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# Evaluating the Level of Pesticides in the Blood of Small-Scale Farmers and Its Associated Risk Factors in Western Ethiopia

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## ABSTRACT

**BACKGROUND:** The presence of agricultural pesticide residue can cause adverse health effects. The main objective of this study was to evaluate the level of pesticides in the blood of small-scale farmers (SSFs) and associated risk factors in western Ethiopia.

**METHODS:** A cross-sectional study was conducted in June 2020 using 240 blood samples, 140 from the exposed small-scale farmers (ESSFs) and 100 from non-exposed small-scale farmers (NESSFs). The blood sample analysis was made for 5 organochlorines (OCs) and 3 synthetic pyrethroids (SPs) pesticides by gas-liquid chromatography with an electron capture detector (GC-ECD) methods. Extraction, and clean up of the samples were made by using standard analytical methods. To define the relationships between the outcomes and explanatory variables, logistic regression models were used.

**RESULTS:** The results show that *p,p'*-DDT, heptachlor and deltamethrin were the most frequently detected pesticides with 96.4%, 95%, and 100% in both ESSFs and NESSFs, respectively. The ESSFs blood samples have shown the highest mean concentrations of permethrin and *p,p'*-DDT ( $1.26 \pm 0.15$ ) and ( $0.28 \pm 0.4$ )  $\text{mgL}^{-1}$ , respectively. SSFs under the age of 40 were 21% less likely to be exposed to permethrin than those above the age of 40 (Adjusted Odd Ratio, AOR, 0.21; 95% CI: 0.1-0.44). Male SSFs were 17 times more likely to be exposed to heptachlor than females (AOR, 17.36; 95% CI: 7.34-41.09) and farmers with no formal education were 18 times more likely to be exposed to deltamethrin than those with primary schools and beyond (AOR, 18.1; 95% CI: 4.53-72.06). Furthermore, SSFs that did not use PPE appropriately were 3.6 and 6.21 times more likely to be exposed to cypermethrin (AOR, 3.6; 95% CI: 1.94-6.54) and *p,p'*-DDE (AOR, 6.21; 95% CI: 3.38-11.41) blood levels than those who did, respectively. SSFs that perform different activities like eating and drinking (11%), chewing (10%), and diverse activities (8%) were more likely to be exposed to *p,p'*-DDT than those farmers who didn't use pesticides.

**CONCLUSIONS:** This study identified a high concentration *p,p'*-DDE, *p,p'*-DDT, heptachlor, cypermethrin, permethrin, and deltamethrin in the blood of small scale farmers. The older age, less education, and farmers with inappropriate PPE use are more likely exposed to pesticides.

**KEYWORDS:** Blood, Ethiopia, farmers, pesticide residues, risk factor

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## Introduction

Pesticides are any substance or combination of substances used to prevent, eliminate, repel, or mitigate pests.<sup>1,2</sup> Chemical pesticides are classified into families, including organochlorine pesticides (OCPs), organophosphates, and synthetic pyrethroids (SPs).<sup>3,4</sup> These chemicals are mostly used in public health, agriculture, and forestry.<sup>4,5</sup> However, OCPs are persistent insecticides that have been worldwide regulated, and their widespread usage causes severe health and environmental problems.<sup>6,7</sup>

Furthermore, farmers in low and middle-income countries have been exposed by misuse, lack of understanding, poor application techniques, and a lack of personal protective equipment (PPE) during application.<sup>7-9</sup> Some reviewed studies revealed that pesticides and their metabolites cause health risks problems. Those reported risks might be due to occupational pesticide exposure, a lack of post-registration monitoring

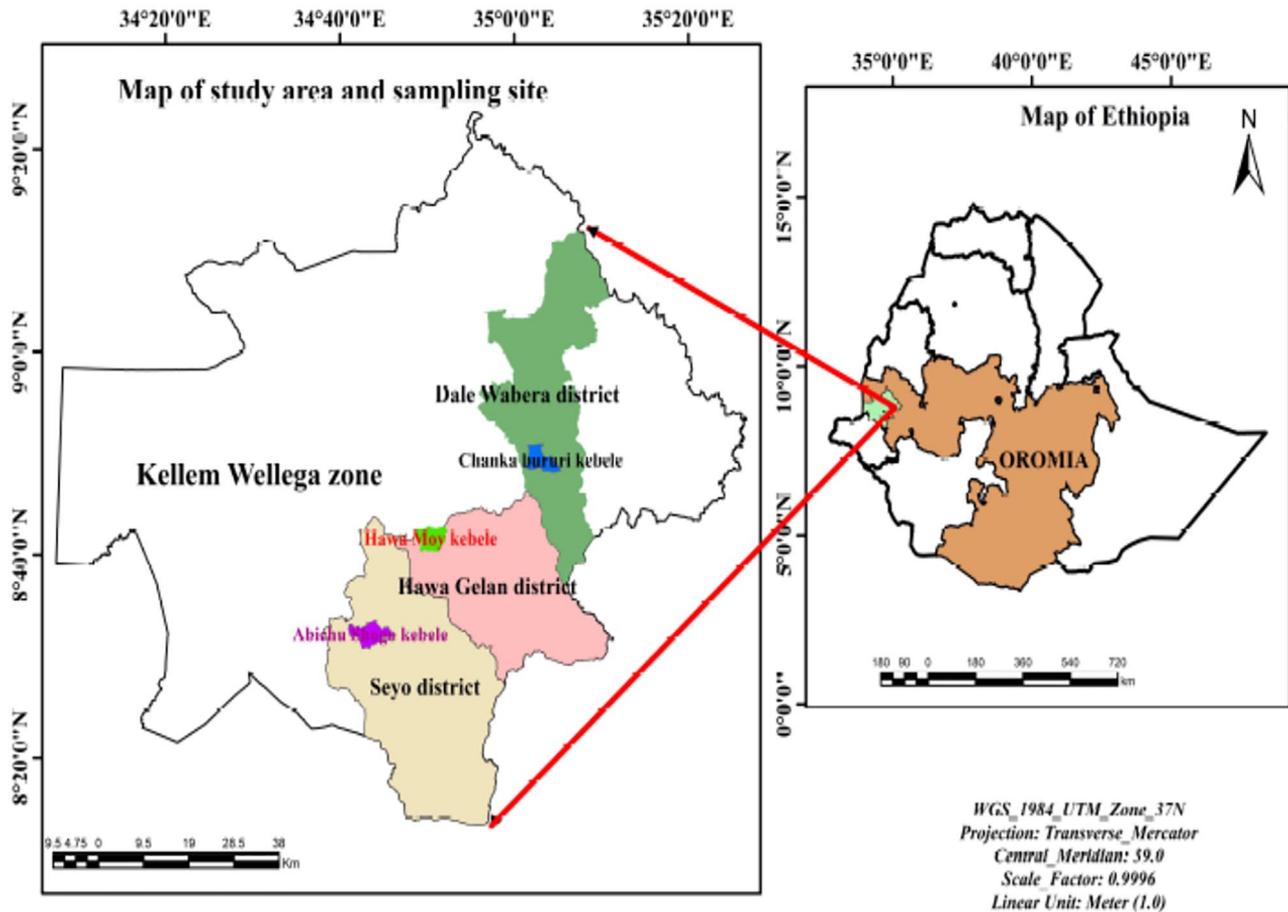
mechanisms, or farmers' lack of awareness of pesticide storage, application, and disposal.<sup>10,11</sup>

Additionally, the use of pesticides without knowing their toxicity and dosage might result in a severe of health problems.<sup>12</sup> For instance, the excessive use of pesticides against pests has been found in the blood of vegetable-producing farmers, causing adverse health effects like headache, loss of consciousness, dark vision, blood pressure, cancer, diabetes, infertility, and Parkinson's disease.<sup>13-16</sup>

Since occupational exposures to OCPs and SPs occur primarily by inhalation and dermal contact.<sup>17</sup> Families living near farms may be more exposed than the usual because of agricultural application drift.<sup>18</sup> Its hydrophobic nature allows it to easily bind the biological membranes of phospholipid bilayers, implying bioavailability in human tissues.<sup>6</sup> As a result, assessing the concentrations of OCPs and SPs in the blood reveals the level of exposure.<sup>19</sup>



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**Figure 1.** Study area map for Sayo, Hawa Gelan, and Dale Wabara districts in Kellem Wellega Zone of Western Ethiopia, 2020.

Small-scale farmers (SSFs) may be exposed to pesticides through occupational exposure, formulation, handling, storage, transportation, and inappropriate application in the agricultural fields or through the food chain.<sup>20,21</sup> Farmers are also exposed to pesticides through the consuming of contaminated food with cypermethrin, permethrin, heptachlor, and deltamethrin.<sup>3,22–25</sup> Correspondingly, pesticide residues found in wheat and bovine milk samples were higher than permissible residue limits.<sup>26–28</sup> On the other hand, farmers engaged in the poor practice of pesticide handling activities have the risk of being exposed to pesticides<sup>29</sup> and accumulate OCPs and SPs concentrations. Since humans hold the top place in the trophic level and become more prone to its harmful effects.<sup>30</sup> For this investigation, blood, plasma, milk, and adipose tissue samples were used to track the degree of exposure of small-scale farmers to lipophilic pesticides.<sup>31</sup> Human blood is the most accessible body fluid for determining pesticide concentration levels.<sup>32</sup>

Ultimately, the blood assessment offers evidence of body pesticide exposure and provides indicators of body load of pesticide concentrations in small-scale farmers for OCPs and SPs. To our knowledge, there are gaps of data available on the level of OCPs and SPs with their associated factor with higher levels of pesticides in blood samples of small-scale farmers (SSFs) in Ethiopia that this study aims to fill in. Therefore, the objective of this study was to determine the blood level of pesticides in

small-scale farmers in the Kellem Wellega zone, western Ethiopia.

## Materials and Methods

### Study setting

The study was conducted in the Kellem Wellega zone of the Oromia region, western Ethiopia. It is 672 km away from Addis Ababa, the capital city of Ethiopia. The zone is divided into 10 districts, with a total population of 965 000 people, 49.75% of them are females.<sup>33</sup> The zone has 175 000 households, with an average household size of 5.5.<sup>34</sup> The present study purposely selected 3 districts, namely Sayo, Hawa Gelan, and Dale Wabara considering their population, density, access to pesticide use, and convenience for the study. The study was conducted in June 2020. The elevation ranges from 1701 to 1830 m above sea level. The area is located at an elevation of 1701–1830 m above sea level. The weather pattern varies between extensive summer rains, a brief wet season, and a winter dry season. The yearly precipitation ranges from 800 to 1200 mm, with daily temperatures ranging from 15°C to 25°C.<sup>35</sup> The region is well-known for its enormous agricultural crop output, which includes coffee, maize, teff, wheat, barley, bean seed, and sorghum. The map of the study area was indicated in Figure 1

### Sample size determination

The sample size was determined using a single population proportion formula by considering 82% of small-scale farmers engaged in the poor practice of pesticide handling activities have the risk of being exposed to pesticides,<sup>29</sup> 95% confidence level, and 5% margin of error with a 10% non-response rate.

$$n = \frac{(Z\alpha/2)^2(p)(q)}{(SE)^2} = \frac{(1.96)^2(0.82)(1-0.82)}{(0.05)^2} = 227, \text{ by adding}$$

10% non-response rate, it becomes  $227 + (227 \times 10\%) = 250$  participants were intended to participate at the household level.

### Sampling techniques

The data were collected from 3 districts: Sayo, Hawa Galan, and Dale Wabara in the Kellem Wellega zone of western Ethiopia, through a household survey, covering 250 small-scale farmers using pesticides. A 3-stage sampling was used to select small-scale farmers for this study. First, 3 districts were chosen purposively out of 10 districts in the zone based on their potential for agricultural products and pesticide use. Second, 3 kebeles, namely Abichu Shogo from the district of Sayo, Hawa Moy from the district of Hawa Galan, and Chanka Bururi from Dale Wabara, were again purposely selected by considering similar criteria in consultation with zonal agricultural experts. Finally, households were selected by proportionally allocating the sample size to each kebele. The considered households from each kebele were randomly chosen for the study.

### Operational definitions

**Small-scale Farming (SFFs):** is the production of crops on a small piece of land without using advanced and expensive technologies.

**Exposed small-scale farmers (ESSFs):** Those farmers that must have used pesticides on their own for at least 1 year and above in the same area.

**Non-exposed farmers (NESSFs):** After training the non-exposed grouped farmers were categorized by organic farming, no chemical exposure background because of using complete PPEs, following the labeled instruction, did not perform any activities during spraying, less frequency of pesticides usage per year, condition of the equipment was not damaged, and never spraying against the wind. Additionally, those farmers who did not use pesticides for at least 1 year before this research, and did not live near vegetable farming were considered.

### Inclusion and exclusion criteria

Individuals under 18 years and those unwilling to give their consent were excluded from the study. SFFs chosen forexposed groups must have used pesticides for at least 1 year and above in

the same area. In addition, for the non-exposed farmers, those who did not use pesticides for at least 1 year before the onset of this research and did not live near pesticides applied farming were considered. Moreover, participants with experiences of smoking and alcohol drinking were not considered.<sup>13</sup>

### Data collection and blood sampling

Data on the blood level of pesticides for SFFs in the Kellem Wellega zone was collected using a structured and pretested questionnaire via face-to-face interviews. A standard questionnaire with some modifications was prepared in the English version, translated to Afan Oromo for a clear understanding, and translated back to confirm the correctness of the translation. During this study, the data collectors explained ambiguous questions to the participants that would not be apparent. Most of the questions were dichotomous, with a yes and no option. Prior to the interview, verbal informed consent was obtained from the study participants after explaining the purpose of the study. Blood samples were simultaneously collected from 140 ESSFs and 100 of NESSFs. The trained lab technician collected 4ml of blood samples with a sterilized syringe, transferred them into polyethylene tubes, and then kept them in the ice-cold box, transported them to the Department of Environmental Health Science and Technology at Jimma University, and stored them at  $-20^{\circ}\text{C}$  for analysis.

### Extraction and cleanup of pesticide residues

OCPs (*p,p'*-DDD (para para 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), *p,p'*-DDE (para para 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene), *o,p*-DDT (ortho para1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, and *p,p'*-DDT (para para 1,1,1,Trichloro-2,2-bis(4-chlorophenyl)ethane and heptachlor) and SPs (permethrin cypermethrin and deltamethrin) pesticides from blood samples were extracted using a method stated by Agarwal et al.<sup>36</sup> A 4 ml of blood samples were diluted with 25 ml of distilled water and 2 ml of saturated brine solution and then transferred to a 60ml separator funnel. The extraction was made by adding 10ml of acetone, 10ml of n-hexane in the same ratio, shaking 3 times for about 3 minutes with occasional releasing pressure, and kept until it forms separate layers. After that, the upper (non-polar) layers were obtained by repeatedly removing the lower (polar) layers 3 times. The 3 combined extracts were passed through anhydrous sodium sulfate to eliminate residual polar solvents.

The extracts' cleanup was made using the USEPA,<sup>37</sup> 3620B method and activating florisil overnight at  $130^{\circ}\text{C}$  and cooled in desiccators until used. One gram of florisil was predetermined and packed into column chromatography by calibration. Anhydrous sodium sulfate was added to 0.5 cm to the top of the florisil column, washed with n-hexane, and discarded. Then the extracts were added to the top of the columns slowly. A 10 and 8.5 ml of hexane was added continuously and followed by

a 1.5 ml of diethylether. Then another 5 ml of hexane was added with 5 ml of the diethylether turn by turn into column chromatography. Finally, the eluent was contained in a single container and evaporated into dryness through the rotary vacuum evaporator. Samples were finally prepared in hexane 1.5ml and analyzed by GC-ECD for pesticides

#### *Chemicals and reagents*

High purity grade of organic solvents (acetone-99.85%, diethyl ether-99%, and n-hexane-99%), saturated brine solution-99%, anhydrous sodium sulfate 99.0%, and filorisil or magnesium silicate with 60 to 100 mesh size used were purchased from Heparin Chemical Private limited company, Addis Ababa, Ethiopia. The standards for permethrin (98%), cypermethrin (98%), deltamethrin (99%), heptachlor (98%), *p,p'*-DDD (99.3%), *p,p'*-DDE (99.9%), *o,p'*-DDT(100%), and *p,p'*-DDT (99%) OCPs and SPs chemicals were obtained from Sigma Aldrich, Germany. In addition, gas chromatography techniques were used to determine pesticide concentration.

#### *Chemical analysis*

The gas chromatography-electron capture detector has determined the pesticides with an auto sampler (GC-ECD, Agilent Technologies7890A). HP-5 capillary column of 30 m × 0.25 mm i.d. × 0.25 μm film thickness was used in combination with the following oven temperature program: The initial temperature was 80°C, ramp at 30°C min<sup>-1</sup> to 180°C, ramp at 3°C min<sup>-1</sup> to 205°C, held for 4 min, ramp at 20°C min<sup>-1</sup> to 290°C, held for 8 min, ramp at 50°C min<sup>-1</sup> to 325°C. The total time of the GC run was 27.92 min. Helium (99.999% purity) was used as a carrier gas at a flow rate of 20 ml min<sup>-1</sup> and high purity nitrogen (N<sub>2</sub> = 99.999%) as a makeup gas at a flow rate of 60 mL min<sup>-1</sup>. An aliquot of 1 μL was injected in split mode with a 50:1 split ratio and a 280°C injection temperature. The pesticide residues were identified with an electron capture detector (μ-ECD) at a temperature of 300°C.

#### *Quality assurance and quality control*

For quality control, procedural blanks were analyzed every 5 samples to check for interferences or contamination from solvent, glassware, and equipment used during the entire analytical procedure. Quantification of the selected OCPs and SPs was performed by the external standard addition method. The calibration curves were obtained by spiking 5 different concentrations of the pesticide standards in the range of 10, 1, 0.1, 0.01, and 0.001 mg l<sup>-1</sup>. The regression coefficient of the standard curve was greater than 0.9994 for all pesticides under study. The detection limit was estimated as the analyte concentration in the sample producing a peak with a signal-to-noise ratio of 3. Limits of quantification (LOQ) were calculated from

signal-to-noise ratios 1:10, which were obtained from the measurement of the samples with the lowest concentration level where peaks of studied pesticides were detected.<sup>38</sup>

#### *Statistical analysis*

The data were cleaned and entered into SPSS software version 24.0 (IM Corp., Armonk, NY, US) for analysis. First, a descriptive analysis was performed using a frequency distribution to understand the characteristics of the study population, followed by further statistical analysis. Given the low proportion (<15%) of individuals with detectable concentrations of OCPs and SPs pesticides, we did not apply any method for dealing with values below the evaluated LOD. However, mean values, detection frequencies, and selected percentiles were used for descriptive analysis. Detection frequencies were calculated separately for the entire samples collected from both ESSFs and NESSFs.

The distributions of the participants' socio-demographics and other explanatory variables were explored using the Chi-square test and parametric tests to examine differences among the ESSFs and NESSFs. The *p,p'*-DDT/*p,p'*-DDE ratio was calculated as an indicator for environmental exposure to DDT.<sup>39</sup> The Chi-square test was used to examine the association between exposed and non-exposed SSFs with their socio-demographics and other descriptive variables. A logistic regression analysis was performed to identify explanatory variables of pesticide residues of OCPs and SPs in blood samples. Regression analysis was conducted only for compounds detected in at least 15% of the study population: *p,p'*-DDE, *p,p'*-DDT, heptachlor, cypermethrin, and permethrin, and deltamethrin.<sup>40</sup> Factors associated with dependent variables (*p,p'*-DDE, *p,p'*-DDT, heptachlor, cypermethrin, permethrin, and deltamethrin) were determined at a significant level of *P*-value less than .20, and chosen in the bivariate were included in regression models. Finally, a stepwise backward elimination was performed based on *P*-values of less than .20 on the variables kept in the model 1-by-1 until only variables with *P*-values below .05 remained for analysis.

#### *Ethical consideration*

The ethical approval letter was obtained from the Institute of Health Institutional Review Board (IRB) of Jimma University on 18/10/2019 (No. IHRPGD/407/2019). Participants have ensured confidentiality of their personal information and were given a chance of leaving the study at anytime. Moreover, written informed consent was obtained from the study participants.

## **Results**

This study included 240 SSFs, that is, 140 participants from the ESSFs and 100 from the NESSFs. The response rate was 96.4% for ESSFs and 100% NESSFs. The study showed that

male SSFs whose age was greater than 40 years and involved in farming activities were significantly exposed to pesticide chemicals ( $P$ -value  $<.00$ ). Farmers with greater or equal to 5 years of working experience in the complete use of PPEs and farmers who sprayed pesticide chemicals against the wind were significantly exposed to pesticide chemicals ( $P$ -value  $<.00$ ). SSFs that were not trained on pesticide chemicals and used frequency of pesticides used per year ( $P$ -value  $<.01$ ) had a significant association with exposure to pesticide chemicals. In addition following the labeled instruction ( $P$ -value  $<0.04$ ) and activities like drinking, chewing, and eating food while spraying had a significant association with exposure to pesticides ( $P$ -value  $<.02$ ) (Table 1).

#### Detection of OCPs and SPs in human blood

The analytical results of OCPs and SPs in the blood are shown in Figure 2. For OCPs,  $p,p'$ -DDT (96.4%), and  $p,p'$ -DDE (85.7%) in ESSFs and heptachlor (95%) in NESSFs were the most frequently detected species, whereas  $o,p$ -DDT, and  $p,p'$ -DDD were detected less frequently in both ESSFs and NESSFs, respectively.  $p,p'$ -DDT has the highest mean concentration ( $0.28 \pm 0.4$ ) and ( $0.25 \pm 0.45$ )  $\text{mgL}^{-1}$  for both ESSFs and NESSFs, respectively. As for ESSFs, deltamethrin (100%) was detected most frequently then followed by cypermethrin (90%) and permethrin (78.6%). Deltamethrin was primarily detected in 60% of the NESSFs and the rest all

**Table 1.** Descriptive statistics for SSFs in Sayo, Hawa Gelan, and Dale Wabara districts of Kellem Wellega zone, western Ethiopia (n=240).

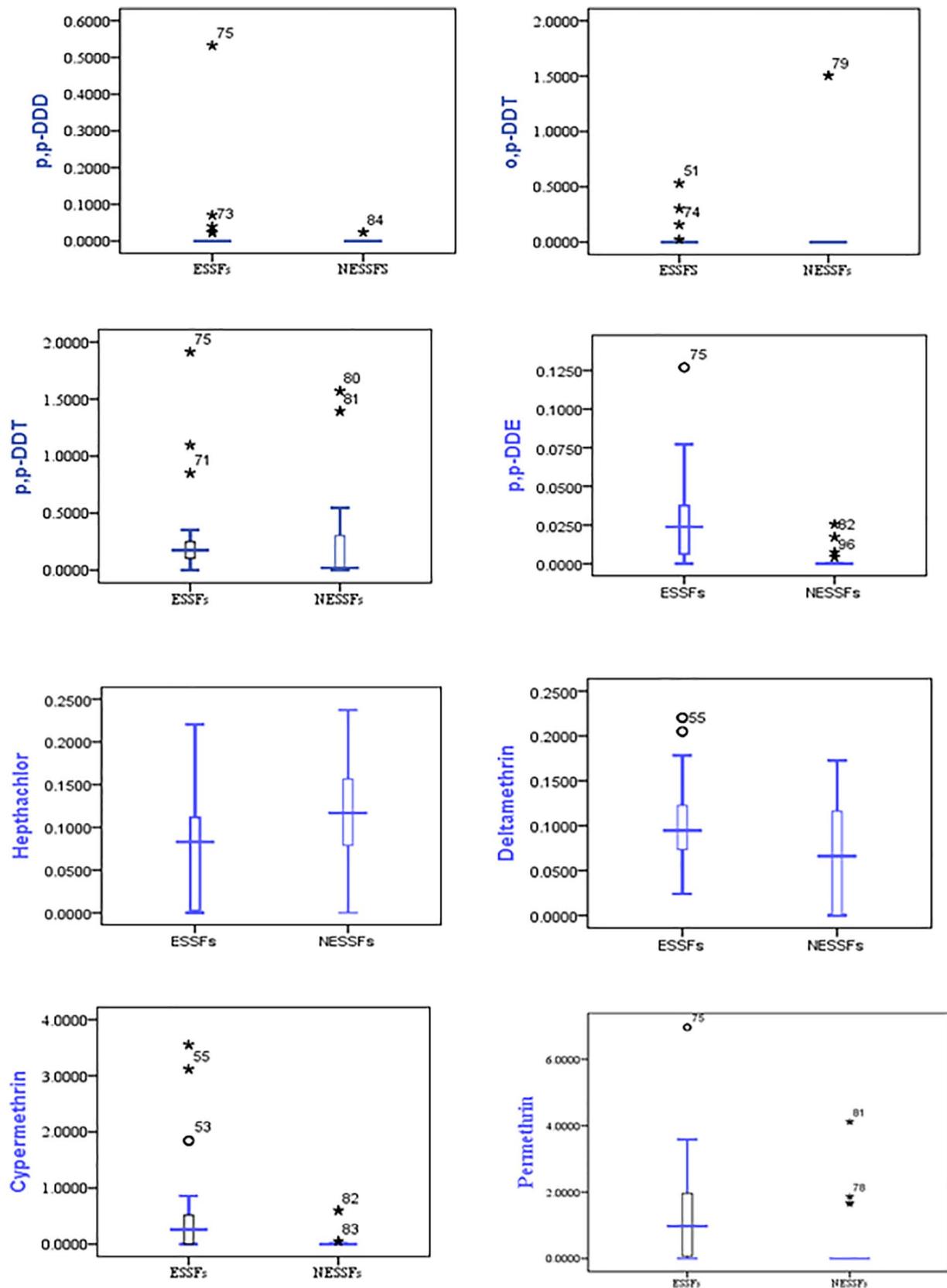
| CHARACTERISTICS OF THE RESPONDENTS | ESSFS (%)  | NESSFS(%) | P-VALUE |
|------------------------------------|------------|-----------|---------|
| Sex                                |            |           |         |
| Male                               | 70 (50)    | 90 (90)   | .00     |
| Female                             | 70 (50)    | 10 (10)   |         |
| Age                                |            |           |         |
| <40y                               | 15 (10.7)  | 50 (50)   | .00     |
| $\geq$ 40y                         | 125 (89.3) | 50 (50)   |         |
| Marital status                     |            |           |         |
| Married                            | 105 (75)   | 70 (70)   | .7      |
| Single and others                  | 35 (25)    | 30 (30)   |         |
| Educational level                  |            |           |         |
| Non-formal education               | 125 (89.3) | 80 (80)   | .36     |
| Primary school and above           | 15 (10.7)  | 20 (20)   |         |

(Continued)

**Table 1.** (Continued)

| CHARACTERISTICS OF THE RESPONDENTS   | ESSFS (%)  | NESSFS(%) | P-VALUE |
|--------------------------------------|------------|-----------|---------|
| Occupation                           |            |           |         |
| Farming                              | 130 (92.9) | 60 (60)   | .00     |
| Farming and others                   | 10 (7.1)   | 40 (40)   |         |
| Working experience                   |            |           |         |
| <5y                                  | 6 (4.3)    | 20 (20)   | .00     |
| $\geq$ 5y                            | 59 (42.1)  | 55 (55)   |         |
| I don't use                          | 75 (53.6)  | 25 (25)   |         |
| Training                             |            |           |         |
| Yes                                  | 59 (42.1)  | 26 (26)   | .01     |
| No                                   | 101 (72.1) | 7 (7)     |         |
| Complete PPEs use                    |            |           |         |
| Yes                                  | 81 (57.7)  | 93 (74)   | .00     |
| No                                   | 39 (27.7)  | 93 (93)   |         |
| Following the labeled instruction    |            |           |         |
| Yes                                  | 50 (35.7)  | 49 (49)   | .04     |
| No                                   | 90 (64.3)  | 51 (51)   |         |
| Activities performed during spraying |            |           |         |
| Eating food/drinking                 | 33 (23.6)  | 34 (34)   | .02     |
| Chewing                              | 15 (10.7)  | 12 (12)   |         |
| Mixed activities                     | 30 (21.4)  | 29 (29)   |         |
| Nothing                              | 62 (44.3)  | 25 (25)   |         |
| Frequency of used per year           |            |           |         |
| 0-2 times                            | 6 (4.3)    | 1 (1)     | .01     |
| 3-4 times                            | 56 (40)    | 32 (32)   |         |
| >4 times                             | 53 (37.9)  | 30 (30)   |         |
| I don't use                          | 25 (17.9)  | 37 (37)   |         |
| Condition of the equipment           |            |           |         |
| Damaged                              | 18 (12.9)  | 19 (19)   | .35     |
| Not damaged                          | 105 (75)   | 67 (67)   |         |
| I don't use                          | 17 (12.1)  | 14 (14)   |         |
| Spraying against the wind            |            |           |         |
| Yes                                  | 66 (47.1)  | 24 (24)   | .00     |
| No                                   | 57 (40.7)  | 34 (34)   |         |
| I don't use                          | 17 (12.1)  | 42 (42)   |         |

Abbreviations: ESSFs, exposed small-scale farmers; NESSFs, non-exposed small-scale farmers.



**Figure 2.** Box plot for some selected OCPs and SPs pesticide residues concentration (mg L<sup>-1</sup>) in the blood of ESSFs and NESSFs. Abbreviations: ESSFs: exposed small-scale farmers; NESSFs: non-exposed small-scale farmers; *o,p*-DDT: ortho para 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; *p,p'*-DDD: para para 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; *p,p'*-DDE: para para 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; *p,p'*-DDT: para para 1,1,1, trichloro-2,2-bis(4-chlorophenyl)ethane.

**Table 2.** Results of logistic regression model for SPs pesticides detected in n > 15% of the study population of both ESSFs and NESSFs.

| EXPLANATORY VARIABLES    | PERMETHRIN         |                   | DELTAMETHRIN                  |                   | CYPERMETHRIN      |                   |
|--------------------------|--------------------|-------------------|-------------------------------|-------------------|-------------------|-------------------|
|                          | COR (95%CI)        | AOR (95%CI)       | COR (95%CI)                   | AOR (95% CI)      | COR (95%CI)       | AOR (95%CI)       |
| Age                      |                    |                   |                               |                   |                   |                   |
| ≤40y                     | 1                  | 1                 | 1                             | 1                 | 1                 | 1                 |
| >40y                     | 0.16 (0.08-0.3)*   | 0.21 (0.1-0.44)*  | 0.05 (0.02-0.12) <sup>b</sup> | 0.04 (0.01-0.11)* | 0.19 (0.09-0.4)*  | 0.24 (0.11-0.54)* |
| Marital status           |                    |                   |                               |                   |                   |                   |
| Married                  | 0.42 (0.23-0.77)*  | 0.32 (0.15-0.67)* |                               |                   |                   |                   |
| Single and others        | 1                  | 1                 |                               |                   |                   |                   |
| Education                |                    |                   |                               |                   |                   |                   |
| Non-formal education     |                    |                   | 4.4 (2.17-8.91)*              | 18.1 (4.53-72.1)* |                   |                   |
| Primary school and above |                    |                   | 1                             | 1                 |                   |                   |
| Occupation               |                    |                   |                               |                   |                   |                   |
| Farming                  | 6.86 (3.23-14.56)* | 4.27 (1.8-10.14)* |                               |                   |                   |                   |
| Other activates          | 1                  | 1                 |                               |                   |                   |                   |
| Training                 |                    |                   |                               |                   |                   |                   |
| Yes                      | 1                  | 1                 | 1                             | 1                 |                   |                   |
| No                       | 1.96 (1.13-3.37)*  | 2.13 (1.08-4.19)* | 3.02 (1.33-6.83)*             | 4.68 (1.5-14.63)* |                   |                   |
| PPEs use                 |                    |                   |                               |                   |                   |                   |
| Yes                      | 1                  | 1                 | 1                             | 1                 | 1                 | 1                 |
| No                       | 5.08 (2.90-8.90)*  | 4.74 (2.47-9.1)*  | 3.54 (1.66-7.54)*             | 2.59 (0.88-7.6)   | 4.38 (2.52-7.6)*  | 3.56 (1.94-6.54)* |
| Working practice         |                    |                   |                               |                   |                   |                   |
| ≤5y                      |                    |                   | 0.07 (0.02-0.23)*             | 0.1 (0.02-0.46)*  | 0.06 (0.01-0.25)* | 0.09 (0.98-0.42)* |
| >5y                      |                    |                   | 0.21 (0.08-0.52)*             | 0.31 (0.1-0.99)*  | 0.27 (0.15-0.48)* | 0.27 (0.15-0.51)* |
| I don't use              |                    |                   | 1                             | 1                 | 1                 | 1                 |

Abbreviations: AOR: adjusted odds ratio; COR: crude odds ratio; CI: confidence interval; Other activities: daily laborers, privately business, non-Government and civil servants.

\*Represent the level of significance at *P*-values < .05.

went under 20%. The highest mean concentration for ESSFs was permethrin ( $1.26 \pm 0.15$ ) followed by cypermethrin ( $0.53 \pm 0.09$ ) mg L<sup>-1</sup>.

#### Factors associated with some SP pesticides

Table 2 shows the logistic regression analysis results for permethrin, cypermethrin, and deltamethrin concentration in the blood of ESSFs and NESSFs. After backward elimination age, marital status, education, occupation, working experience, training, and complete PPE used were significantly associated with some residues of SPs found in the blood of both ESSFs and NESSFs. Thus, SSFs whose ages were less than 40 years

were 21% less likely to be exposed to permethrin than those whose age was older than 40 years (AOR, 0.21; 95%CI: 0.1-0.44). Most of the SSFs that did not use complete PPE were 3.6 times more likely to increase cypermethrin exposure than those using complete PPEs. Finally, according to educational level, farmers who did not have formal education were 18.1 times more likely to be exposed to deltamethrin than those having primary school were and above

#### Factors associated with some OCPs in blood samples

Table 3 shows the logistic regression analysis for *p,p'*-DDE, *p,p'*-DDT, and heptachlor concentrations in the blood of both

**Table 3.** Results of logistic regression model for OCPs detected in  $n > 15\%$  of the study population of both ESSFs and NESSFs.

| EXPLANATORY VARIABLES    | $P_p$ -DDT         |                     | $P_p$ -DDE       |                    | HEPTACHLOR         |                     |
|--------------------------|--------------------|---------------------|------------------|--------------------|--------------------|---------------------|
|                          | COR (95%CI)        | AOR (95%CI)         | COR (95%CI)      | AOR (95%CI)        | COR (95%CI)        | AOR                 |
| Sex                      |                    |                     |                  |                    |                    |                     |
| Male                     | 0.11 (0.04-0.29)*  | 0.3 (0.09-0.83)*    |                  |                    | 11.67 (5.36-25.4)* | 17.36 (7.34-41.09)* |
| Female                   | 1                  | 1                   |                  |                    | 1                  | 1                   |
| Age                      |                    |                     |                  |                    |                    |                     |
| ≤40y                     | 1                  | 1                   |                  |                    | 1                  | 1                   |
| >40y                     | 0.18 (0.1-0.33)*   | 0.2 (0.09-0.48)*    |                  |                    | 3.56 (1.34-9.46)*  | 3.62 (1.17-11.21)*  |
| Educational level        |                    |                     |                  |                    |                    |                     |
| Non-formal education     | 10.31 (4.59-23.2)* | 23.37 (6.97-78.49)* | 3.46 (1.29-9.3)* | 4.22 (1.49-11.99)* |                    |                     |
| Primary school and above | 1                  | 1                   | 1                | 1                  |                    |                     |
| Working practice         |                    |                     |                  |                    |                    |                     |
| ≤5y                      | 0.24 (0.07-0.89)*  | 0.24 (0.06-0.89)*   |                  |                    |                    |                     |
| >5y                      | 1.95 (0.83-4.57)   | 1.95 (0.83-4.57)    |                  |                    |                    |                     |
| I don't use              | 1                  | 1                   |                  |                    |                    |                     |
| Training                 |                    |                     |                  |                    |                    |                     |
| Yes                      | 1                  | 1                   |                  |                    |                    |                     |
| No                       | 2.8 (1.42-5.51)*   | 4.68 (1.5-14.63)*   |                  |                    |                    |                     |
| Complete PPEs use        |                    |                     |                  |                    |                    |                     |
| Yes                      | 1                  | 1                   | 1                | 1                  |                    |                     |
| No                       | 4.75 (2.42-9.34)*  | 6.15 (2.23-16.95)*  | 5.8 (3.2-10.5)*  | 6.21 (3.38-11.41)* |                    |                     |

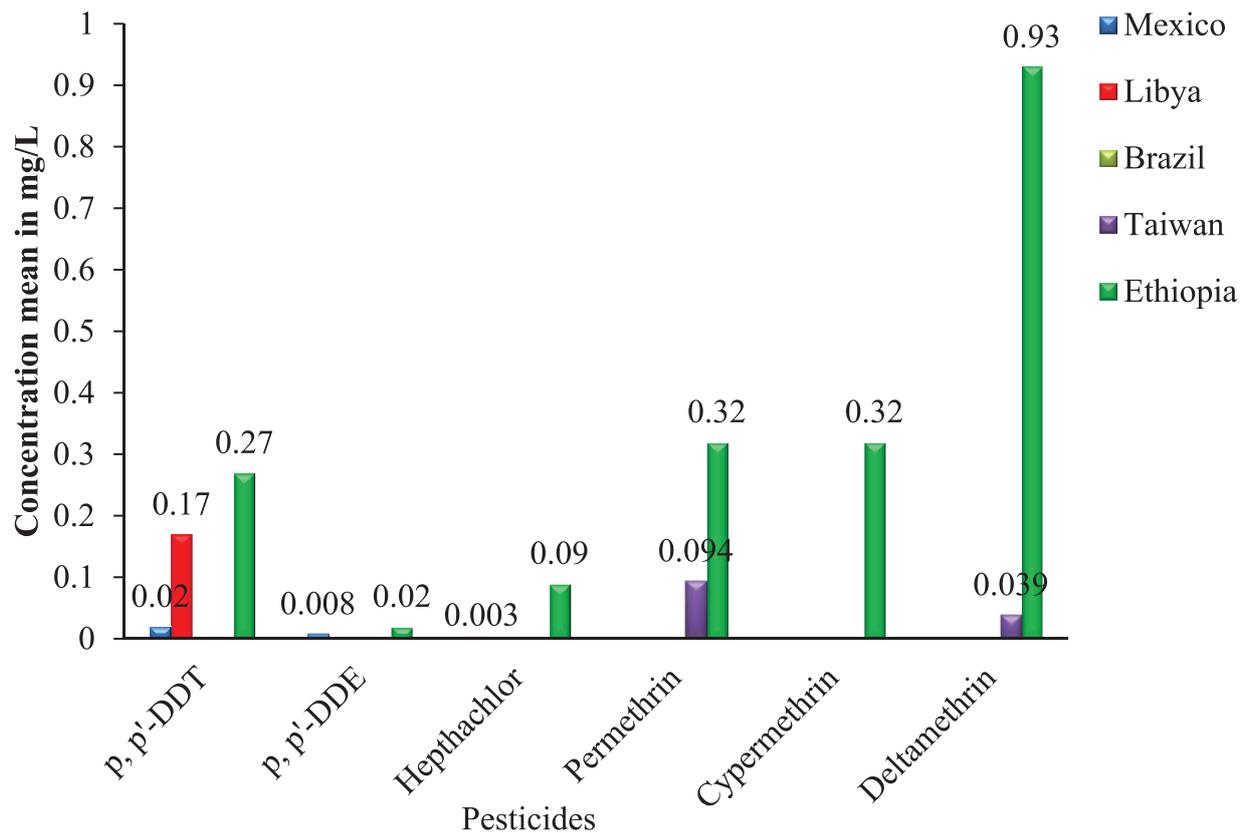
(Continued)

**Table 3.** (Continued)

| EXPLANATORY VARIABLES                | P,P'-DDT          |                    | P,P'-DDE    |             | HEPTACHLOR        |                   |
|--------------------------------------|-------------------|--------------------|-------------|-------------|-------------------|-------------------|
|                                      | COR (95%CI)       | AOR (95%CI)        | COR (95%CI) | AOR (95%CI) | COR (95%CI)       | AOR               |
| Activities performed during spraying |                   |                    |             |             |                   |                   |
| Eating and drinking                  | 0.5 (0.24-1.04)*  | 0.11 (0.02-0.51)*  |             |             |                   |                   |
| Chewing                              | 0.58 (0.22-1.54)  | 0.1 (0.02-0.55)*   |             |             |                   |                   |
| Mixed activities                     | 0.55 (0.26-1.19)  | 0.08 (0.02-0.41)*  |             |             |                   |                   |
| I don't use                          | 1                 | 1                  |             |             |                   |                   |
| Condition of the equipment's         |                   |                    |             |             |                   |                   |
| Damaged                              | 3.73 (1.21-11.5)* | 32.41 (4.22-26.3)* |             |             |                   |                   |
| Not damaged                          | 1.98 (0.9-4.36)*  | 13.93 (2.5-77.23)* |             |             |                   |                   |
| I don't use                          | 1                 | 1                  |             |             |                   |                   |
| Marital status                       |                   |                    |             |             |                   |                   |
| Married                              |                   |                    |             |             | 0.28 (0.11-0.75)* | 0.09 (0.03-0.28)* |
| Single and others                    |                   |                    |             |             | 1                 | 1                 |

Abbreviations: AOR: adjusted odds ratio; COR: crude odds ratio; CI: confidence interval.

\*Represent the level of significance at P-values <.05.



**Figure 3.** Comparison of OCPs and SPs concentration means level ( $\text{mgL}^{-1}$ ) in blood with different countries (reference for Mexico,<sup>41</sup> Libya,<sup>42</sup> Brazil,<sup>40</sup> and Taiwan<sup>45</sup>).

exposed and non-exposed SSFs. After backward elimination, age, sex, marital status, educational level, working practice, training, complete PPE, activities performed during spraying, and condition of the equipment used were significant.

Thus, SSFs whose age was less than 40 years were 80% less likely to be exposed to *p,p'*-DDT concentration in blood than those whose age was greater than 40 years old. Females SSFs were 70% less likely to be exposed to *p,p'*-DDT concentration in blood than males, and small-scale farmers who did not have non-formal education were 23.37 times more likely to be exposed to *p,p'*-DDT than those having primary schools and above.

In addition, SSFs that perform different activities like eating and drinking (11%), chewing (10%), and diverse activities (8%) were more likely to be exposed to *p,p'*-DDT than those farmers who didn't use pesticides. Likewise, damaged and not damaged equipment is more likely to cause exposure of SSFs to *p,p'*-DDT in 32.4 and 13.93 times than those who did not use pesticides. Moreover, SSFs not using complete PPE were 6.21 times more likely to increase the exposure to *p,p'*-DDE blood levels than those who were using complete PPE.

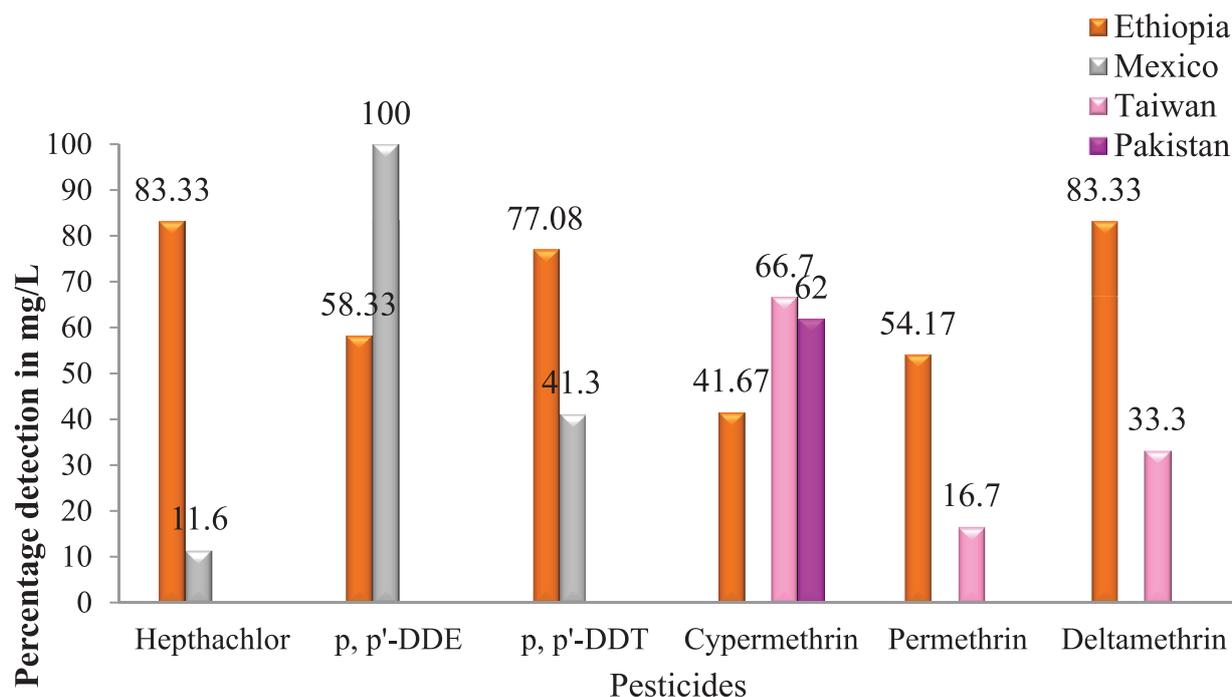
## Discussion

This study observed that *p,p'*-DDE, *p,p'*-DDT, heptachlor, cypermethrin, permethrin, and deltamethrin compounds had been detected in more than 15% of all blood samples. As a

result, the mean concentration of *p,p'*-DDE, *p,p'*-DDT, heptachlor, cypermethrin, permethrin, and deltamethrin in blood samples of both ESSFs and NESSFs was 0.02, 0.27, 0.09, 0.32, 0.32, and 0.93  $\text{mgL}^{-1}$ , respectively.

The mean values of *p,p'*-DDE in this investigation were greater than the levels reported on pesticide residues in human blood samples from India<sup>14</sup> and Mexico.<sup>41</sup> Similarly, the mean concentrations of *p,p'*-DDT and heptachlor in blood were greater in Libyan agricultural workers,<sup>42</sup> serum levels of blood donors of Brazil,<sup>40</sup> and malaria epidemic populations of blood plasma in Mexico<sup>41</sup> (Figure 3). This exposure to high-level pesticides by small-scale farmers is associated with some alterations in the hematological parameters and abnormalities in the urine<sup>43</sup> and oxidative stress that may cause severe diseases in the exposed populations.<sup>44</sup> Due to persistence, and bioaccumulation, the mean concentration of SPs in blood samples of SSFs was higher than that of the same samples of Taiwan<sup>45</sup> and Benin.<sup>13</sup>

A plausible reason for this difference is the extensive use of DDT for controlling malaria among farmers currently, and Ruiz-Suárez et al.<sup>41</sup> used to distinguish between current and historical exposure to DDT. Suppose the ratio of *p,p'*-DDT/*p,p'*-DDE is less than one. In that case, it indicates high persistence in the environment and ongoing bio-magnifications, whereas if it is greater than one, it implies both continuous and continued exposure to DDT.<sup>39</sup> The mean ratio across



**Figure 4.** Comparison of OCPs and SPs percentage detection in blood samples ( $\text{mgL}^{-1}$ ) of different countries (references for Taiwan,<sup>45</sup> Pakistan,<sup>49</sup> and Benin<sup>13</sup>).

the entire blood samples was 9.33, which supports the hypothesis of an active and continuous source of the banned pesticides; despite the considerable legal restrictions introduced against the use of DDT in Ethiopia. Because of their convenience and effectiveness in controlling pests, prohibited pesticides are still in demand from some farmers. Studies have shown that compounds were still being illegally imported without the formal Ethiopian registration process.<sup>46</sup> Furthermore, the remaining stockpiles of DDTs in Ethiopia are stored under inadequate conditions and are currently a source of severe pollution.

The high detection rate of heptachlor, *p,p'*-DDT, permethrin, and deltamethrin in Figure 4 implies that the individuals were exposed to pesticides from surrounding agricultural regions or simply by dietary digestion of contaminated food.<sup>24,28</sup> Moreover, this study depicts that SSFs whose age was less than 40 years were less likely to be exposed to *p,p'*-DDT, *p,p'*-DDE, heptachlor, permethrin, cypermethrin, and deltamethrin than those above 40 years. This is due to bioaccumulation of pesticides from past exposure and decreasing excretion rates with increasing age for SSFs.<sup>32,47</sup> In another way, the younger groups would be convenient for the recommended PPE on pesticide use compared to older SSFs who could use more pesticides to maximize their products.<sup>48</sup>

Moreover, male SSFs dominate the farming activities than females due to the hardness and difficulty of work that might naturally limit the involvement of females. Comparatively, males are more of a household leader and energetic than females and they are more actively involved in farming practices than female SSFs. This causes a distinct accumulation of pesticide chemicals at the workplace, which typically differs by

gender, as men and women.<sup>32,41,50</sup> This might be related to breastfeeding and the menstrual cycle, which can cause significant variations in OCP levels between males and females, resulting in lower blood concentrations in females.<sup>12,51</sup>

In addition, SSFs who got training regarding handling pesticides were more aware of how to use PPE, understand the side effect, and observe the weather conditions before spraying. Since educated farmers are more knowledgeable about pesticide safety, they can better understand, follow the labeled instruction, and conceptualize the side effect of poor practices on pesticide chemicals.<sup>44,48,52,53</sup> Furthermore, this study found that farmers who spent more time working with pesticides were exposed to more pesticides than those who spent less time working with pesticides, that is, the experience of pesticide use influences the exposure of SSFs. The finding of this study was consistent with studies done in Brazilian blood donors and Cameroon tomato farmers in which occupational exposure was associated with previously used pesticides.<sup>54,55</sup>

Furthermore, the hand pump was the most often used pesticide spraying device, which exposes sprayers to health hazards through skin contact, inhalation, and ingestion. Due to limited use of PPE, farmers spray pesticides by dressing T-shirts, shorts, and slippers that offer little protection.<sup>56</sup> In addition, SSFs may be exposed to pesticides occupationally during spraying, loading, maintaining, cleaning equipment, and entering sprayed farmland. This is due to knowledge gaps among respondents, poor practice, and inadequate safety measures.<sup>48,57,58</sup>

The study has a number of drawbacks. The data from non-exposed households of SSFs had a higher level of pesticide

concentrations leading to a positive bias in our estimates of pesticide levels. In addition, we did not have information on food intake from animal origin and body mass index (BMI) to adjust our estimates OCPs and SPs concentrations level. Likewise, there might be poor reporting on the history of previous use, frequency, training, and working experience of pesticide chemicals. Additionally, past exposure assessed by using a questionnaire might be subject to recall bias and lack of consistent reporting from both ESSFs and NSSFs. Due to the inabilities of instruments, the organophosphate pesticides did not include in this report.

## Conclusion

This study identified a high concentration of *p,p'*-DDE, heptachlor, cypermethrin, permethrin, and deltamethrin in the blood sample of small-scale farmers. Older age, less educated, and farmers with inappropriate PPE use are more likely exposed to the identified pesticides. The study identified a high concentration of pesticides from potentially exposed and non-exposed farmer blood samples. Since this study is the first report on pesticides use and its concentration in the blood of small-scale farmers in the study area, we recommend further studies with larger sample sizes focusing on identifying the sources of exposure to pesticides.

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## Author Contributions

Tariku Neme Afeta: Methodology, Formal analysis, Visualization, Writing an original draft, Dr. Gudina Terefe Tucho: Methodology, Formal analysis, Visualization, Writing-review, Editing. Dr. Seblework Mekonen Shegen: Methodology, Formal analysis, Visualization, Writing- review Editing

## Availability of Data and Materials

The datasets analyzed during the current study were available from the corresponding author on reasonable request.

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