*Pak. J. Agri. Sci., Vol. 57(4), 1189-1195; 2020*
*ISSN (Print) 0552-9034, ISSN (Online) 2076-0906*
*DOI: 10.21162/PAKJAS/20.9819*
http://www.pakjas.com.pk

# AN APPRAISAL OF THE EFFECTS OF ORGANOPHOSPHATE ON BIOCHEMICAL PARAMETERS OF FARMERS

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This research aimed to study the effects of organophosphate insecticides on biochemical parameters of small farmers who were engaged in the mixing and spraying of pesticides in Punjab, Pakistan. In this context, blood samples of 110 farmers, having similar socio-economic position, in the cotton and mango belt of Rahimyar Khan (RYK), Punjab, Pakistan were collected to detect the levels of the acetyl-cholinesterase, glutathione, and lipid peroxidation in the blood samples of both groups (pesticide exposed and unexposed controls). All of the selected farmers were also interviewed about age, social interests, habits (smoking), genetic diseases, education, marital status, occupation, spraying experience, types of pesticides, use of personal protection equipment, and diseases. The descriptive results of socioeconomic factors were presented in terms of means ± SD, and non-parametric chi-squared test was used to test their association with case-control groups. Student t-test and ANOVA were used to compare the enzymes between and within groups respectively. The mean level for all enzymes was significantly higher in exposed group with p-values $<0.001$. The impact of all enzymes was different on the farmers in exposed group with p-value $<0.001$. The farmers were most affected with glutathione. Based on the findings of this study, it is strongly recommended that the rural health workers should visit the farmers regularly to monitor their health conditions. Furthermore, the local governing authorities must send their social mobilizers (SMs) to fields in order to promote the importance of self and environmental protection measures.
**Keywords:** Agriculture; Pesticides; Organophosphates; Environmental Protection; Rahimyar Khan.

## INTRODUCTION

The agriculture sector plays a major role in Pakistan's economy. It accounts for more than 21 percent of the country's GDP and consumes more than 44 percent of the country's workforce (Batool et al., 2019). Insects and diseases are the main threats in the agriculture sector; and therefore, management practices based on strong scientific information are required to promote both effective pest management and protection of farmer health. It is well documented that pests are destroying almost half of the world's crop yields (Lamberth et al., 2013). However, indiscriminate applications of pesticides in the crops are causing severe environmental complications along with public or farmers health problems (Coronado et al., 2004).

The most commonly used agrochemicals in the agriculture sector in many parts of the world are Organophosphates (Coronado et al., 2004). These chemicals are mostly used to increase the crop yields by reducing the damage caused by insects in different crops such as cotton, maize, and vegetables. Contrary to their advantages, the occupational exposures to these Organophosphates can cause diverse effects for human health and environment (Gul et al., 2019). Several studies, mostly from Asia, have reported that the uncontrolled availability and unsupervised (unattended) handlings of Organophosphates by poorly trained farmers or workers at the farms are causing serious health risks. These include symptoms or conditions such as biliousness, faintness, tiredness, infertility, diarrhea and migraine, along with other chronic diseases including respiratory impairment, cancer, diabetes, premature ageing, heart problems, skin herpes, and kidney diseases (Gul et al., 2019), affecting not only farm workers but also their families, particularly children. It is also well documented that annually about 250,000 people died worldwide because of Organophosphate misuse or self-poisoning (Avilla and Gonzalez-Zamora, 2009). Exposure to some pesticide groups has the potential to cause enzymatic changes in the body. Insecticides may inhibit the secretion and formation of acetyl-cholinesterase enzyme (Ahmad et al., 2007), resulting in the contraction of smooth muscles of bronchioles, and increased salivary and tear secretions (Kachaiyaphum et al., 2010; Haque et al., 2012). Pesticides are inhibited by the acetyl-cholinesterase accumulation of neurotransmitter which causes the intoxication of the organophosphate.

Excessive application (i.e. exceeding manufacturer's guidelines) of organophosphate pesticides in the agriculture and gardening sectors may result in adverse effects on human health, including both physical and mental disorders. In Pakistan, most of the farming communities are illiterate and unaware of either these possible health effects, or of precautionary measures that are recommended during pesticide application to ameliorate risk. Several studies have documented individual adverse effects of organophosphate pesticides on human health, in particular farmer's health, in different areas of Pakistan (Tariq et al., 2007; Kachaiyaphum et al., 2010; Batool et al., 2019) but comprehensive assessments of the side effects are lacking. Some studies have investigated the impact of the pesticides on the glutathione enzyme, while others have considered adverse effects on the levels of acetyl-cholinesterase and lipid peroxidation separately (Parveen et al., 2011).

Although comprehensive investigations of the impacts of most commonly used insecticides on the farmer's health in Pakistan are essential, on their own they are not sufficient to fully address the problem. Reducing the impact of misuse of pesticides on both operator and bystander health also relies on improving knowledge and understanding of their side effects amongst users. In this context, the present study was conducted to investigate the negative effects of the most commonly used Organophosphates in the farmers and farm workers, to support conclusions on how their safe use can be achieved by promotion and adoption of good practice when applying the products in the field.

## MATERIALS AND METHODS

***Study Area*****:** The present study was carried out in the Rahimyar Khan (RYK) district which is situated in southern part of the Punjab province of Pakistan (Fig. 1). Rahimyar Khan City is the capital of the district and lies between 28º25'11.5" N latitudes and 70º18'12.4" E longitudes (Anonymous, 2019). The total area of the RYK District is about 11,880 km$^2$ with a total population of almost 4.8 million. The current literacy rate of the RYK is about 38%. There are four tehsils in the RYK, namely Liaqatpur, Khanpur, Rahimyar Khan, and Sadqabad. The climate of the district is favourable for cotton, wheat, rice, sugar cane, vegetables, and orchards of mangoes. The annual average temperature and precipitation in the RYK are 26.2 ºC and 101 mm, respectively (Anonymous, 2017). As a consequence of the burgeoning increase in the population and food demand of the RYK, farmers are using advanced techniques and pesticides to enhance the crop yields. The majority of the people of the RYK are connected to the agricultural sector.

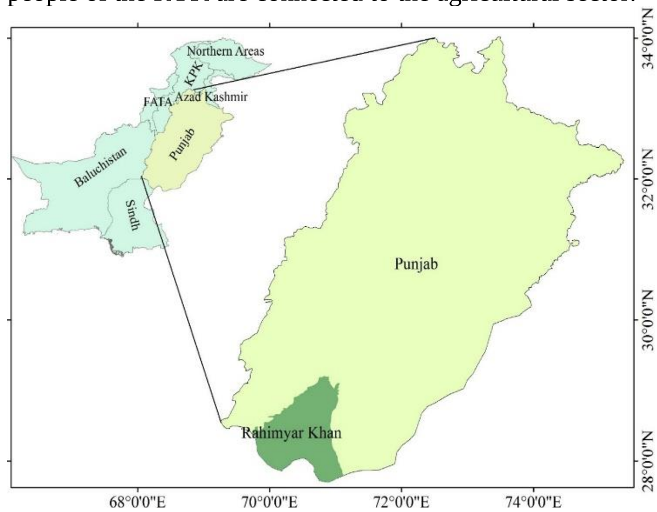


**Figure 1. Location map of the Rahimyar Khan District of Punjab Province of Pakistan.**

***Collection of Blood Samples*****:** In this study, 110 farmers or workers were selected at random (without reference to their names and village inhabited) from different rural areas of Rahimyar Khan (RYK) district of Punjab, Pakistan. All of the selected farmers were interviewed individually. During interviews, several standard questions including age, social interests, habits (smoking), genetic diseases, education, marital status, occupation, spraying experience, types of pesticides, use of personal protection equipment, diseases, types of crops, and job titles were asked.

Blood samples were taken from 100 farmers who had been exposed to organophosphate pesticides, while 10 samples were collected from a control group. The blood samples were taken in 5 ml Ethylenediaminetetraacetic acid (EDTA) vials following the method of Haque et al. (2012). Samples from both the exposed and control group farmers were then further categorized on the basis of education, marital status, smokers and non-smokers. After blood samples were taken the EDTA vials were returned to the laboratory and stored in a refrigerator (Panasonic MPR-721) at 4°C ± 1°C before analysis at the Zoological Laboratory of the Govt. College University, Faisalabad, Pakistan. This work was approved by the Departmental Ethical Review Committee.

***Estimation of Glutathione*****:** The glutathione level in the blood of farmers of both the exposed and control groups, was estimated using the method of Haque et al. (2012). A standard 0.05 ml blood sample from each participant was placed in a 110 test tubes, and 1.5 ml of distilled water was added to each before shaking (KS130, IKA$^{TM}$) for a standard period of time (10 min) to ensure it was well mixed. Following the shaking process, 2 ml of a 10% TCA (Trichloroacetic acid) solution was added and after mixing, the test tubes were placed in the centrifuge (Sigma 2-16K) and spun at 2000 rpm for 15 minutes. After 15 minutes, 1 ml of supernatant was taken from 4 ml solution and a phosphate buffer, (maintaining 7.4 pH), was added to the sample, followed by, 0.1 ml of a 0.04 % DTNB (Ellman's reagent) solution. The colour and absorbance were noted with photospectrometer at 412 nm.

***Estimation of Lipid Peroxidation*:** For the estimation of lipid peroxidation (Haque et al., 2012), 0.05 ml blood samples of farmers of both groups (exposed and control) were collected, placed into test tubes, and then mixed with 4.5 ml of phosphate buffer (which maintained pH 7.4). The solution was kept in an incubator (ASR 332M) for 30 minutes at 37 ºC temperature, then after mixing, the test tubes were spun in the centrifuge at 2000 rpm for 15 minutes. After centrifuging, 3 ml of supernatant was taken and 1 ml of TBA (thiobarbituric acid) solution was added to each test tubes. Test tubes were placed in a water bath for 15 minutes at 100ºC. After 15 minutes, the tubes were placed in ice water (25 min) before being spun in the centrifuge at the rate of 2500 rpm for 15 minutes. Absorbance was noted at 532 nm.

***Determination of Acetyl-cholinesterase Activity*:** In order to establish the activity of the acetyl-cholinesterase in the blood samples of both groups (exposed and control) of farmers (Haque et al., 2012), a 0.025 ml blood sample was taken and then 25-fold diluted blood (by mixing with SDS solution) was mixed with 0.015 ml diluting buffer which was made of Tris-HCl and maintained the 7.4 pH of the solution. Following the dilution, 0.1 mM acetylcholine iodide solution was added in each test tube. The reaction mixture was incubated for 15 minutes with constant shaking at 37ºC. After 15 minutes, the test tubes were removed from the incubator. In the next step, both DTNB (0.04%) and Sodium dodesyle sulphate (SDS) (44%) were mixed in a flask. 0.5 ml solution of DTNB and SDS was put in each of the test tubes. The absorbance of each test tube was noted at 412 nm.

***Data analysis*:** The data were analyzed with statistical software IBM SPSS version 21.0. The descriptive statistics were presented as means ± SD for continuous variables, and in terms of frequencies and percentages for categorical variables. The association of socioeconomic factors with the case-control groups was tested using non-parametric $\chi^2$ test of independence. The means of different enzymes were compared between exposed and control groups using student t-tests. The means of different enzymes were compared in exposed group using a Kruskal-Wallis H test, a non-parametric alternative of one-way ANOVA. The non-parametric test was used due to the violation of the assumption of normality that is required in ANOVA.

## RESULTS

***Socioeconomic Values of Farmers in the Rahimyar Khan District*:** Before performing the tests, exposed and control groups of farmers were categorized according to the age, marital status, education and the number and proportion falling into a range of sub-categories calculated (Table 1).

Results showed that 39% of the farmers in the exposed group were between 15-25 years, 28% were between 26-35 years, 16% were 36-45 years old, 6 % were between 46-55 years, and 11% of farmers were above 56 years (Table 1). Similar demographic characteristics were found in the control, group, providing a firm basis for comparison of neurological data. There was no significant difference in the proportion of farmers that were married in the exposed (68%) and control (70%) group. There were about 50% of the farmers in the exposed group who were uneducated compared to none of the farmers in the controlled group ($P$<0.05; Table 4).

**Table 1. Number and percentage of the 10 and 100 people the exposed and control group respectively, falling with in each age, marital status or education category.**

| Factor | Category | Exposed group | | Control group | |
|---|---|---|---|---|---|
| | | Frequency | % | Frequency | % |
| Age (years) | 15-25 | 39 | 39 | 3 | 30 |
| | 26-35 | 28 | 28 | 3 | 30 |
| | 36-45 | 16 | 16 | 3 | 30 |
| | 46-55 | 6 | 6 | 1 | 10 |
| | 56-65 | 7 | 7 | 0 | 0 |
| | 66-75 | 2 | 2 | 0 | 0 |
| | 76-85 | 2 | 2 | 0 | 0 |
| | Total | 100 | 100 | 10 | 100 |
| Marital Status | Single | 32 | 32 | 3 | 30 |
| | Married | 68 | 68 | 7 | 70 |
| | Total | 100 | 100 | 10 | 100 |
| Education | Uneducated | 50 | 50 | 0 | 0 |
| | Primary | 28 | 28 | 3 | 30 |
| | Elementary | 13 | 13 | 0 | 0 |
| | Matric | 7 | 7 | 3 | 30 |
| | F.A. | 2 | 2 | 1 | 10 |
| | B.A. | 0 | 0 | 3 | 30 |
| | Total | 100 | 100 | 10 | 100 |

Table 2 describes the personal particulars of exposed and control groups of farmers. There were a similar number of samples (25) from all the tehsils (administration territories) collected for this study. However, the 10 samples in the controlled group of farmers were collected from the entire study area, irrespective of the tehsil. The means of the ages of the farmers of both groups (Table 2), revealed that average age of those who were spraying the Liaqatpur Tehsil of the RYK was about 33 years, similar to that in the Khanpur, Rahimyar Khan, and Sadiqabad tehsils were 30, 35.17, and 32.41 years, respectively. It was also found that majority of the farmers in the study area were smokers. The highest percentage of smokers was found in the Liaqatpur and Sadiqabad (64%), followed by Khanpur (60%).

Significant differences in the use of personal protective equipment (PPE) was found between exposed and control groups (Table 2). None of the farmers in the exposed group

**Table 2. Description of personal particulars (mean ±S.D) of exposed and controlled group of farmers in four tehsils of RYK District.**

| Areas | Exposed Group | | | | Controlled Group |
|---|---|---|---|---|---|
| | Liaqatpur | Khanpur | RYK | Sadiqabad | |
| No. of farmers | 25 | 25 | 25 | 25 | 10 |
| Mean age (years) | 33.07± 14.1 | 30.03±10.3 | 35.17± 13.4 | 32.41± 15.3 | 32.50±10.5 |
| Smoking(%) | 64 | 60 | 56 | 64 | 45 |
| Mean exposure (years) | 10.73±9.26 | 12.33±10.15 | 9.21±8.17 | 11.24±10.32 | 8.01±6.70 |
| Use of personal protections (%) | | | | | |
| Hand gloves | 0 | 0 | 0 | 0 | 80 |
| Goggles | 12 | 4 | 20 | 8 | 70 |
| Face mask | 16 | 20 | 28 | 20 | 90 |
| Boots | 24 | 24 | 28 | 24 | 80 |
| BMI (kg $m^{-1}$) | 21.01±2.99 | 21.17±2.81 | 23.16±3.04 | 20.21±3.01 | 19.11±3.31 |

**Table 3. The duration of spray application (hours/week) and frequency of spraying (No./week) of different types of Organophosphate products used by farmers in different tehsils of RYK.**

| Pesticide Name | Khanpur | | Liaqatpur | | Sadiqabad | | Rahimyar Khan | |
|---|---|---|---|---|---|---|---|---|
| | Duration | Frequency | Duration | frequency | duration | frequency | duration | frequency |
| Triazophos | 7 – 8 | 2 – 4 | 6 – 8 | 2 – 3 | 6 –7 | 2 – 3 | | |
| Phorate | 12 – 14 | 3 – 5 | 6 – 8 | 3 – 4 | | | 5 – 6 | 2 – 3 |
| Diazinon | 9 – 12 | 2 – 4 | 9 – 11 | 2 – 4 | | | | |
| Cypermethrin | 6 – 8 | 3 – 4 | 10 – 12 | 3 – 4 | 4 – 5 | 1 – 2 | | |
| Chlorofuran | 6 – 8 | 2 – 4 | 9 – 10 | 1 – 2 | | | 6 – 7 | 2 – 3 |
| Malathione | 5 – 8 | 3 – 4 | 7 – 8 | 1 – 3 | | | 7 – 8 | 3 – 4 |
| Dichlorvos | | | | | 6 – 7 | 2 – 3 | 7 – 9 | 3 – 4 |
| Fenthion | | | | | 5 – 6 | 1 – 2 | | |
| Methyl Parathion | | | | | 6 – 8 | 2 – 3 | | |
| Quinalphos | | | | | 7 – 9 | 3 – 4 | | |
| Fenitrothion | | | | | | | 6 – 8 | 3 – 4 |
| Profenofos | | | | | | | 3 – 4 | 1 – 2 |

used hand gloves during spraying pesticides, whereas 80% of the control group wore gloves. Furthermore, few farmers in the exposed group were using goggles (11%) compared with 70% in the control group ($p<0.001$), face masks (21% exposed, 90% control; $P<0.001$), and safety boots (25% exposed, 80% control; $p<0.001$) while spraying pesticides (Table 2). The percentage of the use of all types of PPE in the control group of farmers was higher than 70%. On the basis of this analysis, it was deduced that most of the farmers in the RYK area are unaware with the use of safety precautions during spraying of pesticides. The mean ages of the farmers of both groups (Table 2) varied between tehsils by a maximum of approximately 5 years, with the figure for the control group being well within this range, making all groups comparable for age. Similarly, the mean length of exposure of "exposed groups" was similar in all four tehsils, but slightly lower in the controls. A similar difference was found in the percentage of the exposed group that smoked (50-64%) and the controls (45%). However, greater variation in availability/use of personal protection equipment occurred between the control and exposed groups. Gloves were not worn by operators in any of the tehsils whereas they were used by 80% of the control group. Goggles were used by between 4 and 20% of operators depending on tehsil, but 70% of the control group reported that they wore goggles. Use of face masks by farm workers also varied between tehsils (16 and 28%) whereas 90% of the control group had masks, and boots were worn by only 24-28% of the exposed groups in the four tehsils compared with 90% of the control group. Thus, clear differences between control and exposed groups in the potential for exposure to pesticides were established in this study. Types of organophosphate pesticide products and the duration of spray applications and frequency per week commonly sprayed by both group of farmers in the four tehsils of RYK District are shown in Table 3. It appears that longer spray application durations are common in Liaqatpur but the range of application frequencies is similar in all tehsils.

In summary, clear associations of different socioeconomic factors with case-control groups (tested using nonparametric $\chi^2$ test of independence) emerge from this study. No Significant differences between exposed and control groups in age, marital status or smoking habits, which were not associated with the case-control groups, providing a firm basis for neurological comparisons. Education, use of goggles, face mask and boots were all found associated with (differ between) the case-control groups ($p < 0.001$) (Table 4).

**Table 4. Results of hypotheses tests to test the association of different socioeconomic factors with case/control groups.**

| Factor | $\chi^2$ | p-value |
|---|---|---|
| Age(<30 & ≥30) | 0.00 | 1.000 |
| Marital status(married & unmarried) | 0.00† | 1.000 |
| Education(uneducated & educated) | 7.26† | 0.007 |
| Smoking (yes & no) | 0.11† | 0.735 |
| Use of goggles (yes & no) | 19.01† | <0.001 |
| Use of face mask (yes & no) | 18.48† | <0.001 |
| Use of boots (yes & no) | 10.61† | 0.001 |

† Pearson's Chi-squared with Yates' continuity correction

The descriptive statistics for different enzymes in control and exposed groups are given in Table 5. The mean levels of different enzymes in blood samples were compared between the exposed and control groups using student t-tests. As the assumption of equal variances was violated with p-value < 0.001 for lipid peroxidation, glutathione and acetyl-cholinesterase a t-test with unequal population variances was used. Significant differences were recorded between control and exposed groups with higher means in the exposed group for all enzymes (Table 6).

The means of different enzymes were also compared within the exposed group to explore which enzyme is most problematic. To perform one-way Analysis of Variance (ANOVA) for testing the equality of means of different enzymes (lipid peroxidation, glutathione and acetyl-cholinesterase), a Shapiro-Wilks test was used to test the normality assumption. The values of Shapiro-Wilk's statistic for lipid peroxidation, glutathione and acetyl-cholinesterase were 0.939, 0.971, 0.970 with p-value < 0.001, 0.027, 0.024, respectively. Since the normality assumption was violated, non-parametric Kruskal-Wallis test was used to compare the three enzymes. The Kruskal-Wallis H test showed that there was a statistically significant difference in the score of different enzymes (lipid peroxidation, glutathione and acetyl-cholinesterase); $\chi^2(2) = 16.458$ $p < 0.001$, with a mean rank enzyme score of 135.76 for lipid peroxidation, 179.23 for glutathione and 136.51 for acetyl-cholinesterase.

The profiles of different diseases in both groups of farmers (exposed and controlled) in different age categories are given in Table 7. It is clear from the results that in the exposed group of farmers, the diseases increase with increasing of age. In both age categories, < 30 years and > 30 years, the incidence

**Table 5. Effect of pesticides on lipid peroxidation, glutathione and acetyl cholinesterase (mean±S.D) in the blood of farmers. The results of t-test to test the hypothesis $H_0$: $\mu_{exposed} = \mu_{control}$ against $H_0$: $\mu_{exposed} > \mu_{control}$.**

| Enzymes | Exposed(n=100) | Control (n=10) | T | df | p-value |
|---|---|---|---|---|---|
| Lipid Peroxidation | 0.25±0.06 | 0.020±0.008 | 12.06 | 108 | <0.0001 |
| Glutathione | 0.31±0.12 | 0.020±0.007 | 7.61 | 108 | <0.0001 |
| Acetyl cholinesterase | 0.25±0.08 | 0.018±0.005 | 9.13 | 108 | <0.0001 |

**Table 6. Results for Leven's test to test the hypothesis $H_0$: $\sigma^2_{exposed} = \sigma^2_{control}$ Vs $H_1$: $\sigma^2_{exposed} \neq \sigma^2_{control}$, and the results of t-test (unequal variances) to test the hypotheses $H_0$: $\mu_{exposed} = \mu_{control}$ Vs $H_1$: $\mu_{exposed} > \mu_{control}$.**

| Enzyme | Leven's test | | t (unequal variances) | p-value | 95% C.I. |
|---|---|---|---|---|---|
| | F | p-value | | | |
| Lipid Peroxidation | 14.878 | < 0.0001 | 33.89 | < 0.0001 | (0.222, 0.249) |
| Glutathione | 12.928 | < 0.0001 | 22.99 | < 0.0001 | (0.266, 0.316) |
| Acetyl cholinesterase | 19.503 | < 0.0001 | 28.12 | < 0.0001 | (0.222, 0.256) |

**Table 7. Profiles of diseases in exposed and controlled groups of farmers according to ages.**

| Disease profile | Exposed group (n=50) | | | | Controlled group (n=5) | | | |
|---|---|---|---|---|---|---|---|---|
| | < 30 years | | > 30 years | | < 30 years | | > 30 years | |
| | n | % | n | % | n | % | n | % |
| Neurological | 10 | 32.3 | 12 | 30.0 | 0 | 0.0 | 0 | 0.0 |
| Peripheral neuropathy | 3 | 9.7 | 4 | 10.0 | 0 | 0.0 | 0 | 0.0 |
| Gastro | 6 | 19.4 | 8 | 20.0 | 0 | 0.0 | 1 | 33.3 |
| Respiratory | 5 | 16.1 | 6 | 15.0 | 1 | 100.0 | 1 | 33.3 |
| Cutaneous | 3 | 9.7 | 5 | 12.5 | 0 | 0.0 | 1 | 33.3 |
| Ocular | 2 | 6.5 | 3 | 7.5 | 0 | 0.0 | 0 | 0.0 |
| Cardio vascular system | 1 | 3.2 | 1 | 2.5 | 0 | 0.0 | 0 | 0.0 |
| Musculoskeletal | 1 | 3.2 | 1 | 2.5 | 0 | 0.0 | 0 | 0.0 |
| Overall disease profile | 31 | | 40 | | 1 | | 3 | |

of neurological diseases were greater than other diseases. However, a larger number of patients with neurological diseases were found in the > 30 years of age category.

The neurological patients in both age categories of exposed groups were significant at the 5% significance level. In case of controlled group of farmers, the diseases were non-significant, at 95% confidence level, in both age categories. Overall, more farmers were suffering in the >30 years age group. Cardiovascular system and Musculoskeletal diseases were exhibited by same proportions of peoples in both > 30 years and < 30 years of age categories.

## DISCUSSION

The burgeoning increase in the demand for food and associated application of pesticides has created serious environmental and human health issues, particularly in the rural areas of Pakistan. Analysis of the level of the lipid peroxide in the blood samples from the exposed and controlled groups of farmers revealed that the serum levels of lipid peroxides (i.e., Malondialdehyde (MDA)) were higher in the exposed group. It is well documented that the pesticides can target the normal role of erythrocyte which depends on an intact cell membrane. Several researchers have reported that the antioxidant defence mechanism of both of these vital enzymes (MDA and glutathione) in the human body can be changed in the pesticides exposed regions of the world (Ognjanovic *et al*., 2008; Bernotiene *et al.*, 2012; Godala *et al.*, 2016). Both of these enzymes are proficiently capable to rummage toxic free radicals. They are moderately capable to prevent from the attack of lipid peroxidation which may occur due to the exposure to the pesticides (Liu et al., 2006; Mahreen et al., 2010). In this study, a decrease in the glutathione enzyme and an increase in the lipid peroxidation activity in the pesticide exposed farmers, are consistent with the previously published results of (Haque et al., 2012). Results of the present study also showed that the red blood cells (RBC) acetyl cholinesterase activities were greatly decreased in the blood samples of the exposed group of farmers when compared to the blood samples of the farmers of the control group. Several researchers have maintained that activates of the acetyl cholinesterase enzymes decreases in the farmers of those regions where exposure to the pesticides are higher (McCanley et al., 2006).

Results of the present study revealed that long term exposure of farmers or farm workers to commonly used pesticides or insecticides in Pakistan leads to the accumulation of pesticides in the body, particularly when appropriate PPE is not routinely worn (as recommended by the major pesticide manufacturers). Such accumulations of pesticides in the body can eventually cause skin damage, liver diseases, damage to the pancreas, and can also cause other chronic conditions such as diarrhea, nausea, allergy, cancer, neurological disorders, respiratory problems, asthma, and eyes problems (Beseler and Stallones, 2009). Exposure to the pesticides can also cause serious diseases such as heart problems, genetic disorder, disturbance in the menstrual cycle of the female, and abortion. The increasing world population will requires 70% increase in worldwide food production is now well accepted and this cannot be achieved without pesticide use. Thus, to avoid widespread food shortages and increasing food prices, the importance of protecting growers by following manufacturer advice to wear protective clothing educating them to use PPE effectively is clearly demonstrated by this study.

***Conclusion*:** The activities of the glutathione and acetyl cholinesterase enzymes were reduced significantly in the blood samples of the farmers who were exposed to the pesticides as compared to those farmers who were using protective equipment and vice versa in case of lipid peroxidation. Diseases are most common in rural areas, the main reason is that farmers are uneducated and do not use the protection equipment during spray application. So, pesticides effects are most common in uneducated farmers. The farmers need to acquire an education and to follow the precautionary measurements. During the spraying of pesticides in the fields, the farmers must need to wear gloves, head caps, coats, glasses, and masks over their mouth.

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**[Received 8 Nov 2019; Accepted 11 July 2020; Published (online) 17 July 2020]**