

## EFFECTS OF PESTICIDES EXPOSURE ON SELECTED BIOCHEMICAL PARAMETERS AMONG AGRICULTURE FARMERS FROM LOCAL POPULATION OF PAKISTAN

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### ABSTRACT

The current study is designed to check the effects of most commonly using pesticides exposure on human health by evaluating especially hematological, liver and renal indicators. Two hundred seventy five (275) farmers which were in close contact with chemicals, selected from the different areas of District Vehari, Punjab. The blood samples were analyzed for complete blood count (CBC), renal function test (RFTs) and liver function tests (LFTs) base on spectrophotometer principles. The obtained results indicated a significant difference observed in certain parameters as compared with control especially in hematological system (CBC) in all groups. The analyzed results of the LFTs show some significant values in group 2 (ALT) and bilirubin each group. The outcomes of study showed that Kidney profile is highly altered in different groups of study population with the increase in level of urea, creatinine and uric acid in group 2. Serum electrolytes and neurological parameters may be helpful for further study.

**Key Words:** Pesticides, Health effects, Liver Function Tests (LFTs), Renal Function Tests (RFTs), CBC, Uric acid.

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### INTRODUCTION

Pesticides chemicals are used in public health or agriculture safety by protecting plants from weeds, diseases and pests, also to protect humans from vector-borne diseases, e.g. dengue fever, malaria and schistosomiasis. The examples are insecticides, fungicides, herbicides, rodenticides, and plant growth regulator. (Nicolopoulou-Stamati *et al.*, 2016).

Pesticide use increased worldwide in crop production approximately twenty times from 1960 to 2000 (Oerke, 2006). Worldwidely, China produced largest amount of pesticides and also utilized in great amount along with its large scale supplier position in global industry. (Zhang *et al.*, 2011). The environmental, food safety and biological pest control function very much archived deleterious impacts of pesticides (Kunz *et al.*, 2011), effects of these agents on health have moreover engaged significant consideration (Huang *et al.*, 2002).

During World War II (1939-1945), advancement of pesticides expanded, in light of the fact that it was dire to upgrade nourishment creation and discover potential chemical warfare agents (Bernardes *et al.*, 2015). Therefore, the 1940s saw a stamped development in manufactured pesticides like DDT, aldrin, dieldrin, endrin, parathion, and 2,4-D. During 1950s, utilization of pesticides in horticulture was that there were no potential risk of these synthetic compounds to earth and human health existed (Unsworth, 2010).

In addition, pesticides have likewise direct advantages to human wellbeing by saving crops and livestock. It is estimated that since 1945, killing pests by using pesticides that carry or transmit disease has stop the deaths of around seven million peoples. Mosquitoes that transmitted disease is malaria. This is one of the most normally known and lethal and deadly diseases that has diminished in prevalence due to utilization of pesticides. Other diseases that controlled with use of pesticides include bubonic plague, which is due to rat fleas, and typhus. Which transmitted by both body lice and fleas (Goulson, 2014).

Many of the pesticides have been concerned with environmental and health issues (Zheng *et al.*, 2016), and use of certain pesticides has been eradicated (Nicolopoulou-Stamati *et al.*, 2016). Pesticides can make contact with skin,

inhalation or ingestion. Possible health outcome can determine by category of pesticide, time period and way of introduction, and individual health status (e.g., malnutrition and healthy/damaged skin). In animal and human body, pesticides may be metabolized, stored, excreted or bio-accumulated in body fat that may causes toxic effects. (Nicolopoulou-Stamati *et al.*, 2016).

Different human wellbeing related concerns are related with pesticides, going from momentary effects, for example, cerebral pains and sickness to chronic impact various cancer, birth imperfections, infertility and endocrine interruption (Bourguet and Guillemaud, 2016). Children specifically, are more endanger by momentary and long-term exposure to pesticides. Furthermore, excessive use of pesticides may lead to the destruction of biodiversity, another pest outbreaks and demolition of non-objective species, soil, water, and air pollution (Recena *et al.*, 2006). Transient health impact include modification in CBC, liver and kidney functions, and nerve conduction speed and amplitudes. Generally, pesticide introduction adversely affects blood indices, the liver and PNS. Long-term pesticide exposure is seen as related with expanded variation of nerve conductions, particularly in sensory nerves. Moreover it influences a wide range of health markers dependent on blood tests and diminish the tibial nerve compound muscle activity potential amplitudes (Jeyaratnam *et al.*, 1987).

Environmental safety and food security are the major concerns infrequently growing human population all over the world. In agricultural serious damage caused by pests, in terms of yield and high price of chemicals that cost billions of dollars annually and increase the agricultural production budget (Chattopadhyay *et al.*, 2017). Regardless of the high costs, the large number of applications of chemical pesticides has been preferred due to the benefits they provide in agriculture by protecting crops from damage caused by pests. On the other hand, pesticides act a serious danger to human health and environment. Especially farmers and individuals who straightforwardly engaged with the treatment of pesticides, are at a high risk of exposure when mixing and applying pesticides or working in treated fields and also from particles on food and drinking water (Lopes and Firpo, 2009).

In agricultural areas, there is great risk of exposure due to the to the lack of knowledge they use toxic chemicals that are banned, inappropriate applying techniques, poorly maintained spraying apparatus, inefficient storage practices, frequently again use of old pesticide containers for food, poor use of personal protection equipment (PPE) and other safety measures (Jallow *et al.*, 2017).

Extensive, inappropriately applied pesticides can present risk to our biological system. These can be a possible health risk and source of our food chain pollution. However, entire world is worried about the issues because of unnecessary utilization of pesticides, but circumstance in developing countries is disturbing when contrasted with developed countries (Jeyaratnam *et al.*, 1987).

While these examinations provided evidence of antagonistic health impacts of pesticide introduction, it was hard to viably control for potentially confounding health-related factors of each participant and precisely define exposure level. The goal of the present investigation is to utilize the most comprehensive neuroelectrophysiological techniques to inspect the clinical and subclinical health effects of pesticide exposures (Hu *et al.*, 2015).

Integrated Pest Management (IPM) system, became an integral factor these frameworks utilized yield creation strategies that attracted predators or parasites that attacked pests and planned pesticide applications to correspond with the most defenseless time of the pest's life cycle, in this manner decreased the measure of applied pesticides (Unsworth, 2010).

Although, IPM or related strategies didn't take out the requirement for pesticide. These chemicals guarantee the creation of good or satisfactory in high quality or quantity pest-free yields, which is very useful to prevents human diseases transmitted by insect or other rodents, also enhance the food supply and positively impacts public health (Fenik *et al.*, 2011)

## MATERIALS AND METHODS

Two hundred seventy five (275) candidates were selected for this study and divided into 5 groups on the basis of time period of exposure to pesticides viz., Lufenuran, Triazophos, Lambda- cyhalothrin, Emamectin benzoate, Acetamidrid, Acephate, Sulfosulfuron, Bromoxynil, Clodinafop-propargyl. Their blood samples collected with 5mL syringe and immediately transferred into two different vacutainers one with ethylenediamine tetraacetic acid (EDTA) used for complete blood count analysis and other with clot activator vacutainer. The blood clot activator vacutainers were later centrifuged for 10 mins at 3000 rpm by using a centrifugation machine. The clear supernatant used for the estimation of renal and liver parameters (Liver Function Tests (LFTs) and Renal Function Tests (RFTs)). The total erythrocyte count (TEC), total leucocyte count (TLC), haemoglobin concentration (HGB), platelet count and other hematological indices determined in the blood by using Sysmex XP-100 automatic hematology analyser in diagnostic laboratory of University of Lahore, Pakistan. The plasma total cholesterol, triglyceride and HDL-Cholesterol were determined by using Roche Cobas C-3111 automatic chemistry analyser.

### Statistical analysis

Data was collected, and preceded on computer software SPSS version 16 for statistical analysis. Significance of means were analyzed by using one way ANOVA and significant group differences were analyzed by using post hoc Tukey test. The benchmark of significance was at least P-value <0.05.

### RESULTS

In previous 100 years the use of pesticides was extensively enhanced in Pakistan. Our study is to evaluate the effects of pesticides on human health. Samples were collected in two seasons in the time period of almost one year. Hematological, liver function tests (LFTs) and renal function tests (RFTs) were analyzed. About 15% of participants took protective measures (e.g. cover mouth and wearing rubber shoes). Different varieties of pesticides were used in both seasons (Table 4).

At the end of the study the results are very significant especially in hematological system. There is significantly ( $p < 0.05$ ) increase in white blood cells (WBC) in all groups except group 5. Group 5 significant value is 0.05 that is equal to the ( $p < 0.05$ ) almost near to significant value. RBCs values of all the groups are insignificant with respect to our results. Hemoglobin (HGB) is significantly ( $p < 0.05$ ) decrease in the group 2 and 4 while in other group there is also decrease in hemoglobin count in some candidates. Hematocrit (HCT) values are declined in group 2 and group 3 significantly ( $p < 0.005$ ) and ( $p < 0.05$ ) respectively while in other groups many some other members are also seen to be decline in HCT. Mean corpuscular hemoglobin concentration (MCHC) value is declined in group 2 only ( $p < 0.05$ ). Lymphocytes (LY) count are declined in group 2 and group 3 are very significant with ( $p < 0.005$ ). Monocytes (MO) count are significantly low in all groups except group 4 with significant value of ( $p < 0.05$ ). Eosinophils (EO) count were significantly decrease in group 2,3,4,6 ( $p < 0.005$ ) and group 5 with (0.05) concern Table 1 for more details.

Table 1. Hematological parameters of candidates (Mean  $\pm$  SEM).

CBC							
	Group 1 (Control)	Group 2 (Zero day)	Group 3 (1 day-30 days)	Group 4 (1 month-3 months)	Group 5 (3 months-6 months)	Group 6 Above 6 months	P- value
<b>WBCs</b> ( $10^3/\mu\text{L}$ )	5.64 $\pm$ 1.21	7.73 $\pm$ 2.09*	7.32 $\pm$ 1.70*	7.61 $\pm$ 1.99*	7.45 $\pm$ 1.56	7.87 $\pm$ 1.71*	0.003
<b>RBC</b> ( $10^6/\mu\text{L}$ )	5.28 $\pm$ 0.48	5.00 $\pm$ 0.488	4.98 $\pm$ 0.48	12.25 $\pm$ 57.76	5.00 $\pm$ 0.41	29.46 $\pm$ 103.95	0.195
<b>HGB(g/dL)</b>	16.00 $\pm$ 1.18	14.22 $\pm$ 2.10*	14.62 $\pm$ 1.46	14.30 $\pm$ 1.20*	14.52 $\pm$ 1.87	14.51 $\pm$ 1.93	0.008
<b>HCT (%)</b>	47.26 $\pm$ 4.26	39.88 $\pm$ 6.60**	41.83 $\pm$ 5.65*	43.06 $\pm$ 3.58	43.38 $\pm$ 5.89	41.73 $\pm$ 5.30	0.001
<b>MCV (fL)</b>	89.00 $\pm$ 5.87	79.79 $\pm$ 12.37	83.84 $\pm$ 10.39	83.69 $\pm$ 8.58	86.14 $\pm$ 10.64	84.75 $\pm$ 9.07	0.065
<b>MCH (pg)</b>	29.66 $\pm$ 1.79	28.41 $\pm$ 3.48	29.35 $\pm$ 3.22	27.85 $\pm$ 3.24	29.02 $\pm$ 3.03	29.50 $\pm$ 3.34	0.044
<b>MCHC</b> (g/dL)	32.80 $\pm$ 1.52	35.85 $\pm$ 3.03*	35.17 $\pm$ 3.16	33.26 $\pm$ 1.85	33.59 $\pm$ 2.38	34.85 $\pm$ 2.73	0.000
<b>PLT</b> ( $10^3/\mu\text{L}$ )	271.26 $\pm$ 71.84	224.59 $\pm$ 54.40	223.34 $\pm$ 56.74	245.82 $\pm$ 57.37	239.94 $\pm$ 50.54	236.50 $\pm$ 46.47	0.013
<b>LY (%)</b>	30.40 $\pm$ 6.31	44.33 $\pm$ 9.16**	41.15 $\pm$ 10.26**	35.61 $\pm$ 10.26	39.71 $\pm$ 9.98	38.72 $\pm$ 7.80	0.000
<b>MO (%)</b>	4.06 $\pm$ 1.57	8.81 $\pm$ 3.34*	8.58 $\pm$ 4.02*	7.58 $\pm$ 4.99	8.76 $\pm$ 4.97*	9.22 $\pm$ 4.10*	0.003
<b>GR (%)</b>	55.46 $\pm$ 7.50	45.59 $\pm$ 10.05	49.00 $\pm$ 11.14	55.64 $\pm$ 13.57	50.05 $\pm$ 11.54	49.94 $\pm$ 8.36	0.000
<b>EO (%)</b>	2.13 $\pm$ 0.99	1.29 $\pm$ 0.46**	1.34 $\pm$ 0.53**	1.32 $\pm$ 0.47**	1.50 $\pm$ 0.50*	1.33 $\pm$ 0.48**	0.000

\* and \*\* represents level of significance at ( $p < 0.05$ ) and ( $p < 0.005$ ), respectively. Abbreviation used White blood cells (WBCs), Hemoglobin (HGB), Red blood cells (RBCs), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Platelets (PLT), Lymphocytes (LY), Monocytes (MO), Granulocytes (GO), and Eosinophil (EO).

The analyzed results of the LFTs show some significant values in each group. There is increase in the level of ALT especially in group 2 there are some member of groups with increase in the level of all the parameters except albumin and protein that are increase significantly. Bilirubin seems to be insignificant in each group while few candidates of each group has increased level of bilirubin. Protein and Albumin are not increase significantly.

After the study the outcome the renal function test shows significant results with the increase in level of urea, creatinine and uric acid in group 2. Uric acid level seems to increase in members of all the groups. Creatinine level may seem to be increased most of the candidates of all groups.

Table 2. Liver function tests (LFTs) with comparison to the control group (Mean  $\pm$  SEM).

LFTs							
	Group 1 (Control)	Group 2 (Zero day)	Group 3 (1 day-30 days)	Group 4 (1 month-3 months)	Group 5 (3 months-6 months)	Group 6 Above 6 months	P-value
<b>BILI</b>	0.69 $\pm$ 0.13	0.70 $\pm$ 0.13	0.74 $\pm$ 0.20	0.77 $\pm$ 0.25	0.72 $\pm$ 0.12	0.76 $\pm$ 0.18	0.509
<b>ALT</b>	26.60 $\pm$ 6.26	41.74 $\pm$ 20.09	35.76 $\pm$ 16.59	30.98 $\pm$ 15.28	34.76 $\pm$ 17.92	33.44 $\pm$ 17.71	0.33
<b>AST</b>	25.53 $\pm$ 5.76	37.66 $\pm$ 21.11	32.73 $\pm$ 17.67	29.83 $\pm$ 16.07	34.05 $\pm$ 19.32	29.66 $\pm$ 9.93	0.206
<b>ALP</b>	186.40 $\pm$ 47.03	218.92 $\pm$ 74.36	199.60 $\pm$ 51.66	196.56 $\pm$ 48.39	196.34 $\pm$ 55.04	201.94 $\pm$ 47.74	0.441
<b>PROTEIN</b>	7.07 $\pm$ 0.40	7.21 $\pm$ 0.56	7.02 $\pm$ 0.50	7.11 $\pm$ 0.54	7.22 $\pm$ 0.54	7.02 $\pm$ 0.51	0.299
<b>ALBUMIN</b>	3.98 $\pm$ 0.41	3.79 $\pm$ 0.21	3.88 $\pm$ 0.27	4.16 $\pm$ 0.51	3.96 $\pm$ 0.39	4.02 $\pm$ 0.39	0.000

Abbreviation used are Bilirubin (BILI), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Protein and Albumin.

Table 3. The renal function tests (RFTs) with comparison to the control group (Mean  $\pm$  SEM).

RFTs							
	Group 1 (Control)	Group 2 (Zero day)	Group 3 (1 day-30 days)	Group 4 (1 month-3 months)	Group 5 (3 months-6 months)	Group 6 Above 6 months	P-value
<b>UREA</b>	26.40 $\pm$ 4.64	28.29 $\pm$ 5.24	26.76 $\pm$ 7.21	24.79 $\pm$ 5.06	26.02 $\pm$ 6.13	25.77 $\pm$ 7.47	0.204
<b>CR</b>	0.79 $\pm$ 0.19	0.92 $\pm$ 0.21	0.98 $\pm$ 0.72	0.86 $\pm$ 0.20	0.94 $\pm$ 0.27	0.90 $\pm$ 0.18	0.572
<b>URIC ACID</b>	5.46 $\pm$ 0.63	5.75 $\pm$ 0.64	5.86 $\pm$ 0.73	5.82 $\pm$ 0.83	5.95 $\pm$ 0.65	6.05 $\pm$ 0.60	0.217

Abbreviation used Creatinine (CR), Urea and Uric acid.

Table 4. Pesticides types with their empirical formulas, their effects on human health according to WHO classification.

Name	Pesticides Empirical formula	Health effects
Lufenuran	C <sub>17</sub> H <sub>8</sub> C <sub>12</sub> F <sub>8</sub> N <sub>2</sub> O <sub>3</sub>	class III (slightly hazardous) (WHO 2002)
Triazophos	C <sub>12</sub> H <sub>16</sub> N <sub>3</sub> O <sub>3</sub> PS	High (class III) for human health and protection. Water hazard class II (Self-assessment): hazardous for water
Lambda- cyhalothrin	C <sub>23</sub> H <sub>19</sub> ClF <sub>3</sub> NO <sub>3</sub>	IPCS hazard classification of Lambda-cyhalothrin moderately hazardous, class II. 1999)
Emamectin benzoate	C <sub>56</sub> H <sub>81</sub> NO <sub>15</sub>	Class 3: Flammable and Combustible Liquids Packing group II: substances presenting medium danger
Acetamidrid	C <sub>10</sub> H <sub>11</sub> ClN <sub>4</sub>	WHO Class II, moderately hazardous
Acephate	C <sub>4</sub> H <sub>10</sub> NO <sub>3</sub> PS	WHO Recommended Classification of Pesticides by Hazard identifies Acephate (technical grade) as Class III: slightly hazardous;
Sulfosulfuron	C <sub>16</sub> H <sub>18</sub> N <sub>6</sub> O <sub>7</sub> S <sub>2</sub>	EPA classification; Class III. It is moderately irritant to skin and non-irritant to eyes.
Bromoxynil	C <sub>7</sub> H <sub>3</sub> Br <sub>2</sub> NO	The WHO Recommended Classification of Pesticides by Hazard identifies bromoxynil (technical grade) as Class II: moderately hazardous;
Clodinafop-propargyl	C <sub>17</sub> H <sub>13</sub> ClFNO <sub>4</sub>	This product is slightly hazardous (WHO Hazard Class III)

## DISCUSSION

Human population and nourishment requests have expanded at an uncommon during the last 2–3 decades, which increased pressure on agrarian framework and natural sanitation (Natasha *et al.*, 2018). Expanded harvest creation has seemed an inescapable component of modern agricultural system to help the increasing population pressures. Utilization of agrochemicals has increased essentially in the ongoing past, which has brought about ecological defilement (Xiong *et al.*, 2017). These days, it is probably not going to acquire high yield production without utilization of pesticide, in addition to water system and chemical manures. If pesticides are not applied according to their suggested dosages, the crop harvesting would diminish essentially, and food costs would take off drastically. Under such conditions, it would get difficult to continue the entire population. Thusly, the use of pesticides has become an essential segment for expanded yield crop production (Ahmad *et al.*, 2019).

Current study shows pesticides put immediate effects on most of the hematological parameters shows significant results with some recent findings. Due to change in the hematological parameter there adverse respiratory and skin allergy affects appears. About 66.66% of the candidates had at least one abnormal indicator. Alteration of CBC parameters are related to pesticides exposure (Fareed *et al.*, 2013). There is an increase in the WBCs level in all the groups that shows pesticides exposure has direct impact on your defense mechanism increase their activity after exposure to pesticides. Despite what might be expected, short-term pesticide presentation diminished monocytes, hemoglobin, and platelets, which recommends a direct poisonous impact of pesticides on peripheral blood cells. Increase white blood cells and significantly decrease hemoglobin, HCT, MCHC, LY and EO counts (Hu *et al.*, 2015). The level of hepatic parameters (LFTs) show some significant increase in the ALT, AST and ALP level that is mostly related to the previous study on pesticides exposure (Awad *et al.*, 2014). Pesticides exposure have some adverse effects when exposed for a long period of time (Pluth *et al.*, 2019). After continuous exposure to pesticides there are also some increase in the renal parameters (RFTs). The level of CR and Urea are seems to high in the farmers that were in exposure to the pesticides for a long time period.

## Conclusion

From the outcomes of present study we concluded that pesticides have very sever effects on human health. The biological parameters including hematological, liver profile and renal profile showed very significant results. WBC (white blood cells) that is related to human defense mechanism showing a significant alteration from normal range. Other parameters of CBC like monocytes, hemoglobin, lymphocytes, granulocytes (GO) and eosinophil (EO) also declined from normal values. Hepatic and renal parameters indicated minor changes as compared to CBC parameters as short range effects. The allergic reactions and dizziness become very common in the farmers that are exposed to pesticides in the field after spraying. After long time exposure the farmers may increase the risk of cytotoxicity and cancer. It is suggested that individuals should be well aware about effects and precautions of pesticides. It is most important to take protective measure during pesticides exposure.

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