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# Research Article
# Male Reproductive Hormonal Profile of Cotton Farmers Exposed to Synthetic Pesticides in the North-East of Benin

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## Abstract

**Background and Objective:** Most chemical pesticides used in agriculture are endocrine disruptors. Several studies report the adverse effect of endocrine disruptors on male sex hormones. This study aimed to investigate the profile of male sex hormones of cotton growers directly exposed to chemical synthetic pesticides in the North-East of Benin. **Materials and Methods:** This study was an observational, cross-sectional, descriptive and analytical study carried out from May 6 to August 30, 2016 in the North-East of the Republic of Benin. Study sample consisted of 95 cotton growers exposed to pesticides (mean age: 33.54±9.43 years) and 95 non-exposed subjects (mean age: 30.97±5.22 years). The level of male hormones: Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and total testosterone was determined in subjects' serum using radioimmunoassay. **Results:** The mean values of LH, FSH and testosterone levels were respectively 7.10±4.73 and 5.31±3.14 UI $L^{-1}$ and 7.03±3.33 ng $mL^{-1}$ in exposed cotton growers versus 7.31±4.20 and 4.95±3.00 UI $L^{-1}$ and 10.23±3.60 ng $mL^{-1}$ in non-exposed subjects. The mean testosterone level of exposed cotton farmers was significantly lower than the one of non-exposed subjects ($p<0.0001$). The prevalences of abnormal concentration of LH, FSH and testosterone in cotton producers were respectively 13.70, 7.37 and 18.94%. **Conclusion:** There are abnormalities in the male hormonal profile of cotton farmers exposed to chemical synthetic pesticides in the North-East of Benin. Among those abnormalities, the one of testosterone level is predominant.

Key words: Pesticides, endocrine disruptors, cotton farmers, hormones, Benin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Many natural and synthetic chemicals are used in agriculture in order to increase production output. Among those chemicals, chemical synthetic pesticides hold an important place. Pesticides are substances aimed at fighting against living organisms considered as harmful for other living organisms. However, they may be endocrine disruptors and substances with hormonal properties or likely to interfere in the synthesis, metabolism, transport and action of hormones[1-3].

The maintenance of fertility and the regulation of male reproductive function are closely associated with reproductive male hormone balance which may be modified by endocrine disruptors[4,5]. The main abnormalities reported as associated with the effect of those endocrine disruptors, are malformations of the genitourinary tract and particularly a declined quality of men's sperm[6]. The effect of endocrine disruptors on male sex hormones is an issue of controversy. For some authors, no effect is observed[7]. For others, disorders of male sex hormone concentrations were noted in variable proportions among pesticide-exposed subjects in many countries[8-12]. If the effects of endocrine disruptors is notable on the animal model, most epidemiological studies focused on man have not clarified them[13,14].

In Benin, huge amounts of those pesticides are used in cotton cultivation which is widespread in the North of the country as the main export commodity. As a result, there is a significant risk of exposure. This risk is not only indirect for the populations in general but is also particularly direct through professional use for cotton farmers who handle those pesticides. Pesticide handling and use result in potential damage of hypothalamic-pituitary-gonadal axis.

This study aimed at investigating the male sex hormone profile of cotton farmers directly exposed to chemical synthetic pesticides in the North-East of Benin so as to now they impact on reproductive function.

## MATERIALS AND METHODS

**Study setting and respondents:** This scientific investigation work was an observational, cross-sectional and descriptive study with analytical purpose based on prospective collection of data. It was conducted from May 6 to August 30, 2016, in the North-East of the Republic of Benin.

This study population consisted of 2 groups of male subjects. Group 1 included cotton farmers directly exposed to synthetic pesticides through professional use, selected in the District of Banikoara (located in the major cotton growing area of Benin); there mean age was 33.54±9.43 years. Group 2 considered as the reference group, consisted of non-cotton farmers, non-exposed to pesticides, recruited in the city of Parakou, with mean age estimated at 30.97±5.22 years.

The sampling method used was two-stage probability random sampling. As regards Group 1, inclusion criteria were male subjects, aged 18-50 years, exposed to synthetic pesticides, who gave their free and informed consent to participate to the survey. They had a farming practice spread over at least the past 5 years and were directly exposed to pesticides at least once during each growing season. Group 2 included male subjects, non-farmers, non-exposed to pesticides, aged 18-50 years and who expressed their free and informed consent to participate to the study. They have been living in Parakou for at least 5 years and had no direct contact with agricultural synthetic pesticides. This study did not include subjects with history of urogenital pathology, those exposed to gonadotoxic substances other than agricultural synthetic pesticides; in addition, it also excluded subjects exposed to radiations, heavy metals and heat. Schwartz formula was used to compute sample minimum size which consisted of 95 subjects.

**Ethical consideration:** This study has been approved by the Local Committee for Biomedical Research of the University of Parakou (Decision notice No. 28/CLERB-UP/P/SP/R/SA). Each respondent participating to this study has given his consent in writing.

**Data collection:** The study data were gathered by means of a questionnaire and superficial venous blood samples were collected into dried tubes. Serums obtained by decantation after centrifugation were stored at -18°C until performance of hormone measurements including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total testosterone by radioimmunoassay (Beckman Coulter, Prague, Czech Republic) using a gamma counter (PC-RIA. MAS Stratec) calibrated for iodin-125. The reference values of the kits were: 1.9-12.47 UI $L^{-1}$ for LH; 1.73-9.37 UI $L^{-1}$ for FSH and 3.67-16.06 ng $mL^{-1}$ for testosterone.

**Statistical analysis:** Mann-whitney U-test helped compare mean values. The threshold of statistical significance was set at 5%. Software Epi Info version 7.0 (CDC, Atlanta, USA) was used for the processing, analysis and interpretation of data.

## RESULTS

The families of organophosphates, pyrethroids and organochlorine pesticides were the most used by cotton farmers. The supply chain for those pesticides was mainly the one authorized by the Government of Benin (98.94%). Combinations of different pesticides during spray application were practiced by 38.90% of cotton growers. Mean duration of cumulative exposure since the first use was estimated at 48.95±13.13 months i.e., 4.08±1.1 years and mean duration of last exposure was 3.44±2.70 months. Only 22.11% of cotton farmers used appropriate protective equipment during pesticide spray applications.

Table 1 compares the mean serum levels of male sex hormones between cotton farmers and non-exposed subjects. It emerges from its analysis that only mean testosterone level of cotton farmers was significantly lower than the one of subjects non-exposed to pesticides ($p<0.0001$).

The abnormalities of serum levels of male sex hormones were variable, but dominated by a decline in total testosterone level compared to the reference values of the assay kit used (Table 2).

## DISCUSSION

The pesticides most frequently used by cotton producers in the North-East of Benin were organophosphates, pyrethroids and organochlorine pesticides. Organochlorine pesticides, including endosulfan, were also used up to 2012 before being officially withdrawn by the Government of the Republic of Benin. The use of the same pesticides in other regions of the world was reported by many authors[10,12,15,16]. In this study, the mean duration of cumulative exposure since first use was estimated at 48.95±13.13 months (4.07±1.09 years). That mean duration of exposure varies depending on studies: more than 5 years in Egypt[17], in Venezuela[8] and in Mexico[10] and 15 years in Mexico[11]. In this study, exposure duration was estimated by aggregation or sum of exposure durations of each growing season; this is not the case in the study of Recio *et al.*[11].

The mean value of LH serum level of exposed subjects (7.10±4.73 UI $L^{-1}$) found in this study is lower than mean values reported in India (10.3±1.1 UI $L^{-1}$)[18] and in Egypt (10.8±5.2)[17]. Blanco-Munoz *et al.*[10] found out values that are variable depending on the degree of exposure of respondents: 7.52±5.17 UI $L^{-1}$ for low exposures and 5.96±2.34 UI $L^{-1}$ for higher exposures without significant difference ($p = 0.25$). LH values that may vary depending on the degree of exposure to pesticides were also reported by Recio *et al.*[11] (4.2±2.7 UI $L^{-1}$ in the low exposure period and 4.8±2.8 UI $L^{-1}$ during the high exposure period). The findings of those studies suggest that the higher the level of exposure to pesticides, the lower LH serum level is. Several reasons explain those differences noted, especially the multiple modes of action of pesticides as endocrine disruptors which are, for ones, inhibitors and, for others, stimulants of hormone secretion[12]. Actually, sometimes the different metabolites of the same pesticide have distinct effects on the endocrine system[12]. Moreover, the study of Blanco-Munoz *et al.*[10] had taken into account that subjects exposed to only one type of pesticide (organophosphates), whereas Recio *et al.*[11] had explored subjects exposed to 3 types of pesticide (organophosphates, organochlorine pesticides and carbamates). Synergistic effect may therefore be another explanation of the results of Recio *et al.*[11].

In the group of exposed subjects, the mean value of FSH plasma concentration was estimated at 5.31±3.14 UI $L^{-1}$. Recio *et al.*[11] found out values comparable to ours: 4.8±2.7 UI $L^{-1}$ for low exposures and 6.1±2.9 UI $L^{-1}$ for high exposures. In contrast, Miranda-Contreras *et al.*[8] and Abdallah *et al.*[17] reported respectively mean values of 7.8±0.5 and 11.3±2.7 UI $L^{-1}$ which are higher than ours. As regards Blanco-Munoz *et al.*[10], they found a concentration of 4.34±1.19 UI $L^{-1}$ for low exposures and 3.5±0.86 UI $L^{-1}$ for high exposures with significant difference ($p = 0.0026$).

Table 1: Comparison of the 2 mean serum levels of male sex hormones between cotton farmers and non-exposed subjects

| Parameters | Exposed cotton farmers (N = 95) | Non-exposed subjects (N = 95) | p-value |
|---|---|---|---|
| FSH (UI $L^{-1}$) | 5.32±3.14 | 4.95±3.00 | 0.416 |
| LH (UI $L^{-1}$) | 7.10±4.73 | 7.31±4.20 | 0.739 |
| Testostérone (ng $mL^{-1}$) | 7.03±3.33 | 10.23±3.60 | <0.0001 |

Table 2: Prevalence of abnormal serum levels of male sex hormones in cotton farmers in the North-East of Benin (n = 95)

| | Low | | High | | Total | |
|---|---|---|---|---|---|---|
| Parameters | Number | Percentage | Number | Percentage | Number | Percentage |
| FSH (UI $L^{-1}$) | 4 | 4.21 | 3 | 3.16 | 7 | 7.37 |
| LH (UI $L^{-1}$) | 5 | 5.28 | 8 | 8.42 | 13 | 13.70 |
| Testosterone (ng $mL^{-1}$) | 17 | 17.89 | 1 | 1.05 | 18 | 18.94 |

These studies point out that as for LH, the higher the level of exposure to pesticides, the more FSH level declines.

In this study, the mean value of testosterone serum level in exposed subjects (7.03±3.33 ng $mL^{-1}$) was higher than the values reported by Blanco-Munoz *et al.*[10] i.e., 3,5±0,64 ng $mL^{-1}$ in poorly exposed subjects and 5.22±1.26 ng $mL^{-1}$ in highly exposed subjects. It is also above the value found out by Recio *et al.*[11] (4.5±1.6 ng $mL^{-1}$ for poorly exposed subjects and 4.8±2.0 ng $mL^{-1}$ for highly exposed subjects). In this study, only the mean testosterone level of cotton farmers was significantly lower than the one of pesticide non-exposed subjects ($p<0.0001$). Lwin *et al.*[18] reported that decline in testosterone level in peanut growers was inversely proportional to urine concentration of pyrethroid derivatives. According to Goncharov *et al.*[19], exposure to bisphenol is associated with low level of circulating testosterone in male Native Americans. Experimental animal models showed that the effect of endocrine disruptors on male sex hormone balance is not dose-dependent[20].

In this study, plasma LH level was abnormal in 13.70% of cotton farmers. This result is higher than the one of Blanco Munoz *et al.*[10] who reported 7.70% in but lower than the one reported by Recio *et al.*[11] i.e., 29%. This difference may be due to the fact that in the study of Recio *et al.*[11], subjects were frequently exposed not only to the same types of pesticide, but also to the use of combination of organophosphates, organochlorine pesticides and carbamates. On the contrary, in this study, the combination of organophosphates and pyrethroids was mostly used.

Plasma FSH level was abnormal in 7.37% of exposed subjects investigated in this study. This finding is lower than the results of Miranda-Contreras *et al.*[8] and Recio *et al.*[11]; they respectively found out 21 and 48%. As an element of the family of organochlorine pesticides and a confirmed endocrine disruptor used in the study of Recio *et al.*[11], endosulfan may explain that high proportion of abnormal FSH level[12]. Unlike the works of Recio *et al.*[11] and Blanco-Munoz *et al.*[10], which respectively reported 11 and 4.5% of cases of abnormal testosterone plasma, this study has highlighted that 18.94% of cotton farmers had those abnormalities. The high propensity for use of organophosphates as androgen inhibitors[12] in the major cotton growing area of Benin, since ban on organochlorine pesticides, may justify our findings.

This study is characterized by some limitations. It should have been a longitudinal study associated with measurements of pesticide residues and with performance of spermiogram in each subject. Those parameters would have helped assess the direct impact of pesticides on male sex hormones on the one hand and the effects of hormone disorders and deficiencies on sperm parameters on the other hand.

## CONCLUSION

From this study aimed at investigating the male sex hormone profile of cotton farmers exposed to pesticides in the North-East of Benin, it emerges that the mean values of LH and FSH sex hormones of pesticide-exposed subjects are not different from those of non-exposed subjects. However, testosterone level is significantly lower in cotton farmers. Those hormonal disruptions may have effects on sperm profile which should be explored in those cotton farmers. A strict regulation of the use of those pesticides in Benin is advisable.

## SIGNIFICANCE STATEMENT

We investigate the profile of male sex hormones of cotton growers directly exposed to chemical synthetic pesticides in the North-East of Benin so as to now they impact on reproductive function. Through a observational, cross-sectional, descriptive and analytical study carried out from May 6 to August 30, 2016 concerning 95 cotton growers exposed to pesticides and 95 non-exposed subjects, we have obtained the following results: The mean testosterone level of exposed cotton farmers was significantly lower than the one of non-exposed subjects ($p<0.0001$). Therefore, a strict regulation of the use of those pesticides in Benin is advisable.

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