

## The Reproductive Health and Liver Functions of Occupationally Exposed Female Pesticide Sprayers of Mango Plantations

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### Abstract

The present study explores the effect of pesticide exposure on the reproductive health and liver function of females participating in agricultural related work. Blood samples were obtained from thirty-five (35) females (out of one hundred females of reproductive age) who were willing donors. Fifty (50) females matched for age and socio economic status was recruited as a control group to compare levels of hormones (estradiol, progesterone and follicle stimulating hormone), liver enzymes-alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Results showed that 56% of the females were illiterate with 48% representing agricultural work. Females who helped in farming operations showed a higher incidence of adverse pregnancy outcome such as abortions and stillbirths compared to non-participants. There was a significant elevation in estradiol and progesterone levels among female agricultural workers. While there was an elevation in serum liver enzymes (AST and ALT) there was a significant decrease in FSH level in females engaged in agricultural activities. The study suggests that participation of females in agricultural activities with potential exposure to pesticides endangers their reproductive health and liver functions.

**Keywords:** Liver enzymes; Reproductive health; Female workers; Agriculture; Occupational exposure

### Introduction

Indian economy is largely based on agricultural products and consequently the output primarily depends on extensive use of pesticides. Agricultural practices involve the use of a diverse group of agro-chemicals that is indiscriminately sprayed by the farmers for pest control. Pesticides are a threat to the environment and capable of eliminating a variety of organism without discrimination. Organophosphorus pesticides (OPs) alone or in combination with organochlorines (OCs) are frequently used and sprayed in mango plantation of Malihabad (Lucknow, U.P) which is a mango belt in north India and therefore remains an important source of poisoning. These agro-chemicals are popularly termed as pesticides and have been broadly classified in to organophosphorus, organochlorines, carbamates and pyrethroids [1]. While India ranks 12th globally, it is the largest manufacturer of basic pesticides in Asia. Of the total pesticide consumption insecticides account for ~75% followed by fungicides (~12%) and herbicides (~ 10%) [2]. In mango field the common pesticide sprayed includes Dichlorovas 76% EC, Monocrotophos 36% EC, Dimethoate 30% EC, Phosphamidon 85% SL Endosulfan 35% EC, Carbaryl, 25% EC, Monocrotophos 36% SL, Methomyl [1].

In rural Malihabad Lucknow, one of the main activities of women, besides their household duties, is extending assistance in agricultural work. This is due to change in the economic conditions of families. A large family in which the chances of increasing cultivated land is remote; a high prevalence of labor migration predisposes the involvement of women in agricultural work. In many parts of the world women constitute a large proportion of farmers and therefore

represents a considerable force in agricultural work [3]. They are exposed to a wide variety of health hazards including toxic chemicals used to boost crop yield. This exposure to pesticides can occur directly from occupational and environmental sources and indirectly through diet. Although there is a growing public concern about the impact of exposure to pesticides on human health, lack of knowledge, careless attitude and appalling safety practices in handling pesticides pose a serious health risk to the farmers. Some pesticides were classified as reproductive and developmental toxins in the Pan American Pesticide Database [4]. Exposure to pesticides has been associated with several disease conditions including hypertension, hepatomegaly, dermatitis, neurological and immunological effects, chromosomal aberrations and elevated cancer risks [5,6]. The actual exposures to insecticides can be assessed by biological monitoring of human tissues and body fluids [7].

The pesticide exposure causes cellular leakage of enzymes from hepatocytes and other affected body organs [8]. A high degree of abnormal liver function in agricultural workers may indicate toxic effects of pesticides as well as its presence in blood. Altered liver enzyme activities have been reported among occupational workers exposed to organophosphorus pesticides alone or in combination with organochlorines [9]. In women, exposure to pesticides have been associated with reproductive malfunctioning which includes decrease in fertility, spontaneous abortions, stillbirth premature birth, low birth weight, developmental abnormalities, ovarian disorders, and disruption of the hormonal function [10,11]. Women's health is gaining attention with the realization that men's and women's bodies react differently to environmental agents. As the number of women participants is increasing worldwide and a considerable proportion of them are of reproductive age; attention is required to note any reproductive dysfunction due to occupational exposure. This study exposes the relevance and influence of pesticides on females engaged in agricultural practices.

## Material and Methods

We conducted a cross-sectional study in female pesticide sprayers of mango plantation in rural Malihabad, Lucknow. This is a cross-sectional comparative study that was conducted at the end of spraying day among the female sprayers. Rural Malihabad village consists of 100 families living in two small satellites (one composed of 60 families and the other of 40 families). The purpose of the study was explained to all the participants and their consent was obtained. The requisite clearance of institutional human ethics committee was obtained for the study.

### Sample collection

All families in the village were surveyed and from each family one female in reproductive age was randomly selected to participate.

### Data collection

Detailed information regarding socio-economic status, family history, type of physical activity, personal habits were recorded for each subject on a pre-tested questionnaire. The questionnaire was in local language and data entered was done by investigators. Questionnaire covers the following points: socio-demographic data, agricultural activities, exposure to pesticides, menarche history, and outcome of pregnancy (abortion, stillbirth, preterm labour, low birth weight or congenital anomalies), dysfunctional uterine bleeding, and tumor of the uterus or ovaries.

Exposed females named a lot of pesticides like monocrotophos, phosphomidon, dichlorvos, malathion, endosulfan, thiodon, methyl parathion, dimethoate and carbaryl etc. used frequently in the form of mixtures by applicators. Exposure was considered chronic because the females included in the study are always engaged in pesticide application (mixing and spraying). The females were considered exposed if they shared in preparing, mixing or spraying pesticides on a regular basis.

The exposed females did not report any co-existing exposure. They did not use any protective measures during spraying or mixing. Fifty females matched for age and socio economic status were recruited as a control group to compare levels of hormones (estradiol, progesterone and follicle stimulating hormone), liver enzymes alanine aminotransferase and aspartate aminotransferase. The control group was chosen from a neighbouring village whose main occupation was not related to any agricultural activities. All the studied populations were non-smokers and non-alcohol drinkers receiving no medications on a regular basis.

Approval consent was obtained from all participants. Under complete aseptic conditions, a blood sample of 5 mL was taken from each subject. Samples were centrifuged for separation of serum to be used for analysis. The estimation of serum liver enzymes (ALT and AST) following the methods of Reitman and Frankel [12]. The hormones (estradiol, progesterone and follicle stimulating hormone) was determined by immunologic methods (Elisa technique) using commercial kits (Panbio, Australia) [13,14]. To overcome variation in hormone levels in different phases of the menstrual cycle, samples were taken from both groups during the follicular phase of the menstrual cycle (starting from the first day of menstruation to mid cycle). Also, pregnant females were not included in the study to exclude high progesterone level during pregnancy.

## Statistical analysis

The statistical significance of mean values of different parameters in exposed and control were performed using the SPSS package system version 16.

## Results

Table 1 show that more than half of the females in the village were illiterate. Only 4% of the females were graduates from the university. In the study, agricultural work represented 48% of the occupations. Females who helped in preparing pesticides represented 67.16%. Females who shared in spraying pesticides represented 13.43%. Duration of work exceeded 10 years in 58.21% of the studied females as shown in table 2.

Socio-demographic status	No.	Percentage
<b>Education</b>		
Can write and read	6	6
Illiterate	56	56
Primary	9	9
Preparatory	2	2
Secondary or diploma	21	21
Two years after secondary school	2	2
Graduation	4	4
<b>Total</b>	100	100
<b>Occupation</b>		
Agricultural workers	48	48
Others	52	52
<b>Total</b>	100	100

**Table 1:** Detailed information regarding socioeconomic status of the female workers in Rural Malihabad, Lucknow.

All studied populations were non-smokers, non-alcohol drinkers, apparently in good health and taking no medication on a regular basis. Agricultural workers showed higher levels of progesterone and estradiol compared to the controls and the difference was statistically significant with p values of <0.001 and <0.001, respectively. However, FSH level was significantly lower in female agricultural workers with p value <0.001. Liver functions were affected as evidenced by high enzyme levels with significant difference at p values <0.001 and <0.05 for AST and ALT, respectively, as shown in table 3. There was no correlation between the duration of work and the tested parameters. It is clear from table 4 that females sharing in agricultural work gave birth to a lower percentage of well-born babies (62.69%) compared to working females (78.79%). Females exposed to pesticides reported higher incidences of uterine bleeding but the difference was not statistically significant compared with those not sharing in the agricultural work.

## Discussion

In the pesticide sprayers of rural Malihabad Lucknow, the health protection has been overlooked for many years despite the health risks associated with occupational exposure to agrochemicals. Of main concern are the most frequently used OP and CB pesticides, such as methamidophos, chlorpyrifos, diazinon, ethyl parathion, dimethoate, mancozeb, zineb, carbendazim, carbofuran, propineb and propamocarb. Most of these pesticides belong to extremely hazardous and highly hazardous category, which have been either banned or strictly controlled in developed countries. With regard to the most common pesticide-related symptoms, pesticide sprayers of mango plantation in Malihabad, Lucknow reported dizziness, headache, nausea, blurring of vision and skin and throat irritation, which were similar to other reports associated with exposure to OP and CB pesticides.

	Yes/No	No.	%
Helping in farming operations	No	33	33
	Yes	67	67
	Total	100	100
Preparation of Pesticides for spraying	Help or do it by herself	45	67.16
	No	22	32.84
	Total	67	100
Helping in spraying pesticides	No	9	13.43
	Yes	58	66.57
	Total	67	100
Duration of exposure	Less than 10 years	28	41.79
	10 years and more	39	58.21

**Table 2:** Agricultural activities conducted by the female workers (studied group) in rural Malihabad, Lucknow.

Parameter	Group	No.	Mean	Std. deviation	P value
FSH (mIU/ml)	Agricultural worker	35	4.229	1.584	<0.001
	Control	50	6.024	2.218	
Progesterone (ng/ml)	Agricultural worker	35	3.283	4.238	<0.001
	Control	50	0.716	0.757	
Estradiol (pg/ml)	Agricultural worker	35	55.369	13.815	<0.001
	Control	50	47.284	10.001	
Aspartate aminotransferase (AST)	Agricultural worker	35	28.343	5.434	<0.001
	Control	50	24.52	3.079	

Alanine aminotransferase (ALT)	Agricultural worker	35	24.429	3.363	<0.05
	Control	50	21.68	5.56	

**Table 3:** Comparison between female agricultural workers and controls concerning hormone levels namely (progesterone, FSH and estradiol) and liver enzymes namely (AST and ALT).

Pregnancy Outcome	Helping in farming operations						P value
	No		Yes		Total		
	No.	%	No.	%	No.	%	
Well born fetus (no preterm labour, low birth weight or congenital anomalies)	26	78.79	42	62.69	68	68	<0.05
Adverse outcome (abortion, Stillbirth, preterm labour, low birth weight or congenital anomalies)	7	21.21	25	37.31	32	32	
<b>Total</b>	<b>33</b>	<b>100</b>	<b>67</b>	<b>100</b>	<b>100</b>	<b>100</b>	

**Table 4:** Pregnancy outcome in females involved in agricultural activities in rural Malihabad, Lucknow.

In undertaking this kind of study, we must first consider the difficulty of quantification and assessment of the effects of the exposure that may lead to chronic intoxication. Multiple exposures of different pesticides which might interact in an additive or multiplicative way and so could affect the pattern of health effects expected in the case of mono-exposures. The other important points that have also affected this study include the large number of spraying done and the usage of various pesticides during the period of study, often in combinations, some commercially available but the majority made by the sprayers themselves. Despite all these limitations, it is possible to draw conclusions about the overall health risks of these complex exposures.

Our study revealed a high percentage of illiterate females in the studied village. Illiteracy, especially among females, still constitutes a major problem in and around rural Malihabad. The study pointed to the large number of females engaged in agricultural activities (67%). The questionnaire showed their unawareness of the health hazards of pesticide exposure on their reproductive health. Only a few of them knew the guidelines for the safe usage and storage of pesticides. None of them gave information about the mixtures of pesticides they use. They only reported that they use different types of pesticides and usually apply them in combinations. Measuring hormone levels in females helping in agricultural activities and exposed to pesticides during their work demonstrated higher progesterone, estradiol and lower FSH levels as compared to controls. Moreover the progesterone level (3.283 ng/ml) is higher than the normal range during the follicular phase, which is 0.2-1.4 ng/ml. This high progesterone level could be attributed to a possible disturbance in hormone level associated with exposure. Those females might be exposed to organophosphorus pesticides in the pesticide mixtures they apply. OPs are suspected to alter reproductive function by reducing brain acetylcholinesterase (AChE) activity and secondarily influencing the gonads. Experiments on animals have shown that repeated doses of OP

significantly decreased brain AChE activity and significantly increased acetylcholine, gamma-aminobutyric acid, epinephrine, nor-epinephrine, dopamine, and 5-hydroxytryptamine concentrations, altering reproductive function by reducing brain acetylcholinesterase activity and monoamine levels, thus impairing hypothalamic and/or pituitary endocrine functions and gonadal processes [15].

The present study, suggest more detailed study of reproductive hormones must be done to clarify the cause for this marked rise of progesterone. The low FSH level is explained by a negative feedback mechanism of the pituitary gland resulting from high estradiol. These findings were in accordance with previous studies that proposed about pesticides induce hormone disruption [16,17]. This alteration of hormone levels may interfere with the menstrual cycle and lead to adverse reproductive health effects [18-20].

It also explains the increased number of females complaining of dysfunctional uterine bleeding in those exposed to pesticides [15] showed negative associations between OP exposure and serum levels of FSH and LH in Mexican agricultural workers, but they did not observe significant associations between testosterone or estradiol serum levels and urinary OP metabolites [21] reported that in adult men, an inverse association exists between urinary levels of OP and CB metabolites and serum LH and testosterone levels.

This study supported the possible association between pesticide exposure and adverse pregnancy outcome. Females involved in agricultural work reported a higher percentage of adverse outcomes of pregnancy (abortion, stillbirth and congenital anomalies) compared to females not engaged in agriculture, which agrees with other studies [22-26]. The intricate processes of the menstrual cycle, ovum production, fertilization, implantation, growth and development of the foetus may be particularly susceptible to low-dose exposures to endocrine disruptors. The study suggests that the reproductive health and liver functions of female pesticide sprayers in agricultural activities is adversely affected and adequate information and safety measures must be imposed to reduce it.

## Conflict of Interest

No conflict of interest.

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