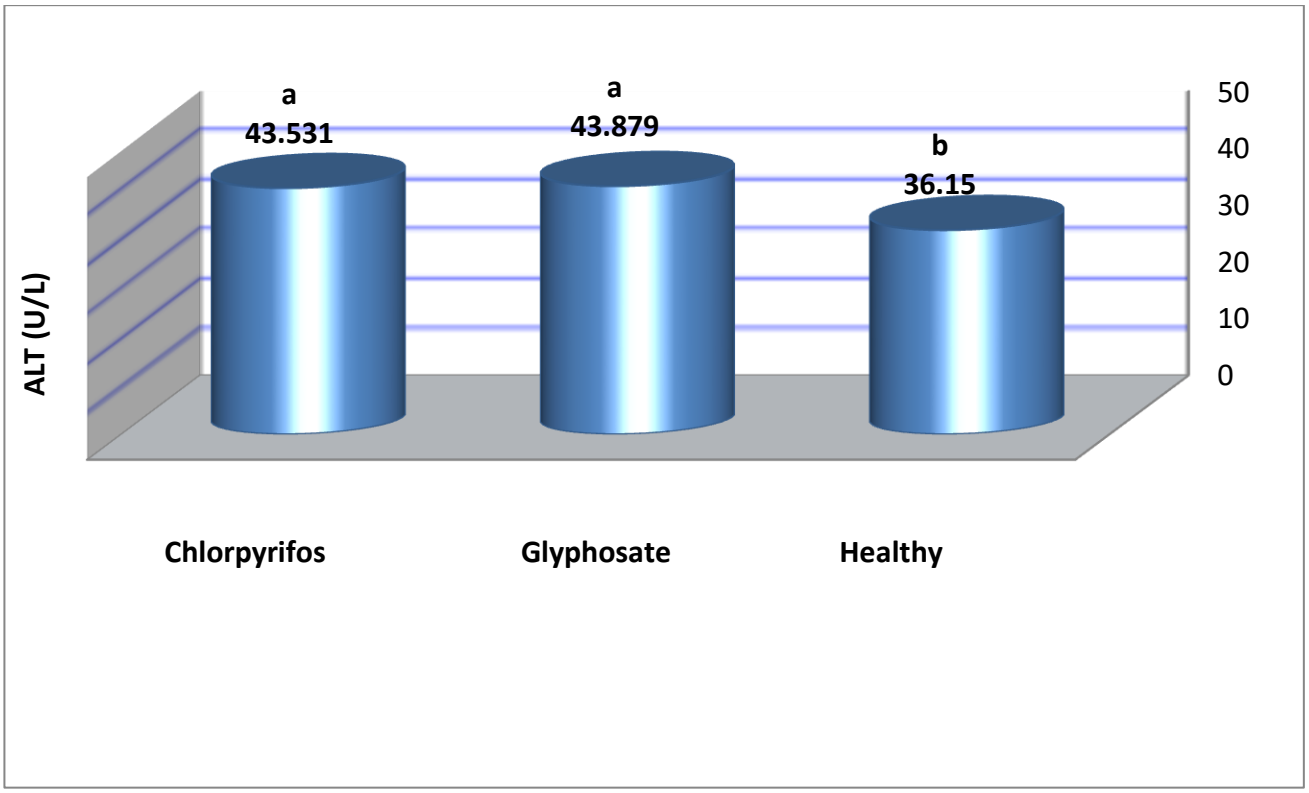

Figure 1: *ALT U/L enzyme activity of farmers and the healthy control group*

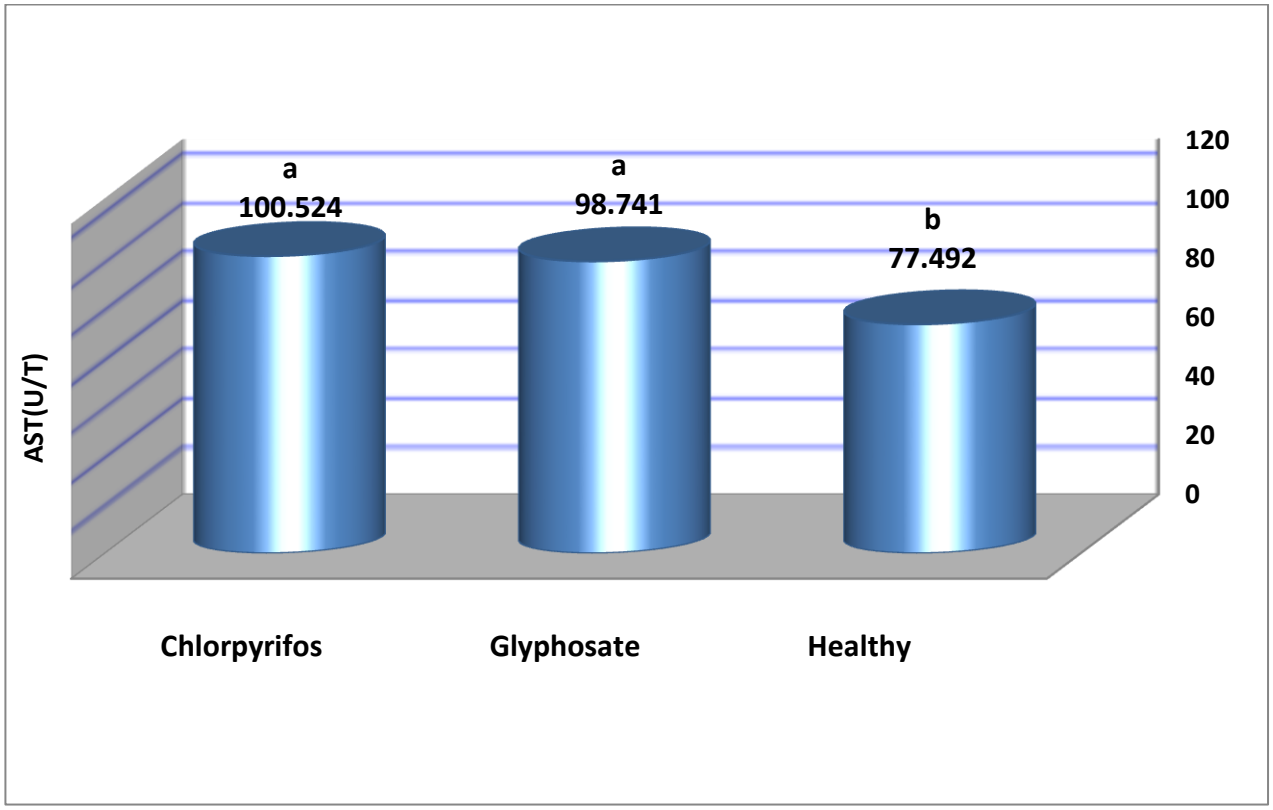

Figure 2: *AST U/L enzyme activity of the farmers and the healthy control group*

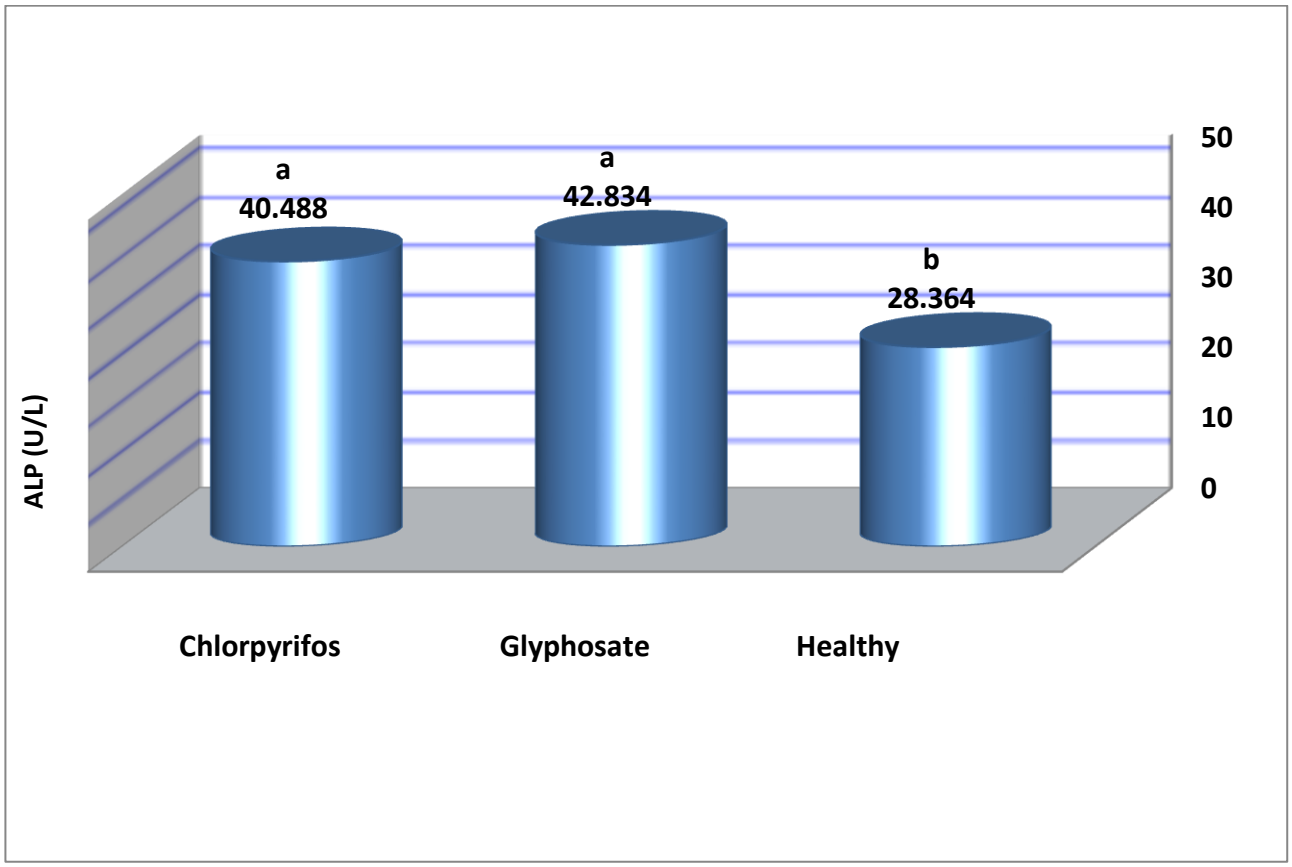

Figure 3: *ALP U/L enzyme activity of the farmers and the healthy control group*

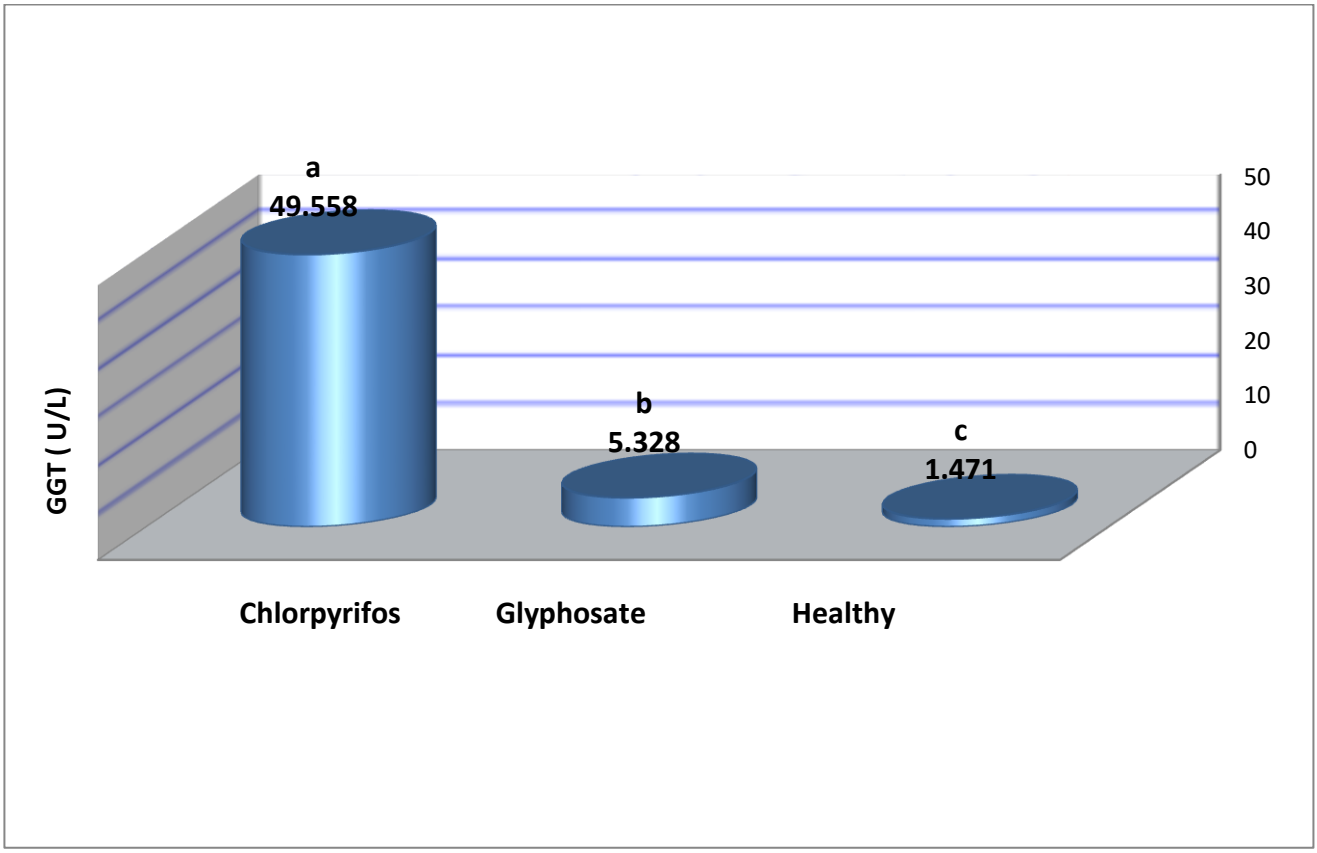

Figure 4: *The activity of the GGT U/L enzyme serum of the farmers and the healthy control group.*

Table 1: Levels of liver enzymes, AST, GGT, ALP, and ALT in serum of study groups

Groups Physiological standards	Control group N=15	Exposed to glyphosate N=15	Exposed to chlorpyrifos N=15
Alanine aminotransferase enzyme (ALT) U/L	36.15 ±3.01 b	43.87 ±2.79 a	43.53 ±2.21 a
Aspartate aminotransferase (AST) U/L	77.49 ±11.43 b	98.74 ±7.93 a	100.52 ±5.11 a
Alkaline phosphatase enzyme ALP)(U/L	28.36 ±3.19 b	42.83 ±5.30 a	40.48 ±2.64 a
Gamma-glutamyl Transpeptidase GGT)(U/L	1.47 ± 0.27 c	5.32 ±1.95 b	49.55 ±5.01 a

- Values represent mean ± standard error Mean ± S.D.

- The different letters (horizontally) indicate the presence of significant differences at a significant level (P≤0.05).

An elevated level of liver enzymes is a sensitive indicator of liver toxicity, so measuring the levels of liver enzymes (ALP, AST, ALT, GGT) is used to evaluate the functional state of the liver. It is known that many soluble serum enzymes in blood, such as these liver enzymes, are indicators of hepatic dysfunction and tissue damage. On one hand, an increase in these enzymes indicates direct damage to the structure of the liver cells. On the other hand, it indicates the leakage of these enzymes from the cytosol in the liver into the bloodstream [6][7].

The current study confirms that exposure to these pesticides leads to pathological changes in the liver, kidneys, heart, and lungs, and perhaps to counteract the stress resulting from prolonged exposure to pesticides. These changes in enzymes indicate cytotoxicity and tissue damage caused by these pesticides, possibly by altering specific molecular pathways[8].

An increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) helps in protein metabolism. When the liver is damaged, ALT rises and is released into the bloodstream. Alanine transaminase is a cytosolic enzyme that is more specific to the liver than aspartate transaminase (AST) [8].

Therefore, levels of AST and ALT in serum are often used to evaluate liver damage, and any increase indicates liver damage and increases oxidative stress, which is associated with the levels of ALT and AST [9]. Other studies have also found a relationship between high levels of enzymes that indicates the relationship of liver damage to pesticides. This reason leads to damage to liver cells and the release of their enzymes into the bloodstream [10].

Also, other studies show that the development of liver disease leads to an increase in the production of reactive oxygen species (ROS). This often leads to greater oxidation of liver fat as a result of the activation of Kupffer cells in the liver. They initiate and perpetuate the inflammatory response and the development of fibrosis. As an increase in inflammatory cytokines affects the vital functions of the liver, it increases iron storage in cells [11].When the liver cell membrane is damaged, a variety of enzymes naturally present in the cytosol are released into the blood, leading to an increase in the level of ALT and AST[12]. AST and ALT are important enzymes because they help in the formation of amine groups necessary to the urea cycle. Estimating these enzymes gives an indicator of amino acid metabolism, and an important indicator of liver function[8][13].Studies have also confirmed that chlorpyrifos is an organophosphorus pesticide that affects differently from one organ to another, and the liver is the first target organ. It causes acute toxicity and increases the level of liver enzymes (AST, ALT) in the blood. Tissue damage, as shown by is not only limited to chronic and acute toxicity. This is shown by the change in the content of carbonyl proteins (CPOs), which is an indicator of the occurrence of oxidative stress and oxidation of proteins. While the rest of the tissues did not show this response, which indicates the sensitivity of the liver, its speed of being affected, and the progress of the stages of stress at its level compared to the rest of the tissues. This is due to the essential role that the liver plays in the various stages of detoxification. This places the liver at risk of high exposure to pesticides and their products. The possibility of liver tissue injury from oxidative stress resulting from exposure to

pesticides remains dependent on the balance achieved between the degree of stress and the enzymatic defense capacity.[14].

During liver damage, it is observed that there is a decrease in the efficiency of the antioxidant system These results confirm that the change in the tissue structure of the liver is a response to pesticides due to the toxic effect of free radicals that cause damage to various components of the membrane. An increase in AST levels may indicate liver damage or disease. As shown in the findings, it is noted that there is a significant decrease in the enzyme present throughout the body and that the highest concentration in the liver is GGT[15]. But when the liver is affected, its level increases. It is the first liver enzyme that rises in the blood when there is a problem in the bile ducts, such as a tumor. The amount of gamma-glutamyl transferase (GGT) in the blood is determined by using the GGT test because the liver is the place where it is most present. The circulation may be contaminated with GGT if the liver is destroyed. Elevated levels of GGT in the blood may indicate liver disease or bile duct injury [16].

The exact cause of liver disease cannot be determined by the GGT test. As a result, it is done concurrently with or after other liver function tests, such as the alkaline phosphatase (ALP) test. Another type of liver enzyme is ALP. It is often used to help diagnose bone problems as well as liver disease [17].

Conclusions

The current research concludes that pesticides greatly affect liver enzymes, and this leads to damage to liver cells and the release of their enzymes into the bloodstream. Farmers must be aware of the dangers of the pesticides they use. It is also necessary to resort to the agricultural authorities specialized in protecting crops in the Ministry of Agriculture and agricultural research centers to get assistance in identifying the infection and choosing the appropriate and recommended pesticide. Farmers should use the recommended pesticides and also know the precautions that must be taken during their application. Farmers' or consumers' awareness regarding these toxins and education is so important and necessary to protect them from diseases and the environment from pollution.

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www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

تقييم تأثير مبيد الجليفوسات والكلوربيريفوس على نشاط إنزيمات الكبد في مصل مجموعة من المزارعين في سامراء

مأرب احمد شوكت[1]*، وجدان ابراهيم عباس[2]
1- قسم علوم الحياة، كلية التربية، سامراء، العراق
2- قسم علوم الحياة، كلية التربية، سامراء، العراق

معلومات البحث:
تاريخ الاستلام: 2024/06/08
تاريخ التعديل: 2024/07/29
تاريخ القبـــول: 2024/08/20
تاريخ الـنشر: 2025/06/30
الكلمات المفتاحية:
مبيدات , كلايفوست , كلوربيريفوس , انزيمات الكبد

معلومات المؤلف
الايميل:
Marib.Ahmed@uosamarra.edu.iq
الموبايل:

الخلاصة:
هـــدفت الدراســـة الـــى تقيـــيم تـــأثير التعـــرض لمبيـــدات الكلايفوســـت والكلوربيريفـوس علـى صـحة المـزارعين فـي المنـاطق الريفيـة والـذين تتـراوح أعمـارهم بـين(20- 45) سـنة اجـراء التجربـة خـلال الفتـرة مـن بدايـة شـهر اذار 2023 و لغايـة شـهر نهايـة حزيـران وقسـمت العينـات ثلاثـة مجـاميع وكـالاتي مجموعـــة مبيـــد كلايفوســـت (15) ومجموعـــة مبيـــد كلوربيريفـــوس (15) ولمجموعـة السـيطرة (15) وتـم جمـع المعلومـات مـن المـزارعين حـول اسـتخدام المبيـدات الزراعيـة وحـالتهم الصـحية. بعـد ذلـك، تـم جمـع عينـات الـدم مـن المـزارعين واسـتخدامها لتقيـيم المتغيـرات الفسـلجية والكيموحيويـة أنـزيم ناقلـة أمـين الألانـين(ALT), إنـزيم ناقلـة الأمـين أسـبارتات(AST), أنـزيم الفوسـفاتاز القلـوي(ALP), ناقلـة الببتيـد غامـا غلوتاميـل(GGT).واظهـرت النتـائج وجـود ارتفـاع معنـوي فـي فعاليـة انزيمـات AST,ALT, ALP, GGT فـي مجمـوعتين مبيـدات الكلايفوسـت والكلوربيريفـوس مقارنـة مـع مجموعـة السيطرة السليمة.