

Original Research Article

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## Influence of Fipronil 5% SC on Nucleic Acids (DNA & RNA) Content in Different Organs of Freshwater Fish *Catla catla*

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### ABSTRACT

Nucleic acids are long-chain polymeric molecules, the monomer (the repeating unit) is known as the nucleotides and hence sometimes nucleic acids are referred to as polynucleotides. Pesticides effects on nucleic acids function. Fipronil 5% SC is a broad-spectrum phenyl pyrazole insecticide. In the present study decreased levels of DNA & RNA were observed in all the tissues of freshwater fish *Catla catla* exposed to 24 hrs lethal and sub lethal & 5days, 10 days sublethal concentrations of Fipronil 5% SC. Pesticides are potential inhibitors of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the pesticide may attack many enzymes responsible for normal metabolic pathway.

### Introduction

Nucleic acids are large biomolecules that play essential roles in all cells and viruses. A major function of nucleic acids involves the storage and expression of genomic information. Deoxyribonucleic acid, or DNA, encodes the information cells need to make proteins. A related type of nucleic acid, called ribonucleic acid (RNA), comes in different molecular forms that play multiple cellular roles, including protein synthesis.

Inhibition of DNA synthesis, thus might affect both protein as well as amino acid levels by decreasing the level of RNA in Protein machinery. Pesticides are potential inhibitors to DNA synthesis, which might result in reduction of DNA level. Because of electrophonic nature, compounds attack on many enzymes which are

responsible for normal metabolic pathways. Thus, it is possible that the enzymes are necessary for DNA synthesis might have been inhibited by toxicant. On compilation of the result, it appears that the disruption of DNA synthesis might have affected RNA synthesis and consequently protein synthesis (Marimuthu *et al.*, 2021).

### Materials and Methods

Fish *Catla catla* of size 6±7 cm and 6.5±7.5 g weight were brought from a local fish farm Kuchipudi, Guntur District of Andhra Pradesh, India and acclimatized at 28 ± 2°C in the laboratory for 15days.

Such acclimatized fish were exposed to sublethal and lethal concentrations of Fipronil 5% SC commercial grade for 24 hr, 5 and 10 days. The vital tissues like

muscle, brain, liver, gill and kidney of the fish were taken for the estimation of Nucleic acids (DNA & RNA), the estimations were conducted along with controls.

### Statistical Analysis

Student's t-test and one way analysis of variance (ANOVA) of SPSS (20.0 version), SPSS Chicago, USA, was employed to calculate the significance of the differences between control and experimental means. P values of 0.05 or greater than were considered statistically significant (Fisher, 1950). Significance level was based on  $P < 0.05$ . Results were expressed as  $\pm S. D$  of five individual.

### Estimation of Nucleic Acids

The nucleic acids, Deoxyribose nucleic acids (DNA) and Ribose nucleic acids (RNA) were estimated by the method of (Searchy and Maclinnis, 1970 a & b). 5% homogenates of gill, brain, muscle, liver and kidney were prepared in 5 ml of 0.5 N perchloric acid and heated at 90°C for 20 minutes. After cooling, the tissue homogenates were centrifuged at 3000 rpm for 10 minutes. The supernatant was separated into two volumes and used for DNA and RNA analysis.

### DNA

The first half or one half of the homogenate was mixed with diphenylamine reagent and kept aside for 20 hrs. After 20 hours the colour developed was read at 595 nm. The standard graph was plotted with standard DNA (calf thymus) supplied by the Sigma Chemical Company with the aforesaid method.

### RNA

The other part of the homogenate was mixed with dischiorcinol and heated at 90°C for 15 minutes. After cooling at room temperature, the colour developed was read at 655 nm. The standard graph was plotted with standard RNA (Baker's yeast) supplied by Sigma chemical company.

## Results and Discussion

### Nucleic acids DNA

The calculated values of nucleic acids along with standard deviation and the percent change over the

control were given in Table.1 & 2 and Fig.V.1-V.2 the DNA content in control fish *Catla catla* in different tissues was in the order of:

Brain > Gill > Liver > Kidney > Muscle

Under exposure to sublethal and lethal concentration of Fipronil 5% SC to fish *Catla catla* for 24 hr, the total DNA was found to decrease in most of the tissues and the lyotropic series in terms of decrement in the DNA content was in the order of:

Fipronil 5% SC sublethal 24 hr: Brain > Gill > Kidney > Muscle > Liver

Fipronil 5% SC lethal 24 hr: Gill > Liver > Muscle > Kidney > Brain

Under exposure to sublethal concentrations of Fipronil 5% SC for 5<sup>th</sup> and 10<sup>th</sup> days. The percent depletion of DNA content in the tissues of *Catla catla* was in the order of:

5 days controls: Brain > Gill > Liver > Kidney > Muscle

Fipronil 5% SC Sublethal 5 days: Gill > Brain > Liver > Kidney > Muscle

10 days controls: Brain > Gill > Liver > Kidney > Muscle

Fipronil 5% SC Sublethal 10 days: Gill > Brain > Muscle > Liver > Kidney

Under control group the total DNA content was present in different tissues of fish *Catla catla* for 24hr, the maximum amount present in Brain (1.773) and followed by Gill (1.614), Liver (1.264), Kidney (1.018) and minimum amount of DNA content was present in Muscle (0.982). Under Fipronil 5% SC for 24 hr sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Gill (29.73) and followed by Brain (29.10), Liver (28.24), Kidney (3.53) and minimum depletion was present in Muscle (1.53).

Under Fipronil 5% SC for 24hr lethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Brain (55.84) and followed by Gill (40.76), Liver (20.46), Kidney (20.13) and minimum depletion was present in Muscle (15.78).

Under control group the total DNA content was present in different tissues of fish *Catla catla* for 5 days, the maximum amount present in Brain (1.243) and followed by Gill (1.108), Liver (1.042), kidney (0.823) and minimum amount of total DNA content was present in muscle (0.738). Under Fipronil 5% SC for 5 days sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Brain (18.50) and followed by Liver (16.12), Kidney (9.11), Muscle (7.99) and minimum depletion was in Gill (6.95). Under control group the total DNA content was present in different tissues of fish *Catla catla* for 10days, the maximum amount present in Brain (1.023) and followed by Gill (0.938), Liver (0.832), Kidney (0.736) and minimum amount of total DNA content was present in Muscle (0.724). Under Fipronil 5% SC for 10days sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Liver (23.44) and followed by Brain (22.87), Kidney (-16.71), Gill (7.03) and minimum depletion was in Muscle (2.90).

## RNA

The calculated values of nucleic acid content RNA, along with standard deviation and the percent change over the control fish were presented in Table.3, V.4 and Fig. V.3, V.4. The RNA content in 24hrs control fish *Catla catla* in different tissues was in the order of:

Liver> Gill> Kidney>Muscle >Brain

Under exposure to sublethal and lethal concentration of Fipronil 5% SC to fish *Catla catla* for 24 hr, the RNA was found to decrease in most of the tissues and the lyotropic series in terms of decrement in the RNA content was in the order of:

Fipronil 5% SC sublethal 24 hours: Liver >Kidney> Gill > Muscle >Brain

Fipronil 5% SC lethal 24 hours: Liver>Kidney> Gill.>Brain>Muscle

Under exposure to sublethal concentrations of Fipronil 5% SC for 5<sup>th</sup> and 10<sup>th</sup> days. The percent depletion of RNA content in the tissues of *Catla catla* was in the order of:

5 days control: Kidney> Gill>Liver> Brain >Muscle

Fipronil 5% SC sublethal 5 days: Gill>Liver >Kidney>Brain > Muscle

10 days control: Liver >Kidney >Gill>Brain > Muscle

Fipronil 5% SC sublethal 10 days: Liver > Kidney> Gill>Brain >Muscle

The results in V. Table. 7 and V. Fig.7. Indicate heterogeneous levels of RNA in the tissue of liver, brain, muscle, gill and kidney. Under control group the total RNA content was present in different tissues of fish *Catla catla* for 24 hr, the maximum amount present in Liver (1.183) and followed by Gill (0.876), Kidney (0.840) Muscle (0.746) and minimum amount of RNA content was present in Brain (0.578). Under Fipronil 5% SC for 24 hr sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Liver (12.43) and followed by Gill (8.56), Muscle (3.35), Kidney (1.78) and minimum depletion was present in Brain (1.03). Under Fipronil 5% SC for 24hr lethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Muscle (35.12) and followed by Gill (18.70), Brain (13.57), Liver (12.17) and minimum depletion was present in Kidney (2.5).

Under control group the total RNA content was present in different tissues of fish *Catla catla* exposed for 5days, the maximum amount present in Kidney (0.804) and followed by Gill (0.763), Liver (0.732), Brain (0.562) and minimum amount of total RNA content was present in Muscle (0.376). Under Fipronil 5% SC for 5days sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Kidney (33.08) and followed by Muscle (29.12), Brain (22.42), Gill (14.02), and minimum depletion was in Liver (13.11). Under control group the total RNA content was present in different tissues of fish *Catla catla* exposed for 10days, the maximum amount present in Liver (0.672) and followed by Kidney (0.647), Gill (0.516), Brain (0.463) and minimum amount of total RNA content was present in Muscle (0.367).

Under Fipronil 5% SC for 10 days sublethal, the percentage of depletion was found in all the tissues of test fish *Catla catla*, maximum percentage of depletion was in Brain (29.59) and followed by Muscle (28.06), Liver (21.42), Kidney (19.94), and minimum depletion was in Gill (19.18).

**Table.1** Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal and lethal concentrations of Fipronil 5% SC for 24hr

| DNA Tissues | Control         | Sublethal       | % Change | Lethal          | % Change |
|-------------|-----------------|-----------------|----------|-----------------|----------|
| Gill        | 1.614<br>±0.011 | 1.134<br>±0.018 | 29.73    | 0.956<br>±0.021 | 40.76    |
| Liver       | 1.264<br>±0.014 | 0.907<br>±0.022 | 28.24    | 0.879<br>±0.023 | 20.46    |
| Kidney      | 1.018<br>±0.019 | 0.982<br>±0.023 | 3.53     | 0.813<br>±0.032 | 20.13    |
| Brain       | 1.773<br>±0.065 | 1.257<br>±0.038 | 29.10    | 0.783<br>±0.043 | 55.84    |
| Muscle      | 0.982<br>±0.042 | 0.967<br>±0.010 | 1.53     | 0.827<br>±0.021 | 15.78    |

Values are the mean of five observations ;(±) indicates the standard deviation:  
Values are significantly at  $P < 0.05$

**Table.2** Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Fipronil 5% SC for 5 and 10 days

| DNA Tissues | 5 days          |                 |          | 10days         |                 |          |
|-------------|-----------------|-----------------|----------|----------------|-----------------|----------|
|             | Control         | Sublethal       | % Change | Control        | Sublethal       | % Change |
| Gill        | 1.108<br>±0.011 | 1.031<br>±0.018 | 6.95     | 0.938<br>±0.06 | 0.872<br>±0.021 | 7.03     |
| Liver       | 1.042<br>±0.014 | 0.874<br>±0.022 | 16.12    | 0.832<br>±0.04 | 0.637<br>±0.023 | 23.44    |
| Kidney      | 0.823<br>±0.023 | 0.748<br>±0.019 | 9.11     | 0.736<br>±0.02 | 0.613<br>±0.032 | 16.71    |
| Brain       | 1.243<br>±0.065 | 1.013<br>±0.038 | 18.50    | 1.023<br>±0.02 | 0.789<br>±0.043 | 22.87    |
| Muscle      | 0.738<br>±0.042 | 0.679<br>±0.010 | 7.99     | 0.724<br>±0.01 | 0.703<br>±0.021 | 2.90     |

Values are the mean of five observations ;(±) indicates the standard deviation:  
Values are significantly at  $P < 0.05$

**Table.3** Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal and lethal concentrations of Fipronil 5% SC for 24hr

| RNA Tissues 24hr | Control        | Sublethal      | % Change | Lethal         | % Change |
|------------------|----------------|----------------|----------|----------------|----------|
| <b>Gill</b>      | 0.876<br>±0.01 | 0.801<br>±0.02 | -8.56    | 0.713<br>±0.01 | -18.70   |
| <b>Liver</b>     | 1.183<br>±0.03 | 1.036<br>±0.15 | -12.43   | 1.039<br>±0.05 | -12.17   |
| <b>Kidney</b>    | 0.840<br>±0.05 | 0.825<br>±0.03 | -1.78    | 1.819<br>±0.03 | -2.5     |
| <b>Brain</b>     | 0.678<br>±0.14 | 0.671<br>±0.24 | -1.03    | 0.586<br>±0.14 | -13.57   |
| <b>Muscle</b>    | 0.746<br>±0.05 | 0.721<br>±0.01 | -3.35    | 0.484<br>±0.05 | -35.12   |

Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at  $P < 0.05$

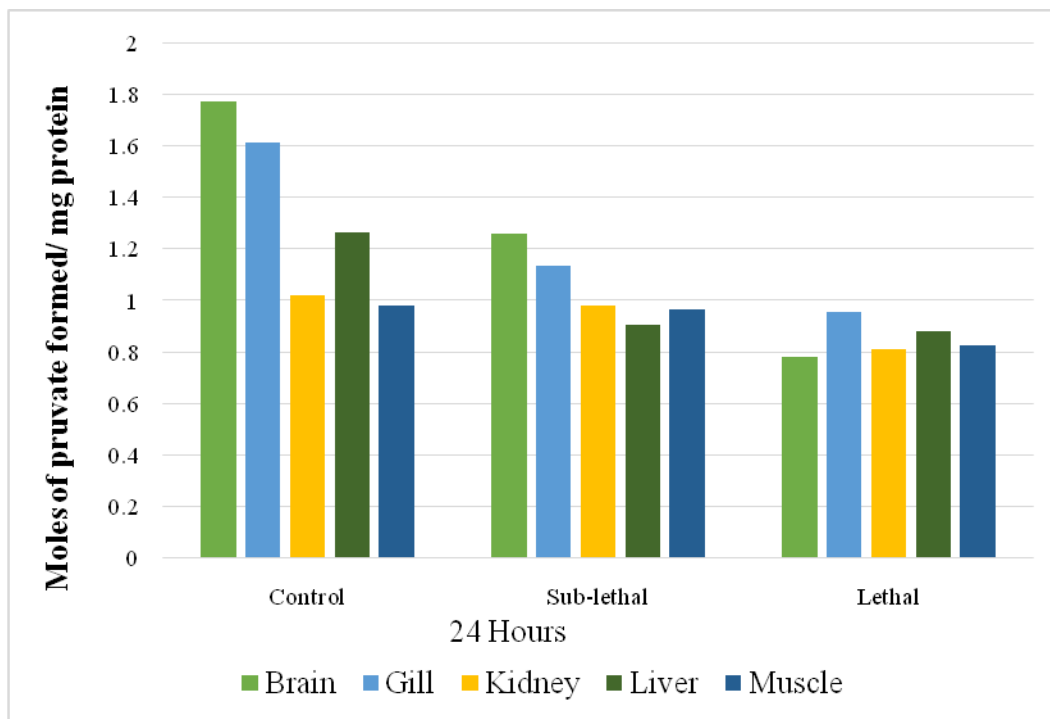
**Table.4** Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Fipronil 5% SC for 5 and 10 days

| RNA Tissues   | 5 days         |                |          | 10 days        |                |          |
|---------------|----------------|----------------|----------|----------------|----------------|----------|
|               | Control        | Sublethal      | % Change | Control        | Sublethal      | % Change |
| <b>Gill</b>   | 0.763<br>±0.01 | 0.656<br>±0.04 | -14.02   | 0.516<br>±0.02 | 0.417<br>±0.32 | -19.18   |
| <b>Liver</b>  | 0.732<br>±0.05 | 0.636<br>±0.08 | -13.11   | 0.672<br>±0.05 | 0.528<br>±0.12 | -21.42   |
| <b>Kidney</b> | 0.804<br>±0.01 | 0.538<br>±0.01 | -33.08   | 0.647<br>±0.08 | 0.518<br>±0.05 | -19.94   |
| <b>Brain</b>  | 0.562<br>±0.08 | 0.436<br>±0.05 | -22.42   | 0.463<br>±0.13 | 0.326<br>±0.07 | -29.59   |
| <b>Muscle</b> | 0.478<br>±0.12 | 0.376<br>±0.32 | -21.34   | 0.367<br>±0.24 | 0.264<br>±0.05 | -28.06   |

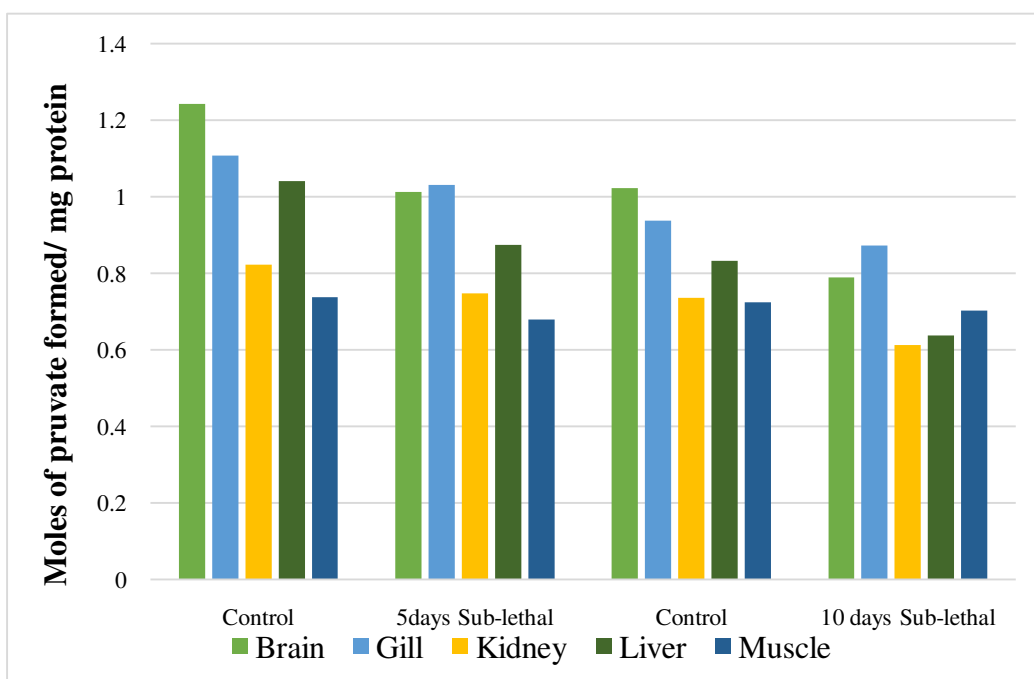
Values are the mean of five observations ;(±) indicates the standard deviation:

Values are significantly at  $P < 0.05$

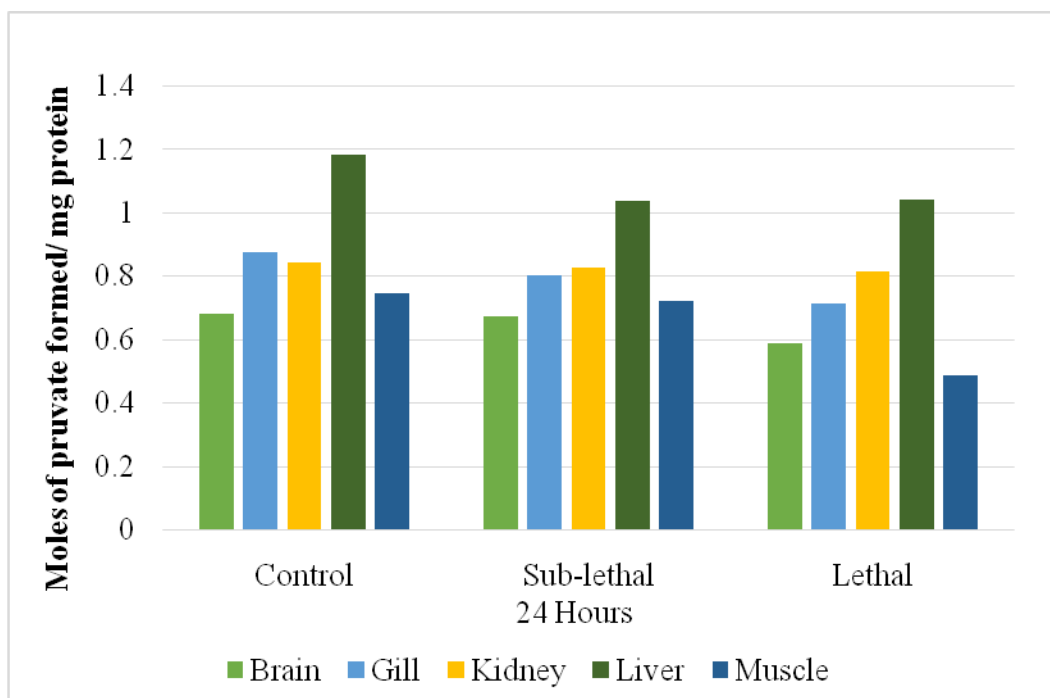
**Figure.1** Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal and lethal concentrations of Fipronil 5% SC for 24hr



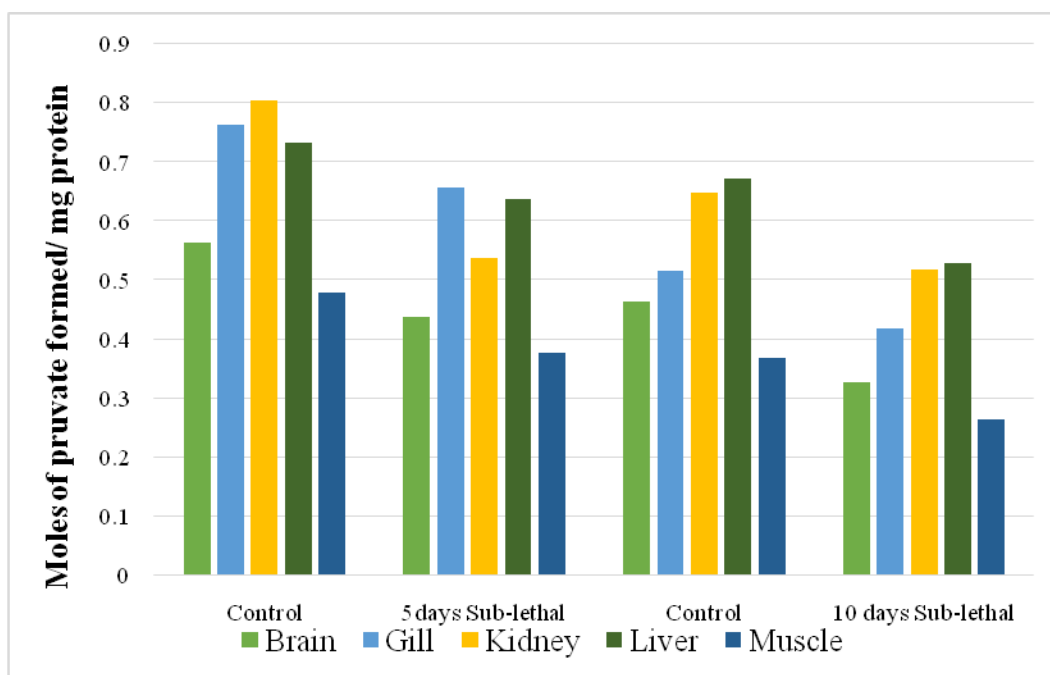
**Figure.2** Changes in the DNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Fipronil 5% SC for 5 and 10 days



**Figure.3** Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal and lethal concentrations of Fipronil 5% SC for 24hr



**Figure.4** Changes in the RNA (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish, *Catla catla* exposed to sublethal concentrations of Fipronil 5% SC for 5 and 10 days



In the present study observed that the chemical contaminants damages DNA by multiple mechanisms and many researchers observed the decreased DNA in the lethal and sub lethal concentration. Anitha (2017) observed decrease DNA content in fish *Labeo Rohita* exposed to Pyraclostrobin. Nirmala (2016) Observed decrease DNA content in freshwater fish *Ctenopharyngodon idella* exposed to Flubendamide. The level of DNA and RNA was found to decreased in brain, liver, gonads and kidney of *N. notopterus* fish due to exposure to copper sulphate indicates toxicity effect on nucleic acid synthesis (Wistrand *et al.*, 2018).

Decreased DNA content was observed in freshwater fish *Ctenopharyngodon idella* exposed to Profenofos, (Hari babu, 2017). Rajeswari (2020) DNA content was decreased in *Ctenopharyngodon idella* exposed to Cyhalothrin. Prassana (2018) decreased DNA content was observed Ethion exposed to *Labeo rohita* lethal and sub lethal concentration. Decreased content of DNA is observed in *Labeo rohita* and *Ctenopharyngodon idella* exposed to Fipronil 5% SC (Anithasmruthi, 2022). Observed decrease DNA content in freshwater fish *Cyprinus carpio* exposed to profenofos (Rahman *et al.*, (2020). All these observations supported by the present study of decreased levels of DNA was observed in all the tissues of freshwater fish *Catla catla* exposed to 24 hrs lethal and sub lethal & 5days, 10 days sublethal concentrations of Fipronil 5%SC broad spectrum insecticide.

Decreased DNA-RNA levels in fish *Ctenopharyngodon idella* exposed to Bifenthrin at different seasons (Chaitanya Kumari, 2022). Similar findings were observed by Anithasmruthi (2022), the DNA and RNA content was decreased in gill, liver, brain and kidney of fish *Catla catla* exposed to different concentrations of Fipronil. The decline DNA content could be due to the disturbance in the normal DNA synthesis. Inhibition of DNA synthesis, thus might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesis machinery. Pesticides are potential inhibitor of DNA synthesis, which might result in reduction of RNA level. Because of electrophilic nature, the pesticide may attack many enzymes responsible for normal metabolic pathway.

Thus, it is possible that the enzyme necessary for DNA synthesis might have been inhibited by toxicant.). Furthermore, a recent study related more than one pyrethroid metabolite to DNA damages in human sperm,

raising concerns about possible negative effects on human reproductive health (Jurewicz *et al.*, 2015). Yuzefovych *et al.*, (2013) a number of chemicals, associate with DNA damage, have been tested on liver of aquatic animals, isolated tissues or different cell types. Chemicals that act directly on DNA, chemicals whose metabolites cause DNA damage, chemicals that cause the production of reactive oxygen species that can damage DNA, chemicals that inhibit DNA synthesis and repair. Inhibition, many chemical contaminants damage DNA by multiple mechanisms (Lalitha Vinnakota, 2022). decreased nucleic acids (DNA and RNA) content liver, muscle, gill tissues of freshwater fish *Labeo rohita* treated with Azoxystrobin, decline in nucleic acid content due to decreased protein synthesis and damage to liver, which is the major tissue for detoxification mechanism.

The pesticide damaged to neuron cells resulting in demyelination (Szepanowski *et al.*, 2019). In the present study decrease in levels of RNA was observed in all the tissues of fish *Catla catla* exposed to 24 hrs sublethal and lethal & 5days, 10 days Sublethal concentrations of Fipronil 5% SC. The decrease of RNA might also be due to interference in the incorporation of precursor in the nucleic acid synthesis or inhibition of the RNA polymerase function (Xu *et al.*, 2019). Minimum depletion of RNA content was observed in brain exposed to phosalone 35% EC (Nirmala, 2016). The pesticide-mediated reduction in protein contents of various tissues including blood of other species of fish reported by Shafat Ali *et al.*, (2020).

DNA damaging agents capable of inducing strand breakage, cross-links and alkali-labile sites (Amunugama and Walter, 2020). The DNA and RNA contents have been studied in gill, liver and brain of a grass carp, *Ctenopharyngodon idella* exposed to Pyraclostrobin decreased DNA content in all the tissues along with RNA content in liver and brain, it was increased in gill due to Pyraclostrobin toxicity (Katti Ravi babu *et al.*, 2021).

The present study reveals that Fipronil 5% SC caused variability in the nucleic acids content in different tissues and the degree of variability or extent of alterations by the Fipronil 5% SC. The reasons for the decreased levels of nucleic acid are the enzyme inhibition, mitotic changes, impairment of nucleic acid metabolism, increased activity of phosphatases and the degradation of cells. On compilation of all these results it appears that the disruption of DNA synthesis might have reflected in RNA synthesis.



Nucleic acids are naturally occurring chemical compounds that serve as the primary information carrying molecules in cells. They play an especially important role in directing protein synthesis. Based on the results obtained from the present investigation, it is concluded that fipronil causes deleterious effects on fishes and much alters the DNA and RNA contents of certain tissues. Significant decrease in both protein and nucleic acids levels would suggest that fipronil impairs the processes of protein synthesis in the tissues of test fish. These changes may be potentially disruptive for the survivability of fish in aquaculture farm and natural resources.

### Author Contributions

Harinath Prathakota: Investigation, formal analysis, writing—original draft. Chittakula Anithasmruthi: Validation, methodology, writing—reviewing. Mangalivijayalakshmi:—Formal analysis, writing—review and editing. V. Venkatarathnamma: Investigation, writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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