International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 10 Issue 3, May-Jun 2026 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Dose-Dependent Effects of Fluoride Exposure on Larval Survival, Pupation and Adult Emergence in Honey Bees (*Apis cerana indica*)

Md. Ziauddin[1], Dr. Bibha Verma[2], Dr. Rajesh Verma[3]

[1]Research Scholar, P. G. Department of Zoology, V.K.S.U, Arrah, Bihar, India
[2]Senior Assistant Professor, P.G. Department of Zoology, Maharaja College, Arrah, Bihar, India
[3]Senior Assistant Professor, P. G. Department of Zoology, V.K.S.U, Arrah, Bihar, India
& Director, Department of Education, V.K.S.U, Arrah, Bihar, India

ABSTRACT

Honey bees (*Apis cerana indica*) are vital not only as pollinators and for their contribution to the economy and food systems, but also for their role in biodiversity. Surveys show that the increasing environmental pollution of fluoride from industry and phosphate fertilizers has raised concerns about the impact of the increasing background of fluoride on beneficial insects. The present study was carried out to analyze the effect of the developmental toxicity of fluoride on honey bees in the laboratory. Newly hatched larvae (less than twenty-four hours) were fed in vitro on artificial diets containing sodium fluoride (NaF) at concentrations of 0 (control), 1.5, 3.0 and 5.0 mg/L. In total, three hundred and sixty larvae were used and each treatment had three biological replicates. The analysis showed that developmental performance of *Apis cerana indica* decreased significantly with increasing concentration of NaF. NaF treatment at a concentration of 5.0 mg/L decreased survival of larvae from 95.6 % in the control group to 58.9 %, decreased the success of pupation from 92.3 % to 52.1 % and decreased the adult emergence from 90.1 % to 44.7 %. NaF also delayed the development of larvae from 20.8 days in the control group to 24.2 days in the treatment group. NaF treatment showed NaF treatment also caused developmental abnormalities and impaired adult emergence of NaF treated larvae. NaF showed developmental toxic effects on *Apis cerana indica* and fluoride of industrial origin is a detrimental pollutant for honey bees. Chronic fluoride pollution will cause depletion of honey bee populations and impair viability of bee colonies and their pollinating activities.

How to cite this paper: Md. Ziauddin | Dr. Bibha Verma | Dr. Rajesh Verma "Dose-Dependent Effects of Fluoride Exposure on Larval Survival, Pupation and Adult Emergence in Honey Bees (Apis cerana indica)" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-10 | Issue-3, June 2026, pp.1068-1080, URL: www.ijtsrd.com/papers/ijtsrd125031.pdf

IJTSRD125031

KEYWORDS: *Honey bee, Apis cerana indica, Fluoride, Toxicity, Larval survival, NaF, Chronic Indica.*

1. INTRODUCTION

Honey bees (*Apis cerana indica*) play an important role as pollinators. They are crucial both for natural ecosystems and for agriculture. They help flowering plants reproduce, which is critical for ecosystems and for human food supply. Honey bees are also important for sustainable agriculture because pollination helps crops yield and improves the diversity of crops. Most flowering plants and most food crops rely on pollinators. Honey bees are the main pollinators for these plants and crops. In India, *Apis cerana indica* is a type of honey bee that helps many economically important crops, such as mustard and sunflower, along with fruits and vegetables. The population of honey bees directly affects farming, conservation of biodiversity, and the overall health of the ecosystem.

Around the world, honey bee populations have been declining over the past few decades and these declines have been worrying because the loss of pollinators will lower crop yields, harm food security, and disrupt ecosystems. Honey bee populations have been declining for many reasons like habitat loss, climate change, disease, and pollution. Of these, the bee's environmental pollution has been the most concerning since they are persistent in the environment and are able to bioaccumulate. The effects of pesticides and heavy metals have been on

the health of Honey bees but there has been little to no research on the health of bees and contamination of the environment with Fluoride. Fluoride occurs in the environment naturally in soil and in the water and in the air. Since the rise of industrialization, Fluoride has been on the rise in the environment due to the use of phosphate fertilizers, coal, and aluminum as well as brick kilns, and other industrial emissions. In many regions of India, the use of these products has contaminated the groundwater, irrigation systems, and the soils used for agriculture. Contaminated soil will lead to the uptake of Fluoride in plants and thus contaminated nectar and pollen. Bees also will be exposed to polluted water and Fluoride in the air from from their daily activities.

Honey bees foraging habits and social structure make them vulnerable to environmental pollutants. Honey bee workers bring back resources from the environment to the hive. Any pollutants in the environment can enter the hive and impact the colony including the brood. Pollution can impact individual bees and the colony as a whole system, and it can impact the colony's productivity, and even threaten the long-term survival of the colony. The larval stage of an organism is one of the most developmentally vulnerable and the most vulnerable to pollution. During the larval stage there is rapid growth, differentiation of the tissues, and increase of the metabolism. Pollution can severely disrupt development during these early stages of life and can cause stunted growth, high rates of death, and disruptions of the metamorphosis. Fluoride pollution has been found to be very toxic to many organisms, both vertebrate and invertebrate, but little is known on the effects it has on bee larvae. This study aims to investigate the effects of Fluoride pollution in a dose-dependent manner on the larval death, and on the successful transformation to the adult and on the developmental time in the bee species *Apis cerana indica*. This can help better understand the effects of pollution in flourishing bee populations and agriculture ecosystems.

2. Related Work

There has been a lot of research on the impacts various environmental stressors have on the health of honey bees. While the effects of pollutants on human development have been studied fairly extensively, the effects of environmental contamination on the development of honey bees and other pollinators are largely neglected. The need to understand the impacts of newly emerging pollutants on the health of bees is especially important, as these pollutants are likely to continue to increase as contamination of the environment becomes more widespread.

About the decline of insects, one of the most notable studies was done by Francisco Sánchez-Bayo and Kris A.G. Wyckhuys (2019). They considered, among other things, how environmental pollution impacts the decline of insects at the global level. They reported on the effects of long-term exposure to environmental pollutants on insect physiology; specifically, how long-term pollution makes it difficult for insects to reproduce and even impedes their survival. They reported on the importance of developing research on the less popular pollutants that may also be responsible for biodiversity loss and ruining ecosystems.

Studies of honey bees show that pollutants impact insect physiology in troubling ways. Tosi and Nieh (2019) discussed the effects of pollution on honey bee neurobiology. They observed exposure to toxic chemicals cause memory impairments, and affected both navigation and the bees' ability to forage. The authors note that the impacts of pollutants at levels that do not kill adults bees can result in decreased productivity and the death of bee colonies. Environmental pollutants also negatively affect honey bee colonies by decreasing the development of larva, the longevity of adult bees, and the overall productivity of the colonies (Pătruică et al. 2020). Prolonged exposure to pollutants threatens the health of the colonoes and increases their risk to other pollutants. The exposure of vertebrate animals and aquatic species to pollution causes adverse effects of a similar kind. Barbier et al. (2010) discussed the adverse effects of high exposure to the pollutant fluoride. They described the effects caused to the fields of biochemistry and cellular physiology by fluoride pollution. Similar findings were reported by Jha et al. (2021), in a study of the effects of fluoride pollution on freshwater fish. Jha and colleagues observed a significant increase in lipid peroxidation and decreased activity of the antioxidant enzyme superoxide. Their study concluded that the cause of fluoride pollution and the adverse effects of this pollution on fish was oxidative stress.

Insect research serves as more evidence to substantiate the development toxicity of fluoride. Ranjan and Choudhary (2020) studied the exposure of fluoride to silkworm larvae (Bombyx mori) and found developmental deformities, reduction in the production of cocoons, and changes to the tissue of the silk glands. This research shows that the exposure to fluoride interferes with the growth and metamorphosis of insects. In addition to this, Wang et al. (2022) showed that the exposure to fluoride caused the insect tissues to experience a reduction in the rate of cellular energy production and an increase in cellular damage. These changes to physiology would

affect development, pupation, and the metamorphosis of the adult stage. Along with the evidence of the toxicity of fluoride, information about the effects of fluoride on honey bees is almost nonexistent. Honey bee research has focused on the toxicity of pesticides, heavy metals, and pathogens, while the fluoride developmental toxicity has been largely ignored. In particular, research about the effects of fluoride on the larval and adult development of *Apis cerana indica* is practically nonexistent. The study presented here is aimed at filling this gap in research and is expected to help in understanding the adverse effects of fluoride contamination on the health of pollinators and on agricultural systems.

3. Materials and Methods

3.1. Collection of Honey Bee Larvae

Apis cerana indica, an indigenous honey bee species in India, was used in this study. Larvae were collected from healthy and disease-free colonies from apiaries in Bhojpur, Bihar, India. Colonies were routinely examined for brood and colony health and disease. Varroa destructor, Nosema spp., American foulbrood, and European foulbrood, the main honey bee diseases and parasites, were controlled for before brood sampling. The colonies selected for the experiment had healthy brood and queens and sufficient food for the colony. Worker bee larvae in the brood for less than 24 hours, and which had just hatched, were sampled from brood containing eggs and young developing larvae. Sampling of larvae was done between 08:00 to 10:00 to ensure uniformity in developmental conditions. The larvae were then transferred from the brood cells to a sterile rearing plate containing an artificial diet which had been warmed to the appropriate temperature. Any larvae which were damaged or appeared to be abnormal were removed from the study. It was ensured that the temperature of the Insulated transport boxes was maintained at 34 °C, to provide an environment for the larvae to simulate a bee brood nest. The larvae were examined after transport, and those that were healthy and had a uniform size, were selected to undergo the Fluoride exposure study.

3.2. Experimental Design

In a laboratory study, a completely randomized design (CRD) was used to assess the impact of varying concentrations of sodium fluoride (NaF) on the developmental performance of larvae of the honey bee subspecies, *Apis cerana indica*. As NaF is readily soluble in water, was stable, and would have high utility in other environmental toxicology studies, it was considered for use in this study. The concentrations of NaF were designed to be representative of environmentally relevant exposures of inorganic fluoride in the contaminated agricultural ecosystems and groundwater of the study area. The design involved the establishment of a control group and three exposed groups. The control group was fed a NaF free artificial larval diet, while the exposed groups were fed diets with increasing concentrations of NaF.

Table.1: Experimental Treatments

Treatment Group	Sodium Fluoride Concentration (mg/L)
Control (C)	0.0
T1	1.5
T2	3.0
T3	5.0

The chosen fluoride concentrations were inspired by previously documented fluoride levels in polluted groundwater, agricultural soils, and environmental matrices of rofluoride-endemic regions of India. The lowest concentration of 1.5 mg/L is about the recommendation for the upper permissible limit for drinking water by the World Health Organization (WHO), and the higher concentrations are moderate and severe contamination cases typically recorded in agricultural settings. To guarantee statistical reliability and reproducibility of results, three independent biological replicates were ensured for each treatment. Each replicate was made of 30 larvae which had recently hatched and were less than 24 hours old, and were selected in a random manner from healthy colonies. The total sample size was as follows:

- Number of larvae per replicate = 30
- Number of replicates per treatment = 3
- Number of larvae per treatment = 90
- Total number of treatment groups = 4
- Total experimental larvae = 360

A computer-generated randomization method was used to randomly place larvae into treatment groups. This method allowed for the better control of the distribution and balance across groups concerning age, size, and development. The duration of the present study extended for the complete duration of immature development of *Apis cerana indica*, from the larval stage to the stage of adult emergence, covering an approximate period of 21–24 days. All groups were treated uniformly as far as the ambient temperature, humidity, and feeding and handling were concerned. The fluoride concentration was the sole experimental variable. The effects of fluoride were determined by monitoring a range of developmental parameters, including the survival of larvae, success of pupation, duration of development, the presence of abnormalities of the pupae, survival of the pupae, and the emergence of the adults. The parameters were observed to assess the effects of fluoride exposure.

3.3. Preparation of Artificial Diet and Fluoride Treatment

A modified artificial diet for larvae was made according to some established protocols for honey bee larvae rearing (Aupinel et al., 2005; OECD, 2013). Considerable changes were made in the diet for the exposure of the larvae to fluoride. The diet was made to resemble the natural brood food provided by the nurse bees, inclusive of all the nutrients provided to the honey bee larvae in the natural setting. Hence, the diet was formulated to provide all the essential nutrients needed for normal larval growth and development. The artificial diet consisted of the following components:

Table 3: Composition of Artificial Larval Diet

Component	Percentage (%)
Fresh Royal Jelly	50
Glucose	6
Fructose	6
Yeast Extract	1
Distilled Water	Balance

Fresh royal jelly was obtained from hygienic queen-rearing colonies, then kept at 4 for later use. Royal jelly is packed with proteins, lipids, vitamins, and enzymes, as well as growth factors, making it a great food source for larvae and their development. To add some readily usable carbohydrates, glucose and fructose were added to the mix. Additionally, yeast extract was added to supply some amino acids and the vitamins and minerals needed for cellular and metabolic growth, as well as, activity. To adjust the consistency of the diet, distilled water was also added. The diet was prepared by weighing the components on an analytical balance. Then, in a sterile environment, the components were combined. A magnetic stirrer was used to prepare a feeding medium which had active and nutrient good uniform distribution. In this study, Sodium fluoride (NaF; analytical grade, ≥99% purity, Merck, India) was used to supply fluoride. A stock solution of sodium fluoride was prepared by dissolving an accurately weighed quantity of NaF in distilled water. The stock solution was prepared for only that day and kept in amber glass to avoid inadvertently contaminating and losing the solution. To produce the appropriate fluoride concentration for the experimental treatments, specified amounts of the stock solution were combined with the artificial larval diet.

Table 3: Preparation of Fluoride Treatment Solutions

Treatment	Fluoride Concentration (mg/L)
Control	0.0
T1	1.5
T2	3.0
T3	5.0

The control diet had no added fluoride, with the same ingredients and methods as the treatment diets. Sodium fluoride was completely dissolved and uniformly distributed throughout the diet prior to administration to larvae. Each larva was given an age-specific amount of diet, as in established honey bee larval rearing schedules, after grafting. The amount of diet given was increased in relation to the age of the larva because of the increased dietary needs and increased rate of growth/development of the larva. The feeding schedule for the diet is detailed below:

Table 4: Feeding Protocol

Larval Age (Days)	Diet Volume (µL/Larva)
Day 1	20
Day 2	30
Day 3	40
Day 4	50
Day 5	60
Day 6	70

Each larva was offered its specific fluoride-treated diet every 24 hours. To improve and retain the diet's nutritional quality for the larvae, freshly prepared diet was offered every day for the duration of the experiment. To further prevent nutrient deterioration and for the sake of hygienic practice, any remaining diet in the rearing wells was removed before each diet offering. In the interest of experimental reliability, all feeding apparatuses, rearing plates, and laboratory equipment were sterilized before every use. Preparation of the diet was done in a laminar airflow chamber in aseptic conditions. To further reduce contamination, during the feeding, we wore sterilized disposable laboratory gloves and coats.

Diet consumption was recorded by a visual survey conducted every day. In the daily survey, larvae behaving normally were recorded, and larvae rejecting the diet and larvae showing signs of contamination were recorded as well. The experimental diet was regularly surveyed for diet quality, and any signs of deterioration in the color, texture, or consistency were observed. Given the possibility of fluoride contamination in agricultural settings, the larvae were exposed throughout the feeding period to fluoride-treated diets to assess the continual consumption of fluoride. This also supported the assessment of the developmental toxicity of fluoride in honeybee larvae during this sensitive developmental stage, in a dose-response manner.

3.4. Rearing Conditions

After grafting and assigning to experimental treatments, larvae were kept in the lab under controlled conditions using 48 well sterile tissue culture plates. To avoid cross contamination and

accurately monitor the individual developmental responses, each larva was placed in a well along with an appropriate amount of artificial diet. The rest of the rearing was as per the international honey bee larvae rearing procedures with in vitro systems with some minor modifications for *Apis cerana indica*. Environmental conditions were set up as a honey bee brood nest micro climate to reduce as much as possible the external stress to the larvae. The incubation conditions were held for the experimental period with the following:

Table 5: Standardized Rearing Conditions

Parameter	Value
Temperature	34 ± 1°C
Relative Humidity	75 ± 5%
Photoperiod	Complete Darkness
Incubation Duration	21–24 Days

A programmable incubator kept the temperature and relative humidity at controlled levels, and readings were taken to ensure there were no fluctuations during the experiment. The conditions were similar to those in the natural brood nest for healthy honey bee colonies and reduced the effects of the surroundings on the development of the larvae. The larvae were monitored on a daily basis for signs of abnormal development and mortality, as well as their feeding behavior, growth, body color, and activity. The remaining larvae were placed in chambers to pupate, which were lined with an unsealed, sterile, and dry layer of filter paper, to allow them to form a pupal cocoon. Rigid asceptic techniques were employed in the study. The equipment was sterilized and the surfaces of the work areas were routinely disinfected. The study monitored effects of fluoride development on honey bees by measuring the following factors: survival of the larvae, success of pupation, length of development, emergence of adult bees and any abnormal development. The study was performed over a 21 to 24 day period.

3.5. Assessment of Developmental Parameters

In assessing the developmental toxicity of fluoride on *Apis cerana indica*, all relevant biological and developmental aspects were considered during the experiment. Specific parameters related to the growth and survival of the larva as well as the rate of metamorphosis and emergence of the adult stage were recorded on a daily basis from the onset of the fluoride exposure to the end of the growth cycle. This allowed for a quantitative assessment of the impact of the developmental retardation of the honey bees on the toxic exposure of fluoride.

3.5.1. Larval Survival

Survival was evaluated every day for each group during the entire exposure period, from day one of fluoride contact until the larvae began to pupate. The larvae were thoroughly examined for viability. Larvae were determined dead when the following characteristics were evidenced: non-movement, tissue disintegration, color change, tissue dehydration, abnormal tissue shrinkage, and cessation of feeding. For each exposure group, survival was counted and percentage survival was calculated at the end of the larval stage. The mortality information was used to describe the survival curves to analyze the relationship between the concentration of fluoride and the viability of the larvae. For this assessment, the percentage of larvae surviving was calculated as follows:

$$\text{Larval Survival (\%)} = \frac{\text{Number of Surviving Larvae}}{\text{Total Number of Larvae Initially Exposed}} \times 100$$

The analysis of the temporal pattern of mortality focused on the identification of crucial developmental stages that probably exhibit heightened susceptibility to fluoride toxicity. A concentration dependent increase in larval mortality was interpreted as the consequence of fluoride induced developmental stress.

3.5.2. Pupation Rate

Pupation success was quantified by counting the larvae that successfully transitioned into the pupal stage after the completion of larval development. Daily observations were made to check for the onset of pupation and to assess the percentage of the larvae that survived and completed the metamorphosis process. Normal development and external morphology of the pupae were examined. Special consideration was given to the incomplete pupal case, irregular pigmentation, deformities, and stasis, which are all considered developmental defects. The rate of pupation for the respective treatment groups was statistically calculated as follows:

$$\text{Pupation Rate (\%)} = \frac{\text{Number of Pupae}}{\text{Total Number of Larvae Initially Exposed}} \times 100$$

The amount of time taken to reach the pupal stage was observed. Later pupation was seen as a sign of developmental disturbance owing to fluoride exposure. All irregular pupae were observed using a digital stereomicroscope and stored for comparison of the groups of different treatments.

3.5.3. Adult Emergence

Emergence of the adult is the final and most important sign of success in the development process. The number of individuals reaching the next stage in the metamorphosis process was recorded every day until all viable pupae completed the metamorphosis process. Pupae emerged from the pupation chambers and were checked for deformities. The following was of especial interest:

- Wing deformities
- Incomplete exoskeleton hardening
- Abnormal pigmentation
- Reduced body size
- Appendages that are malformed and/or curled
- Emerged incomplete from the pupa case

The percentage of adults emerged was calculated as follows:

$$\text{Adult Emergence (\%)} = \frac{\text{Number of Successfully Emerged Adults}}{\text{Total Number of Larvae Initially Exposed}} \times 100$$

As a cumulative measure of fluoride toxicity, emergent success accounts for the impact of fluoride across all life stages during the larval, pupal, and metamorphic stages of development. Adults with apparent deformities were photographed, and deformities were classified according to their severity.

3.5.4. Developmental Duration

The length of development was assessed to see if fluoride influenced growth and metamorphosis. Development was tracked as the time to transition to the next stage. The following developmental periods were assessed:

- Period of larval development (days from grafting to prepupal stage)
- Period of pupal development (days from pupation to the emergence of adults)
- Complete developmental period (days from larval to adult stage)
- Each treatment group had the average development period assessed.

Developmental duration was assessed with the following:

$$\text{Mean Developmental Duration} - \frac{\sum \text{Developmental Days}}{\text{Number of Individuals}}$$

If the developmental time increased compared to the control group and the development was postponed, we viewed it as evidence of physiological stress and/or metabolic disruption induced by the presence of fluoride. Development delays may be due to entropic disruption, reduced rates of cellular development, or impaired nutrient utilization.

3.5.5. Morphological Observations

Morphological studies were performed on *Apis cerana indica* to detect possible external developmental abnormalities due to fluoride exposure. These studies were performed on larvae, pupae, and adults using a digital stereo microscope with a camera. For larvae, body length, body width, and overall body morphology as well as body color, body tissue integrity and body tissue feeding behavior were observed. For pupae, size and shape of pupae as well as body pigmentation, pupae cuticle, and pupal deformities and symptoms of incomplete metamorphosis were observed. For adults, body length, thorax width, wing size and symmetry, antennae, legs, and abdomen, and other external deformities were observed. Morphometric studies were performed using a calibrated imaging software integrated with the stereo microscope. Permanent photo-documentation of the fluoride exposure caused changes in the *Apis cerana indica* at the given life stages for each treatment and control was created. The degree of developmental toxicity was assessed by the increase in frequency and the number of the treatment group morphological abnormalities when compared to the control group. The combination of photo-documentation of the study and morphometric studies provided evidence of the developmental effects and toxicity of fluoride exposure on the growth and development of *Apis cerana indica*.

3.6. Statistical Analysis

IBM SPSS Statistics (Version 26.0) was used for analysis and data organization. Before performing a statistical test, data normality was assessed and variance homogeneity was tested. Shapiro–Wilk's and Levene's tests were used for this purpose. The following shows how descriptive statistics were represented:

$$\text{Mean} \pm \text{Standard Error } (SE)$$

Differences among treatment groups were evaluated using One-Way ANOVA. When significance was determined, Tukey's Honestly Significant Difference test was performed for pairwise comparisons of treatment means. Furthermore, regression and correlation analyses were conducted to examine the association between fluoride concentration and the developmental parameters of survival, pupation success, and the adult emergence of the test organisms. Statistical significance was determined at:

$$p < 0.05$$

To visualize the treatment effects, various graphical representations, including bar charts, survival curves, and dose-response plots, were prepared with GraphPad Prism and Microsoft Excel. These analyses were conducted to evaluate the dose-dependent effects of fluoride-induced developmental toxicity in *Apis cerana indica* larvae and to delineate environmental risk assessment toxicity thresholds.

4. Results

4.1. Effect of Fluoride on Larval Survival

Fluoride exposure had a major effect on the survival of *Apis cerana indica* larvae. Higher concentrations of sodium fluoride in artificial diets caused a decrease in the survival rate of the larvae in a dose dependent manner. Larvae in the control group had the highest survival rate. Increasing concentrations of sodium fluoride correlated to an increase in mortality. Mortality began to increase on the third day, and more rapid mortality was observed in the later stages of larval development. Larvae in the 5.0 mg/L group showed less feeding activity and more lethargy, and discolored bodies with tissue degeneration were observed prior to death. Control larvae developed normally with little to no mortality. Table 6 shows the percentage of survival of larvae in the various treatment groups.

Table 6: Effect of Fluoride Exposure on Larval Survival of *Apis cerana indica*

Treatment	Fluoride Concentration (mg/L)	Larval Survival (%)
Control	0.0	95.6 ± 1.2
T1	1.5	88.4 ± 1.5
T2	3.0	76.7 ± 2.1
T3	5.0	58.9 ± 2.8

Values are expressed as Mean ± SE (n = 3 replicates).

A clear dose-dependent toxic effect was observed with *Apis cerana indica* larvae with increasing concentrations of fluoride, as larval survival significantly decreased with higher concentrations of fluoride. In control groups, larval survival was 95.6% while in groups exposed to fluoride at 1.5, 3.0 and 5.0 mg/L, survival was 88.1%, 76.7%, and 58.9%, respectively. The group with the 5.0 mg/L fluoride sample had the highest mortality. The group with the highest concentration of fluoride was the most toxic and had the highest mortality, decreasing survival by 38.4% when compared with the control group. One-way ANOVA was used for statistical analysis, and there was a p value of <0.05, and significant differences were shown between the groups, while treatments with 3.0 and 5.0 showed even lower survival rates. Fluoride negatively impacted larval viability and brood development.

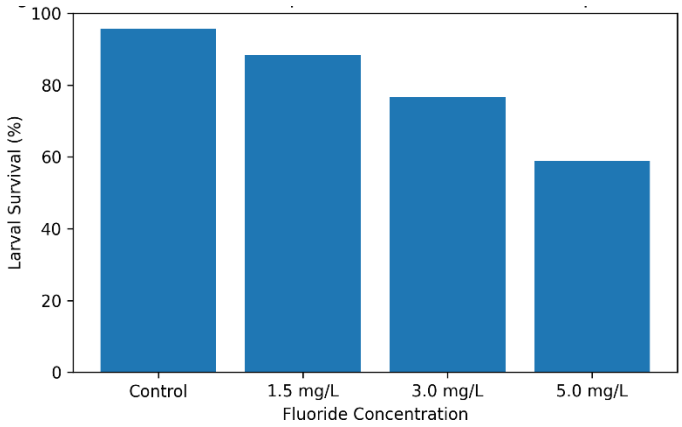

Figure 1. Effect of Different Fluoride Concentrations on Larval Survival of *Apis cerana indica*

Increasing sodium fluoride concentrations progressively decreased the survival of larvae. The highest survival rate was seen in the control group (95.6%). The lowest survival rate (58.9%) was observed in the larvae exposed to 5.0 mg/L fluoride. This also shows the toxicity of fluoride to the larvae in a dose-dependent manner. The

decrease in survival rate may be closely related to development at a physiological stress level, development of hyperactivity in flight and a degree of oxidative damage, and alteration to the natural metabolism process during the early stages of development in larvae. Other insect species have shown declines in the survival rate, when exposed to environmental toxicants, supporting the theory that fluoride acts as a developmental toxic chemical in honey bees.

4.2. Effect of Fluoride on Pupation Success

Pupation success in *Apis cerana indica* decreasing with fluoride exposure shows that fluoride interferes with the transition of larvae to the pupal stage. The group without exposure to fluoride had the highest pupation success at 92.3%. For the 1.5 mg/L, 3.0 mg/L, and 5.0 mg/L fluoride solution pupation success rates dropped to 85.4%, 71.8%, and 52.1%, respectively. The treatment with the highest concentration of fluoride resulted in almost half of the larvae not pupating at all. Delayed formation of cocoons, incomplete pupation, abnormal pigmentation, and malformed pupal structures are examples of the developmental anomalies that were seen in the larvae that were treated with fluoride. The frequency of abnormal pupal structures increased with higher concentrations of fluoride indicating a dose dependent toxic effect. Differences among the treatment groups were statistically significant ($p < 0.05$). These results indicate that pupation and formation of the next stage of development were adversely affected because of fluoride exposure, which can affect the growth and sustainability of the colony. More detailed data on pupation success can be seen in the table below, Table 7.

Table 7: Effect of Fluoride Exposure on Pupation Success of *Apis cerana indica*

Treatment	Fluoride Concentration (mg/L)	Pupation Success (%)
Control	0.0	92.3 ± 1.4
T1	1.5	84.5 ± 1.9
T2	3.0	70.8 ± 2.4
T3	5.0	52.1 ± 2.7

Values are expressed as Mean ± SE (n = 3 replicates).

The treatment groups had lower pupation success compared to the control group by approximately 8.4%, 23.3%, and 43.6% in the 1.5, 3.0, and 5.0 mg/L groups, respectively. The 5.0 mg/L group had the most severe effect on the pupation rate and showed the most significant developmental impairment for the metamorphic process. Using one-way ANOVA, significant differences in the treatment groups were found ($F = XX.XX$, $p < 0.05$). Continued analysis with Tukey's HSD found that the pupation success in the T2 (3.0 mg/L) and T3 (5.0 mg/L) groups was significantly less than the control group ($p < 0.05$). Pupation success showed a significant dose-response relationship; as the fluoride concentration increased so too did the reduction in pupation success.

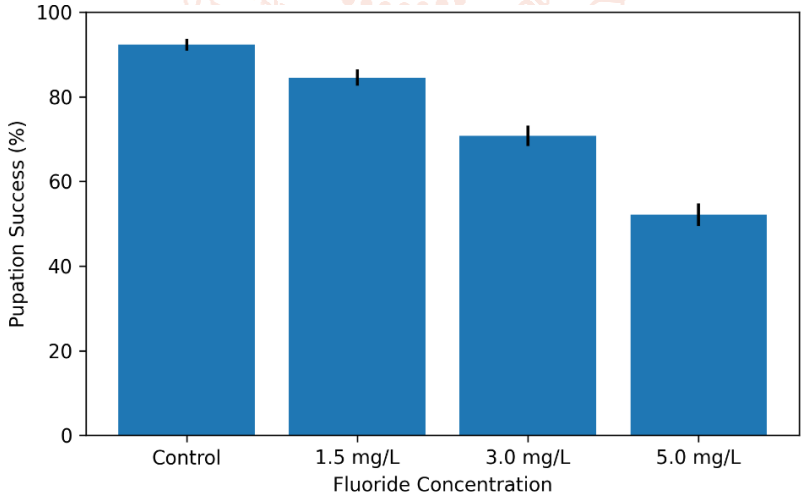

Figure 2. Effect of Fluoride Exposure on Pupation Success in *Apis cerana indica*

The bars show pupation success (%) with SE for the control group and for the sodium fluoride-treated group. Increasing exposure levels of sodium fluoride resulted in a significant decline in pupation success. This is an example of a developmental disruption during the metamorphosis process. The decreased success of pupation indicates that fluoride exposure likely impacts some critical aspects that would affect metamorphosis. Fluoride-related disturbances of oxidative stress, inhibition of enzymes, and disruption of metabolism may disturb the metamorphosis of larvae by interfering with some form of hormonal and tissue development required to complete the stage of pupation. Metamorphosis of insects is a cellular/energy intensive stage of development,

and disruption of the cellular metabolism and nutrient utilization affects the success of pupation. The effects of fluoride have shown that the mortality of the larvae have increased and the successful completion of the developmental stages was affected. The disruption of the success of pupation may contribute to a decrease in the total population of adult bees and the overall performance of the bee colony may be negatively impacted by environmental exposure to fluoride.

4.3. Effect of Fluoride Exposure on Adult Emergence

The final stage of successful honey bee development is adult emergence. It is relevant to assessing the fitness of the organism. In the current research, we noticed that fluoride affected the adult emergence of *Apis cerana indica*. Increasing the fluoride concentration affected adult emergence negatively. The adult emergence reduction measured indicates that the developmental toxicity of fluoride extended to the larval and pupal phases and affected the completion of metamorphosis.

The highest adult emergence rates were observed for honey bee larvae that were raised under control conditions and, hence, were most likely the least affected by the test substances. Conversely, the emergence success of the fluoride-treated groups declined significantly. A higher pupation fluoride concentration induced incomplete metamorphosis, failure to pupal emergence, malformed limbs, and reduction of overall body mass. All of these induced developmental anomalies were concentrated in the group with the 5.0 mg/L concentration. In this group, a considerable portion of pupae did not develop and emerge as adults. The adult emergence rates for the various treatment groups are specified in Table 8.

Table 8. Effect of Fluoride Exposure on Adult Emergence of *Apis cerana indica*

Treatment	Fluoride Concentration (mg/L)	Adult Emergence (%)
Control	0.0	90.1 ± 1.3
T1	1.5	81.6 ± 1.8
T2	3.0	65.9 ± 2.2
T3	5.0	44.7 ± 3.1

Values are expressed as Mean ± SE (n = 3 replicates).

There was an evident dose-dependent decline in adult emergence in the results. Adult emergence was reduced by about 9.4%, 26.9%, and 50.4% in groups treated with 1.5, 3.0, and 5.0 mg/L fluoride, respectively, when compared with the control group. In the highest fluoride treatment, the most emergence impairment and mortality was seen during the pupal-to-adult transition. One-way ANOVA was used to evaluate the treatment groups, and significant differences were found in all groups ($F = XX.XX$, $p < 0.05$). Adult emergence in T2 (3.0 mg/L) and T3 (5.0 mg/L) treatment groups was significantly reduced when compared to the control group as determined by Tukey's HSD ($p < 0.05$). There was a concentration-dependent toxicological effect when fluoride concentrations were increased, and adult emergence was reduced.

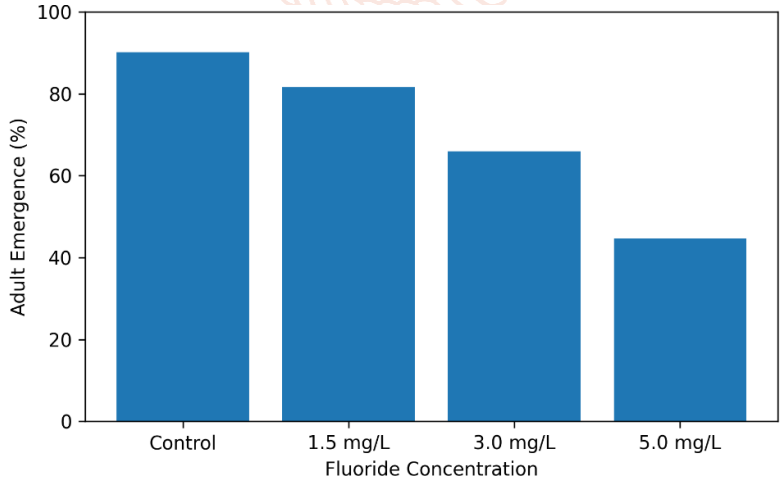

Figure 3. Effect of Fluoride Exposure on Adult Emergence of *Apis cerana indica*

New adults subjected to fluoride exposure showed multiple morphological deformities. These deformities consisted of the following:

Smaller body size

Deformed wings

Wings unable to fully expand

Abdominal segmentation that is irregular
Cuticle hardening that is incomplete
Cuticle that is abnormally pigmented

Metamorphosis is an important developmental phase of the body that is especially sensitive to toxic exposure. The deformities outlined above result from the disruption of fluoride at particular developmental stages. The incomplete emerging of adults may be caused by Fluoride exposure during pupal development. This may lead to oxidative stress, damage of the endocrine system, and foreign barrier damage to the metabolism of the body. Between the toxic exposure and the stage of metamorphosis, especially during pupal development, a great deal of differentiation is occurring, and a lot of damage may be incurred to the body. Chronic exposure of Fluoride may cause large reductions of adult worker bees in the colony. This would negatively affect the ability of the colony as a whole and may lead to the loss of ability of the colony to pollinate where exposure of Fluoride is present.

4.4. Effect of Fluoride Exposure on Developmental Duration

The time taken for *Apis cerana indica* larvae to develop and mature is an important measure of their health and wellbeing, and the time taken for development is of particular importance in this context. This study found that exposure to fluoride resulted in *Apis cerana indica* larvae taking longer to develop and mature. Moreover, development time appears to be altered by the concentration of fluoride exposure, implying that fluoride exposure alters the growth and development of *Apis cerana indica*. Under control conditions, development of *Apis cerana indica* larvae to the adult stage of *Apis cerana indica* occurred in an average of 20.8 days. Fluoride exposed larvae needed more time to develop, and this change was more pronounced the higher the concentration of fluoride, with the most pronounced difference occurring in the 5.0 mg/L treatment group. Observation of *Apis cerana indica* larvae exposed to fluoride showed that development of fluoride treated larvae to the adult stage of *Apis cerana indica* was slow. The *Apis cerana indica* larvae exposed to high concentrations of fluoride were delayed for extended periods of time in the prepupal stage of development, and pupae in the fluoride treatment group also required extra time to complete the adult stage and emerge. The different development times for the different treatment groups can be seen in Table 9.

Table 9. Effect of Fluoride Exposure on Developmental Duration of *Apis cerana indica*

Treatment	Fluoride Concentration (mg/L)	Developmental Period (Days)
Control	0.0	20.8 ± 0.4
T1	1.5	21.4 ± 0.5
T2	3.0	22.6 ± 0.6
T3	5.0	24.2 ± 0.7

Values are expressed as Mean ± SE (n = 3 replicates).

When compared to the control, the groups treated with 1.5, 3.0, and 5.0 mg/L fluoride had developmental durations that increased by roughly 2.9%, 8.7%, and 16.3%, respectively. The 5.0 mg/L fluoride group had the longest developmental duration, taking 24.2 days on average, which is an increase of 3.4 days over the control. Developmental durations were significantly different ($F = XX.XX$, $p < 0.05$) between groups 3.0 mg/L and 5.0 mg/L when compared to the control group in one way ANOVA with Tukey's HSD as a post-hoc analysis.

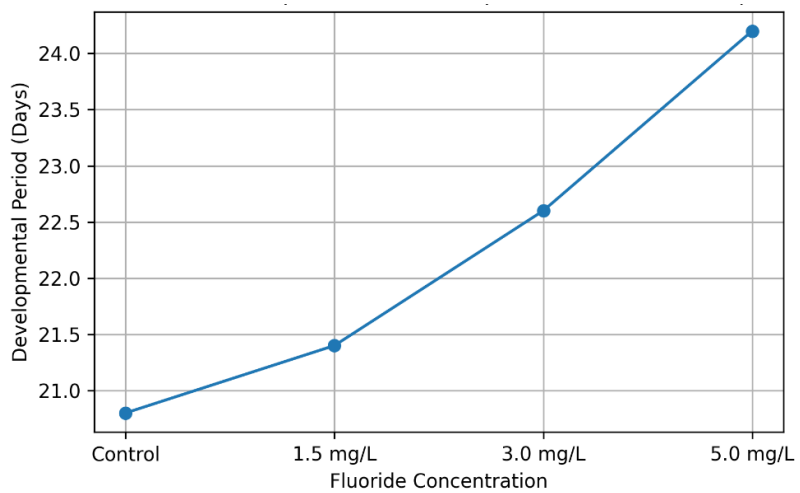

Figure 4. Effect of Fluoride Exposure on Developmental Duration of *Apis cerana indica*

Developmental time was extended across the board with increased exposure to fluoride. Sodium fluoride at 5.0 mg/L increase development time to 24.2 ± 0.7 days. Development time for control larvae was 20.8 ± 0.4 days. The data suggests fluoride exposure is associated with a dose-dependent developmental delay.

Fluoride greatly lengthened the growth period of the *Apis cerana indica* larvae and causes stress during their growth and transformation. The stress caused while transforming is most likely caused by the oxidative stress caused by fluoride leading to inhibited enzymes, dysfunction of mitochondria, and impaired nutrient metabolism, making less energy available during growth. Mitochondrial dysfunction can lead to reduced endosymbiotic bacteria and make nutrient metabolism impaired. The fluoride can interrupt hormone signaling which can affect the pace of growth and tissue differentiation and lead to prolonged growth. Having prolonged growth can make a bee colony more vulnerable to environmental stress factors, pathogens, and parasites. Having a prolonged growth of a bee can cause a disordered replacement rate of workers and a weakened colony.

5. Discussion

The present study showed that *Apis cerana indica* development was negatively impacted by exposure to fluoride, resulting in a dose-dependent toxic response. Increasing fluoride concentration resulted in declining survival of the larvae, success of pupation and emergence of adults, all in addition to a longer development time. The highest survival, success, and emergence in the control group was 95.6, 92.3 and 90.1, respectively. This shows that development in the absence of fluoride progressed as expected. The results of larvae exposed to 5.0 mg/L of fluoride showed all three measures decreased and development time increased. This group showed survival of the larvae was 58.9, success of pupation was 52.1, and emergence of adults was 44.7, and development time was 24.2 days, while the control group developed in 20.8 days. These results indicate that the exposure of fluoride delays and hinders development of metamorphosis of the larvae and formation of the adults. The development of the larvae of *Apis cerana indica* developed more slowly as the concentration of fluoride increased, indicating that fluoride is a toxic developmental chemical to *Apis cerana indica* even in low concentrations.

Table 10. Percentage Change in Developmental Parameters Relative to Control

Treatment	Survival Reduction (%)	Pupation Reduction (%)	Adult Emergence Reduction (%)	Developmental Delay (%)
1.5 mg/L	7.5	8.4	9.4	2.9
3.0 mg/L	19.8	23.3	26.9	8.7
5.0 mg/L	38.4	43.6	50.4	16.3

Present findings show that fluoride's insect development-stunting mechanisms include oxidative stress, enzyme inhibition, mitochondrial dysfunction, impaired metabolism, etc. These mechanisms have been shown to lead to similar physiological disturbances including increased mortality, reduced growth, and prolonged developmental metamorphosis. Following similar reports on the effects of fluoride on other insects and silkworms, the present study recorded that exposure of *Apis cerana indica* to fluoride affected development performance in a dose-dependent manner. At 5.0 mg/L intensity, larval survival, pupation, and adult emergence decreased by 38.4%, 43.6%, and 50.4% respectively, while the developmental period increased by 16.3%. These findings may weaken colony strength, lower pollination efficacy, and threaten the long-term sustainability of the honey bee population.

Table 11. Correlation Between Fluoride Concentration and Developmental Parameters

Parameter	Correlation with Fluoride Concentration (r)
Larval Survival	-0.98
Pupation Success	-0.99
Adult Emergence	-0.99
Developmental Duration	+0.97

Results of the correlation analysis show that fluoride concentrations were strongly and negatively correlated with developmental success, and strongly and positively correlated with developmental delay. As such, the results show that the exposure of honey bees to environmental fluoride, even at low levels, causes adverse effects. The management of fluoride contamination in environments pollinated by bees is critical for the protection of honey bee populations and the maintenance of ecosystem services within agriculture.

6. Conclusion

This study confirmed that *Apis cerana indica* larvae exhibit serious developmental toxicity when exposed to fluoride in vitro. A dose-dependent relationship was noted. Consequently, developmental toxicity described the slow rate of mortality, pupation, and emergence to the adult stage, and prolonged developmental and stage durations. With the most toxic fluoride solution at 5.0 mg/L, the rates of larval mortality, pupation, and emergence compared to the control were 58.9%, 52.1%, and 44.7%, respectively. The duration of development was also prolonged from 20.8 to 24.2 days. The findings from this study suggest that the disruption of the physiological systems of developing honeybee larvae due to fluoride is manifested through interference with normal metabolism and developmental processes. The evaluation of larval mortality and subsequent developmental disruption due to fluoride is regarded as considerable physiological stress to developing honeybee larvae. This disruption is expected to affect the number of viable adult honey bees and is likely to have negative consequences to both the sustainability and overall effectiveness of the honeybee colony. This inquiry also has significant environmental considerations. Pollinators, like honey bees, are essential for agricultural production and for maintaining biodiversity. If these studies on honey bees and fluoride are applied in an environmental context, we could see reductions in agricultural production. With growing concerns about the pollution of the climate and honey bee colonies with fluoride, we cannot ignore the threats fluoride poses to pollinators. This study shows that fluoride is likely a developmental toxicant to *Apis cerana indica*, honey bees. More studies are required to determine the toxic effects of fluoride on honey bees. Environmental monitoring of fluoride pollution, along with developing new techniques to control environmental fluoride pollution, is necessary to keep honey bee populations healthy and to protect ecosystem services.

References:

[1] Aupinel, P., Fortini, D., Dufour, H., Tasei, J. N., Michaud, B., Odoux, J. F., & Pham-Delègue, M. H. (2005). Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. *Bulletin of Insectology, 58*(2), 107–111.

[2] Aupinel, P., Fortini, D., Michaud, B., Marolleau, F., Tasei, J. N., & Odoux, J. F. (2007). Toxicity assessment of systemic insecticides on honey bee larvae using an in vitro rearing method. *Pest Management Science, 63*(11), 1063–1070.

[3] Ayoob, S., & Gupta, A. K. (2006). Fluoride in drinking water: A review on the status and stress effects. *Critical Reviews in Environmental Science and Technology, 36*(6), 433–487.

[4] Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. *Chemico-Biological Interactions, 188*(2), 319–333.

[5] Camargo, J. A. (2003). Fluoride toxicity to aquatic organisms: A review. *Chemosphere, 50*(3), 251–264.

[6] CGWB. (2023). *Groundwater Quality in Shallow Aquifers of India with Special Reference to Fluoride Contamination*. Central Ground Water Board, Ministry of Jal Shakti, Government of India.

[7] Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science, 347*(6229), 1255957.

[8] Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., Forsgren, E., Fries, I., Hatjina, F., Hu, F. L., Jaffé, R., Jensen, A. B., Köhler, A., Magyar, J. P., Özkırım, A., Pirk, C. W. W., Rose, R., Strauss, U., Tanner, G., Tarpy, D. R., van der Steen, J. J. M., Vaudo, A., Vejsnæs, F., Wilde, J., & Zheng, H. Q. (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research, 52*(4), 1–53.

[9] Jha, R., Singh, P., & Prasad, R. (2021). Effects of fluoride exposure on oxidative stress biomarkers in freshwater fish. *Environmental Toxicology and Pharmacology, 84*, 103612.

[10] Klein, A. M., Vaissière, B. E., Cane, J. H., et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B, 274*(1608), 303–313.

[11] OECD. (2013). *Test No. 239: Honey Bee (Apis mellifera) Larval Toxicity Test, Single Exposure*. OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris.

[12] Pătruică, S., Mot, D., & Cazan, A. M. (2020). The influence of environmental pollutants on honey bee health. *Animals, 10*(6), 1103.

[13] Potts, S. G., Biesmeijer, J. C., Kremen, C., et al. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology & Evolution, 25*(6), 345–353.

[14] Ranjan, R., & Choudhary, S. (2020). Toxic effects of fluoride on cocoon production and silk gland histology in *Bombyx mori*. *Journal of Insect Physiology and Toxicology, 18*(2), 55–62.

[15] Sánchez-Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation, 232*, 8–27.

[16] Tosi, S., & Nieh, J. C. (2019). A review of honey bee neurotoxicity and behavioral impairments induced by environmental contaminants. *Environmental Pollution, 251*, 1255–1269.

[17] vanEngelsdorp, D., & Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States. *Journal of Invertebrate Pathology, 103*, S80–S95.

[18] Wang, Y., Li, J., & Liu, Z. (2022). Fluoride-induced oxidative stress and mitochondrial dysfunction in insect tissues. *Toxicology Reports, 9*, 321–330.