academicJournals

Vol. 7(8), pp. 846-851, August 2013
DOI: 10.5897/AJEST2013.1510
ISSN 1996-0786 © 2013 Academic Journals
http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

Full Length Research Paper

Impairment in biochemical indices in sprayers exposed to commonly used organophosphorus and pyrethroids: A case study

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Accepted 1 August, 2013

This study was carried out to evaluate the effect of spraying organophosphate, carbamate and synthetic pyrethroid pesticides on biochemical parameters viz., red blood cell (RBC) acetylcholine esterase (AchE) and plasma butyrylcholinesterase (BChE) activities, oxidative stress parameters viz., plasma T-BARs, catalase activity and antioxidant levels such as reduced glutathione, and α-tocopherol and pesticide residues (analysed using the GC/LC-MS/MS) in sprayers cultivating cotton, chilly and paddy crops in Guntur district for a period of >10 years. The study population included sprayers (120) with a mean age of 37.8 ± 10.8 years and controls (60) with a mean age of 37.3 ± 12.2 years. A significant difference was observed for AchE, BChE activities, thiobarbituric acid reactive substances (TBARs), catalase activity, reduced glutathione and the concentration of pesticide residues in the sprayers cultivating different crops for a period of >10 years as compared to the controls. However, there is no such significant difference observed for α-tocopherol.

Key words: Pesticide residues, chronic exposure, biochemical indices, sprayers running head, biochemical indices, pesticide residues in sprayers

INTRODUCTION

Several groups of pesticides are being used by farmers worldwide for a long time now in order to protect crops from pests and to enhance food production (Issa et al., 2010). According to WHO (2009), approximately 25 million pesticide poisoning cases occur annually among agricultural workers in developing countries. Reports on the effects of pesticide exposure on the health of pesticide applicators have been studied earlier (Ismail et al., 2010). A review of these studies demonstrated a wide range of neuromuscular disorders, which include paresthesia, increased sensitivity to vibration, disturbed balance, tremors and acetylcholine esterase (AchE) inhibition and deterioration of the hematological, hepatic and renal functions (Ismail et al., 2010). Similarly, in our earlier studies, significantly lower level of AchE activity, oxidative stress and antioxidant levels in the cotton cultivating farmers engaged in spraying pesticides in cotton fields was observed. Such observations were not found in non- agricultural workers who were not occupationally exposed (controls) to the spraying

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operations during any time of their life (Jonnalagadda et al., 2010). Further, earlier studies indicated the alterations in biochemical parameters in pesticide exposed population (Bhatnagar et al., 2003; Khan et al., 2008). However, pesticide poisoning in agricultural farmers due to occupational exposure is poorly documented in developing countries (Smit et al., 2003).

Cultivation of commercial crops such as cotton and chilly is more prevalent in Guntur district of Andhra Pradesh, India and the use of pesticides for those crops is also high as compared to the other crops. The farmers are repeatedly exposed to different types of pesticides, either simultaneously or serially during their work in the field. There is paucity of data on the incidences of pesticides in blood samples of sprayers of Guntur District (AP) and their effect on biochemical parameters if any. Therefore, the present study was carried out with an objective to determine the pesticide residues in the serum of farmers cultivating cotton, chilly and paddy crops due to chronic exposure to multiple pesticides for a period of >10 years and their effect on biochemical parameters as compared to control subjects.

MATERIALS AND METHODS

Study population

A study was carried out in Guntur District (AP), India, targeting mainly the agricultural sprayers involved in pesticide spraying on three types of crops viz., cotton, chilly and paddy for short term (<5) and long term (>10) exposure (n=120). Control group (n = 60) matched for age and gender was also selected. It was ensured that they do not have any occupational history of spraying pesticides during their life time. The objective of the study was explained to the subjects and the informed consent was also obtained. Ethical clearance was obtained from Institutional Ethical Committee to include human subjects for drawing of blood samples. About 49% of the farmers were found to have used organophosphates viz., monocrotophos (dimethyl (E) -1-mehtyl-2-(methylcarbonyl) vinyl phosphate - $C_3H_{14}NO_5P$, dichlorovos, 2,2-dichlorovinyl dimethyl phosphate - $C_4H_7C_{l2}O_4P$, quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothioate $C_{12}H_{15}N_2O_3PS$) etc. (supplied by M/S. Bayer India Pvt. Ltd., M/S. Dhanuka Pvt. Ltd., M/S. Rallies India Pvt. Ltd.,) and synthetic pyrethoroids (12%) such as (RS) - α cyano-3-phenoxybenzyl (1RS, 3RS; 1RS, 3SR)-3-(2,2-dichlorovinyl) -2,2-dimethyl cyclopropane carboxylate - $C_{22}H_{19}C_{12}NO_3$), Imidacloprid (18%) (6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine- $C_9H_{10}ClN_5O_2$). About 11% have used each of spinosad $C_{41}H_{65}NO_{10}$ (spinosyn A) + $C_{42}H_{67}NO_{10}$ (spinosyn D) and a slightly hazardous group of carbamate (11%) like indoxacarb methyl (S)-N-[7-chloro-2,3,4a,5-tetrahydro-4a-(methoxycarbonyl)indeno[1,2-e][1,3,4]oxadiazin-2-ylcarbonyl]-4-(trifluoromethoxy-$C_{22}H_{17}ClF_3N_3O_7$).

Laboratory methods

Determination of erythrocyte AchE, α-tocopherol (vitamin E) and TBAR levels

About 10 ml (each) of the blood samples from all the three types of sprayers (n = 120) spraying pesticides for more than a period of 10 years was collected in EDTA (ethylenediamine tetra acetic acid) vials (used for reduced glutathione and AchE tests) and heparin vials for analyzing the RBC catalase activity, thiobarbituric acid reactive substances (TBARs) and α-tocopherol levels in plasma. All the samples were transported to the laboratory within 24 h in frozen condition using gel packs. Out of the 10 ml of the blood samples, 3 ml was taken in a separate tube for the purpose of obtaining serum. Serum was obtained by centrifuging 3 ml of the blood sample at 1500 to 2000 RPM and the same was used for the estimation of pesticide residues. The erythrocyte AchE levels and plasma BChE activity were measured immediately (Ellman et al., 1961). The samples were centrifuged at 2000 rpm for 15 min and the plasma and leukocyte layers were separated and used for other assays. The erythrocyte sediment in the tubes was washed thrice with 0.9% saline for carrying out the catalase and glutathione (GSH) assays. All the samples for the remaining tests were stored at -80°C until assayed. Catalase activity was determined spectrophotometrically and reduced glutathione was determined fluorimetrically by Op-Thalaldehyde (OPT) method (Aebi et al., 1974). Vitamin E was determined using high performance liquid chromatography (HPLC) method (Allard et al., 1998). Lipid peroxidation was quantified by plasma thiobarbituric acid reactive substances by spectrophotometric method (Bhat, 1991).

Extraction of pesticide residues from the human biological samples

One milliliter (each) of serum sample from all the farmers cultivating cotton, chilly and paddy crops was processed for the analysis of the pesticide residues using solid phase extraction (SPE) cartridges. Serum samples were loaded into the SPE cartridges after pre-conditioning with methanol and then the residues were eluted as per the standard procedures (Jonnalagadda et al., 2010). To determine the quality of the methodology, a recovery study was performed on 10 spiked replicates of blank human (not occupationally exposed) blood sera, which presented contamination levels below the detection limits. The spike studies were conducted for standard compounds of monocrotophos (36%) (dimethyl (E) -1-methyl-2-(methyl carbonyl) vinyl phosphate), $C_3H_{14}NO_5P$, dichlorovos (76%), quinalphos (25%) and synthetic pyrethoroids such as (RS) - α cyano-3-phenoxybenzyl (1RS,3RS;1RS, 3SR)-3-(2,2-dichlorovinyl) -2,2-dimethyl cyclopropane carboxylate $C_{22}H_{19}C_{12}NO_3$ etc., and a slightly hazardous carbamate like indoxacarb and a nicotinoid like spinosad. After extraction from the serum, one µL was injected into GC (for synthetic pyrethroid compounds) and LC-MS/MS (for organophosphorous compounds). It revealed that the mean recovery values ranged from 90.5 to 93.8%. To confirm the determination of synthetic pyrethroid compounds that corresponded to synthetic pyrethroid pesticide residue peaks, a gas chromatograph-mass spectrometer (GC-MS, Perkin Elmer and Turbomass with auto system XL-GC) was used. The peaks eluted were confirmed by comparing the results of mass spectra of substances from blood sera with those of standard compounds selecting specific ions obtained from an ion trap detector. The linearity curves were drawn for each compound. The minimum detection limit for the synthetic pyrethroid pesticide studied was 1.0 ng/mL. Simultaneously, linearity curves for organophosphorous, carbamate and nicotinoid pesticides were also drawn. The minimum detection limit for the compounds detected using LC.MS/MS ranged from 0.1 to 1.0 ng/mL.

Statistical analysis

Descriptive statistics such as mean and standard deviation of various biochemical parameters were calculated. The duration of exposure to spraying is divided broadly into two categories, that is,

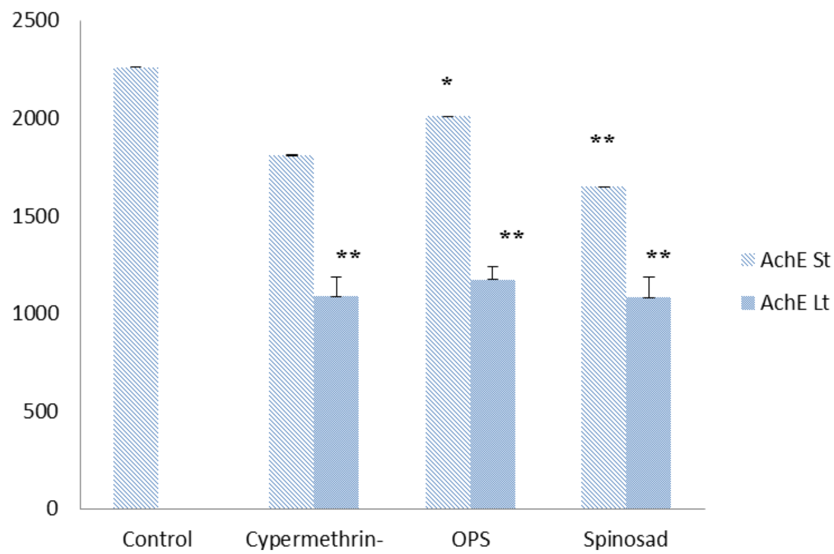

Figure 1. Comparison of mean AchE (U/L) among the sprayers exposed to cypermethrin, OPs and spinosad. * significant P<0.05, ** significant P<0.01.

short and long term of exposure depending on duration. Pesticide residues levels were compared between short, long term and controls using one way of ANOVA with post hoc analysis (least square difference). Since the variation is large between exposed and control subjects, non-parametric "µ" test was applied to assess the difference in pesticide residues between short and long term exposed groups. Significance was calculated at p = 0.05 and p = 0.01 level. The mean values of biochemical parameters were presented using bar charts.

Operating conditions of LC-MS/MS

Model: Agilent 6460 Triple Quad, Agilent Gradient Liquid chromatograph.
Mobile Phase A: 9:1 methanol: water containing 5 mM ammonium formate, Mobile Phase B: 9:1 water containing 5 mM ammonium formate: methanol. Mobile phase A, B were pumped in gradient mode at a flow rate of 0.6 ml/min, Column: Purosphere Star Column 4.6 x 150 mm, 5 µm,Column oven temperature 35°C, run time: 15 min. Reconstitution of the extracted sample has been done with the composition of mobile phase A and 5 µL was injected.

Operating conditions of GC

Model: Varian CP - 3800 equipped with electron capture detector (ECD). 5 phenyl, 95% methyl polysiloxane column was used with an an internal diameter of 0.50 µm, thickness of 0.53 µm and with 30 m length with split less system. GC-operating conditions: Column oven temp (°C) initially at 100 (hold for 2 min); 190 at 5°C per min (hold for 5min); 250 at 10°C per min (hold for 4 min); Injector port: 270°C, Detector temperature: 300°C using Iolar-1 nitrogen as a carrier gas with a flow rate of 1.5 ml/min with a total run time of 35 min.

RESULTS

The mean age of the exposed subjects was 37.8 ± 10.8, while that of the unexposed (control) subjects was 37.3 ± 12.2 years. Different biochemical parameters were estimated among the sprayers (exposed) cultivating different types of crops for short and long terms of exposure and the results were compared with controls who were not occupationally exposed to any type of the pesticides during any time of their lives. It was observed that the difference in the inhibition of AchE activity was highly significant (P<0.01) among the sprayers exposed to synthetic pyretheroid such as cypermethrin, organophosphorous pesticides (monocrotophos, dichlorvos, quinalphos) and significant to nicotinoid like spinosad for long term as compared to the control (Figure 1).

While the BchE activity inhibition was highly significant (P<0.01) among the sprayers exposed for long time to organophosphorous pesticide compounds like monocrotophos, dichlorovos, quinalphos when compared with the controls (Figure 2). It is also interesting to note that the reduction in the catalase activity was highly significant (P<0.01) among the sprayers exposed to cypermethrin and OPs for a long term exposure as compared to the controls (Figure 3). However, the reduced glutathione among the sprayers exposed for a long time to cypermethrin was significant while for OPs it was not significant as compared to the controls (Figure 4). Further, an increase in TBARs (P<0.01) for the sprayers exposed for long time to cypermethrin and organophosphorous compounds was observed when compared with the controls (Figure 5). It is interesting to note that there was no such significant difference observed for α-tocopherol.

The average levels of pesticide residues detected among the sprayers were high as compared to control subjects. However the levels of pesticide residues viz.,

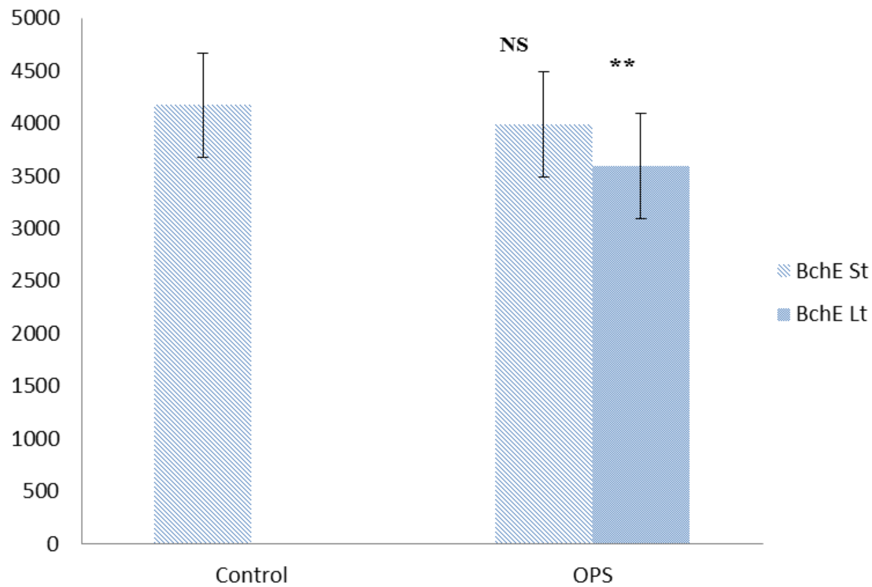

Figure 2. Comparison of mean BChE (U/L) among the sprayers exposed to OPs. NS. No sigificance. P>0.5, **significant P<0.01.

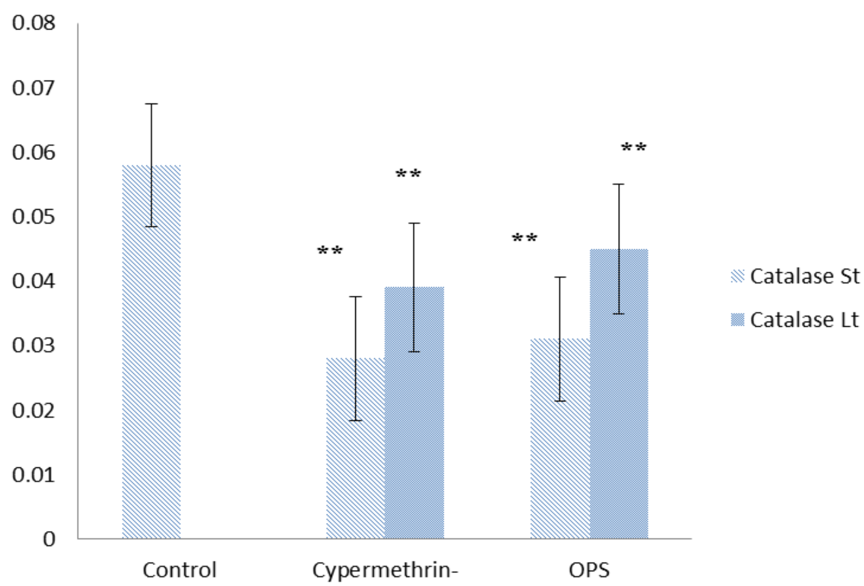

Figure 3. Effect of cypermethrin and OPs on mean catalase (mg protein/min) among the sprayers with control. ** significant P<0.01.

indoxacarb, spinosad and op group of compounds were high as compared to the rest of residues (Table 1).

DISCUSSION

Pesticides not only affect the ecosystem, but also affect the health of the sprayers, due to multiple exposures to different pesticides used by the sprayers against pests/weeds. The present study results indicate that chronic and multiple exposures to different complex mixture of pesticides suggest the accumulation of acetyl-choline resulting in production of cholinergic effects and inhibition of red blood cell (RBC) AchE activity and significant inhibition of plasma cholinesterase in the sprayers could be due to the significant levels of pesticide residues in their blood/plasma as reported earlier (Khan et al., 2008) and are in conformity with earlier reports (Safi et al., 2005; Ismail et al., 2010; Issa et al., 2010).

Pesticides not only decreased the AchE and BchE activities, but also altered antioxidant and lipid peroxi-dation parameters in the sprayers spraying them for a

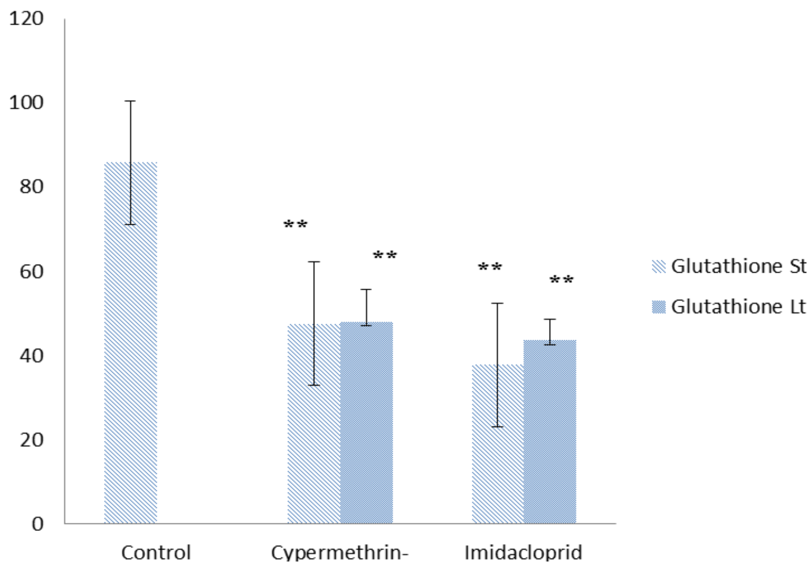

Figure 4. Comparison of mean glutathione (µg/mg protein) among the sprayers exposed to cypermethrin and imidacloprid with the control. ** significant P<0.01.

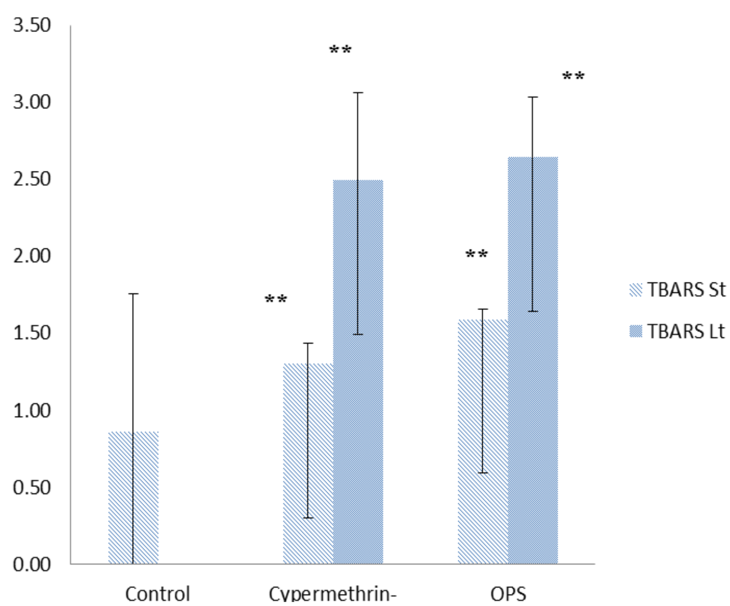

Figure 5. Comparison of cypermethrin and OPS on the mean plasma TBARs (nM/mL) among the sprayers and compared with control. **significant P<0.01.

Table 1. Serum concentrations (ng/ml) of different pesticide residues among the sprayers and control.

Pesticide	Control		Sprayer	
	no.	Mean ± SD	no.	Mean ± SD
Cypermethrin		0.0031±0.0017	14	15.93±12.89**
Monocrotophos, dichlorovos, quinalphos		0.0026±0.0016	59	20.35±16.08**
Imidacloprid	61	0.0027±0.0016	21	17.47±12.55**
Indoxacarb		0.003±0.0017	13	26.46±22.78**
Spinosad		0	13	23.87±15.96**

**Significant P<0.01 by using non parametric "µ" test.

prolonged period (Prakasam et al., 2001; Sulak et al., 2005; Gokalp et al., 2005; Ogutcu et al., 2006; Jonnalagadda et al., 2010). The increase in plasma TBARs among the sprayers was also accompanied by reduction in the catalase activity as a compensatory mechanism. Decrease in the antioxidant level such as reduced glutathione in the sprayers in the present study is also suggestive of increased utilization of antioxidants to scavenge the reactive oxygen species (Jonnalagadda et al., 2010). However, there was no significant difference in the levels of α-tocopherol in the sprayers engaged in spraying the pesticides for both short and long term exposure.

The multiple pesticide residues that were detected in the plasma of the sprayers, may be due to their extensive and repeated use for the crops for >10 years and were found in the order indoxacarb followed by spinosad, OP group of compounds, imidacloprid and cypermethrin (Klein and Karl, 1990). Pesticide exposure may produce biochemical changes even before the clinical health manifestations that may appear in the sprayers. However, low quantities of pesticide residues were detected in the control subjects indicating no significant alterations in the biochemical parameters (Jonnalagadda et al., 2010). Lack of knowledge and awareness on using the protective devices also would have increased the risk due to repeated exposure in the sprayers unlike in the developed countries. Whereas in India, a developing country extensive education and awareness are essential for sprayers, on the beneficial effects of using protective devices while spraying the pesticides so as to protect themselves from the adverse effects due to the exposure.

ACKNOWLEDGEMENTS

Financial assistance provided by Department of Science and Technology, Ministry of Science and Technology, New Delhi, India is gratefully acknowledged.

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