

Potential efficacy of *Syzygium cumini* against toxic impact of propargite on haematological alteration in the fresh water fish *Channa striata*

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ABSTRACT: Pesticides are stable compounds and they enter the aquatic ecosystem through the agriculture run off. The evaluation of nature and degree of harmful effects produced by the toxic substance in the aquatic organisms are evaluated by toxic tests. 96 hour LC₅₀ values have generally been found to be satisfactory for the measurement of acute toxicity. Therefore, in this present study is an attempt to study the toxicity of the pesticide with respect to the haematology, of fish *Channa striata*. The Propargite affects not only fish but also organisms in the food chain through the process of consumption of one by the other. The cytometric measurements of erythrocytes of sublethal exposure showed that there are not many differences from the control. In the control fish, the erythrocytes were oval in shape with elongated nucleus. Fish, exposed to sublethal concentration of Propargite showed abnormal size Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration. In the hematology, the total Red Blood Corpuscle and Haemoglobin content were decreased with the increasing hours of exposure of the Propargite. The amount of the Mean Corpuscular Haemoglobin (MCH) also was increased. The constant increase in the differential count clearly indicates that the pesticide stress certainly stimulates the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provision of useful diagnostic procedure to assess the physiological stress in the fish.

KEYWORDS: *Syzygium cumini*, Propargite, Haematology, *Channa striata*.

I. INTRODUCTION

Environmental pollution occurs when the environmental degradation crosses the limit so that. It becomes lethal to living organisms. In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding [1]. Contamination by pesticides in the aquatic ecosystem is a serious problem and fish are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated food [2]. Fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. Propargite is a widely used organophosphate pesticide, second largest selling in India and used for more than a decade to control pests on cotton, paddy fields, pasture and vegetable crops [3]. Pesticides are one of the most potentially harmful toxic chemicals introduced into the environment. Though they have contributed considerably to human welfare, their adverse effects on non-target organisms are quite significant. Aquatic ecosystems that run through agricultural or industrial areas have a high probability of being contaminated by runoff and ground water leaching by a variety of toxic pesticides which pose a potential direct threat to freshwater organisms, particularly to sensitive animals, such as fish. Propargite is an organophosphate insecticide and is highly toxic to freshwater fish. Fishes have an important role in the food chain; therefore, investigation of the effects of toxic pesticides such as Propargite on fish has a diagnostic significance in the evaluation of negative effects of pesticides on human health [4].

Fish immune system, important for defense against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defense mechanism against pathogens [5]. Effects of Propargite on the immune factors of fish such as mRNA levels of IL-1 β and IFN- γ 2b in immune organs of common carp have been reported by [6-7], studied the immune response parameters of *Oreochromis niloticus*. Results indicated that Propargite at 0.051 mg/L induced a diminishing in concentration of IgM in plasma. On the other hand, organisms exposed to high concentration of the pesticide showed an increase in the lysozyme activity [8]. Propargite during 96h caused a significant decrease in the phagocytic capacity and in the percentage of phagocytic cells present in blood. They cause inhibition of the nervous tissue acetylcholinesterase (AChE) activity [9]. AChE is an important enzyme that can be measured environmental bio-indicator in the animal body. Cholinesterase is involved in the signal transmission at neuromuscular junctions and is also intensely expressed in the organism nervous system. The main role of AChE is to catalyze the hydrolysis of acetylcholine into cholin and acetic acid at cholinergic synaptic sites [8-9]. Erythrocytes are the dominant cell type in the blood of fish; it is widely accepted that fish like most other vertebrates. The high erythrocyte number was

associated with fast movement, predaceous nature, and high activity with streamlined body [9] Leucocytes are involved in the regulation of immunological function in many organisms and the increase in WBC in stressed animals indicates a protective response to stress [10]. Similarly the significant increase of WBC count may be due to generalized immune response. In the present study is a haematology study of cytometric measurement, red blood corpuscle estimation, total white blood corpuscle estimation, haemoglobin content and mean of corpuscle haemoglobin in the *Syzygium cumini* against toxic impact of Propargiteon fresh water fish *Channa striata*.

II. MATERIALS AND METHODS

Collection and maintenance of the experimental Animal

The freshwater fish *Channa striata* were collected from the VGM fish farm located in Kurinjipadi, Cuddalore district. The fishes were brought to the laboratory and transferred to the rectangular cement tanks 100 (175) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of their sex for the experiments.



Figure 1. *Syzygium cumini*

Syzygium cumini

Syzygium cumini, commonly known as jambolan, Java plum, black plum or jamun, is an evergreen tropical tree in the flowering plant family Myrtaceae. It is native to the Indian Subcontinent, adjoining regions of Southeast Asia, China and Queensland. The name of the fruit is sometimes mistranslated as blackberry, which is a different fruit in an unrelated family. *Syzygium cumini* has been spread overseas from India by Indian emigrants and at present is common in former tropical British colonies (Figure 1).

Collection and preparation of *Syzygium cumini*

The dried *S.cumini* was collected from Thiruvankadu Village near to Chidambaram. The *Syzygium cumini* powder was kept in carefully.

Preparation of *Syzygium cumini*

Dried *Syzygium cumini* powder weight of 50g and rice brand powder was weighted at 50g for both the powders mixed well then adds sterilized water make titer paste. After this paste to make pellets (1gram) according to fish feed (artificial feed) sizes. Pellets were dried inside the room at normal temperature and avoid the direct sunlight. Pellets were packed in airtight bottle for experimental use.

Selection of Herbicide

In the present investigation, the herbicide Propargite has been selected. Propargite is a broad leaf herbicide that has been used historically on agriculture and forestry. Herbicide is a chemical used to kill or otherwise manage certain species of plants considered to be pests. It is used most extensively on corn crops and to a lesser extent on other food crops such as asparagus, sugarcane and macadamia, pineapple as well as in conifer restoration plantings ("Propargite" pesticide Information profile, 1996). Propargite is a herbicide that does not occur naturally.

Toxicity Studies

Acute toxicity tests were conducted to measure the impact of toxicant on aquatic animals within a short period of five hours. In the toxicity studies, the renewal technique of acute static test was adopted, in which fish were periodically exposed to the concentrations of the same composition, usually once in every 24 hours by transferring the animals from one test chamber to another (Committee on methods for toxicity tests with aquatic organisms, 1975). The LC_{50} is a statistical estimate of the concentration of toxic material in the water that kills 50 percent of the test animals under experimental conditions with specific time intervals [9]. This value is ideally suited for toxicity studies as it gives

more acceptable and reproducible concentration required affecting 50 per cent of the organisms than any other value [10].

Hematological Studies

The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), Total Leucocyte Count (TLC) and Haemoglobin content (Hb). For RBC count, a method devised by Yokayama and later modified by Christensen *et al.*, was followed. For counting the total number of WBC, the pipette with white lead was used. The number of cells present in the four large corner squares marked by capital letter ‘L’ was counted and multiplied by 10³ which give the total number of WBC per cubic millimeter of blood. Haemoglobin determination is the quickest means for detecting anaemia. However, many factors are known to influence the haemoglobin level. The Sahli Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20mm mark.

Statistical Analysis

The data obtained from the quantitative study were expressed as the mean S.E. The mean values were calculated from 6 individual observations. P-values were calculated by two tailed students ‘t’ test. The two mean values obtained were considered significant from each other. Since the calculator student’s ‘t’ ration was greater than ‘t’ 0.05 or ‘t’ 0.01 value at the respective degree of freedom (df=n1+n2+-2). The ‘t’ values were calculated by using the formula [8].

$$T = \frac{X_1 - X_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Where X₁ and X₂ represent the means of control and treated values, SE₁ and SE₂ were their respective standard errors. Standard error was calculated from the formula:

$$SE = \frac{S.D}{\sqrt{n}} \text{ Where 'n' was total number of observations.}$$

III. RESULTS

Haematological parameters

The quantitative changes of haematological parameters like RBC, WBC, Hb, PCV, MCV, MCH and MCHC of the blood cells in the freshwater fish *Channa striata* in all groups control (group 1), sublethal concentration of Propargite (group 2), Propargite along with *Syzygium cumini* (group 3) and *Syzygium cumini* alone (group 4) exposed to 24, 48, 72, 96 and 120 hrs are observed.

Red blood corpuscles (RBC)

In the present investigation Propargite treated fish (group 2) show the decrease in RBC (red blood cells) content when compared to control (group 1). The percentage changes are -22.71, -37.60, -14.61, -31.92 and -20.82 for 24, 48, 72, 96 and 120 hrs respectively. Whereas in the Propargite and *Syzygium cumini* treated fish (group 3) record significant recovery from the effect of group 2. The increased percentage recoveries are 53.39, 67.93, 75.93, 122.8 and 167.67 for 24, 48, 72, 96 and 120 hrs respectively. While in fish of *Channa striata* exposed to *Syzygium cumini* alone, when compared with control, a slight variation is noticed. Percentage changes in *Syzygium cumini* (group 4) are -11.20, -2.91, -11.74, -4.23 and -5.47 for 24, 48, 72, 96 and 120 hrs respectively. The recorded RBC content in blood cell counts of four groups is statistically significant at 1% and 5% levels (Table 1).

Table 1. Changes in the level of RBC (× 10⁶/mm³), WBC (× 10³/mm³), Hb (g/L) and PCV (%) in *Channa striata* fingerlings exposed to 120 hrs sublethal concentration of Propargite and antidote *Syzygium cumini*

| Groups | | Hours of exposure | | | | |
|---|--|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| | | 24 | 48 | 72 | 96 | 120 |
| RBC (× 10 ⁶ /mm ³) | Group 1 – Control | 4.39 ± 0.02 | 3.43 ± 0.03 | 3.49 ± 0.03 | 3.54 ± 0.04 | 3.65 ± 0.04 |
| | Group II - Propargite % COC | 2.62** ± 0.01 -22.71 | 2.14** ± 0.02 -37.60 | 2.98* ± 0.04 -14.61 | 2.41* ± 0.05 -31.92 | 2.89* ± 0.05 -20.82 |
| | Group III - Propargite+ <i>Syzygium cumini</i> % COC | 5.20* ± 0.03 +53.39 | 5.76* ± 0.03 +67.93 | 6.14* ± 0.04 +75.93 | 7.89* ± 0.05 +122.88 | 9.77* ± 0.06 +167.67 |
| | Group IV - <i>Syzygium cumini</i> % COC | | | | | |

| | | | | | | |
|--|--|--------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
| | Group IV - <i>Syzygium cumini</i> % COC | 3.01** ± 0.02 -11.20 | 3.33** ± 0.02 -2.91 | 3.08** ± 0.03 -11.74 | 3.39** ± 0.04 -4.23 | 3.45** ± 0.05 -5.47 |
| WBC (× 10 ³ /mm ³) | Group I – Control | 22.14 ± 0.08 | 22.16 ± 0.07 | 22.19 ± 0.08 | 23.12 ± 0.07 | 23.15 ± 0.09 |
| | Group II - Propargite % COC | 27.54** ± 0.05 +24.39 | 29.15** ± 0.06 +31.54 | 30.14** ± 0.07 +37.08 | 34.54** ± 0.09 +49.39 | 37.79** ± 0.08 +60.64 |
| | Group III - Propargite+ <i>Syzygium cumini</i> % COC | 24.12** ± 0.07 +8.94 | 28.12** ± 0.08 +26.89 | 28.48** ± 0.06 +28.34 | 29.11** ± 0.07 +25.90 | 22.90* ± 0.06 +29.15 |
| | Group IV - <i>Syzygium cumini</i> % COC | 20.18** ± 0.05 -8.85 | 20.27** ± 0.06 -8.52 | 21.45** ± 0.05 -3.33 | 21.89** ± 0.04 -5.32 | 22.46** ± 0.057 -2.98 |
| Hb (g/L) | Group I – Control | 9.65 ± 0.12 | 9.69 ± 0.14 | 9.75 ± 0.18 | 9.83 ± 0.16 | 9.94 ± 0.16 |
| | Group II - Propargite % COC | 8.93* ± 0.23 -7.46 | 8.42* ± 0.21 -13.10 | 8.65* ± 0.26 -11.22 | 8.12* ± 0.19 -17.39 | 8.22* ± 0.23 -17.30 |
| | Group III - Propargite+ <i>Syzygium cumini</i> % COC | 10.11* ± 0.40 +4.76 | 10.22* ± 0.19 +5.46 | 11.21* ± 0.42 +14.97 | 11.49* ± 0.21 +16.88 | 12.33* ± 0.46 +24.04 |
| | Group IV - <i>Syzygium cumini</i> % COC | 9.12* ± 0.13 -5.49 | 9.19* ± 0.14 -5.15 | 9.67* ± 0.18 -0.82 | 9.47* ± 0.17 -3.66 | 9.65* ± 0.19 -2.91 |
| PCV (%) | Group I – Control | 48.5 ± 0.236 | 48.17 ± 0.437 | 59.12 ± 0.256 | 60.45 ± 0.237 | 62.16 ± 0.437 |
| | Group II - Propargite % COC | 24.41* ± 0.312 -17.44 | 44.75* ± 0.239 -10.80 | 49.15* ± 0.241 -15.86 | 52.45* ± 0.245 -13.23 | 56.12* ± 0.233 -10.03 |
| | Group III - Propargite+ <i>Syzygium cumini</i> % COC | 20.49* ± 0.412 -20.78 | 43.56* ± 0.317 -13.17 | 49.78* ± 0.256 -15.79 | 49.18* ± 0.255 -18.64 | 54.12* ± 0.247 -12.93 |
| | Group IV - <i>Syzygium cumini</i> % COC | 47.65* ± 0.242 -1.75 | 49.18* ± 0.473 -2.09 | 52.17* ± 0.484 -2.82 | 60.43* ± 0.495 -0.03 | 61.87* ± 0.455 -0.46 |

Mean ± S.E. – Mean of six individual observations; (+/-) indicate the percentage change over control; * Significant at 5% level; **Significant at 1% level; %COC – % change over control.

White blood corpuscles (WBC)

The Propargite exposed fish (group 2) show a slight increase in the WBC count when compared to control fish (group 1). There are no noticeable changes in the control fish. The increased per cent changes are 24.39, 31.54, 37.08, 49.39 and 60.64 for 24, 48, 72, 96 and 120 hrs respectively. Whereas in the Chlorpyrifos, along with *Syzygium cumini* treated fish (group 3) record significant recovery from the effect of Propargite (group 2). The percentage recoveries are 8.94, 26.89, 35.55, 38.88 and 43.41 for 120 hrs respectively. While in fish exposed to *Syzygium cumini* alone (group 4), no changes are noticed and it is equal to normal. Percentage changes in *Syzygium cumini* alone (group 4) are -8.85, -8.52, -3.33, -5.32 and -2.98 for 24, 48, 72, 96 and 120 hrs respectively. WBC content in blood cells counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 1).

Haemoglobin (Hb)

Hb levels of *Channa striata* fingerlings exhibit remarkable changes. In group 2, the values of Hb decrease than into control (group 1). The per cent changes are -7.46, -13.10, -11.22, -17.30 and -17.30 for 24, 48, 72, 96 and 120 hrs respectively, whereas the Propargite along with *Syzygium cumini* treated fish (group 3) record significant recoveries from the effect of Propargite (group 2). Increased percentage recoveries are 4.76, 5.46, 14.97, 16.88 and 24.04 for 24, 48, 72, 96 and 120 hrs respectively. While in fish exposed to *Syzygium cumini* alone (group 4), slight variations are noticed compared with control (group 1). Percentage changes in *Syzygium cumini* alone (group 4) are -5.49, -5.15, -0.82, -3.66 and -2.91 for 24, 48, 72, 96 and 120 hrs respectively. Hb content in blood cell counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels (Table 1).

Packed cell volume (PCV)

In the present investigation, group 2 shows decrease in the level of PCV content when compared to group 1, group 3 and group 4. The per cent changes for group 2 are -17.44, -10.80, -15.86, -13.23 and -10.03 for 24, 48, 72, 96 and 120 hrs respectively. While in the fish exposed to group 3, PCV content decreases when compared to group 2. The per cent changes are -20.78, -13.17, -15.79, -18.64 and -12.93 for 24, 48, 72, 96 and 120 hrs respectively. Remarkable minimum value is noticed in group 4 and the percentage changes are -1.75, -2.09, -2.82, -0.03 and -0.46 for 24, 48, 72, 96 and 120 hrs respectively. The level of PCV content for the four groups is statistically significant at 1% and 5% levels (Table 1).

IV. DISCUSSION

Fish blood is a patho-physiological reflector of the whole body and therefore blood parameters are important in diagnosing the structural and functional status of the fish exposed to toxicants [11]. Pesticide toxicants are known to induce anaemia in fish. In the present study, it is found that a reduced RBC count and Hb contents in the

Propargite exposed fish, may be due to inhibition of erythrocyte production or increase in the rate of erythrocyte destruction [12] have reported that the anaemia is caused due to reduction in the erythrocyte production which supports the present findings [13-14] have reported that the RBC count and haemoglobin content values significantly decrease resulting macrocytic anaemia in *Heteropneustes fossilis* exposed to zinc. In the present study, *Propargite* along with *Syzygium cumini* (group 3) exposure induces a noticeable concentration dependent on decreased Hb level may impair oxygen supply to various tissues, thus resulting in a slow metabolic rate and low energy production [15]. The significant decrease in the Hb concentration may also be due to either in increase in the rate at which Hb is destroyed or due to a decrease in the rate of Hb synthesis [17].

Vasantharaja *et al.* [18] have observed that the reduction in Hb content of toxicant exposed fish could be due to the inhibitory effect of the toxic substance on the enzyme system in the synthesis of Hb [18] have reported decreasing haemoglobin, haematocrit level and erythrocyte count in *Channa punctuata* after exposure to either copper alone or copper and cadmium [19] have reported a decrease in RBC count with an increase in WBC and Hb level after chronic exposure of *Cyprinus carpio* to sublethal concentration of sodium selenite. Increase in the total leucocyte count has been attributed to several factors like increase in thrombocytes, lymphocyte or squeezing of WBC in peripheral blood in biochemical changes related from fish body [20]. In the present study, MCH is decreased from 24 to 120 hrs of sublethal treatment of propagate. The decreased MCH and MCV levels may be a sign of hypochromic microcytic anemia. MCH is significantly decreased in *C. idella* after 48 hrs exposure fenvalerate [21] also report an increase in MCV and MCH of *Channa striata* after treatment with paper and pulp mill effluent, increase in MCHC together with MCV and MCH after exposure to zinc which is indicative of megaloblastic anaemia [22]. Total Hb concentration is reduced in the blood of triploids containing significantly fewer erythrocytes. The average volume of red blood cells (MCV) is greater in triploids, such that the mean cellular haemoglobin content (MCH) in erythrocytes of triploids is significantly greater than in diploids and the mean cellular haemoglobin concentrations (MCHC) are equivalent. Reported values for total blood haemoglobin (Hb) and MCHC concentrations in diploid and triploid fish are not consistent, whereas the mean cellular haemoglobin content (MCHC) is commonly reported to be higher in polyploids [21-22] report no differences in total Hb but higher MCH values in triploid shidrum (*Umbrina cirrosa*) compared to their diploid counterparts, since the mean cellular haemoglobin (MCHC) is significantly reduced at that time.

V. CONCLUSION

It is evident that Propargite presented in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of the delicate balance of the environment, entry into the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms. Finally, we conclude that Propargite is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. Altered haematological responses can be used as tools in bio assessment to monitor ecotoxicological risks associated with pesticides such as Propargite to various fish.

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