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Toxicological and genotoxic evaluation of dichlorvos on multivoltine races of silkworm (*Bombyx mori* L.)

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Abstract
The silkworm *Bombyx mori* L. is essential for silk production. This species may be at risk from dichlorvos (DDVP), an organophosphate pesticide commonly used in agriculture and sericulture. This study evaluates the harmful effects of dichlorvos on the Pure Mysore and C-nichi multivoltine races of B. mori. We exposed larvae to sublethal doses of dichlorvos at 2, 5, 10, and 15 ppm using a leaf-dip method. We measured protein content, adult lifespan, dominant lethal mutations, and economic traits like larval weight, cocoon and shell weight, shell ratio, and filament length. The results showed a dose-dependent decline in economic traits and total protein concentration, with C-nichi being more sensitive than Pure Mysore. The adult lifespan gradually decreased with higher doses of Dichlorvos. Dominant lethal experiments confirmed significant genotoxic effects at 10 and 15 ppm. These findings suggest that even low levels of Dichlorvos can harm silkworm health, productivity, and genetic integrity. The study highlights the importance of responsible pesticide use in sericulture and the role of *Bombyx mori* as a useful bioindicator for pesticide toxicity in ecosystems.

Keywords: *Bombyx mori*, dichlorvos, pesticide toxicity, sericulture, economic traits, genotoxicity

Introduction

Humans, as the dominant member of the ecosystem, have greatly influenced the natural world. Over centuries, humanity has changed the ecosystem through colonization, urbanization, industrialization, agriculture, mining, and technology. These changes have certainly contributed to social prosperity, but they have also caused serious ecological imbalances (Chu & Karr 2017) [3]. One of the most significant problems resulting from this shift is environmental degradation, especially due to chemical agents like pesticides. Rapid advancements in industry and agriculture have introduced large amounts of synthetic chemicals into the ecosystem. While these chemicals can be beneficial for food production and disease control, they also pose serious threats to biodiversity and ecological stability (Ahmad *et al.*, 2024) [1].

Pesticides play a crucial role in modern farming by boosting crop yields and controlling pests. However, their extensive use has led to significant environmental issues. Organophosphates are the most widely used group of insecticides because they are highly effective and break down quickly in the environment. Dichlorvos (DDVP), a popular organophosphate, is used in farming, gardening, and animal care under various brand names, including Nuvan, Vapona, and Duravos (Tudi *et al.*, 2021) [12].

Sericulture depends on raising Bombyx mori L., which only eats mulberry (*Morus* spp.) leaves. Therefore, pesticide use must be very careful. Chemical treatments aimed at controlling mulberry pests, like *Diaphania pulverulentalis*, can accidentally affect silkworm growth, cocoon quality, and reproduction (Singh *et al.*, 2025) [11]. Dichlorvos is necessary to protect mulberry crops, but it can also harm silkworm health and lead to sub-lethal and genotoxic effects. This study aims to evaluate the impact of Dichlorvos on the biological and economic traits of two multivoltine *Bombyx mori* races (Muthusamy & Rajakumar, 2016), (Yeshika *et al.*, 2020) [13].

Dichlorvos (O, O-dimethyl 2, 2-dichlorovinyl phosphate; $C_4H_7Cl_2O_4P$; MW: 220.98) is a common organophosphate pesticide used in agriculture and sericulture. It appears as a colour less to amber liquid with a slight odour. It dissolves easily in water (10, 000 mg/L) and other organic solvents.

Farmers use it widely to control pests in food storage areas, greenhouses, and on cattle. It is also used in mulberry production (Okoroiwu & Iwara, 2018) [9]. Despite its common use in sericulture, research on its direct and indirect effects on *Bombyx mori* is limited. Preliminary studies have shown potential toxic risks. Dichlorvos orally led to significant physiological changes in bivoltine silkworm races. Previous research stressed the need to monitor pesticide use in mulberry fields and suggested specific safety intervals: 0.01% for 7 days and 0.05% for 11 days. Later research (Yeshika *et al.*, 2020) [13] found that a concentration of 0.076 ppm with a 7-day safety period could be effective and generally safe. However, these studies lacked detailed toxicogenetic research and offered little insight into Dichlorvos mutagenic effects on silkworms (Singh *et al.*, 2025) [11], (*Munhoz et al.*, 2013) [6].

Several model species have been used in pesticide toxicity studies, including bacteria, yeast, Drosophila, Neurospora, and rats. These models have helped identify potential issues like mutagenicity, teratogenicity, carcinogenicity, and general toxicity. Dichlorvos at an LC_{50} of 3.5 ppm caused cell death in Euglena gracilis (Richardson *et al.*, 2019) [10]. These findings highlight the susceptibility of a wide range of organisms to organophosphate insecticides.

Despite its common use in agriculture and sericulture, the genotoxic effects of dichlorvos on silkworms have not been thoroughly investigated, especially across different exposure pathways and genetic strains (Mishra *et al.*, 2014) [5]. This is a significant gap, since *Bombyx mori* is not only an important silk-producing species but also a well-known model organism in genetics, biochemistry, and toxicology research. Its sensitivity to environmental changes and chemicals makes it a great choice for studying the ecological and biological impacts of agro-chemicals (Chanda, 2024) [2] (Nguku *et al.*, 2009) [8].

The current study seeks to fill this gap by thoroughly assessing the toxicity and genotoxic potential of Dichlorvos in multivoltine *Bombyx mori* races. It aims to determine how LC_{50} values affect silkworm rearing performance and genetic stability through various delivery methods, including topical and oral. These findings should clarify the safe and regulated use of pesticides in sericulture and enhance understanding of silkworms as bioindicators in environmentaltoxicology.

Materials and Methods

Silkworm rearing

The multivoltine silkworm races Pure Mysore (PM) and C-nichi were obtained from the germplasm bank of the Department of Studies in Sericulture Science, University of Mysore, India. Eggs were incubated at 25 ± 1 °C and 80 ± 5% relative humidity, with black boxing performed on the 8th day to ensure uniform hatching. Three layings from each race were selected, and larvae were reared separately under standard laboratory conditions (Chanda, 2024) [2] using high-quality mulberry leaves from the departmental garden. The key characteristics of the two races are presented in Table 1.

Table 1: Characteristic features of two silkworm races used in present study

Strain	Voltinism	Origin	Larval pattern	Cocoon color	Cocoon shape
Pure Mysore	Multivoltine	India	Plain	Greenish yellow	Spindle
C-nichi	Multivoltine	India	Plain	White	Dumbbell

Table 2: Eleven common quantitative traits were used to evaluate the performance of the races. These traits are briefly explained in table below

Sl. No.	Traits	Unit	Definition
1.	Fecundity	No.	Total number of eggs laid by a single healthy female moth
2.	Hatching Percentage	%	Percentage of eggs hatched from a disease free layings
3.	Larval weight	g	Avg weight of V-instar larva
4.	Larval duration	h	Time from hatching to spinning (in hours)
5.	Cocoon weight	g	Avg weight of 25 male and 25 female
6.	Shell weight	g	Avg weight of silk shell from same cocoons
7.	Shell ratio	%	Percentage of shell weight in total cocoon $\frac{\text{Shell wt}}{\text{Cocoon wt}} \times 100$
8.	Pupation rate	%	Percentage of larvae that pupate successfully $\frac{\text{Live pupae}}{\text{Larvae after 3rd moult}} \times 100$
9.	Filament length	m	Avg silk length reeled from a single cocoon
10.	Denier	d	Thickness of silk filament $\frac{\text{Silk wt}}{\text{Silk length}} \times 9000$
11.	Renditta		Weight of cocoon needed to produce 1kg of raw silk $\frac{\text{Cocoon wt}}{\text{Reeled silk wt}}$

Experimental design

Silkworm Strains and Stage Selection

Second-day-old fifth instar larvae of two multivoltine silkworm (Bombyx mori) races, Pure Mysore (PM) and C-nichi, were used in this investigation.

Evaluation of dichlorvos toxicity

a) Administration Method

100 individuals per duplicate, three replicates per treatment, were used to separate the larvae into treatment groups. Following the leaf-dipping method described by Kuwana *et*

al. (1967) [4], we administered dichlorvos orally. We prepared four concentrations in 1000 milli-liters of distilled water: 2 ppm, 5 ppm, 10 ppm, and 15 ppm.

b) Method of Treatment (oral)

The larvae received fresh mulberry leaves suitable for late instars. We surface dried the leaves and dipped them in the corresponding dichlorvos concentrations. After one feeding session with treated leaves, all batches were given untreated leaves until spinning the next day. The control groups received leaves dipped in double-distilled water.

c) Post-treatment observations

After 24 hours of exposure, we measured larval mortality to assess acute toxicity. We raised all treated and control groups under standard laboratory conditions until cocooning. We reported the larval weight based on 10 randomly selected larvae, the duration of the larval stage, and survival rates to pupal and adult stages. We calculated viability by using cumulative mortality at various phases, which indicated the level of toxic impact.

Haemolymph collection

On the fifth day of the fifth instar, we extracted haemolymph from larvae by cutting the first proleg and drawing the fluid into pre-chilled Eppendorf tubes containing a thiourea crystal to prevent melanization. We centrifuged the samples at 5000 rpm for 10 minutes to separate haemocytes from debris. The clear supernatant served to estimate total soluble protein, which used crystalline Bovine Serum Albumin (BSA) as the standard. Protein content was stated as mg protein per milli-liter of haemolymph.

Protein estimation

Protein determination is required to quantify the amount of protein in a sample, normalize it against the protein concentration, or during the purifying process. The method utilized must be determined by the volume of sample, accuracy, and presence of interfering substances. To ensure accurate measurement, the sample protein is compared to a known amount of a standard protein, such as bovine serum albumin (BSA).

The team calculated the protein concentration using Lowry's technique. They used BSA to create a standard curve, ranging from 0.2 to 1.0 mg/ml. They prepared the working reagent (Reagent D) by mixing alkaline copper sulphate and tartrate solutions. For each test tube, they added 1 mL of Reagent D (and 100 µL of the sample or standard. After mixing, they incubated the solution for 10 minutes. Then, they added 100 µL of 1N Folin-Ciocalteu reagent, vortexed the mixture, and incubated it at room temperature for 30 minutes. Finally, they measured the absorbance at 660 nm and determined the protein concentrations in unknown samples using the standard curve.

Longevity

After a month of emergence, researchers collected the two multivoltine races, Pure Mysore and C-nichi, from each replicate at 11 a.m. They placed them on a rearing tray covered with a cellule. Observations took place every day at 10 a.m. and 4 p.m. Adult moths died when they did not react biologically to being prodded with a pencil point. The average adult lifespan for each race and sex was calculated.

Results

The table 3 shows how different concentrations of Dichlorvos (2-15 ppm) impact key economic traits in two silkworm races: Pure Mysore and C-nichi. As the concentration increased, both races experienced a drop in larval weight, cocoon and shell weight, and filament length, which suggests poor growth and lower silk quality. In Pure Mysore, larval weight went down from 18.29 g (control) to 6.68 g at 15 ppm. Filament length decreased from 335 m to 260 m. For C-nichi, larval weight fell from 20.82 g to 7.80 g, while filament length dropped from 340 m to 245 m. The findings indicate a dose-dependent decline in performance, with C-nichi showing greater sensitivity to Dichlorvos.

Table 3: Rearing performance of treated and control batches for eleven economic traits in the two multivoltine races of Silkworm *Bombyx mori* L.

Economic traits Race	Conc. In ppm	Hatching %	10 Larval weight (g)	Larval Duration (h)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)	Filament Length (m)
Pure Mysore	Control	95	18.29±1.25	696±3.5	0.970±.016	0.120±.007	12.37±.015	335±4.1
	2	-	16.75±2.25	678±2.5	0.860±.014	0.100±.012	11.02±.020	332±3.2
	5	-	13.53±2.13	744±2.3	0.820±.018	0.100±.028	12.19±.018	325±3.8
	10	-	07.91±2.13	744±2.8	0.780±.023	0.080±.022	10.25±.016	295±3.1
	15	-	06.68±4.13	720±2.3	0.750±.083	0.110±.018	14.66±.017	260±3.3
	Mean	-	12.63±2.18	716.4±3.2	0.836±.05	0.102±.011	12.09±.021	309.4±4.1
C-nichi	Control	94	20.82±1.18	528±3.3	0.950±.015	0.120±.006	12.63±.014	340±3.9
	2	-	19.26±2.17	514±2.3	0.910±.012	0.100±.009	10.98±.018	316±3.1
	5	-	15.87±2.06	576±2.00	0.830±.015	0.100±.024	12.04±.015	305±2.9
	10	-	09.22±2.02	624±2.6	0.800±.021	0.090±.020	11.25±.013	290±2.7
	15	-	07.80±3.36	600±2.1	0.650±.076	0.080±0.17	12.30±.016	245±3.1
	Mean	-	14.59±2.26	568.4±2.8	0.828±.04	0.098±.009	11.84±.018	299.2±3.6

Fig 1: Mortality in the larvae of Pure Mysore race (15 ppm)

Fig 2: Mortality in the larvae of C.nichi race (15 ppm)

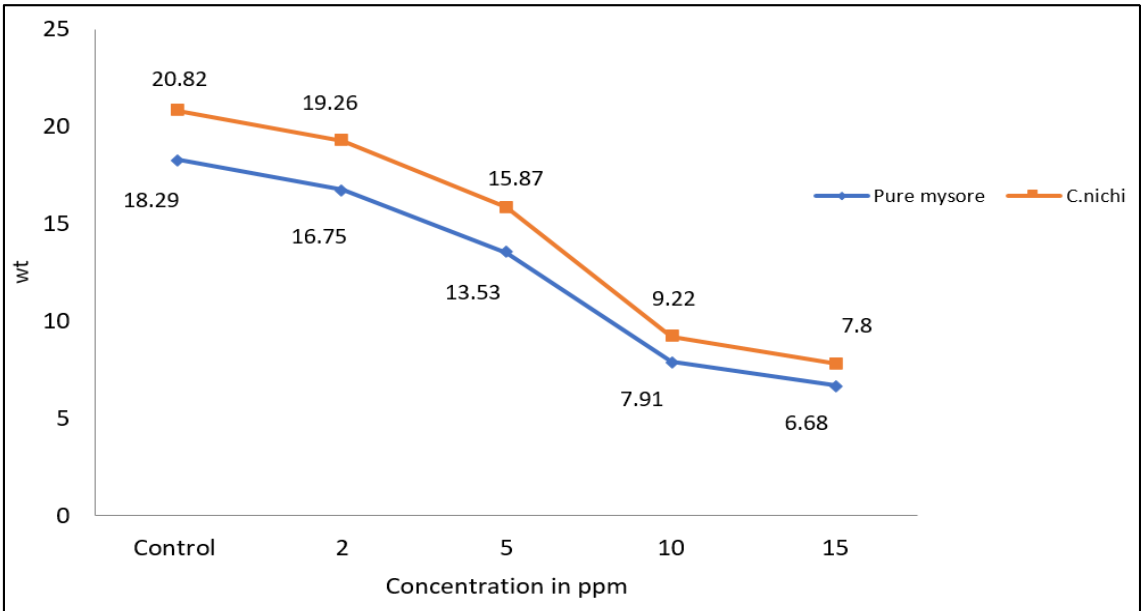

Fig 3: V[th] instar larval weight (g) of the treated and control batches of two races silkworm *Bombyx mori*.

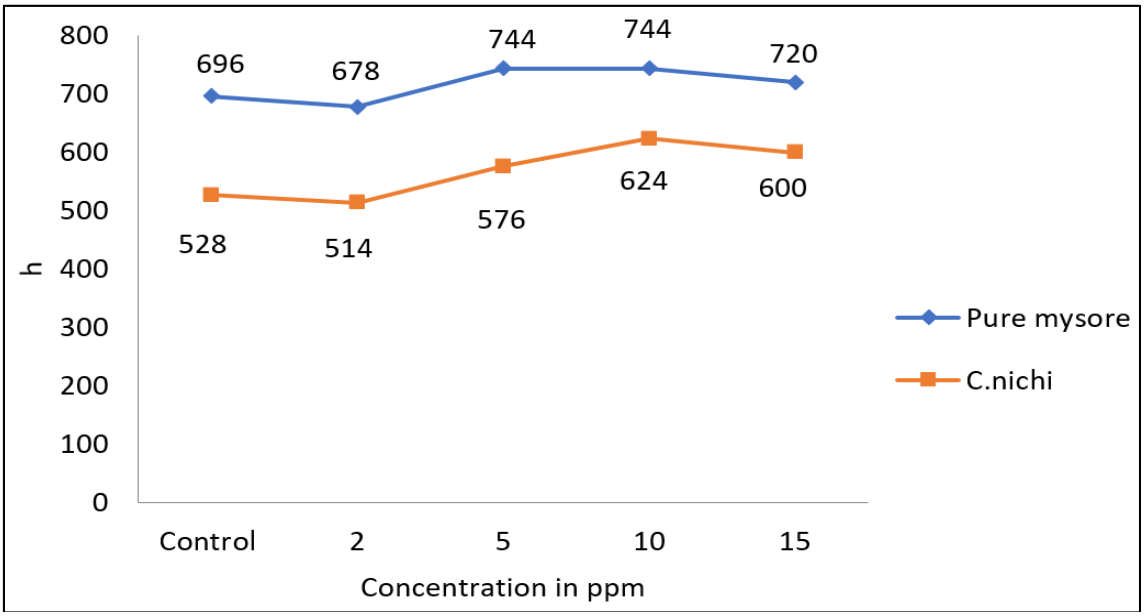

Fig 4: V[th] instar larval duration (h) of the treated and control batches of two races of silkworm *Bombyx mori*.

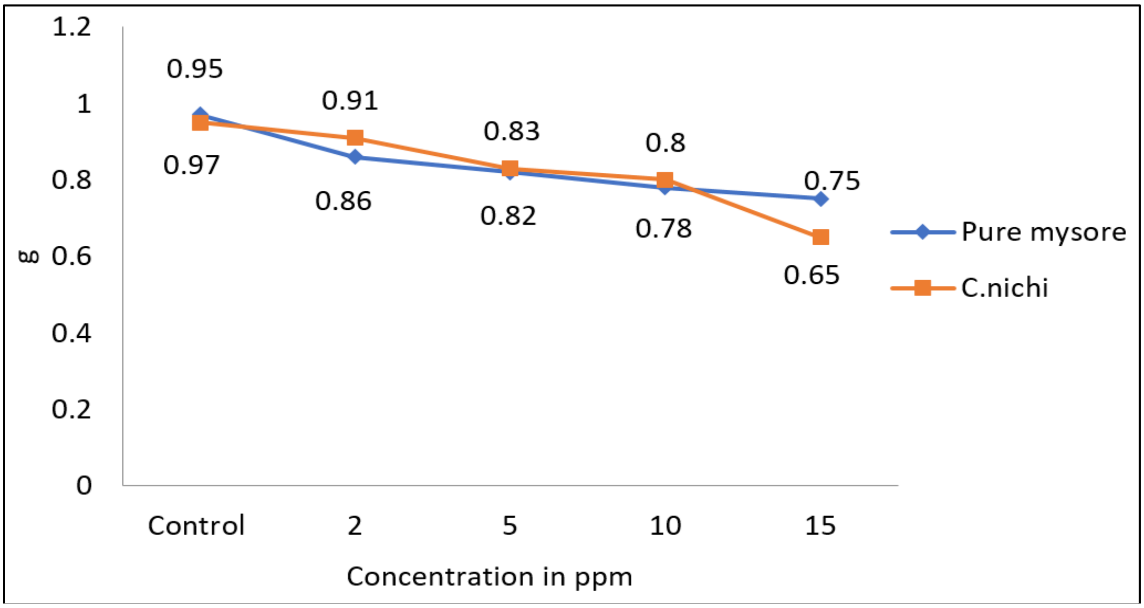

Fig 5: Vth instar cocoon weight (g) of the treated and control batches of two races of silkworm *Bombyx mori*.

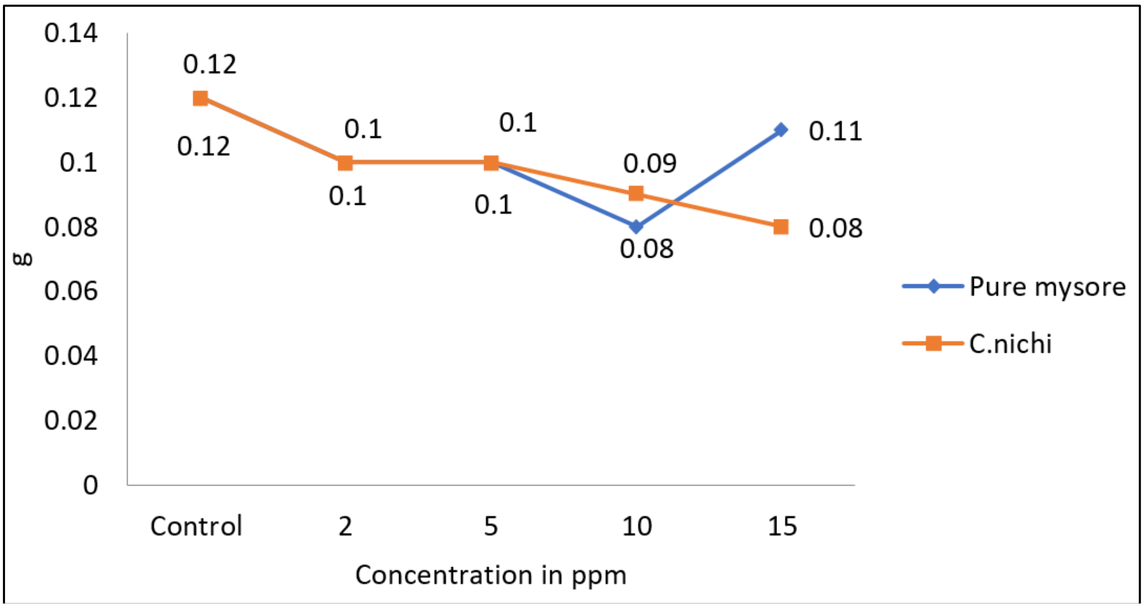

Fig 6: Vth instar shell weight (g) of the treated and control batches of two races of silkworm *Bombyx mori*.

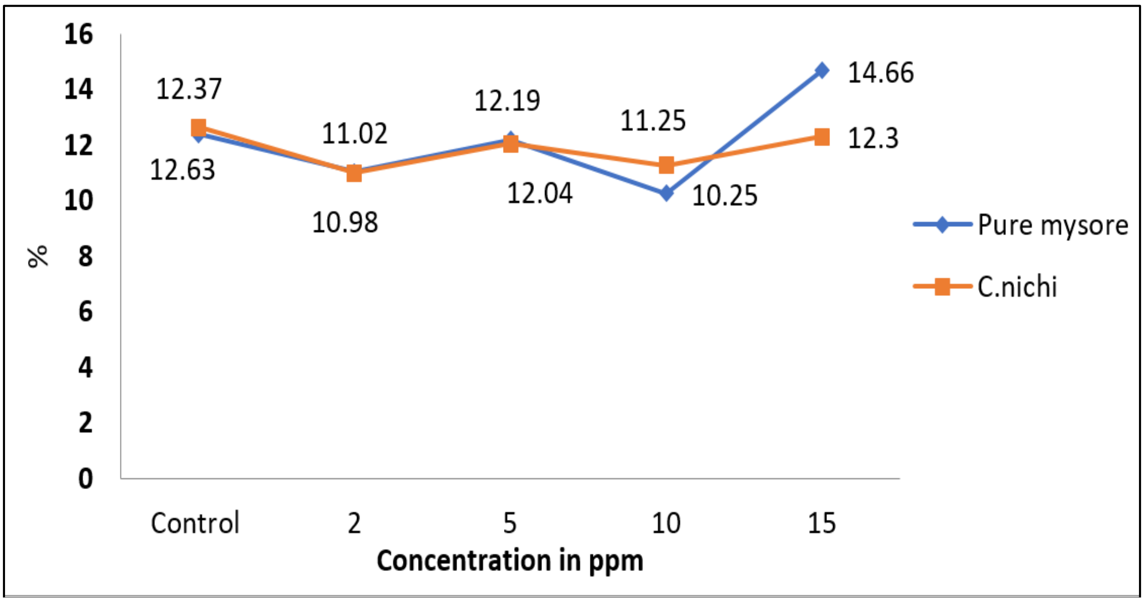

Fig 7: Vth instar shell ratio (%) of the treated and control batches of two races of silkworm *Bombyx mori*.

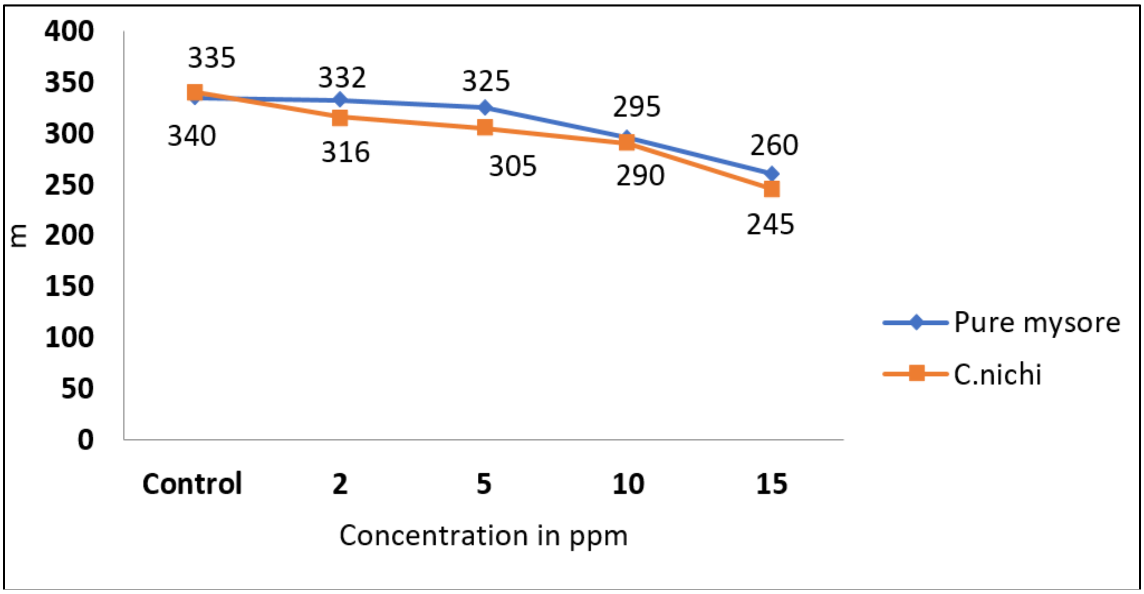

Fig 8: Vth instar filament length (m) of the treated and control batches of two races of silkworm *Bombyx mori.*

Protein content

As pesticide exposure rose, the protein content in both races gradually decreased. In the control group, Pure Mysore and C-nichi had protein levels of 38.18 mg/mL and 36.22 mg/mL, respectively. However, at the highest concentration of 15 ppm, the protein content dropped significantly to 18.25 mg/ml in Pure Mysore and 11.11 mg/ml in C-nichi. This trend indicates that the harmful effect of Dichlorvos is dose-dependent, with C-nichi being more sensitive than Pure Mysore. The decline in protein levels shows a decrease in metabolic activity and increased physiological stress caused by the pesticide. Depicted in table 4 and graph fig 9.

Table 4: Protein content (mg/ml) in the whole body homogenate of the Pure Mysore and C.nichi

Concentration in ppm	Pure Mysore	C.nichi
	Mean ± SD	Mean ± SD
Control	38.18±0.01	36.22±0.01
2	36.12±0.02	35.11±0.11
5	30.41±0.01	22.72±0.02
10	25.52±0.01	15.10±0.01
15	18.25±0.01	11.11±0.02

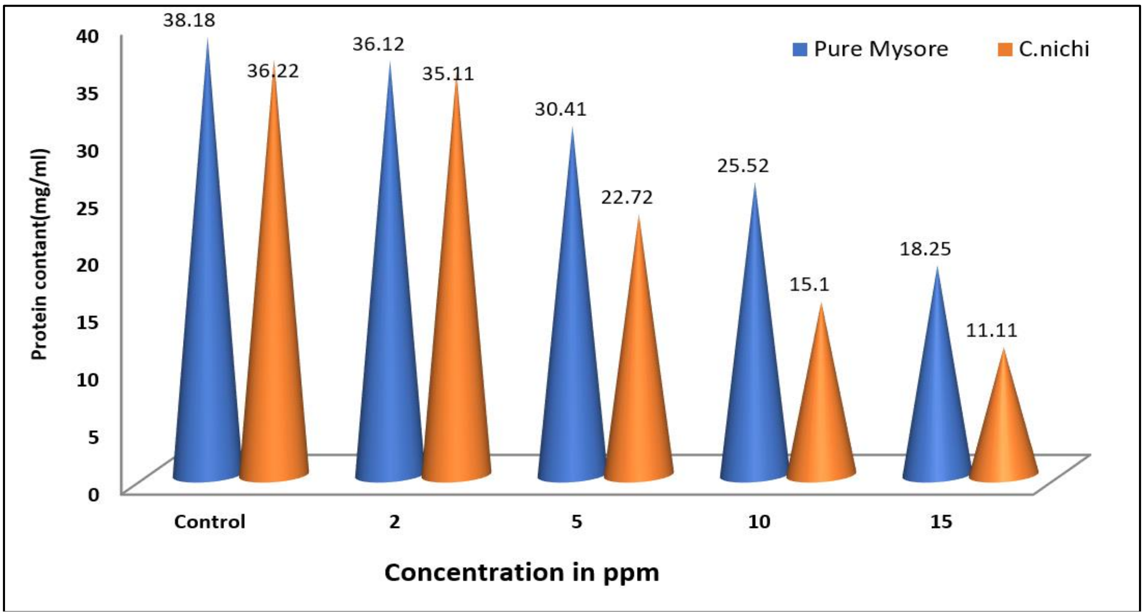

Fig 9: Graphical representation of the Protein content (mg/ml) in the whole body homogenate of the treated and control batches of the two multivoltine races.

Life span

The average adult lifespan of male and female moths from two multivoltine silkworm races, Pure Mysore and C-nichi, after exposure to different Dichlorvos concentrations of 2, 5, 10, and 15 ppm is depicted in table 5 and represented in a graph (Fig10, Fig 11). Both races experienced a steady decline in lifespan as pesticide levels increased, showing a harmful effect that depends on the dose. In the Pure Mysore race, female lifespan dropped from 6.80 days in the control group to 5.85 days at 15 ppm, while male lifespan fell from 5.20 to 4.83 days. Similarly, in the C-nichi race, female lifespan decreased from 5.91 days in the control group to 4.60 days, while male lifespan declined from 5.38 to 4.20 days.

Table 5: Mean adult life span in the control and treated batches the two multivoltine races.

Race	Concentration	Mean adult life span	
		Female	Male
Pure Mysore	Control	6.80±1.90	5.20±1.90
	2	6.20±1.00	5.10±1.38
	5	6.25±2.20	5.40±1.40
	10	6.00±1.20	4.93±2.10
	15	5.85±2.10	4.83±1.00
C-nichi	Control	5.91±1.81	5.38±1.98
	2	5.33±2.10	5.00±1.90
	5	5.30±2.20	4.96±1.80
	10	4.80±1.90	4.90±1.31
	15	4.60±1.98	4.20±2.03

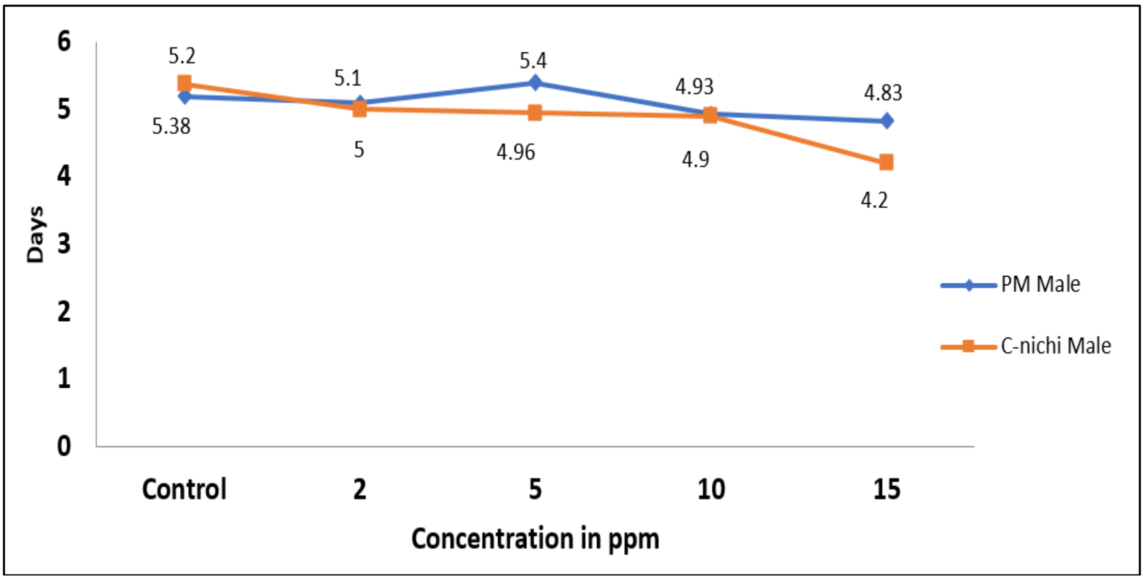

Fig 10: Mean adult life span of males of PM & C.nichi treated with different concentration of Dichlorvos.

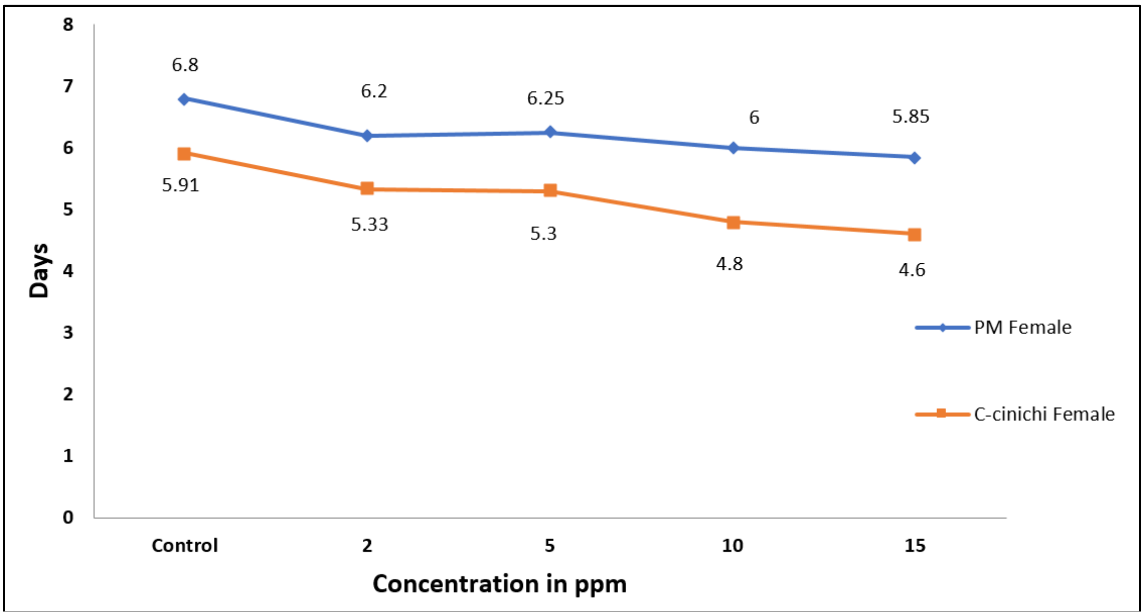

Fig 11: Mean adult life span of females of PM & C.nichi treated with different concentration of Dichlorvos.

Treatment with Dichlorvos

Higher concentrations of Dichlorvos (2-15 ppm) led to a dose-dependent rise in dominant lethal mutations in both the Pure Mysore and C-nichi silkworm races(table 6)represented in graph fig 12. In Pure Mysore, mutation rates increased from 1.26% (control) to 10.16% at 15 ppm. For C-nichi, the rates rose from 1.87% to 11.81%. Significant differences were noted between 10 and 15 ppm. Overall, C-nichi showed slightly greater sensitivity, suggesting that Dichlorvos had a stronger genotoxic effect in this race.

Table 6: Dominant lethal induced by Diclorvors in two multivoltine races

Race	Conc. In ppm	Total no of egg counted	Total no. of egg hatched	Hatching%	Total no of dead eggs	% of dominant lethal
Pure Mysore	Control	4480	4423	98.71	56	1.26
	2	4385	4323	98.50	62	1.43
	5	2298	2200	95.73	98	4.45
	10	2185	2060	94.27	125	6.06 *
	15	1983	1800	90.77	183	10.16 *
CD at 5%						0.28
C-nichi	Control	3963	3890	98.15	73	1.87
	2	4010	3927	97.93	83	2.11
	5	2612	2500	95.57	112	4.48
	10	2320	2140	92.24	180	8.41 *
	15	1230	1100	89.43	130	11.81 *
CD at 5%						0.32

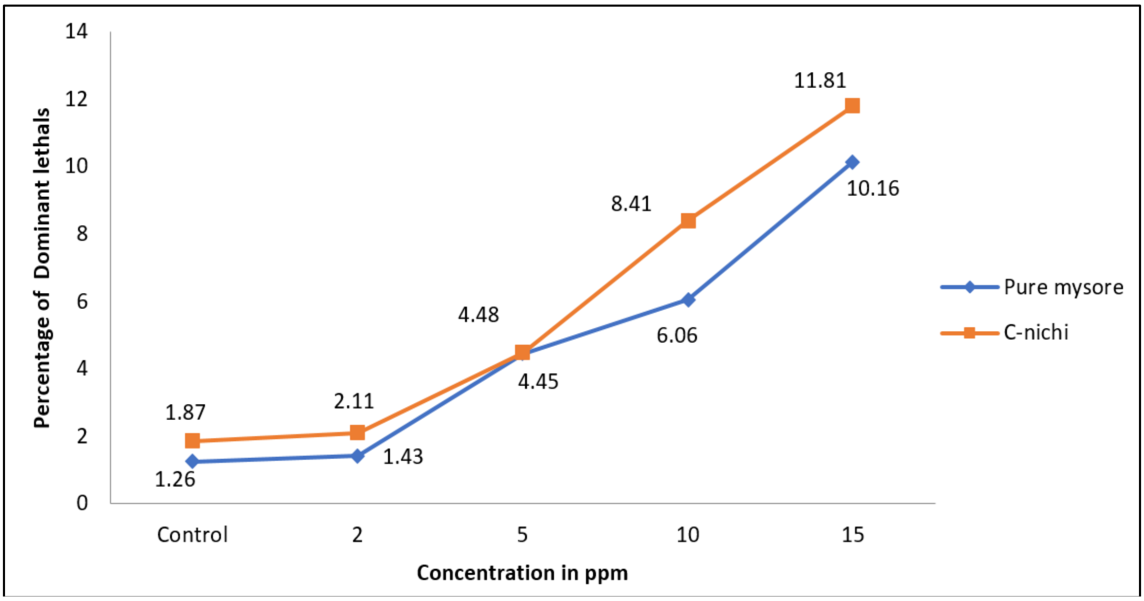

Fig 12: Graphical representation of effect of Dichlorvos on induction of dominant lethality in two races of silkworm *B. mori.*

Conclusion

The current study examined the toxic and genotoxic effects of Dichlorvos, an organophosphate pesticide, on two multivoltine silkworm races, *Bombyx mori*: Pure Mysore and C-nichi. The researchers used dominant lethal experiments to assess major economic features, protein content, adult longevity, and the potential for mutations.

The results showed that exposure to Dichlorvos significantly affected important biological and commercial factors in both silkworm races. Higher concentrations led to notable decreases in larval weight, cocoon and shell weights, shell ratio, filament length, and protein content. C-nichi was more sensitive than Pure Mysore. Adult longevity decreased, and the rates of dominant lethal mutations increased with higher pesticide concentrations. This highlights the negative impact of the chemical on reproductive and genetic stability. These findings highlight the important need for careful and controlled use of Dichlorvos in sericulture. While it effectively kills mulberry pests, its harmful effects on silkworm health and productivity suggest that we should prefer alternative, eco-friendly pest management methods to maintain sustainable sericulture practices. Additionally, this study confirms that *Bombyx mori* serves as a sensitive and reliable bioindicator in environmental toxicology research.

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