DOI: 10.24996/ijs.2024.65.7.8

ISSN: 0067-2904

Evaluation of the Impacts of Pesticides Exposure in Different Durations on the Neural System and Antioxidants in Iraqi Farmers

Haneen Abdulsalam, Maan Abdul Azeez , Jamela Jouda*
Department of Biology, Collage of Science, Mustansiriyah University, Baghdad, Iraq

Received: 4/3/2023 Accepted: 29/6/2023 Published: xx

Abstract

The objective of this research was to investigate the effects of pesticides in different durations on some neurotransmitters and oxidative parameters compared to controls. A total of 120 male farmers participated in this study, 30 not exposed to pesticides served as control group and 90 exposed to pesticides and were split into two groups based on their exposure durations (<5 & ≥5 years) and three age-groups (<20, 20-40 and ≥41 years). Blood was collected to determine acetylcholinesterase (ACHE), serotonin (5-TH), dopamine (DA), reactive oxygen species (ROS), total antioxidant capacity (TAC) and malondialdehyde (MDA) levels using ELISA kits. The results of ACHE, serotonin, dopamine and TAC levels increased, whereas MDA and ROS levels decreased among farmers exposed to the pesticides. According to the exposure duration and farmers age, only 5-TH, DA and TAC levels were affected. It can be concluded that pesticides exposure may have an acute effect on acetylcholine and a chronic effect on the serotonin and dopamine via change in the antioxidant system which may play an important role in the neural diseases.

Keywords: Pesticide, Farmers, Neurotransmitters, Oxidative stress.

تقييم تأثير التعرض للمبيدات في فترات مختلفة على الجهاز العصبي ومضادات الأكسدة لدى المزارعين العراقيين

حنين عبد السلام، معن عبد العزيز شفيق، جميلة جودة*
قسم علوم الحياة، كلية العلوم، جامعة المستنصرية، بغداد، العراق

الخلاصة

كان الهدف من هذا البحث هو التحقق من تأثير المبيدات الحشرية على بعض النواقل العصبية وعوامل الاكسده في الدم لفترات مختلفة ومقارنتها بمجموعة السيطرة،. اجمالي120 من المزارعين ذكور شاركوا في هذه الدراسة، 30 لم يتعرضوا للمبيدات وعملوا كمجموعة ضابطة، و90 تعرضوا للمبيدات تم تقسيمهم إلى مجموعتين بناءً على مدة تعرضهم (أقل من 5 سنوات وأكثر من 5 سنوات) وإلى ثلاث فئات عمرية (20، 20−40 و41 سنة). تم جمع عينات الدم لتحديد مستوى كل من استايل كولين استريز (ACHE), دوبامين (DA), سترتونين (5−TH), مركبات الاوكسجين التفاعلية (ROS), سعة المواد المضادة للتاكسد الكلية (TAC), قياس اكسدة الدهون (MDA) باستخدام طريقة الإمتزاز المناعي المرتبط بالانزيم ELISA. أظهرت النتائج انخفاضًا في مستويات ACHE و5−TH و DA و TACبالإضافة الى زيادة في مستويات MDA

* Email: jamela.jouda@uomustansiriyah.edu.iq

و ROS بين المزارعين المعرضين للمبيدات مقارنة بمجموعة التحكم. وفقًا لمدة التعرض وعمر المزارعين تأثر فقط TH−5 و DA و TAC. نستنتج إن التعرض للمبيدات يؤثر على الجهاز العصبي ويمكن أن يكون له تأثير حاد على الأسيتيل كولين استريز وتأثير مزمن على السيروتونين والدوبامين من خلال تأثيره على نظام مضادات الأكسدة، والذي يمكن أن يلعب دورًا مهمًا في الأمراض العصبية.

1. Introduction

Pesticides are a class of naturally toxic components that are frequently used to protect crops against weeds, diseases and pests [1]. Pesticides are described as "any compound, or mixture of substances of chemical or biological elements intended for repelling, eliminating, or controlling any pest, or regulating plant growth," by the Food and Agriculture Organization of the United Nations [2].

Since pesticides mainly target the nervous system of the pest, studies have indicated that acetylcholinesterase (AChE) activity in plasma, for example, may be used as one of the most prevalent biomarkers of pesticide exposure [3]. Moreover, pyrethroid and other pesticides exposure may depress serotonin levels in the brain which may be related to the loss in serotonergic neurons and decreased serotonin synthesis [4]. However, pesticides can also cause oxidative stress by increasing free radical generation or altering antioxidant defence mechanisms such as detoxifying and scavenging enzymes. Organochlorines (OPs), carbamates and pyrethroids, among other pesticides, have been shown to be toxic in large parts due to oxidative stress [5].

According to another study, manufacturing workers exposed to various doses of carbamate showed increased malondialdehyde (MDA) levels, decreased thiol levels and decreased overall antioxidant capacity. Thus, oxidative stress biomarkers are also utilized to determine exposure to pesticides such as organophosphates and carbamates [6]. Pesticide exposure may alter antioxidant capacity and disrupt defence mechanisms, as well as increase free radicals' formation which causes protein, nucleic acid and lipid peroxidation [3]. Various biochemical changes are known to occur as a result of low-level pesticide exposure, some often might be caused due to the harmful biological impacts that have been linked to people [7]. In fact, prolonged exposure to low concentrations of residue pesticide results in a variety for harmful biological risks on the immunological, haematological and neurological systems [8].

In Iraq, farmers spray the pesticides without wearing the personal protective equipment (PPE) and without using the recommended dosage, hence increase the risk of harmful effects mentioned above. For this reason, the purpose of this research was to study the effects of different pesticides durations on some blood circulation neurotransmitters, as well as to elucidate some underlying mechanisms of pesticide toxicity through measuring some oxidative parameters.

2. Materials and Methods

The current study was carried out in March and April 2022 on a through six field excursions to Iraq's districts with intense vegetable farming in Hilla, Kute and Baghdad. In this study, questioner forms were given to a very large number of farmers to make sure that they were healthy and any one with sickness was excluded. Then, 120 healthy adults between the ages of 15 and 45 were selected, 30 men who were not exposed to pesticides served as the control group, and 90 male farmers who were exposed to pesticides were split into:

1. Two groups based on their exposure duration (less than 5 years & more than 5 years)
2. Three groups based on their age (<20, 20-40 and ≥41years).

Ten milliliters of venous blood samples were collected from each participant using a disposable syringe and then placed in a sterile test tube. The samples were later centrifuged for 15 min at 3000 rpm. The serums were separated and used to estimate the level of neurotransmitters such as ACHE, serotonin (5-TH), and dopamine (DA) and oxidative stress markers such as reactive oxygen species (ROS) by Sandwich enzyme-linked immunosorbent assay (ELISA), as well as MDA and total antioxidant capacity (TAC) levels by competitive ELISA technique, using the kits commercially available from My BioSource/ USA.

Results were expressed as mean ± SE or a percentage (%) of case frequency. The data was examined for many comparisons after one-way analysis of variance (ANOVA), using Fisher's test or t-test. A regression analysis based on analysis of combined variance was done (ANCOVA). StatView 5.0 was used to conduct all the experiments data analysis. The differences were considered significant when $p< 0.05$.

3. Results

The levels of ACHE, 5-TH and DA in the serum of farmers exposed to pesticides and control groups are displayed in Table 1. ACHE, serotonin and dopamine levels significantly decreased ($p<0.0001$) in farmers exposed to pesticide compared to control group. In comparison to the control group which had serum concentrations of 95.002 ± 2.924 U/L, 21.795 ± 0.884 ng/mL and 20.300 ± 0.865 ng/mL respectively, the ACHE, serotonin and dopamine levels were 43.768 ± 1.183 U/L, 12.051 ± 0.718 ng /mL and 12.316 ±0.386 ng /mL respectively.

Table 1: Levels of serum neurotransmitters in farmers exposed to pesticide and controls.

Group	ACHE (U/L) (Mean ± SE)	5-TH (ng/mL) (Mean ± SE)	DA (ng/mL) (Mean ± SE)
Control	95.002 ± 2.924*	21.795 ± 0.884*	20.300 ± 0.865*
Exposed	43.768 ± 1.183	12.051 ± 0.718	12.316 ± 0.386
***P*-value**	<0.0001	<0.0001	<0.0001

* The significant difference between farmers exposed to pesticide and in controls.

According to the duration of pesticides exposure levels of 5-TH and DA decreased significantly ($p<0.05$) in the groups exposed for less than five years (7.656 ± 0.409 and 10.177 ± 0.326 ng/ml respectively) compared to the groups exposed for more than five years (16.446 ± 0.906 and 14.455 ± 0.488 ng/ml respectively). There was, however, no significant difference between the AChE levels in the group exposed ≥ 5 years and <5 years (43.443 ± 1.300 and 41.092 ± 1.892 U/L respectively) (Table 2).

Table 3 shows no significant difference ($p\geq0.05$) in ACHE and that the DA depended on the farmers', exposed to pesticide, age groups. The concentrations of these parameters in 21-40 age group were 43.665 ± 1.322 U/L and 12.168 ± 0.457 ng/ml respectively, in <21 were 47.056 ± 5.077 U/L and 14.134 ± 1.271 ng/ml respectively and in >40 were 42.032 ± 2.928 U/L and 11.857 ± 0.737 ng/ml respectively. However, the 5-TH level significantly ($p<0.05$) increased in <21 age group compared to 21-40 and >40 (16.744 ± 1.944, 11.395 ± 0.806 and 12.377 ± 2.027 ng/ml respectively).

Table 2: Levels of serum neurotransmitter based on duration of pesticide exposure

Group	ACHE (U/L) (Mean± SE)	5-TH (ng/ml) (Mean± SE)	DA (ng/ml) (Mean ±SE)
< 5 Years	41.092 ±1.892	16.446± 0.906*	14.455 ±0.488*
≥ 5 Years	43.443± 1.300	7.656 ± 0.409	10.177± 0.326
***P*-value**	0.0526	<0001	<0001

* The significant difference between farmers exposed to pesticide ≥ 5 and < 5 years

Table 3: Levels of serum neurotransmitter based on the age of farmers exposed to pesticide.

Group	ACHE (U/L) (Mean ±SE)	5-TH (ng/ml) (Mean ±SE)	DA (ng/ml) (Mean ±SE)
< 21	47.056 ± 5.077	16.744 ± 1.944*	14.134± 1.271
21-40	43.665 ± 1.322	11.377 ± 2.027	12.168± 0.457
> 40	42.032 ± 2.928	12.395 ± 0.806	11.857 ± 0.737
***P*-value**	G1 vs. G2=0.4059 G2 vs. G3= 0.6398 G1 vs. G3=0.3166	G1 vs. G2=0.0287 G2 vs. G3=0.6337 G1 vs. G3=0.1421	G1 vs. G2=0.1384 G2 vs. G3=0.7825 G1 vs. G3=0.1619

* The significant difference between farmers exposed to pesticide age groups

The current results showed a highly significant (p<0.0001) decrease in TAC levels and a highly significant (p<0.0001) increase in MDA and ROS levels in exposed groups (12.140 ± 0.681 U/ml, 11.056 ± 0.803 nmol/ml and 1433.23 ± 58.711 U/L respectively) compared to the control group (43.033 ± 0.855 U/ml, 7.941 ±0.882 nmol/ml and 622.839 ± 37.018 U/L respectively) (Table 4).

Table 4: Oxidation parameters levels in farmers exposed to pesticide and in controls

Group	TAC (U/ml) (Mean ± SE)	ROS (U/L) (Mean ± SE)	MDA (nmol/ml) (Mean ± SE)
Control	43.033 ± 0.855*	622.839 ±37.018	7.941 ±0.882
Exposed	12.140 ± 0.681	1433.23 ±58.711*	11.056 ± 0.803*
***P*-value**	< 0. 0001	< 0. 0001	< 0. 0001

* The significant difference between farmers exposed to pesticide and in controls.

Table 5 shows that TAC levels in ≥5 years exposure duration group significantly (p<0.0001) decreased while MDA and ROS level significantly (p< 0.0001) increased (9.668 ± 0.644 U/ml, 14.357 ± 1.216 nmol/ml and 753.716 ± 66.812 U/L respectively) compared to <5 years exposure duration group (60.256 ± 4.784 U/ml, 7.754 ± 0.717 nmol/ml and 491.963 ± 10.821 U/L respectively).

This study also revealed that there was no significant difference in the ROS and MDA levels depending on the age of farmers exposed to pesticides: in 21-40 age group, the values were 603.290 ± 41.646 U/L and 11.968 ± 0.970 nmol/ml respectively; in age <21, the values were 717.572 ± 134.290 U/L and 7.188 ± 1.159 nmol/ml respectively; and in age >40, the values were 664.048 ± 105.194 U/L and 8.749 ± 1.640 nmol/ml respectively. However, TAC increased significantly ($p<0$.05) in the age group <21 compared to 21-40 and >40 (19.565 ± 3.210, 11.439 ± 0.661 and 10.800 ± 1.532 U/ml respectively) (Table 6).

Table 5: Levels of oxidation parameters based on duration of exposure of pesticide

Group	TAC (U/ml) (Mean ±SE)	ROS (U/L) (Mean ± SE)	MDA (nmol/ml) (Mean ± SE)
<5 years	14.612 ± 1.058*	491.963 ± 10.821	7.754 ± 0.717
≥ 5 years	9.668 ±0.644	753.716 ± 66.812*	14.357 ± 1.216*
P-Value	0.0002	0.0002	<0.0001

* The significant difference between farmers exposed to pesticide ≥ 5 and <5 years

Table 6: Levels of oxidation parameters based on an ages of farmers exposed to pesticide.

Group	TAC (U/ml) (Mean ± SE)	ROS (U/L) (Mean ± SE)	MDA (nmol/ml) (Mean ± SE)
< 21	19.565 ± 3.210*	717.572± 134.290	7.188 ± 1.159
21-40	11.439 ± 0.661	603.290 ± 41.646	11.968 ± 0.970
> 40	10.800 ± 1.532	664.048± 105.194	8.749 ± 1.640
***P*-Value**	G1 vs. G2 = 0.0003 G2 vs. G3= 0.727 G1 vs. G3= 0.0013	G1 vs. G2= 0.371 G2 vs. G3= 0.578 G1 vs. G3= 0.732	G1 vs. G2= 0.0796 G2 vs. G3= 0.1662 G1 vs. G3= 0.6377

* The significant difference between farmers exposed pesticide age groups

The current findings showed that MDA had significant negative correlation while ROS had significant positive correlation with the neurotransmitters (ACHE, 5-TH and DA). However, TAC had significant positive correlation with the DA and 5-TH but not with ACHE. On the other hand, significant negative correlation of TAC with the age of the farmer exposed to pesticide was recorded, however no correlation of MDA and ROS with the age was noticed. Also, there was a negative correlation of TAC and ROS and positive correlation of MDA with the duration of pesticide exposure. On the other hand, ages of farmers and their exposed durations had significant negative correlations with serotonin and dopamine but not with ACHE (Table 7).

Table 7: The correlation of oxidation parameters with neurotransmitters levels, ages of farmers and durations of exposure.

	TAC (U/ml)	ROS (U/L)	MDA (nmol/ml)	Age	Duration
ACHE(U/L)	R=0.077 P=0.5248	+R=0.238 P=0.0429	-R=-0.349 P=0.0026	R=-0.0316 P=0.7695	R=0.045 P=0.7420
5-TH (ng/ml)	+R=0.384 P=0.0009	+R=0.518 P=<0.0001	-R=-0.467 P=<0.0001	-R=-0.256 P=0.0295	-R=-0.518 P=<0.0001
DA (ng/ml)	+R=0.376 P=0.0011	+R=0.431 P=0.0002	-R=-0.456 P=<0.0001	-R=-0.242 P=0.0392	-R=-0.502 P=<0.0001
Age	-R=-0.289 P=0.0135	R=-0.122 P=0.3022	R=0.114 P=0.3409	------	-------
Duration	-R=-0.356 P=0.0022	-R=-0.285 P=0.0156	+R=0.322 P=0.0057	------	--------

4. Discussion

It is generally known that mixtures of pyrethroids, carbamates and organophosphates have synergistic effects and should be avoided whenever possible. These effects may result in problems with the central nervous system such as depression, anxiety, obsessive-compulsive behavior [9], and neuroinflammation in Parkinson's disease [10].

Since Iraqi farmers always use a mixture of different pesticides and spray it without wearing PPE, this study aimed to investigate the impact of the pesticides on the nervous system. The first major findings of our research were that farmers exposed to pesticides had

lower levels of AChE, 5-TH and DA in their serum compared to controls. These findings correspond with numerous other findings that suggested that the level of ACHE significantly decreased in the serum of farmers exposed to pesticides than in controls in different communities such as Mexico, Tunisia, Thai orchid [11, 12, 13]. Moreover, these results support previous research suggesting that pesticides reduced levels of the monoamine neurotransmitters (DA and 5-TH) [14, 15].

Researchers have discovered that cholinesterase activity inhibition by organophosphates and carbamates, as well as persistent activation of voltage-sensitive sodium channels by pyrethroids and organochlorine pesticides, to be the most common sources of severe acute pesticide poisonings [16]. The main cause of organophosphate poisoning is AChE inhibition of the nervous system which leads to an excessive stimulation of cholinergic tone through acetylcholine buildup within synapses as well as neuromuscular junctions [17]. The two most extensively marketed neonicotinoids are imidacloprid and acetamiprid. Neonicotinoids bind to mammalian nicotinic acetylcholine receptors (nAChRs) differently from nicotine, serving as selective agonist in a completely different manner [18].

However, it has been found that pyrethroids and other insecticide changes may alter brain's levels of neurotransmitters, as well as metabolites of monoamine neurotransmitters which agrees with our results that found a reduction in the 5-TH and DA [13]. Same results were reported by other researchers who found that cyfluthrin (Pyrethroid insecticides) caused a statistically significant decrease in 5-HT and dopamine levels in brain region in dose-dependent manner [4, 15]. The decreased brain serotonin levels caused by pesticide exposure could be related to reduced serotonin production and destroying the serotonergic neurons [4]. However, an *in vivo* exposure to permethrin and deltamethrin increased dopamine transporter (DAT) as well as (DAT)-mediated dopamine absorption in striatal synaptosomes. It has also been revealed that continuous high-level exposure reduces the dopamine uptake in SK-DAT cells which could be due to apoptosis [19].

Interestingly, while there was no change in AChE levels, however serotonin and dopamine levels significantly decreased in farmers who had been exposed to the pesticides for more than 5 years, compared to those exposed for less than 5 years in the current study. Rahman *et al*. suggested that the pesticide's high concentration had major effects on consumers, and the longer duration effect was more physiologically significant [20].

Pesticide exposure, both acute and chronic, inhibits AChE activity. Following that, neurological symptoms are brought on by delayed absorption which inhibits AChE and causes an excess accumulation of ACh in muscarinic and nicotinic sites, including the neuromuscular junction [21]. These facts could be the reason why there was no variation in the AChE levels according to the duration of pesticide exposure in the current investigation. However, it has been discovered that rats exposed to rotenone develop neuro-degeneration within a peripheral nervous system over time, as well as a reduction in motor nerve conduction velocity, especially in the sciatic nerves, as a result of a lack of monoamines (AD and 5-TH) and a disruption of chemical synapses [22]. These pieces of evidence might explain the significant decrease in serotonin and dopamine levels with increasing exposure duration in the present study.

Taken together, these evidences might explain the significantly negative correlation of duration with the DA and 5-TH levels but not with the AChE levels that were found in this study.

In current data, there was a high tendency differences in the DA and AChE levels reaching to be significant in the 5-TH levels between pesticide-exposed age groups. The lowest levels were in the >40 years group and the highest in the <20 years group. There was no significant difference between a quantitative density of AChE-positive neurons in elderly brains and young-adult brains [23]. Same results were found in the dopamine and serotonin systems. The dopamine system in the brain undergoes significant alterations with aging that have an impact on cognitive performance [24]. The quantitative changes take place in the pre- and post-synaptic components for the serotonin and dopamine systems throughout nonpathological aging [25].

Taken together, these evidences might explain the significantly negative correlation of age with DA and 5-TH levels and non-significant negative correlation with AChE level found in this study.

Reactive oxygen species as byproducts of regular cellular metabolism, are required for the generation of energy for life functions [26]. However, as signaling molecules that control biological and physiological processes, ROS are created in cells in relatively low amounts under normal circumstances [27]. Pesticides are an example of an environmental stressors that may lead to oxidative stress by forcing cells to produce excessive amounts of ROS which can harm biological macromolecules like DNA and lead to cellular damage [28]. On the other hand, when the cell membrane's lipid peroxidation occurs as a result of an excess of reactive free radical production, it causes cell damage or death by generating changes in cellular integrity [29]. As a result, the body employs a defensive response including an anti-oxidation system [30]. The enzymes of endogenous antioxidant work harder to eliminate the constantly produced free radicals at first due to an induction, but subsequently enzyme depletion occurs, leading to oxidative cell damage [31]. So, studying the level of oxidative stress and antioxidant status in employees exposed to pesticides for an extended period of time is interesting. Particularly, the significant negative effects on health caused by an imbalance between the body's antioxidant defenses and oxygen free radical (ROS) generation might lead to a variety of serious consequences, including neurotoxicity [32].

ROS, MDA and TAC were all measured in this study. In the current study, ROS and MDA levels significantly increased whereas TAC levels significantly decreased. The findings of this investigation were in line with those obtained from previous research that found the human exposure to pesticides is mainly linked to increased production of free-radical species which resulted in an increase in lipid peroxidation and a reduction in antioxidant activities [33]. Many studies agree with our results and show a statistically significant decrease in TAC after insecticides and pesticides exposure [34, 35, 30]. On the other hand, another study reported that MDA levels in fish increased when exposed to eprinomectin (a member of an ivermectin family) [35]. While another study reported that because of the increased air contamination, workers had a generally larger increase in MDA [36]. The considerably damaging increase in MDA level supports the hypothesis that oxidative stress persistence has a crucial role in the progression of physiological issues [37].

Furthermore, the current study found a significant difference in ROS, MDA and TAC levels depending on the duration of pesticides exposure and a significant correlation between them which agrees with other studies that found long-term pesticides exposure may be a significant contributor to the progression of vascular diseases via lipid peroxidation, oxidative stress, and metabolic disturbances of lipoproteins. [12]. These results were explained by the

simple fact that repeated pesticides exposure depletes reactive oxygen species which exhausts the antioxidants in the body [38, 29, 32].

As previously mentioned, the significance of oxidative stress for pesticide-induced neurotoxicity has been widely documented and is linked to a variety of neurological disorders such as Alzheimer and Parkinson diseases [33]. This study found links between oxidative stress parameters and neurotransmitters levels which might explain the findings. Our results agreed with many researchers who suggested that ROS has significant positive correlations with the neurotransmitters and this finding backs up previous research that found the inhibition in significantly (AchE) correlated with the reduction of TAC [34, 39]. Moreover, our findings agree with other research that found that exposure to pesticides decreased AChE activity and lipid peroxidation [40, 41, 42].

5. Conclusion

Despite the fact that the majority of farmers use excessive levels of mixed pesticide types without wearing the PPE, no localized toxicological research has been conducted on them under these conditions. The current investigation revealed that some biochemical markers had changed in these farmers, particularly sprayers who often came into touch with pesticides mixes. Although these alterations might not have clinical significance in the near future but then it could be very dangerous. It is strongly suggested that farmers should take greater caution when handling pesticides, particularly when it comes to using appropriate and efficient PPE.

References

[1] Z. B. Doğanlar, O. Doğanlar, H. Tozkir, F. D. Gökalp, A. Doğan, F.Yamaç, Ü. E. Aktaş: "Nonoccupational exposure of agricultural area residents to pesticides: pesticide accumulation and evaluation of genotoxicity ". *Archives of environmental contamination and toxicology*, vol.75, no .4, pp.530-544, 2018.

[2] FAO Food and Agriculture Organization: " The International Code of Conduct on Pesticide Management "; Rome, Italy; ISBN 978925108548, 2014.

[3] A. Lukaszewicz-Hussain: "Role of oxidative stress in organophosphate insecticide toxicity–Short review". *Pesticide biochemistry and physiology*, vol .98, no. 2, PP.145-150, 2010.

[4] M. R. Martínez-Larrañaga, A. Anadón, M. A. Martínez, M. Martínez, V. J. Castellano and M. J. Díaz: "5-HT loss in rat brain by type II pyrethroid insecticides". *Toxicology and industrial health*, vol.19 , no.7, pp. 147-155, 2003.

[5] M. Abdollahi, A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie: "Pesticides and oxidative stress: a review". *Med Sci Monit*, vol.10, no.6, pp.141-147, 2004.

[6] A. K. Ranjbar, P. Pasalar and M. Abdollahi: "Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers". *Human & experimental toxicology*, vol. 21, no .4, pp.179-182, 2022

[7] W. Tayeb, A. Nakbi, M. Trabelsi, N. Attia, A. Miled and M. Hammami: "Hepatotoxicity induced by sub-acute exposure of rats to 2, 4-Dichlorophenoxyacetic acid based herbicide". *Journal of hazardous materials*, vol.180, no.1, pp.225-233, 2010.

[8] J. Martin-Rein, G. Casanova, B. A. Dahiri, I. Fernández, A. Fernández-Palacín, J. Bautista and l. Moreno: "Adverse health effects in women farmers indirectly exposed to pesticides". *International Journal of Environmental Research and Public Health*,vol.18, no.11, pp.590, 2021.

[9] P. M. Motsoeneng and M. A. Dalvie: "Relationship between urinary pesticide residue levels and neurotoxic symptoms among women on farms in the Western Cape, South Africa". *International Journal of Environmental Research and Public Health*, vol.12, no.6, pp.6281-6299, 2015.

[10] B. Vellingiri, M. Chandrasekhar, S. Sabari, A. V. Gopalakrishnan, A. Narayanasamy, D. Venkatesan and A. Dey: "Neurotoxicity of pesticides–A link to neurodegeneration". *Ecotoxicology and Environmental Safety*, vol. 243, pp.113972, 2022.

[11] J. R. von Osten, R. Tinoco-Ojanguren, A. M. Soares and L. Guilhermino: "Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. Archives of Environmental Health". *An International Journal*, vol. 59, no.8, pp.418-425, 2004.

[12] T. Wafa, K. Nadia, N. Amel, C. Ikbal, T. Insaf, K. Asma and H. Mohamed: " Oxidative stress, hematological and biochemical alterations in farmers exposed to pesticides". *Journal of Environmental Science and Health*, Part B, vol.48, no.12, pp.1058-1069, 2013.

[13] S. Aroonvilairat, W. Kespichayawattana, T. Sornprachum, P. Chaisuriya, T. Siwadune and K. Ratanabanangkoon: " Effect of pesticide exposure on immunological, hematological and biochemical parameters in Thai orchid farmers—a cross-sectional study". *International journal of environmental research and public health*, vol .12, no. 6, pp. 5846-5861, 2015.

[14] D.Milatovic, R. C. Gupta and M. Aschner: " Anticholinesterase toxicity and oxidative stress". *The Scientific World Journal*, vol. 6, pp. 295-310, 2006.

[15] J. L. Rodríguez, I. Ares, V. Castellano, M. Martínez, M. R. Martínez -Larrañaga , A. Anadón and M. A. Martínez: " Effects of exposure to pyrethroid cyfluthrin on serotonin and dopamine levels in brain regions of male rats". *Environmental research*, vol.146, pp.388-394, 2016 .

[16] M. M. Hossain and J. R. Richardson: "Mechanism of pyrethroid pesticide–induced apoptosis: role of Calpain and the ER stress pathway". *Toxicological Sciences*, vol.122, no.2, pp. 512-525, 2011.

[17] J. Y. Chan, S. H. Chan, K. Y. Dai, H. L. Cheng, J. L. Chou and A. Y. Chang: "Cholinergic receptor-independent dysfunction of mitochondrial respiratory chain enzymes, reduced mitochondrial transmembrane potential and ATP depletion underlie necrotic cell death induced by the organophosphate poison mevinphos". *Neuropharmacology*, vol.51, no.7-8, pp. 1109-1119, 2006 .

[18] A. Anadón, I. Ares, M. Martínez, M.R. Martínez-Larrañaga, and M.A. Martínez: "Neurotoxicity of Neonicotinoids" . *In Advances in Neurotoxicology*. Vol. 4, pp. 167-207. 2020.

[19] M. A. Elwan, J. R. Richardson, T. S. Guillot, W. M., Caudle, and G. W. Miller: "Pyrethroid pesticide-induced alterations in dopamine transporter function", *Toxicology and applied pharmacology*, vol. 211, no.3, pp.188-197, 2006.

[20] S. A. H. A. Rahman and D. A. Sattar: "Effect of different concentration of Super Cyren pesticide on some physiological and histological traits of mice after different periods of oral administration". *Iraqi Journal of Science*, Vol. 58, No.4C, pp. 2291-2300,2017.

[21] M. O'Malley: "Clinical evaluation of pesticide exposure and poisonings". *The lancet*, vol .349 no.9059, pp.1161-1166, 1997.

[22] Z. K. Binienda, S. Sarkar, L. Mohammed-Saeed, B. Gough, M. A. Beaudoin, S. F Ali and S. Z. Imam: " Chronic exposure to rotenone, a dopaminergic toxin, results in peripheral neuropathy associated with dopaminergic damage". *Neuroscience Letters*, vol.541, pp.233-237, 2013.

[23] M. Janeczek, T. Gefen, M. Samimi, G. Kim, S. Weintraub, E. Bigio and C. Geula: "Variations in acetylcholinesterase activity within human cortical pyramidal neurons across age and cognitive trajectories". *Cerebral cortex*, vol.28, no. 4, pp.1329-1337, 2018.

[24] A. S. Berry, V. D. Shah, S. L Baker, J. W. Vogel, J. P. O'Neil, M. Janabi and W. J. Jagust: "Aging affects dopaminergic neural mechanisms of cognitive flexibility". *Journal of Neuroscience*, vol.36, no .50, pp.12559-12569, 2016.

[25] D. G. Morgan: "The dopamine and serotonin systems during aging in human and rodent brain". *Progress in Neuro-psychopharmacology and Biological Psychiatry*, vol.11, no .2-3, pp.153-157, 1987.

[26] A. V. Snezhkina, A. V. Kudryavtseva, O. L. Kardymon, M. V. Savvateeva, N. V. Melnikova, G. S. Krasnov and A. A. Dmitriev: "ROS generation and antioxidant defense systems in

normal and malignant cells". *Oxidative medicine and cellular longevity*, vol.2019, pp.1-17, 2019.

[27] M. Schieber and N. S. Chandel: "ROS function in redox signaling and oxidative stress". *Current biology*, vol .24, no.10, pp. R453-R462, 2014.

[28] A. Etemadi-Aleagha, M. Akhgari and M. Abdollahi: "A brief review on oxidative stress and cardiac diseases". *Mid. East. Pharmac*, vol. 10, pp.8-9, 2002.

[29] J. Vidyasagar, N. Karunakar, M. S Reddy, K. Rajnarayana, T. Surender and D. R. Krishna: "Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning". *Indian journal of pharmacology*, vol. 36, no .2, 76, 2004.

[30] Y. Huang, Y. Hong, Z. Huang, J. Zhang, and Q. Huang:" Avermectin induces the oxidative stress, genotoxicity, and immunological responses in the Chinese Mitten Crab, Eriocheir sinensis ". *PloS one*, vol.14, no. 11, pp.e0225171, 2019.

[31] J. Kalra: "Oxygen free radicals: key factors in clinical diseases". *Lab. Medica. International*, vol. 1, pp.16-21 ,1994.

[32] A. Ranjbar, H. Solhi, F. J. Mashayekhi, A. Susanabdi, A. Rezaie and M. Abdollahi:"Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study". *Environmental Toxicology and Pharmacology*, vol. 20, no. 1, pp.88-91, 2005.

[33] D.S Rohlman, W. K. Anger and P. J. Lein: "Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure". *Neurotoxicology*, vol.32, no. 2 ,pp. 268-276, 2011.

[34] A. A. Mecdad, M. H. Ahmed, M. E. ElHalwagy and M. M. Afify, "A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers". *Egyptian Journal of Forensic Sciences*, vol.1, no. 2, pp. 93-98, 2011.

[35] L. Weifen, Z. Xiaoping, S. Wenhui, D. Bin, L. Quan, F. Luoqin and Y. Dongyou:" Effects of Bacillus preparations on immunity and antioxidant activities in grass carp (Ctenopharyngodon idellus) ". *Fish physiology and biochemistry*, vol.38, no.6, pp.1585-1592, 2012.

[36] S. H., Al–Dulaimi and A. M., Rabee : "Measurement of Pollution Level with Particulate Matter in Babylon Concrete Plant and Evaluation of Oxidative Stress and Hematological Profile of Plant Workers". *Iraqi Journal of Science*, Vol. 62, No. 11, pp. 3834-3841, 2021.

[37] A. H. Abdulameer and S. Z. Hussein: " Assessment of Oxidative Stress Parameters for some of Baghdad City Fuel Stations Workers". *Iraqi Journal of Science*, Vol. 64, No. 6, pp. 2669-2680, 2023.

[38] K. Soltaninejad and M. Abdollahi:"Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review". *Med Sci Monit*, vol.15, no.3, pp.75-90,2009.

[39] A. K. R. A. M. Ranjbar, P. Pasalar and M. Abdollahi:" Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers". *Human & experimental toxicology*, vol. 21, no.4, pp. 179-182. 2002.

[40] Y. A. Surajudeen, R. K. Sheu, K. M. Ayokulehin and A.G. Olatunbosun: "Oxidative stress indices in Nigerian pesticide applicators and farmers occupationally exposed to organophosphate pesticides". *International journal of applied and basic medical research*, vol.4, Suppl. 1, pp. S37, 2014.

[41] A. Mehta, R.S. Verma and N. Srivastava: "Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver". *Pesticide biochemistry and physiology*, vol. 94, no.2, pp.55-59, 2009.

[42] F. N. Bebe and M. Panemangalore: "Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats". *Journal of Environmental Science and Health*, Part B, vol.38, no. 3, pp.349-363, 2003.