

# Assessment of Pesticide Exposure in Female Population Living in Cotton Growing Areas of Punjab, Pakistan

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**Abstract** The exposure assessment study was conducted in the southern Punjab, the cotton growing area of Pakistan among two female groups, one as cotton pickers (occupationally exposed) and the other group as non-pickers. The average blood cholinesterase activity before the spraying in non-pickers were found to be  $5.32 \pm 0.70$  kU/L with the average activity of female cotton pickers  $5.31 \pm 1.12$  kU/L. Whereas, due to environmental contamination the average activity of enzyme cholinesterase (ChE) was calculated to be  $4.80 \pm 1.08$  kU/L in non picker's blood samples, collected after exposure to pesticides (post-season) as compared to  $2.81 \pm 1.32$  kU/L in female cotton pickers. All these values were found be in the normal range. The mean values of ChE were found significantly different (1.95) among various groups when statistically analyzed by Z-test ( $\alpha = 0.05$ ). The effects of pesticides found equal in both groups of female were discussed in detail with common symptoms observed in majority of cotton pickers.

**Keywords** Cholinesterase · Pesticides · Female cotton pickers · Risk

Indiscriminate use of pesticides may cause considerable hazards to health and environment. As pesticides are inherently toxic to living organisms, they are more likely to

affect the health of human beings than other agricultural chemicals. Exposure to pesticides in agriculture occurs during loading, mixing, application of pesticides and manual activities in treated crops. The pesticide poisoning cases are observed in developing countries, while malnutrition and dehydration are likely to increase the susceptibility to pesticide poisoning (WHO 1990) and annually 10,000 farmers and field worker get poisoned by pesticides in Pakistan while unintentional acute pesticide poisoning cases are observed due to occupational exposure (Anwar et al. 2006; Hashmi and Khan 2011). Human exposure to pesticides is usually estimated by measuring the levels in the environment i.e. soil, water and food (Anwar et al. 2005, 2011, 2012; Anwar 2011).

Cotton is a cash crop of Pakistan growing on 2.9 million hectares and plays a vital role in the country's economy but receive tremendous amount of organophosphate (OP), carbamate and pyrethroid pesticides to protect against insect pests. Bungush and Anwar (2000) Tariq et al. (2007) and Hashmi and Khan (2011) reviewed acute pesticide poisoning cases and identified the factors related to occupational workers in Pakistan.

The pesticide exposure intentional, accidental or via food chain is followed by medical symptoms (Ali et al. 2012; Anwar et al. 2006; Khan et al. 2010; Zhang et al. 2011) and poisoning starts with vomiting, headache, nausea, sweating, suffocation, restlessness, muscle pain and fasciculation of muscles. The WHO recognizes the ChE bio monitoring as a preventive measure against OP exposure and there is a good correlation between exposure and ChE reduction (Khan et al. 2005). Acute clinical poisoning is likely to appear when the ChE activity is inhibited by 50 % or more and 30 % inhibition is considered as a hazard level (WHO 1990). Acute pesticide illness with off target pesticides (Lee et al. 2011) cancer incident and pesticides

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exposure (Weichenthal et al. 2010) have been reported in the literature from different countries. In Pakistan due to illiteracy or other associated social problems the studies on effect and illness due to pesticides are scanty. Khan et al. (2005) reported the ChE activities among farmers and non farmers living in Multan, Pakistan. Recently Anwar et al. (2011) discussed the pesticides impact on human health in district Nawabshah, Sindh, Pakistan.

Present study was conducted in the southern Punjab i.e. Multan and Bahawalpur Divisions, the major cotton growing areas of Pakistan. The field study was limited to a manageable geographical area where female cotton pickers are living and have a great potential to be exposed to pesticides. The villages selected on the willingness of the female workers that participate in the study from Lodhran district were Mozah Lodhran, Kallowala, Nizamabad (Vehari-Khanewal Road), Basti Gewniwala, Basti Bahowala, Lodhran ward-7, Khan Waghawala and Chak Miranwala and Mozah Salah, Bakirpur, Dera Bhaka and Abbas Nagar villages from Bahawalpur. The present work had been carried out to provide base line data on the impact of hazardous effects of pesticides on female health living in the cotton growing areas which helps in implementing the pesticide policy in Pakistan.

## Materials and Methods

After preliminary survey two female groups (13–35 years of age) were selected as cotton pickers and non-pickers (30–37 female in each group) from the selected area. Blood samples (5 cc each) were collected in a heparinized tube to prevent from clotting in pre-season (i.e. before the spray from June to July) and post-season (i.e. after the exposure in the month of October) from the identified female groups comprising of total 80 samples. For quantitative analysis the samples were centrifuged then separated serum and the whole blood samples were kept in a refrigerator and then transported in icebox to Islamabad laboratory.

Quantitative blood cholinesterase (ChE) activity was determined by the calorimetric method in duplicate (Knedel and Bottger 1967) with the help of diagnostic kit supplied by Diagnostica Merck. This method is based upon the hydrolysis of cholinesterase. *S*-butyrylthiocholine iodide (6 mmol/L) is used as substrate which was readily dissociated by serum ChE into butyrate and thiocholine. Butyrate reacts with dithiobisnitrobenzoate (0.25 mmol/L) an indicator forming a yellow coloured compound 2-nitro-5-mercaptop benzoate, the rate of which was proportional to the concentration of ChE present in the serum. For analysis chromogen mixture (phosphate buffer 50 mmol/L pH7.7 and indicator) was prepared in 100 mL distilled water. Substrate solution was prepared by adding 5 mL of distilled water to the substrate and shake well. Working

reagent was prepared by dissolving 2.00 mL of chromogen solution with 0.01 mL of substrate solution (stable for 2 h at 25°C) and add 0.01 mL of serum mixed well and after approx. 1 min measure the increase in absorption every min for 3 min on Perkin Elmer lambda 3 Double Beam UV-visible, Model R100A Spectrophotometer at 405 nm wavelength. Reproducibility was assessed from repeated parallel determination. ChE activity kU/L =  $\Delta A/\text{min. } 15.2$  (normal range kU/L for women 2.0–6.7 at 25°C).

## Results and Discussion

Female cotton pickers became nauseated and fainted in the field in some areas due to last spray was done before 3–15 days of picking. Spray affect the health of pickers as they spend long working hours 6 a.m., to 6 p.m., i.e. 12 h approximate in the field, but are not aware of the risk they face or are too poor to take protective measures and thus face health problems. In the blood samples before exposure to pesticide, the average activity of ChE in non-pickers was calculated to be 5.32 kU/L with the average activity of female cotton pickers 5.31 kU/L. These values are in the normal range (2.0–6.7 kU/L) but, are closer to the maximum of the range. Whereas, due to environmental contamination the average activity of ChE was 4.80 kU/L in non picker blood, collected after exposure to pesticides (post-season) were found to be significantly different with the average activity 2.81 kU/L of female cotton pickers (Table 1). The activities of ChE can be shown in Fig. 1.

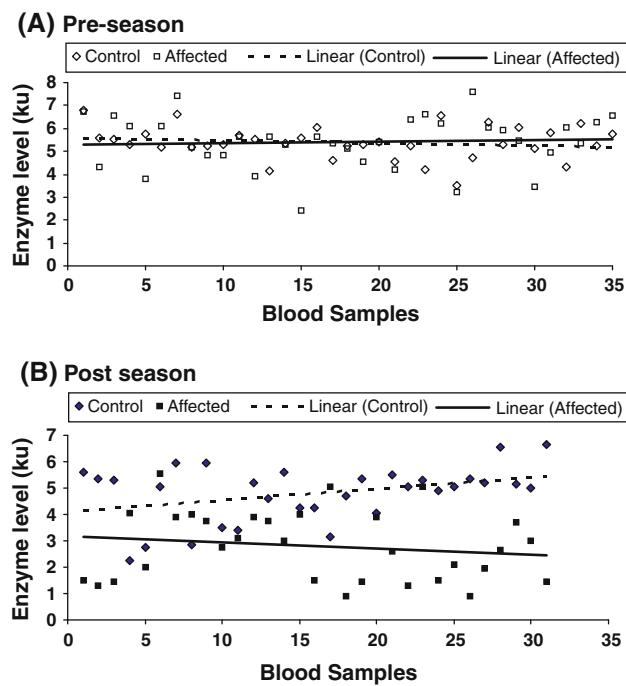
The 30 female cotton pickers during post season survey complained a variety of symptoms like headache, nausea, vomiting, skin irritation, general weakness, fever, dizziness, stomach pain and blisters. Some (46 %) women had cuts on hands and broken skin. Other (11) females with low ChE activity had reported headache, vomiting, nausea, itching, fever, stomach cramps and muscular pain.

During picking cotton crop was still contaminated with pesticide residues, and female pickers were found to eat and drink in the field without washing their hands. Residue of pesticides may enter their gastrointestinal tract through mouth and caused vomiting and nausea. It was observed that after spray the activities of ChE was decreased and

**Table 1** Average cholinesterase enzyme activities (kU/L) in female pickers during pre spray and post spray season in Punjab, Pakistan

Surveys	Female groups	
	Non-pickers	Cotton pickers
Pre-season	5.32 $\pm$ 0.7	5.31 $\pm$ 1.12
Post-season	4.80 $\pm$ 1.08	2.81 $\pm$ 1.32

Normal range kU/L 2.0–6.7 for women at 25°C



**Fig. 1** Cholinesterase Enzyme activities in blood of cotton picker of Multan division, before (a) after spry (b)

females (100 %) suffered from headache, vomiting and nausea. Some had problem like stomach cramps and some reported to have skin problem like skin peeling, itching and blisters and skin burning. The young females who are energetic and they spent 9 h in the fields for 90 days/season. Long picking hours also enhanced the residues to enter the body through respiratory tract and caused coughing, breathing deficiency and fainting. A similar finding with 50 %–80 % ChE inhibition was reported in 25 % female pickers in cotton growing area of Punjab (Khan et al. 2005). Although the methodology of these two studies was different in two ways: first, a comprehensive socio-economic survey was carried out to co-relate the enzyme activities with the perception of female pickers about hazards of pesticides and second, ChE was determined both in terms of quality and quantity. The occupational group like cotton picker is in high risk to pesticide poisoning than non-occupational group. The long working hours during picking season exposed female pickers more in danger to hazards of pesticide than males (Khan et al. 2005). The assessment of ChE, a biological monitor confirms the adverse effect of pesticides on cotton pickers health. During picking in the contaminated field pesticide residues enter into their blood (Azmi et al. 2006) through skin, inhalation or ingestion and is accumulated in adipose tissue resulting in health damage (Khan et al. 2010) which causes economic losses in term of wages (days lost) and medical treatment.

In a study carried out in Sindh by Anwar et al. (2006) reported that 89 % farmers suffered from dizziness, headache, tiredness, excessive sweating, salivation nervousness, short breath, cold legs and hands at night, stomach cramps, vomiting, red eyes, coughing and unconsciousness similar to present study. Moreover all types of pesticides effect on the ChE activity that also found to be lowered in farm workers (Khan et al. 2005, 2010; Zhang et al. 2011). Exposure of organophosphate (OP) pesticides even after 3 days brought adverse effects on ChE inhibition and extensive use of OP results in acute intoxication and symptoms starts with vomiting excessive sweating restlessness and fasciculation of the muscles (Anwar et al. 2006). Azmi et al. (2006) reported pesticide residues (cypermethrin, deltamethrin, diazinon, monocrotophos, DDT, DDE and Polytrin-C) in blood of 14 families related to vegetable farming of Gadap, Karachi, Pakistan and its significant effect on enzyme levels i.e. glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP) that increased in farmers with complaints about liver and kidney dysfunction and renal tract infection (RTI). During picking in the pesticides contaminated field residue enters into the blood through skin, inhalation or ingestion resulting in poisoning of pesticides with symptoms (Khan et al. 2010) reported mild to moderate pesticide poisoning, which was correlated with depression on plasma ChE levels during a study conducted among the 105 tobacco farmers in Sawabi, Pakistan. Among the 58 (55 %) had positive-exposure reduction in ChE level < 20 % from baseline, 35 (53 %) had mild poisoning (20 %–40 %) reduction and 12 (11 %) had moderate poisoning (>40 %) reduction with little knowledge about the safety measures, casual attitude and unsatisfactory safety practices with regards to the use of basic protective equipments during pesticide applications on the tobacco crop.

It is concluded that the training for safety procedures in handling, storage and disposal of chemicals are required for farmers and occupational workers especially female cotton pickers in Pakistan. This relation of health workdays lost and treatment cost with ChE activity of female cotton pickers indicates that pesticide related illness has a negative impact on their work ability.

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