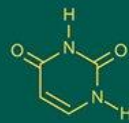


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## Assessment of organophosphorus pesticides residues in raw milk from Jabalpur city

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### Abstract

Pesticides are widely used in agriculture and public health leading to their entry into the food chain. Due to their lipophilic nature these compounds can accumulate in milk and pose potential health risks to consumers. The present study was conducted to evaluate organophosphorus pesticide residues in raw milk samples collected from Jabalpur, Madhya Pradesh, India. A total of 100 raw milk samples were obtained from dairy farms (n = 30), vendors (n = 35) and retail shops (n = 35) during November 2025 to January 2026. Sample extraction and cleanup were carried out using acetonitrile extraction followed by dispersive solid-phase extraction. The analysis of eleven organophosphate pesticides was performed using gas chromatography equipped with a flame photometric detector (GC-FPD) with matrix-matched calibration. The results revealed that none of the analyzed samples contained detectable levels of the selected organophosphorus pesticides. The absence of residues may be attributed to the rapid degradation and metabolism of these compounds in animals, along with improved pesticide management practices. The findings indicate that the raw milk samples from the study area were within safe limits with respect to organophosphate pesticide residues. However, continuous monitoring and regular surveillance are recommended to ensure sustained food safety and protection of public health.

**Keywords:** Raw milk, Organophosphorus pesticides, Gas chromatography (GC-FPD), Food safety

### Introduction

Pesticides such as organochlorines, organophosphates and synthetic pyrethroids are widely used in agriculture and public health programs to enhance crop productivity and control vector-borne diseases. Their extensive use has resulted in their persistence in the environment and subsequent entry into the food chain. Due to their lipophilic nature many of these compounds bioaccumulate in animal tissues and are eventually excreted in milk (World Health Organization, 2020) [10]. Continuous exposure to pesticide residues has been associated with adverse health effects including endocrine disruption, neurotoxicity, immune-suppression and carcinogenic outcomes.

Raw milk is an essential component of the human diet providing high-quality proteins, fats, vitamins and minerals. However, it may also act as a potential vehicle for pesticide residues particularly lipophilic compounds that accumulate in milk fat. This raises significant food safety concerns, especially for vulnerable populations such as infants and children who consume milk in higher quantities relative to their body weight (Food and Agriculture Organization, 2021) [4].

Several studies from India have reported the presence of pesticide residues in milk. For instance, a multicenter study by (Gill *et al.*, 2020) [6] detected organochlorines and organophosphate residues in bovine milk samples collected from different regions of India, highlighting regional variability and potential health risks. Similarly, (Bedi *et al.*, 2018) [11] reported the occurrence of multiple pesticide residues such as DDT and HCH in milk indicating environmental contamination and transfer through feed and fodder. Other Indian studies have also demonstrated the presence of pesticides like chlorpyrifos and endosulfan in milk emphasizing the need for strict monitoring and control measures.

Monitoring pesticide residues in milk is therefore essential to ensure food safety and compliance with regulatory standards such as Maximum Residue Limits (MRLs) established by agencies like the European Food Safety Authority (2022) [3] and Food Safety and Standards Authority of India (2023) [5]. Continuous surveillance and improved agricultural practices are crucial to minimize contamination and safeguard public health.

### Materials and Methods

The present study was conducted in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, Nanaji Veterinary Science University, Jabalpur, Madhya Pradesh. The investigation was carried out from November 2025 to January 2026 covering different areas of Jabalpur city. A total of 100 raw milk samples were collected from different sources (Dairy Farms - 30, Vendors - 35 and Retails shops - 35) within Jabalpur city. All samples were collected in sterile containers, transported under refrigerated conditions and processed for pesticide residue analysis.

### Sample preparation and extraction

A certified multi-residue standard of organophosphate pesticides, comprising eleven compounds viz. azinophos-methyl, chlorpyrifos, diazinon, disulfoton, dimethoate, ethion, malathion, monocrotophos, parathion-methyl, phorate and parathion-ethyl-was procured from Lab India Instruments Pvt. Ltd., Thane, Maharashtra, India.

The organophosphorus pesticides from the buffalo and cow raw milk samples were detected as per the FSSAI protocol (*Manual of Methods of Analysis of Foods Pesticide Residues*, 2016) [4]. The protocol was specifically optimized for the high-moisture and high-fat matrix of milk to ensure efficient removal of lipids and proteins as these substances might interfere with chromatographic analysis or contaminate the GC column. Briefly 10 -15 ml of homogenized milk sample was transferred into a 50 ml centrifuge tube followed by the addition of 10 ml acetonitrile. A salt mixture ( $\text{MgSO}_4$ , NaCl, and sodium citrate) was added to facilitate phase separation. The mixture was vortexed for 1 min and centrifuged at 5,000 rpm for 5 min.

For cleanup, the acetonitrile layer was transferred to a tube containing  $\text{MgSO}_4$ , PSA, and C18 sorbents. After vortexing and centrifugation (10,000 rpm), the supernatant was filtered through a 0.22  $\mu\text{m}$  syringe filter. The extract was evaporated under nitrogen and reconstituted in ethyl acetate prior to analysis (FSSAI, 2016).

Optionally, the acetonitrile extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in ethyl acetate or toluene to enhance the stability of organophosphate pesticides during GC injection. The final extract was subsequently analyzed using a flame photometric detector (FPD) or a nitrogen-phosphorus detector (NPD).

### Gas chromatography - Flame photometric detector (GC - FPD)

The analysis of the eleven organophosphate pesticides was performed using a Thermo Scientific TRACE™ 1300 gas chromatograph equipped with an Instant Connect Flame Photometric Detector (IC-FPD). Chromatographic

separation was achieved on a TG-5MS capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness). The oven temperature program was set initially at 100  $^{\circ}\text{C}$ , ramped at 25  $^{\circ}\text{C min}^{-1}$  to 180  $^{\circ}\text{C}$ , followed by an increase at 4  $^{\circ}\text{C min}^{-1}$  to 200  $^{\circ}\text{C}$  and finally at 10  $^{\circ}\text{C min}^{-1}$  to a final temperature of 250  $^{\circ}\text{C}$ . A post-run temperature of 290  $^{\circ}\text{C}$  was maintained for 2 min to ensure column cleanliness. The injector and detector temperatures were maintained at 250  $^{\circ}\text{C}$ . Helium (99.99% purity) was used as the carrier gas at a constant flow rate of 1.0  $\text{ml min}^{-1}$ . A 1  $\mu\text{l}$  aliquot of the sample was injected in split less mode, with a total chromatographic run time of 16-20 min, following FSSAI guidelines (FSSAI, 2016).

**Extraction:** Fresh milk (500 ml) was placed in a 250 ml reparatory funnel. Acetone (150 ml) was immediately added to the milk and the flask was manually shaken for 10 min. The entire contents were filtered through a Buchner funnel with filter paper in a 500 ml Erlenmeyer flask. An additional 25 ml acetone was used to wash the filter cake in the Buchner funnel and this was added to the filtrate. The filtrate was extracted first with 100mL methylene chloride and then with 50 ml methylene chloride. The extract was dried over anhydrous sodium sulfate at 50-60 $^{\circ}\text{C}$ , under reduced pressure.

The sample was transferred quantitatively to disposable extrelut 3 mini column with Ca 3 ml n hexane. The solution was allowed to drain into filling material. For even distribution waiting was done for 10 min and then elute 3 times with 5 ml acetonitrile equilibrated with n-hexane. Methanol (4ml) was added to elute and concentrate to dryness in rotary evaporator of 50-55 $^{\circ}\text{C}$  reduced pressure. Benzene (1ml) was added and analyzed by GC (FSSIA, 2016).

### Preparation of calibration standards

(A) Stock solutions 1 mg/ml: The organophosphorus (10mg) reference standard were transferred into 10 ml volumetric flasks and dissolved in ethyl acetate to attain and fill the volume to 10 ml. (B) Intermediate solution 10  $\mu\text{g/ml}$  pipette: Each stock solution (1ml) was transferred to 100 ml volumetric flasks and diluted with ethyl acetate. (C) Working solution 0.5  $\mu\text{g/ml}$ ; 5 ml of each intermediate solution was transferred into a 100 ml volumetric flask and diluted with ethyl acetate.

To compensate for matrix-induced enhancement or suppression effects during Gas Chromatography (GC) analysis, a matrix-matched calibration approach was utilized. A seven-point calibration curve was established by fortifying 2 ml aliquots of representative working matrices (bovine milk) with varying volumes (5, 10, 15, 20, 25, 50 and 100  $\mu\text{l}$ ) of the intermediate pesticide standard mixture. Additionally, each calibration point was spiked with 20  $\mu\text{l}$  of a 1  $\mu\text{g/ml}$  internal standard to monitor analytical consistency.

The fortified calibrates underwent the identical extraction and dispersive-SPE cleanup protocols as the experimental samples. Following the cleanup phase, the extracts were evaporated to dryness under a nitrogen stream and subsequently reconstituted in 100  $\mu\text{l}$  of HPLC-grade ethyl acetate. A 1  $\mu\text{l}$  volume of each reconstituted calibrates was introduced into the GC-FPD system for analysis (FSSIA, 2016).

Calculation formula

Peak height of sample

Peak height of the standard

suL of the standard

uL of the samples

Final volume of the samples extract concentration in (ml)

Mass in g of the sample solution

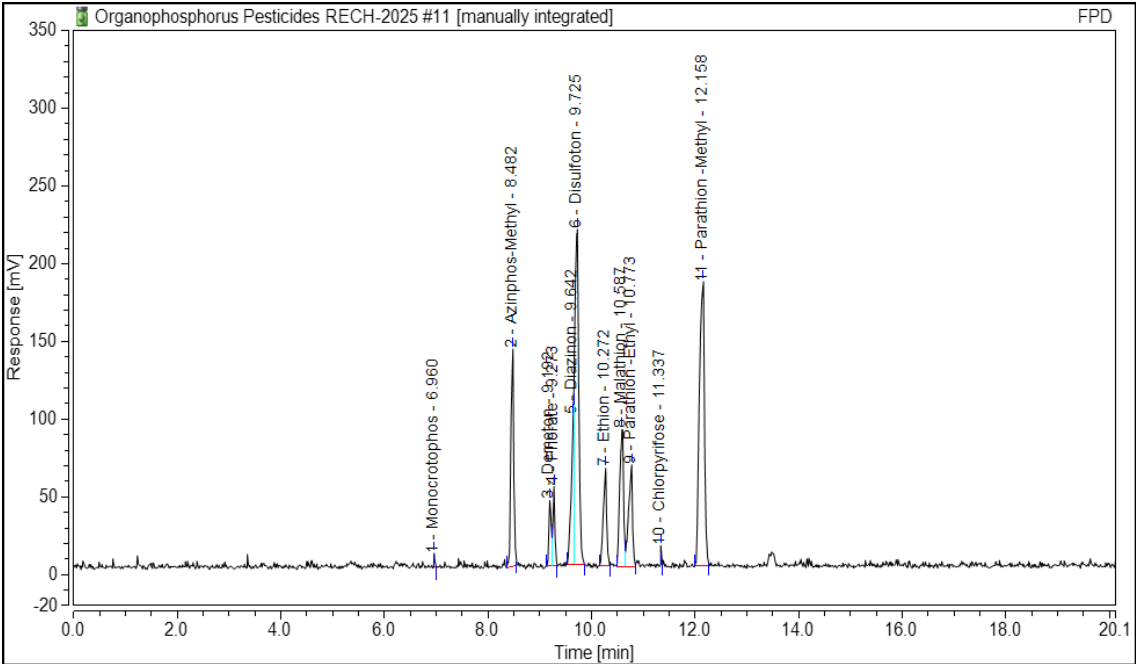
Residue level (mg/kg) =

x

x

Concentration in ppm of reference std.

Chromatogram



| Integration Results |                   |                |        |         |               |                 |
|---------------------|-------------------|----------------|--------|---------|---------------|-----------------|
| No.                 | Peak Name         | Retention Time | Area   | Height  | Relative Area | Relative Height |
|                     |                   | min            | mV*min | mV      | %             | %               |
| 1                   | Monocrotophos     | 6.960          | 0.157  | 8.609   | 0.20          | 0.89            |
| 2                   | Azinphos-Methyl   | 8.482          | 8.741  | 139.822 | 11.38         | 14.49           |
| 3                   | Demeton           | 9.192          | 2.188  | 42.294  | 2.85          | 4.38            |
| 4                   | Phorate           | 9.273          | 2.106  | 51.083  | 2.74          | 5.29            |
| 5                   | Diazinon          | 9.642          | 5.064  | 96.015  | 6.59          | 9.95            |
| 6                   | Disulfoton        | 9.725          | 19.965 | 215.608 | 25.99         | 22.35           |
| 7                   | Ethion            | 10.272         | 4.562  | 62.692  | 5.94          | 6.50            |
| 8                   | Malathion         | 10.587         | 7.527  | 87.963  | 9.80          | 9.12            |
| 9                   | Parathion -Ethyl  | 10.773         | 5.515  | 65.439  | 7.18          | 6.78            |
| 10                  | Chlorpyrifose     | 11.337         | 0.162  | 12.455  | 0.21          | 1.29            |
| 11                  | Parathion -Methyl | 12.158         | 20.820 | 182.839 | 27.11         | 18.95           |
| Total               |                   |                | 76.807 | 964.818 | 100.00        | 100.00          |

Results and Discussion

Pesticides are widely used to protect crops and enhance agricultural productivity; however, their persistence in the environment poses potential risks to human health including endocrine disruption, neurotoxicity and developmental effects particularly in prenatal and postnatal stages. Due to their high persistence, bioaccumulation and biomagnifications potential, many organochlorines pesticides have been banned or restricted in India and globally.

The present study focused on the detection of eleven organophosphorus pesticides, namely monocrotophos, azinphos-methyl, demeton, phorate, diazinon, disulfoton, ethion, chlorpyrifos, malathion, parathion-ethyl and parathion-methyl in raw milk samples collected from different sources in Jabalpur city. Analysis was carried out using GC-FPD following Food Safety and Standards Authority of India (2016) guidelines.

The results revealed that none of the analyzed milk samples (n = 100) showed detectable levels of the selected organophosphorus pesticides (Tables 01). This indicates that all samples were within the permissible limits and suggests minimal contamination of raw milk in the study area.

The absence of detectable residues may be attributed to the rapid degradation and metabolism of organophosphates in animal systems resulting in limited accumulation in milk. Additionally, improved pesticide usage practices, adoption of safer and targeted agrochemicals and better feed management may have contributed to reduced exposure in dairy animals.

These findings are consistent with some earlier reports although variations exist across regions. Dhanger and Patil (2021) [2] detected chlorpyrifos in 3 out of 60 milk samples and reported phorate residues in 1.20% of samples (Melgar *et al.*, 2010) [7]. They also reported organophosphate residues in 6.73% of total milk samples and 8.67% of raw milk

including 0.83% parathion-methyl. Similarly, (Salas *et al.*, 2003) <sup>[9]</sup> observed organophosphate residues in 39.60% of pasteurized milk samples, while (Ravichandran *et al.*, 2015) <sup>[8]</sup> detected monocrotophos in raw milk samples.

The absence of residues in the present study suggests improved compliance with pesticide regulations and better

agricultural practices in the study region. However, considering the variability in findings across different geographical areas, continuous monitoring and surveillance of pesticide residues in milk are essential to ensure food safety and protect public health.

**Table 1:** Organophosphorus in raw milk by Gas chromatography

| S. No. | Source of samples | Number of samples | Name of organophosphates |                 |         |         |          |            |        |              |           |                 |                  |
|--------|-------------------|-------------------|--------------------------|-----------------|---------|---------|----------|------------|--------|--------------|-----------|-----------------|------------------|
|        |                   |                   | Monocrotophos            | Azinphos-Methyl | Demeton | Phorate | Diazinon | Disulfoton | Ethion | Chlorpyrifos | Malathion | Parathion-Ethyl | Parathion-Methyl |
| 1.     | Dairy farms       | 30                | 00.00                    | 00.00           | 00.00   | 00.00   | 00.00    | 00.00      | 00.00  | 00.00        | 00.00     | 00.00           | 00.00            |
| 2.     | vendors           | 35                | 00.00                    | 00.00           | 00.00   | 00.00   | 00.00    | 00.00      | 00.00  | 00.00        | 00.00     | 00.00           | 00.00            |
| 3.     | Retails           | 35                | 00.00                    | 00.00           | 00.00   | 00.00   | 00.00    | 00.00      | 00.00  | 00.00        | 00.00     | 00.00           | 00.00            |
|        | Total             | 100               | 00.00                    | 00.00           | 00.00   | 00.00   | 00.00    | 00.00      | 00.00  | 00.00        | 00.00     | 00.00           | 00.00            |

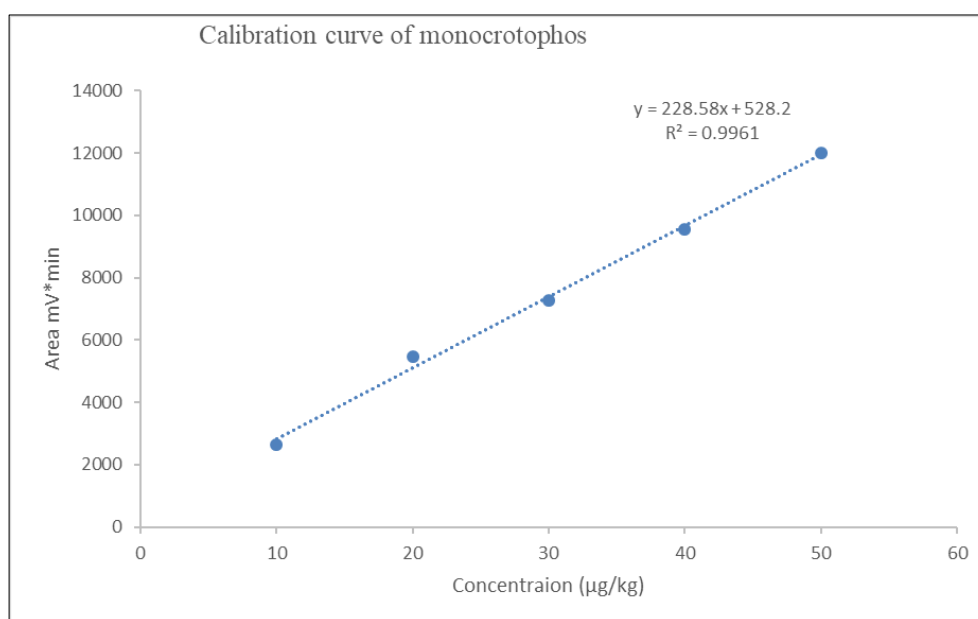


**Fig 1:** Calibration curve of monocrotophos

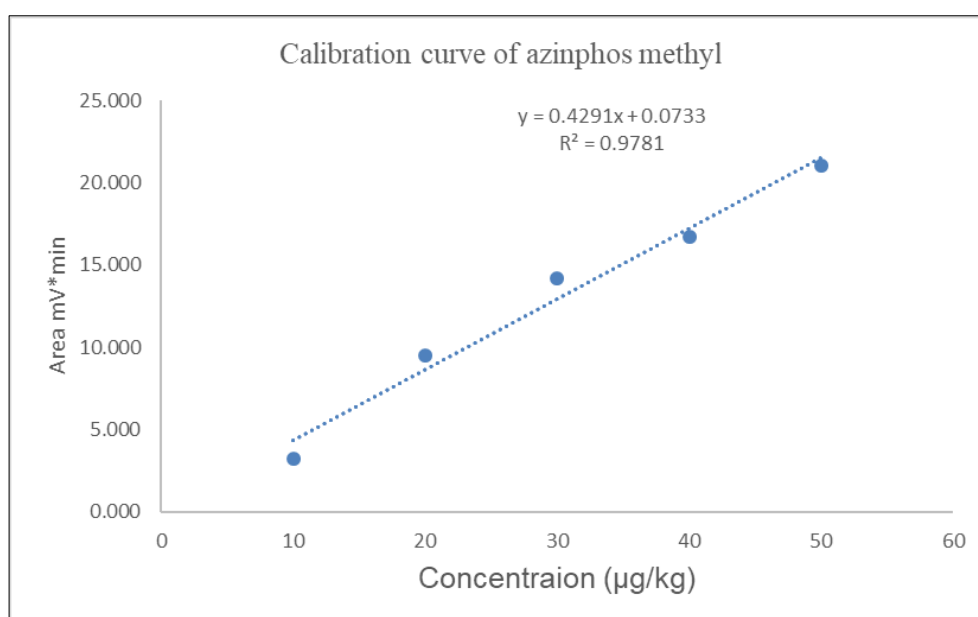
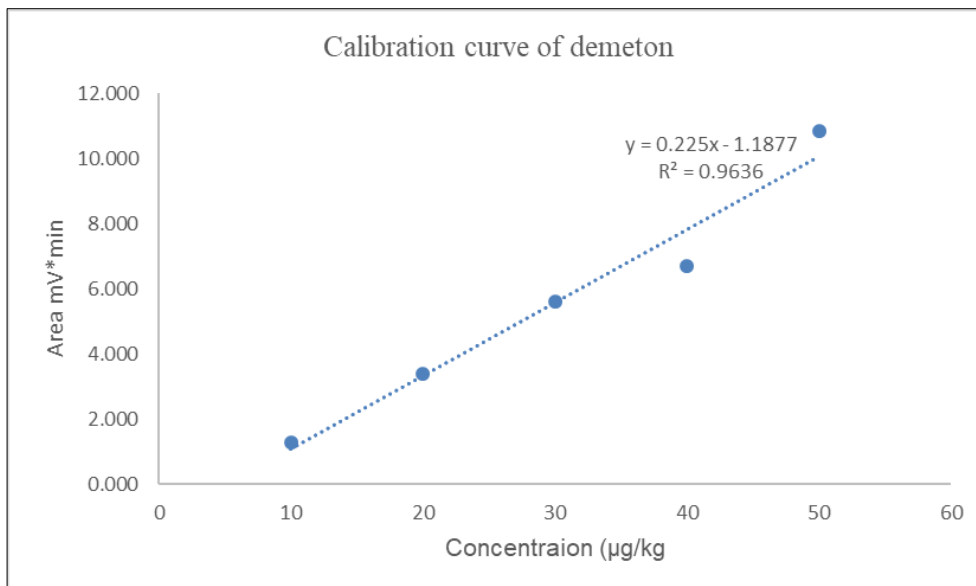
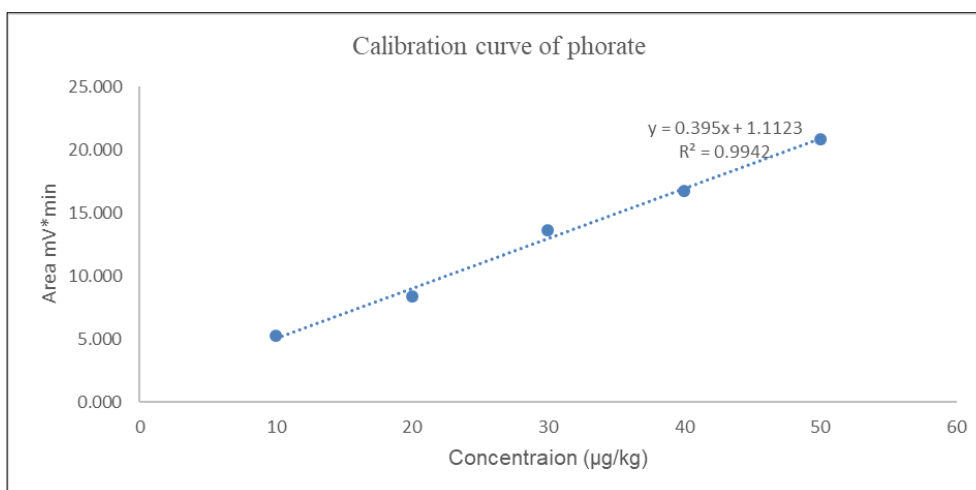
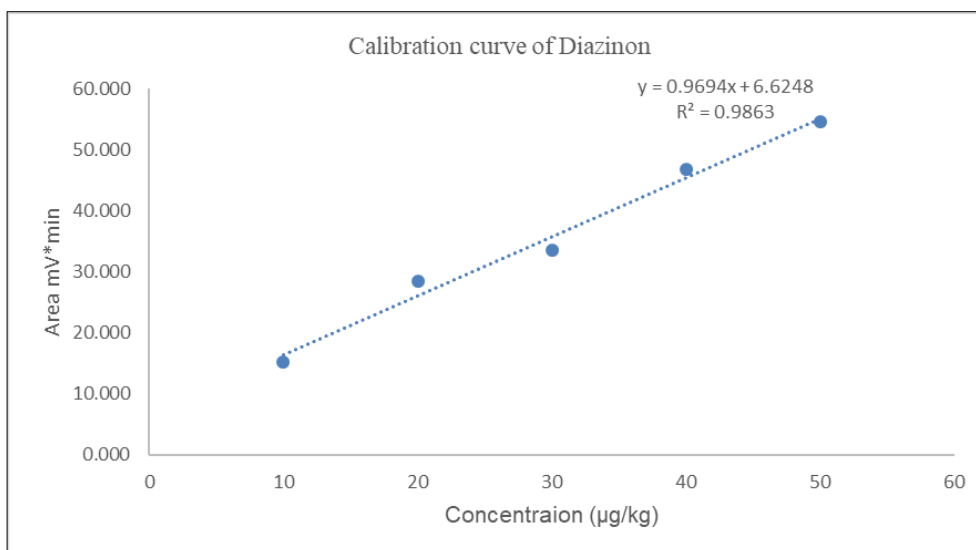
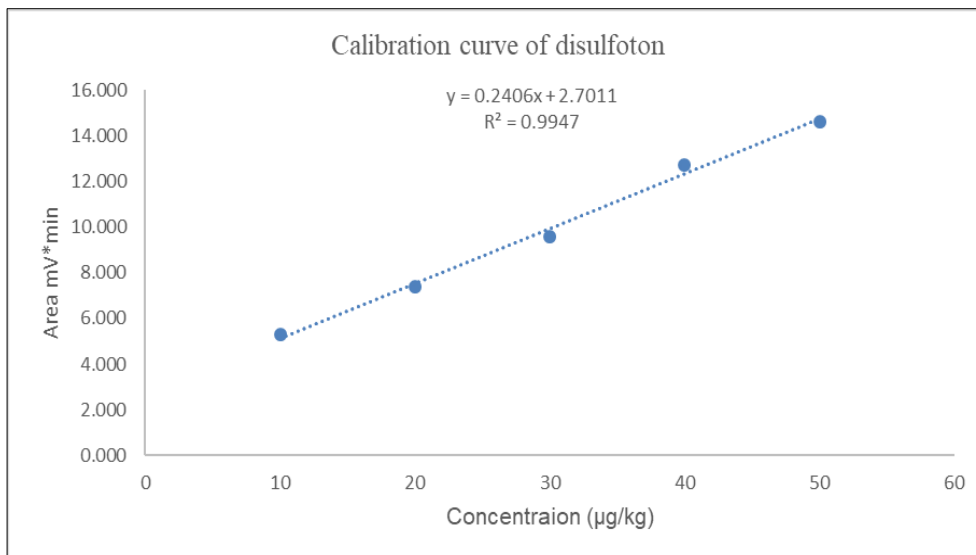
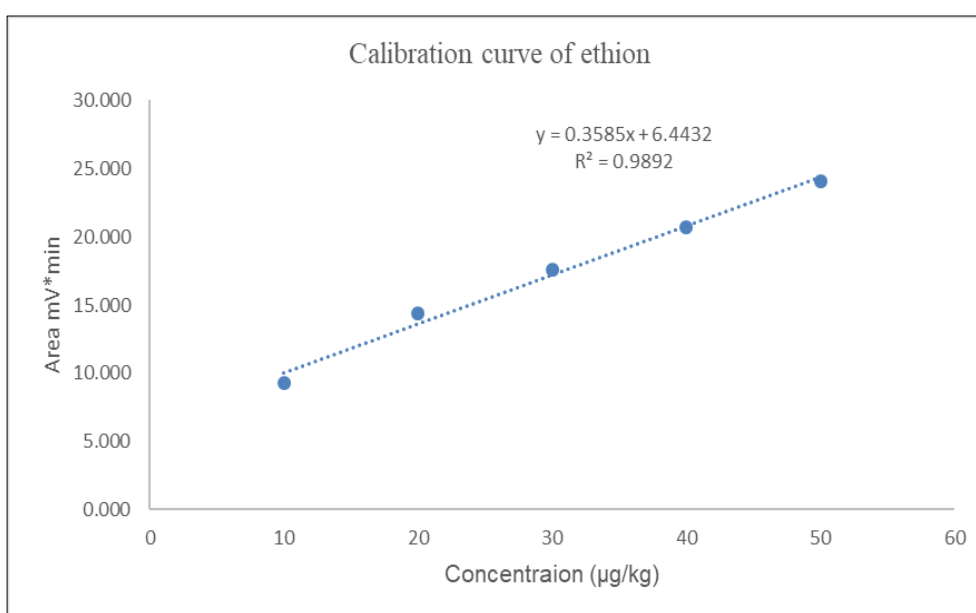
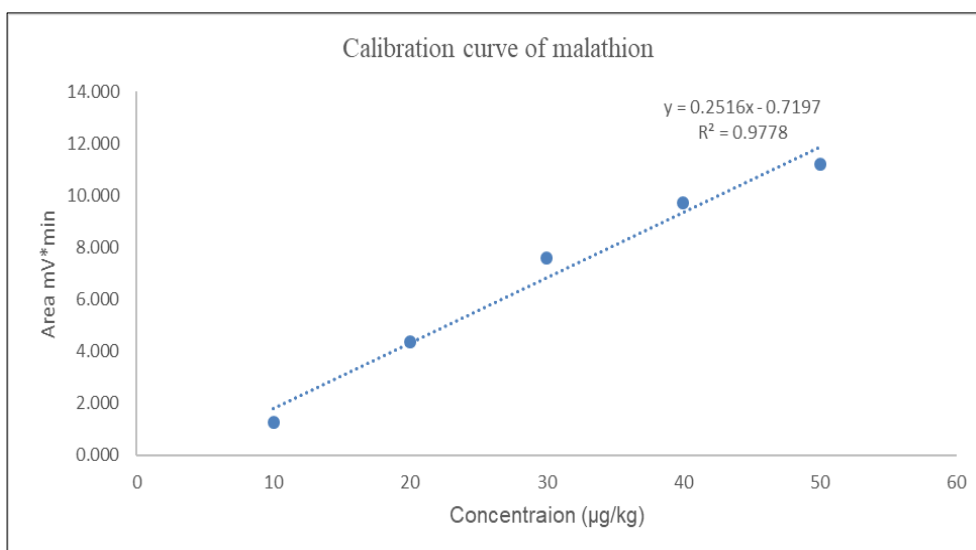
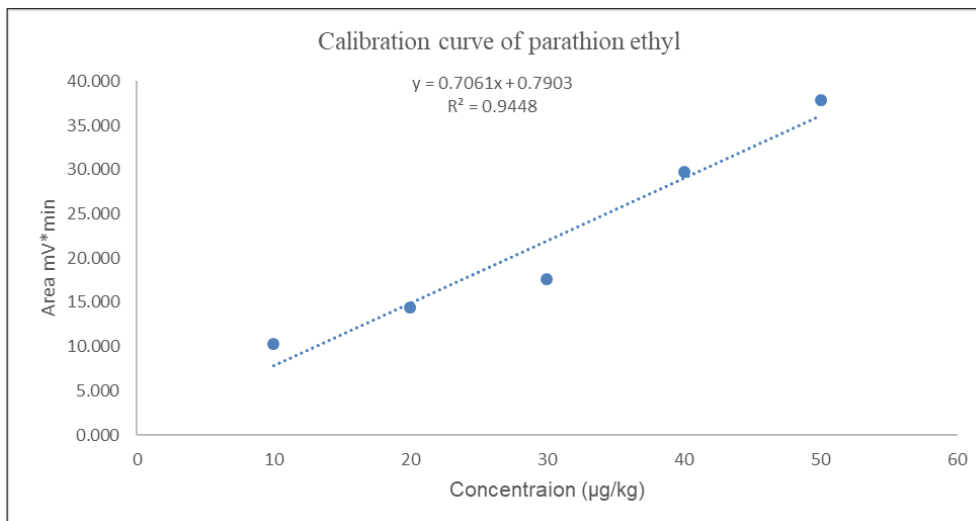
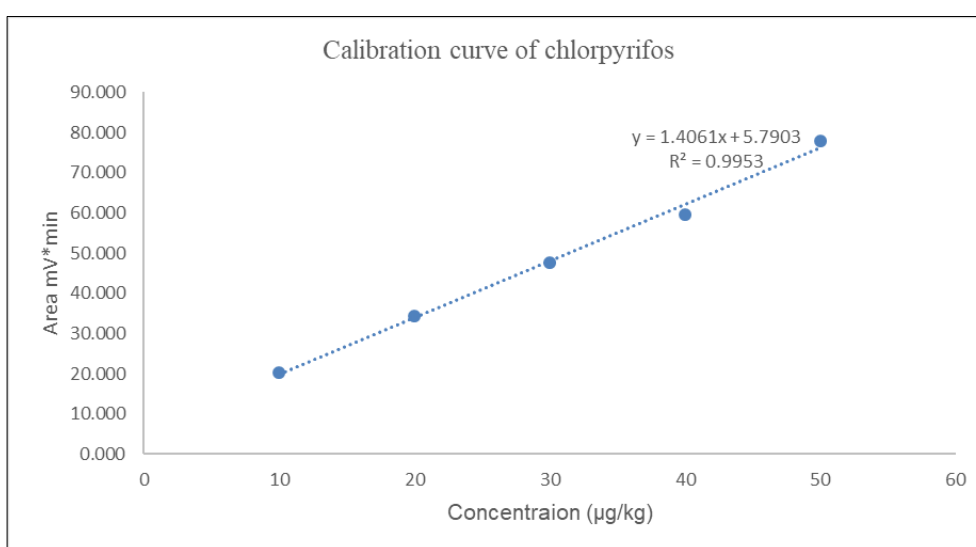
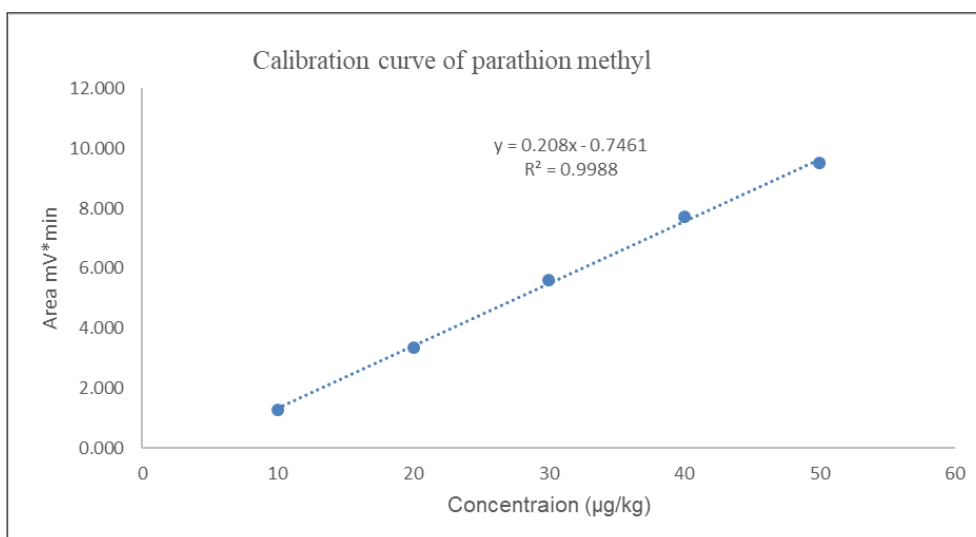


**Fig 2:** Calibration curve of azinphos-methyl

**Fig 3:** Calibration curve of demeton**Fig 4:** Calibration curve of phorate**Fig 5:** Calibration curve of Diazinon

**Fig 6:** Calibration curve of disulfoton**Fig 7:** Calibration curve of ethion**Fig 8:** Calibration curve of malathion

**Fig 9:** Calibration curve of parathion ethyl**Fig 10:** Calibration curve of chlorpyrifos**Fig 11:** Calibration curve of parathion methyl

### Conclusion

The present study demonstrated that none of the analyzed raw milk samples from Jabalpur contained detectable levels of selected organophosphorus pesticides. This indicates that

the milk samples were within safe limits and pose minimal risk to consumers. The absence of residues may be attributed to the rapid metabolism of organophosphates in animals and improved pesticide management practices.

These findings reflect better compliance with food safety standards and agricultural regulations. However, continuous monitoring of pesticide residues in milk is essential to ensure sustained food safety and public health protection.

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