

## Growth promotion and chloramine T amelioration of *Senna auriculata* leaf and flower on the fry of the common carp *Cyprinus carpio*

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### Abstract

Chloramine T (Cl-T) is a low toxic disinfectant used in the aquaculture industry both culture and processing. However, it is toxic to fishes and prawns. Therefore in this study, a 28 day growth trial was conducted on the fry of *Cyprinus carpio* (initial length:  $2.25 \pm 0.029$  cm; initial weight:  $0.43 \pm 0.039$  g) using three types of formulated diets. The diet-I was formulated without *Senna auriculata* as an ingredient, diets-II, and III were with *S. auriculata* leaf, and flower, respectively. These three diets were fed to the fish to assess the growth promotion potential of *S. auriculata*, and to understand the amelioration of Cl-T by *S. auriculata* while assess the sub-lethal toxicity of Cl-T at three different concentrations (25, 50, and 75% of the 96 hr LC<sub>50</sub>, i.e., 6.16, 12.33 and 18.50 mg/L, respectively). The diet formulated using *S. auriculata* has yielded better growth and survival of fish in comparison to control ( $p < 0.05$ ); among the two plant parts, the flower has performed better than that of the leaf. Regarding the sub-lethal toxicity of Cl-T, the concentration dependent decrease ( $p < 0.05$ ) in growth and survival of the fish was recorded. Regarding amelioration of Cl-T toxicity, *S. auriculata* flower has mitigated better than that of its leaf when the fish was fed, respective formulated diets when compared with control. However, the elevated response in growth and survival between leaf and flower of *S. auriculata* included groups was not statistically significant. Actually, the growth and survival in *S. auriculata* included diet fed fish groups were improved over control (*S. auriculata* un-included group). These observations indicated the fact that *S. auriculata* flower has more growth promoting potency than that of its leaf in *C. carpio*. Similarly, *S. auriculata* flower has more ameliorative potency against chloramine T than that of its leaf. Therefore *S. auriculata* can be used in aquaculture of fishes to promote sustainability.

**Keywords:** Carp, growth, survival, chloramine, amelioration, tea tree

### Introduction

Ammonia (NH<sub>3</sub>) bonded to chlorine is chloramine. In acid conditions (below pH, 7.0) NH<sub>3</sub> is not toxic, because free hydrogen ions convert it to ammonium (NH<sub>4</sub>). As pH rise above 7.0, these hydrogen ions are less available, leaving more toxic NH<sub>3</sub>. Chlorine does not occur in natural waters in its active form. Chlorination of water leaves residual chlorine, which includes hypochlorous acid (HOCl), its dissociation releases hypochlorite ions (OCl<sup>-</sup>), the element chlorine (Cl) as a greenish-yellow poisonous gas (which is dangerous to use because of the possibility of accidental release) and its molecular Cl (Cl<sub>2</sub>), and chloramines. OCl<sup>-</sup> are introduced to water as disinfectants. Chlorine gas (Cl<sub>2</sub>) can dissolve in water to form HOCl, which can then react with ammonia to form chloramines that have less disinfecting power than free chlorine residuals. HOCl and Cl<sub>2</sub> are both used as disinfectants, while chloramines are sometimes used as a more stable alternative secondary disinfectant. Therefore, chloramines are called combined chlorine residuals. Chlorine gas and HOCl are about 100 times more potent than OCl<sup>-</sup>. Liquid chlorine (sodium hypochlorite; NaOCl), the bleach is used in concentrations ranging from 2 to 15%. Powdered chlorine (calcium hypochlorite; Ca(OCl)<sub>2</sub>) available in 65-100% chlorine, can be dissolved in water to use in shrimp farms. Cl<sub>2</sub> is more commonly used to treat the water in farming and processing

of shrimp and other seafood. The residual Cl<sub>2</sub> in the processing water usually present about 1.0 ppm. Sodium or calcium hypochlorite can generate toxic chlorine gas if put into highly acidic water. Therefore, applications of chlorine products should be dealt with at most care and it spills can be avoided.

Chlorine is unstable and easily dissipates in water. Chloramine is stable than chlorine, does not dissipate from the water as easily, and it isn't as likely to combine with other chemicals. But chloramine isn't as good at killing off the microorganisms in the water as chlorine, so higher levels of chloramine are often used (1.0 ppm). Small doses of chlorine and chloramine are safe for human. But these chemicals are very toxic to fish. They can also kill the nitrifying bacteria that keep the aquarium water in good quality. Therefore chemical de-chlorinators are used. Chlorine can be removed by adding sodium thiosulfate, which reacts with the chlorine (or the chlorine portion of the chloramine) to form harmless chloride ions. But, the de-chlorinator removes the chlorine portion of the chloramine and leaves the toxic NH<sub>3</sub> free in the water. The nitrifying bacteria convert toxic NH<sub>3</sub> to non-toxic NH<sub>4</sub>.

In aquaculture of fishes, chloramine T (Cl-T) applied in baths or flow-through systems during the early stages of a disease outbreak at a concentration of 10 ppm typically for 1 hour. It breaks down in water to release hypochlorous acid,

which is so reactive and readily able to bind to organic compounds, denature the genetic material of the pathogen via oxidation. It is most often used to treat disease caused by infectious agents like *Flavobacterium columnare* and *Tenacibaculum maritimum* (Ostland *et al.* 1995; Altinok, 2004; Harris *et al.* 2005; Leef *et al.*, 2007<sup>[4, 34, 48, 66]</sup>; Mainous *et al.* 2012; Gourzioti *et al.* 2016; Quezada-Rodriguez *et al.* 2022; Maskill *et al.* 2026)<sup>[31, 51, 54, 75]</sup>. It is also used to treat gill and skin flukes (*Gyrodactylus*), fungal (branchiomycosis) infections, parasites like *Ichthyobodo* (costiasis), *Chilodonella* (chilodonellosis), trichodina and *Neoparamoeba*, and non-infectious agents like harmful algae and jellyfish blooms (Florent *et al.* 2010; Mitchell and Rodger, 2011; Nowak *et al.* 2014; Powell *et al.* 2015; Oldham *et al.* 2016; Marcos-Lopez and Rodger, 2020; Padros and Constenla, 2021; Larcombe *et al.* 2024)<sup>[29, 47, 53, 57, 63, 65, 68, 71]</sup>. Therefore, Cl-T provides an effective treatment against viruses including white spot syndrome virus, bacteria, fungi, parasites and flukes. As chloramine-T is extremely reactive, and so should only be used in small doses and as a last resort rather than a first response since even low doses can cause issues such as gill burns and stress. Actually, Cl-T has low toxicity but its high concentrations can be toxic to fishes, amphibians and reptiles, and these pass through the gills into the bloodstream inhibiting the ability of the red blood cells to carry oxygen causes histopathological damages in gills (and other vital organs as well), which in turn leading to hypoxia and anoxia, which ultimately causes even death of organism (Miller and Mitchell, 2009)<sup>[56]</sup>.

The following are some of the medicinal plants enhancing fish growth, health, physiology and resistance to infections as well as stress. These are *Ocimum basilicum*, *Ocimum sacnctum*, *Thymus vulgaris*, *Azadiracta indica*, *Allium sativum*, *Curcuma longa*, *Zingiber officinale*, *Aloe vera*, *Ulva lactuca*, *Sargassum ilicifolium*, *Moringa oleifera*, *Mentha piperita*, *Pelargonium sidoides*, *Withania somnifera*, *Eclipta alba*, *Mangifera indica*, *Nigella sativa*, *Psidium gujava*, *Urtica dioica*, *Citrus depressa*, *Glycyrrhiza glabra*, *Trigonella foenum graecum*, *Allium sativum*, *Punica granatum*, *Cynodon dactylon*, *Hygrophila spinosa*, *Solanum trilobatum*, *Andrographis paniculata*, *Psoralea corylifolia*, *Eclipta erecta*, *Picrorhiza kurooa*, *Phyllanthus niruri*, *Tinospora cordifoliaare*, *Syzygium cumini*, *Echinacea purpure*, *Zataria multiflora*, and *Populus tomentosa* (Nya and Austin, 2009; Awad and Austin, 2010; Harikrishnan *et al.* 2011, 2021; Kaleeswaran *et al.* 2011; Elkamel and Mosaad 2012; Talpur *et al.* 2013; Talpur and Ikhwanuddin, 2013; El-Dakar *et al.* 2015; Awad *et al.* 2015; Wankhede *et al.* 2015; Ngugi *et al.* 2015; Elabd *et al.* 2016; Ghehdarijani *et al.* 2016; Shiu *et al.* 2016; Awad and Awaad, 2017; Christyapita *et al.* 2017; Shakya, 2017; Aly *et al.* 2019; Rath *et al.* 2019; Sanchez *et al.* 2020; Setijaningsih *et al.* 2021; Prabu *et al.* 2021; Li *et al.* 2022; Hasani, 2022; Tuong *et al.* 2023; Ergene *et al.* 2023; Aly *et al.* 2023; Yousefi *et al.* 2024; Choudhary *et al.* 2024; Abu-Zahra *et al.* 2024, 2025; El-Gammal *et al.* 2025)<sup>[1, 2, 5, 6, 8, 9, 10, 19, 24, 25, 26, 27, 30, 32, 33, 35, 40, 61, 64, 72, 77, 81, 82, 85, 87, 92, 94, 95]</sup>.

Xenobiotics, such as heavy metals, pesticides, drugs, pollutants etc., induce oxidative stress in both lower and

higher animals including human beings by generating reactive oxygen species, as metabolic byproducts, which are unstable, oxygen containing molecules with unpaired electrons (superoxide anion, O<sub>2</sub><sup>-</sup>, an oxygen with an extra electron; hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, a signaling molecule; hydroxyl radical, OH<sup>o</sup>, an extremely reactive free radical that causes instant cell damage; singlet oxygen, <sup>1</sup>O<sub>2</sub>, a higher energy, non-radical form of oxygen). These oxidative toxins cause damage to the cells, including cell death. Plant-derived phytochemicals (curcumin, a curcuminoid of turmeric maintains glutathione system; catechins, a polyphenol as well as a flavonoid of green tea suppress lipid peroxidation; resveratrol, a stilbene of grapes, apples and berries reduce radical chain reactions and inflammation; quercetin, a flavonoids of onions, apples and berries provides primary cellular defense; anthocyanins, a polyphenolic group flavonoid of fruits, vegetables and flowers provides anti-inflammation. vitamins C and E of citrus fruits, nuts and greens scavenge lipophilic and hydrophilic radicals) combat this damage through direct free radical scavenging (by donating electrons or hydrogen atoms to free radical for breaking the destructive oxidative chain reaction), metal chelation (by binding metal ions of copper and iron to prevent radical generation), and up-regulation of cellular antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (by converting the free radicals into water (H<sub>2</sub>O) and molecular oxygen (O<sub>2</sub>) (Chaudhary *et al.* 2023; Khan *et al.* 2025; Chandimali *et al.* 2025; Egwu *et al.* 2026)<sup>[17, 23, 26, 43]</sup>.

In the background of the above facts, this study aimed to understand the continuous chronic exposure of *Cyprinus carpio* fry to sub-lethal concentrations of 96 hr LC<sub>50</sub> of chloramine-T for a period of 4 weeks (28-days) whether affect the survival and growth of the fish. If it is so, this study further aimed to see that whether it can be ameliorated by the leaf and flower of *Senna auriculata* as all parts of the plant have been reported for their medicinal uses in indigenous system of medicines like Ayurveda and siddha against various ailments, since it contains alkaloids, quinone, glycosides, saponins, phenols, terpenoids, flavonoids, tannins, steroids, palmitic acid, linoleic acid, benzoic acid 2-hydroxyl methyl ester, 1-methyl butyl ester, resorcinol, α-tocopherol-β-D-mannosidase, epicatechin, ferulic acid, quercetin-3-O-rutinoside, quercetin, proanthocyanidin B1 etc. These compounds possess a wide range of pharmacological properties such as antidiabetic, antioxidant, anti-inflammatory, antihyperlipidemic, hepatoprotective, nephroprotective, cardioprotective, anti-atherosclerotic, anticancer, antimutagenic, antimicrobial, antiulcer, antipyretic, anthelmintic, immunomodulatory, antifertility, anti-venom, anti-melanogenesis etc. Its preparations also prescribed against skin disorders, rheumatism, ophthalmia, conjunctivitis etc. (Uma *et al.* 2017; Shafeeq *et al.* 2018; Nille *et al.* 2021; Alshehri *et al.* 2022; Shreya *et al.* 2023; Baluchamy and Subramanian, 2023; Kavita *et al.* 2024; Nandhini *et al.* 2024; Poornasundari *et al.* 2024; Tilak *et al.* 2024; Pushpalatha *et al.* 2025; Mohamed and Ramesh, 2026)<sup>[3, 11, 41, 59, 60, 62, 69, 73, 83, 86, 90, 93]</sup>.

Materials and method

**1. Purchase of chloramine trihydrate (Chloramine -T)**  
Chloramine-T ( $C_7H_7ClNNaO_2 \cdot 3H_2O$ ; MW, 281.69), a white crystalline powder manufactured by Ottokemi, Works-101, Aarkay Ruby Industrial Estate (1B), Opp. Shree Narayan Industrial Estate, Chinchpada, Vasai East, Waliv, Maharastra-401208, India, was purchased from a local chemical supplier.

**2. Procurement and acclimation of *C. carpio* fry**  
The fry of the common carp *Cyprinus carpio* (L.) Roxb, with an average length of  $1.84 \pm 0.36$  cm and an average weight of  $0.290 \pm 0.06$  g were procured from The Tamil Nadu Fisheries Department, which implements various welfare schemes for fishermen in Edappadi, Salem, India, stocked in clean fiberglass aquarium tanks (120 x 80 x 40 cm) irrespective of the sex and acclimatized to laboratory conditions for three weeks (21 days) with groundwater (Temperature, 28 °C; pH, 7.4; Salinity, 0.54 ppt; total hardness, 135.0 mg/l; total dissolved solids, 0.85 g/L; dissolved oxygen, 7.754 mg/l; BOD, 32.0 mg/l; COD, 135.30 mg/l; ammonia, 0.321 mg/l; nitrate, 1.14 mg/l), during which they were fed with rice bran, groundnut oil cake and corn flour mix twice a day. In order to maintain a healthy environment, which devoid of accumulated metabolic state, the medium was adequately aerated, every day at least 50% of the tank water was replaced, the feces, and unfed feed if any were removed by siphoning method causing minimum disturbance to the fishes. During acclimation, the fish stock was maintained at natural photoperiod and ambient temperature. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic state. These fish fingerlings were served as the stock for the experimental schedule.

**3. Collection and processing of *S. auriculata***  
A medicinal shrub identified as *Senna auriculata* (Linnaeus, 1758) also known as Tanner's Cassia/ Avertaki/ Avaram) was gathered from Bharathiar University campus, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2024<sup>[60]</sup>-25/Tech/191). The leaves and flower samples of *S. auriculata* were separated and dried under shadow. The fully dehydrated (well-dried) leaves and flower were ground well using a mechanical blender into fine powders and kept in airtight containers with proper labeling for further use. *S. auriculata* leaf and flower showed a good amount of proximate composition (crude protein, crude fat, total ash, total nitrogen free content and energy) and quality of secondary phytochemicals (alkaloids, flavonoids, terpenoids, polyphenols, saponins, tannins cardiac glycosides and quinines), which contributes to its traditional medicinal uses. It exhibited antibacterial activity, ROS production and apoptotic potential (Pushpalatha *et al.* 2025)<sup>[73]</sup>.

**4. Feed formulation (Pearson square method)**  
The basal ingredients, such as fish meal, soybean meal, corn flour, tapioca flour, hen egg and sunflower oil were purchased from local merchants. Vitamin B-complex with vitamin-C (Pfizer Ltd., Mumbai, India) was purchased from local medical shop (each vitamin capsule contains, Thiamine Mononitrate IP, 10 mg; Riboflavin IP, 10 mg; Pyridoxine Hydrochloride IP, 3mg; vitamin B12 (as tablets 1:100) IP, 15 mcg; Niacimide IP, 100 mg; calcium pantothenate IP, 50 mg; folic acid IP, 1.5 mg; Biotin USP, 100 mcg; Ascorbic acid IP, 150 mg). The basal ingredients were pulverized separately using a micro pulverizer and hand sieved through ingredient severs.

Basal diet (Fishmeal, and Soybean meal)

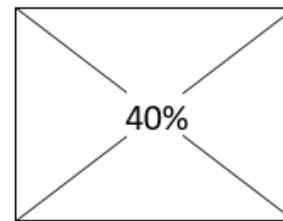
|                            |                                                                                     |                                                                                                                                      |
|----------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| Fishmeal Protein = 59.39%  |  | Fishmeal: 42.16 - 40.00 = <b>2.16</b>                                                                                                |
| Soya meal Protein = 42.16% |                                                                                     | Soya meal: 59.39-40 = <b>19.39</b>                                                                                                   |
|                            |                                                                                     | <b>To make the feed with 40% protein</b><br>Fishmeal: 2.16/21.55×100 = <b>10.02</b> g<br>Soya meal: 19.39/21.55×100 = <b>89.97</b> g |

Fig 1: Basal diet formulation with fishmeal, and soybean meal by Pearson square method.

The sieved ingredients were taken in different proportion based on the calculations made through Pearson square method fixed for 40% protein in each diet by adjusting the quantity of fishmeal, soya meal, *S. auriculata* leaf and flower based on their protein values (Figs. 1-3; Table 1) to prepare isonitric diets. The crude protein value of the fishmeal and soya meal was analysed in our lab previously,

which was reported as 59.39% and 42.16%, respectively (Manjula, 2023)<sup>[52]</sup>. The crude protein value of *S. auriculata* leaf and flower was 15.60% and 16.56%, respectively (Pushpalatha *et al.* 2025)<sup>[73]</sup>.

4.2. *S. auriculata* leaf incorporated diet (Fishmeal + Soybean meal)

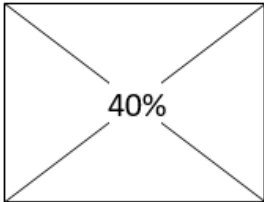
|                                                                              |                                                                                                                                                                                                                                   |                                                      |
|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Fishmeal Protein +<br>Soya meal Protein =<br>(59.39+42.16)=101.55/2 = 50.77% |                                                                                                                                                  | <i>C. auriculata</i> leaf: 40 - 15.60 = <b>24.44</b> |
|                                                                              |                                                                                                                                                                                                                                   |                                                      |
| <i>C. auriculata</i> leaf Protein =15.60%                                    |                                                                                                                                                                                                                                   | Fishmeal + Soya meal:<br>(50.77 - 40) = <b>10.77</b> |
|                                                                              | <b>To make the feed with 40% protein</b><br>Fishmeal:<br>(24.44/35.21)×100=69.41/2= <b>34.70 g</b><br>Soya meal:<br>(24.44/35.21)×100=69.45/2= <b>34.70 g</b><br><i>C. auriculata</i> leaf:<br>(10.77/35.21)×100 = <b>30.58 g</b> | Total: <b>35.21</b>                                  |

Fig 2: *S. auriculata* leaf incorporated experimental diet formulation with fishmeal, and soybean meal by Pearson square method.

#### 4.3. *S. auriculata* flower incorporated diet (Fishmeal + Soybean meal)

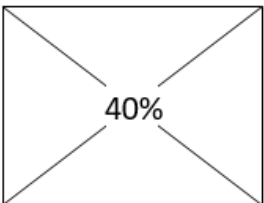
|                                                                              |                                                                                                                                                                                                                                   |                                                      |
|------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Fishmeal Protein +<br>Soya meal Protein =<br>(59.39+42.16)=101.55/2 = 50.77% |                                                                                                                                                 | <i>C. auriculata</i> leaf: 40 - 16.56 = <b>23.44</b> |
|                                                                              |                                                                                                                                                                                                                                   |                                                      |
| <i>C. auriculata</i> flower Protein =<br>16.56%                              |                                                                                                                                                                                                                                   | Fishmeal + Soya meal:<br>(50.77 - 40) = <b>10.77</b> |
|                                                                              | <b>To make the feed with 40% protein</b><br>Fishmeal:<br>(23.44/34.21)×100=68.51/2= <b>34.25 g</b><br>Soya meal:<br>(23.44/34.21)×100=68.51/2= <b>34.25 g</b><br><i>C. auriculata</i> leaf:<br>(10.77/34.21)×100 = <b>31.48 g</b> | Total: <b>34.21</b>                                  |

Fig 3: *S. auriculata* flower incorporated experimental diet formulation with fishmeal, and soybean meal by Pearson square method.

Table 1: Composition of ingredients used in preparation of different iso-nitrous diets

| Ingredients (proportion in g)      | Normal (Basal diet) | <i>S. auriculata</i> incorporated diets |        |
|------------------------------------|---------------------|-----------------------------------------|--------|
|                                    |                     | Leaf                                    | Flower |
| Fish meal                          | 10.02               | 34.70                                   | 34.25  |
| Soya meal                          | 89.97               | 34.70                                   | 34.25  |
| Corn flour                         | 25.00               | 25.00                                   | 25.00  |
| Tapioca flour                      | 5.00                | 5.00                                    | 5.00   |
| Egg albumin                        | 7.00                | 7.00                                    | 7.00   |
| Sunflower oil                      | 2.00                | 2.00                                    | 2.00   |
| Vitamin B-complex with vitamin C   | 1.00                | 1.00                                    | 1.00   |
| <i>S. auriculata</i> (leaf/flower) | --                  | 30.58                                   | 31.48  |
| Total                              | 139.99              | 139.98                                  | 139.98 |

The fishmeal and soya meal were used as protein sources. The *S. auriculata* leaf and flower were also taken to equate the protein in the respective diets in addition to their incorporations to see their ameliorative potency against Cl-T toxicity. Corn flour was taken as a carbohydrate source. Egg albumin and tapioca flour (as a carbohydrate source as well)

were used as binding sources. The sunflower oil was taken as a source of lipid. Vitamin B complex with vitamin C was also added as a supplement.

The fishmeal, soya meal, corn flour, and tapioca flour were mixed together. The mixed feed ingredients devoid of egg albumin, sunflower oil and vitamin B- complex were stream

cooked for 15 minutes at 95-100 °C and allowed to cool at room temperature. After egg albumin, sunflower oil and vitamin B-complex with vitamin C mix were added and mixed well. The dough was prepared with 10% boiled water and pellatised in a manual pellatiser fixed with 3 mm diameter dice and the pellets were sun dried until the moisture content reached less than 10%. The feed was physically examined for visual appearance, such as uniformity, colour and fragrant smell. The pellets were with smooth surface. In such a way the normal diet was prepared devoid of any form of *S. auriculata* leaf and flower inclusions and stored in sterilized airtight containers for further usage. Similarly the other isonitric diets by incorporation of *S. auriculata* leaf, and flower were prepared.

##### 5. Effect of chloramine T on the growth of *C. carpio*, and its amelioration by *S. auriculata*

Based on the 96 h LC<sub>50</sub> value (24.67 mg/L), three sub-lethal concentrations (25, 50 and 75% of the 96 h LC<sub>50</sub>; 6.16, 12.33 and 18.50 mg/L, respectively) were chosen (Pushpalatha *et al.* 2026) [74]. The fry of *C. carpio* (2.25±0.60 cm size and 0.405±0.07 g weight) was starved 24h before commencing the feeding trial. Three sets each with four groups, totally twelve groups of fish, each with 20 fingerlings were maintained in 20L water in plastic tanks under a triplicate experimental set-up. The first set of fish represents chloramines toxicity alone with a control (fed with basal diet devoid of *S. auriculata*). The second set of fish represents chloramines toxicity amelioration by *S. auriculata* leaf with a control (fed with *S. auriculata* leaf incorporated diet). The third set of fish represents chloramines toxicity amelioration by *S. auriculata* flower with a control (fed with *S. auriculata* flower incorporated diet). The toxic medium was renewed daily by freshly prepared sub-lethal concentrations of chloramines T. During the course of the experiment, the toxic medium was not aerated. The experiment was extended for a period of four weeks (28 days). During the exposure period, the percentage mortality was found to be negligible (about 2%); dead fish

were removed and replaced with fish of similar size that had been separately reared in the same medium. The feed was allocated to the fish for two times a day (10:00 am and 4:00 pm) at 10% of body weight. The feces, and unfed feed if any were collected separately by siphoning method causing minimum disturbance to the fishes, and taken into the account of calculation of nutritional indices. The survival, morphometry (length gain (LG) and weight gain (WG) were calculated in each set and compared with respective controls using the following formulae.

$$\text{Survival (\%)} = \frac{\text{Total No. of live fish on the final day}}{\text{Total No. of fish introduced initially}} \times 100$$

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}.$$

$$\text{Length gain (cm)} = \text{Final length (cm)} - \text{Initial length (cm)}.$$

## Results and Discussion

### 1. Growth promotion of *S. auriculata*

Table 2 depicts the growth and survival performance of *C. carpio* under three different diet types (type-I: without *C. auriculata* (control); type-II: with *S. auriculata* leaf; type-III: with *S. auriculata* flower). The morphometric analysis of *C. carpio* revealed that among the three diets, the diet in which *S. auriculata* flower was used as an ingredient showed the best growth performance ( $p < 0.05$ ) in terms of length and weight gains, followed by the leaf of *S. auriculata*. These observations indicated the fact that *S. auriculata* flower has more growth promoting potency than that of the leaf. Therefore, *S. auriculata* can be used in aquaculture of fishes to promote the sustainability. Table 3 represents the significant status of t-values in respect of table 2. The increased weight gain and survival rate has been reported in the milkfish, *Chanos chanos* fed *Cassia auriculata* leaf powder supplemented diet (Chandrasekar *et al.* 2017; Taştan and Salem, 2021<sup>[16, 89]</sup>; Mahmoodi *et al.* 2023; Sakthibalan *et al.* 2026)<sup>[50, 78]</sup>.

**Table 2:** Morphometric details, growth and survival of the fish *C. carpio* fry fed formulated feed with *S. auriculata* leaf, and flower as an ingredient, for a duration of 28 days (Initial length: 2.25 ± 0.029 cm; Initial weight: 0.43 ± 0.039 g).

| Diet Type | Feeding Group                               | Growth and survival of <i>C. carpio</i> fry on <i>S. auriculata</i> |                  |                       |                         |                       |
|-----------|---------------------------------------------|---------------------------------------------------------------------|------------------|-----------------------|-------------------------|-----------------------|
|           |                                             | Final Length (cm)                                                   | Final Weight (g) | Length Gain (LG) (cm) | Weight Gain (WG) (g)    | Survival rate         |
| I         | Diet without <i>S. auriculata</i> (Control) | 3.40 ± 0.50                                                         | 1.047 ± 0.155    | 1.15 ± 0.16           | 0.617 ± 0.034           | 72.0 ± 2.0            |
| II        | Diet with <i>S. auriculata</i> Leaf         | 3.60 ± 0.40                                                         | 1.083 ± 0.170    | 1.35 ± 0.19           | 0.650 ± 0.042           | 74.0 ± 3.0            |
|           | Difference between diets I and II           | (0.20) ↑                                                            | (0.036) ↑        | (0.20) ↑              | (0.033) ↑ <sup>NS</sup> | (2.0) ↑ <sup>NS</sup> |
| III       | Diet with <i>S. auriculata</i> Flower       | 3.80 ± 0.40                                                         | 1.095 ± 0.186    | 1.55 ± 0.21           | 0.665 ± 0.053           | 78.0 ± 3.0            |
|           | Difference between diets I and III          | (0.40) ↑                                                            | (0.048) ↑        | (0.40) ↑              | (0.048) ↑ <sup>NS</sup> | (6.0) ↑ <sup>S</sup>  |
|           | Difference between diets II and III         | (0.20) ↑                                                            | (0.012) ↑        | (0.20) ↑              | (0.015) ↑ <sup>NS</sup> | (4.0) ↑ <sup>S</sup>  |

Statistics represents column wise and diet type wise t-test ( $p < 0.05$ ).

Values in parentheses (↑) are actual differences over controls.

Growth promotion response: diet type-I control vs. diet type-II; diet type-I control vs. diet type-III; diet type-II vs. diet type-III; ↑<sup>NS</sup>, elevation not significant; ↑<sup>S</sup>, elevation significant.

**Table 3:** Summary of the t-value ( $p < 0.05$ ) status for the growth promoting response of the fish *C. carpio* fry to *S. auriculata* leaf, and flower as an ingredient

| Parameter   | Growth promoting potency of <i>S. auriculata</i> in <i>C. carpio</i> fry                                                    |                                                                                                                                |                                                                                                                                  |
|-------------|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
|             | Diet-I control (without <i>S. auriculata</i> )<br>vs.<br>Diet-II control (with <i>S. auriculata</i> leaf)<br>of the table 2 | Diet-I control (without <i>S. auriculata</i> )<br>vs.<br>Diet-III control (with <i>S. auriculata</i> flower)<br>of the table 2 | Diet-II control (with <i>S. auriculata</i> leaf)<br>vs.<br>Diet-III control (with <i>S. auriculata</i> flower)<br>of the table 2 |
|             |                                                                                                                             |                                                                                                                                |                                                                                                                                  |
| Weight gain | ↑NS                                                                                                                         | ↑NS                                                                                                                            | ↑NS                                                                                                                              |
| Survival    | ↑NS                                                                                                                         | ↑S                                                                                                                             | ↑S                                                                                                                               |

↑NS, elevation not significant; ↑S, elevation significant.

Phytochemicals like alkaloids, flavonoids, terpenoids, saponins, polyphenols, essential oils etc., and their secondary metabolites (active principle compounds) that stimulate digestive enzymes in fish and facilitate nutrient absorption, thereby improve food conversion and accelerate growth through enhanced protein synthesis via up-regulation of growth hormones and insulin-like growth factors, in addition to antioxidant, anti-inflammatory, immune, wound healing, stress tolerance, disease resistance etc., responses (Chakraborty *et al.*, 2011; Taştan and Salem, 2021; Hassaan *et al.* 2021; Mahmoodi *et al.* 2023; Moezzi *et al.* 2025; Sakthibalan *et al.*, 2026) [13, 36, 50, 58, 78, 89]. It has been reported that luxurious presence of alkaloids and

flavonoids in *S. auriculata* leaf and flower, respectively, in addition to many secondary bioactive metabolic compounds (Pushpalatha *et al.* 2025) [73]. When optimum levels of these compounds containing plant parts are supplemented, they elevate blood cell count, hemoglobin content, and activities of lysozyme and phagocytosis in order to provide antiviral, antibacterial and antiparasitic as well as prophylactic activities (Chakraborty *et al.* 2014; Chandrasekar *et al.* 2017; Rashidian *et al.* 2021; Zhang *et al.* 2022; Dadras *et al.* 2023; Oz *et al.* 2024; Zanganeh *et al.* 2026) [14, 16, 21, 76, 96, 97].

## 2. Toxicity of chloramine-T and its amelioration by *S. auriculata*

**Table 4:** Ameliorative potency of formulated feed with *S. auriculata* leaf, and flower as an ingredient against chloramine T toxicity over growth and survival of the fish *C. carpio* fry for a duration of 28 days (Initial length:  $2.25 \pm 0.029$  cm; Initial weight:  $0.43 \pm 0.039$  g).

| Diet Type    | Feeding Group                                               | Toxic effect of Cl T on <i>C. carpio</i> fry, with and without <i>S. auriculata</i> |                   |                       |                         |                       |
|--------------|-------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------|-----------------------|-------------------------|-----------------------|
|              |                                                             | Final Length (cm)                                                                   | Final Weight (g)  | Length Gain (LG) (cm) | Weight Gain (WG) (g)    | Survival rate         |
| I            | Basal Diet without <i>S. auriculata</i> (Control)           | $3.40 \pm 0.50$                                                                     | $1.047 \pm 0.155$ | $1.15 \pm 0.16$       | $0.617 \pm 0.034$       | $72.0 \pm 2.0$        |
|              | Basal Diet + Cl-T (lower) without <i>S. auriculata</i>      | $3.30 \pm 0.40$                                                                     | $0.973 \pm 0.166$ | $1.05 \pm 0.19$       | $0.543 \pm 0.022$       | $70.0 \pm 3.0$        |
|              | Difference between control-I and Cl-T (lower)               | (0.1) ↓                                                                             | (0.074) ↓         | (0.1) ↓               | (0.074) ↓ <sup>S</sup>  | (2.0) ↓ <sup>NS</sup> |
|              | Basal Diet + Cl-T (middle) without <i>S. auriculata</i>     | $3.10 \pm 0.60$                                                                     | $0.819 \pm 0.171$ | $0.85 \pm 0.19$       | $0.389 \pm 0.030$       | $68.0 \pm 3.0$        |
|              | Difference between control-I and Cl-T (middle)              | (0.3) ↓                                                                             | (0.228) ↓         | (0.3) ↓               | (0.228) ↓ <sup>S</sup>  | (4.0) ↓ <sup>NS</sup> |
|              | Basal Diet + Cl-T (higher) without <i>S. auriculata</i>     | $3.00 \pm 0.60$                                                                     | $0.729 \pm 0.112$ | $0.75 \pm 0.19$       | $0.299 \pm 0.017$       | $65.0 \pm 2.0$        |
|              | Difference between control-I and Cl-T (higher)              | (0.4) ↓                                                                             | (0.318) ↓         | (0.4) ↓               | (0.318) ↓ <sup>S</sup>  | (7.0) ↓ <sup>S</sup>  |
| II           | Basal Diet with <i>S. auriculata</i> Leaf (Control)         | $3.60 \pm 0.40$                                                                     | $1.083 \pm 0.170$ | $1.35 \pm 0.19$       | $0.650 \pm 0.042$       | $74.0 \pm 3.0$        |
|              | Basal Diet + Cl-T (lower) with <i>S. auriculata</i> Leaf    | $3.40 \pm 0.50$                                                                     | $1.070 \pm 0.131$ | $1.15 \pm 0.11$       | $0.640 \pm 0.029$       | $72.0 \pm 3.0$        |
|              | Difference between control-II and Cl-T (lower)              | (0.2) ↓                                                                             | (0.013) ↓         | (0.2) ↓               | (0.010) ↓               | (2.0) ↓               |
|              | Basal Diet + Cl-T (middle) with <i>S. auriculata</i> Leaf   | $3.30 \pm 0.30$                                                                     | $1.040 \pm 0.141$ | $1.05 \pm 0.12$       | $0.610 \pm 0.020$       | $70.0 \pm 10.0$       |
|              | Difference between control-II and Cl-T (middle)             | (0.3) ↓                                                                             | (0.043) ↓         | (0.3) ↓               | (0.040) ↓               | (4.0) ↓               |
|              | Basal Diet + Cl-T (higher) with <i>S. auriculata</i> Leaf   | $3.10 \pm 0.40$                                                                     | $1.022 \pm 0.105$ | $0.85 \pm 0.14$       | $0.592 \pm 0.022$       | $69.0 \pm 3.0$        |
|              | Difference between control-II and Cl-T (higher)             | (0.3) ↓                                                                             | (0.061) ↓         | (0.5) ↓               | (0.058) ↓ <sup>NS</sup> | (5.0) ↓ <sup>NS</sup> |
| III          | Basal Diet with <i>S. auriculata</i> Flower (Control)       | $3.80 \pm 0.40$                                                                     | $1.095 \pm 0.186$ | $1.55 \pm 0.21$       | $0.665 \pm 0.053$       | $78.0 \pm 3.0$        |
|              | Basal Diet + Cl-T (lower) with <i>S. auriculata</i> Flower  | $3.50 \pm 0.40$                                                                     | $1.085 \pm 0.126$ | $1.25 \pm 0.20$       | $0.655 \pm 0.028$       | $76.0 \pm 2.0$        |
|              | Difference between control-III and Cl-T (lower)             | (0.30) ↓                                                                            | (0.010) ↓         | (0.30) ↓              | (0.010) ↓               | (2.0) ↓               |
|              | Basal Diet + Cl-T (middle) with <i>S. auriculata</i> Flower | $3.40 \pm 0.30$                                                                     | $1.060 \pm 0.176$ | $1.15 \pm 0.14$       | $0.630 \pm 0.035$       | $75.0 \pm 3.0$        |
|              | Difference between control-III and Cl-T (middle)            | (0.40) ↓                                                                            | (0.035) ↓         | (0.40) ↓              | (0.035) ↓               | (3.0) ↓               |
|              | Basal Diet + Cl-T (higher) with <i>S. auriculata</i> Flower | $3.20 \pm 0.40$                                                                     | $1.040 \pm 0.145$ | $0.95 \pm 0.22$       | $0.610 \pm 0.034$       | $74.0 \pm 2.0$        |
|              | Difference between control-III and Cl-T (higher)            | (0.60) ↓                                                                            | (0.055) ↓         | (0.60) ↓              | (0.055) ↓ <sup>NS</sup> | (4.0) ↓ <sup>NS</sup> |
| Amelioration | Diet I + Cl-T (higher) vs. Diet II + Cl-T (higher)          | (0.10) ↑                                                                            | (0.293) ↑         | (0.10) ↑              | (0.293) ↑ <sup>S</sup>  | (6.0) ↑ <sup>S</sup>  |
|              | Diet I + Cl-T (higher) vs. Diet III + Cl-T (higher)         | (0.20) ↑                                                                            | (0.311) ↑         | (0.20) ↑              | (0.311) ↑ <sup>S</sup>  | (9.0) ↑ <sup>S</sup>  |
|              | Diet II + Cl-T (higher) vs. Diet III + Cl-T (higher)        | (0.10) ↑                                                                            | (0.018) ↑         | (0.10) ↑              | (0.018) ↑ <sup>NS</sup> | (5.0) ↑ <sup>NS</sup> |

Cl T, chloramine T.

Diet-I, Cl-T toxicity.

Diet-II, Cl-T toxicity amelioration by *S. auriculata* leaf.

Diet-III, Cl-T toxicity amelioration by *S. auriculata* flower.

Statistics represents column wise and diet type wise t-test.

Values in parentheses (↓) represent actual differences between respective controls and experiments, and (↑) represent actual differences between experiments.

↑<sup>NS</sup>, elevation not significant; ↑<sup>S</sup>, elevation significant.

↓<sup>NS</sup>, decrease not significant; ↓<sup>S</sup>, decrease significant.

Table 4 depicts the growth and survival performance of *C. carpio* under three different diet types (type-I with Cl-T but without *S. auriculata*; type-II with Cl-T and *S. auriculata* leaf; type-III with Cl-T and *S. auriculata* flower) along with respective control diets devoid of chloramine T. When the fish was fed the diet without *S. auriculata*, a dose dependent reduction ( $p < 0.05$ ) in survival and growth was recorded against Cl-T in comparison to control. Similarly, the dose dependent reduction ( $p < 0.05$ ) was also recorded against Cl-T when the fish was fed *S. auriculata* leaf diet as well as *S. auriculata* flower diet in comparison to respective controls. The actual reduction in survival and growth was found to be

considerably decreased against Cl-T when the fish was fed with *S. auriculata* flower followed by *S. auriculata* leaf in comparison to respective control diets. These observations indicated the fact that *S. auriculata* flower has more ameliorative potency against Cl-T than that of the leaf. Furthermore, when the fish was unexposed to Cl-T toxicity, the flower and leaf of *S. auriculata* were acted as growth promoters. Therefore, *S. auriculata* can be used in aquaculture of fishes to promote the sustainability, as water pollution is predominant in the present day environment. Table 5 represents the significant status of t-values in respect of table 4.

**Table 5:** Summary of the t-value ( $p < 0.05$ ) status for the ameliorative potency of formulated diets with *S. auriculata* leaf, and flower as an ingredient against chloramine T toxicity over growth and survival of the fish *C. carpio* fry

| Parameter   | Toxic effect of Cl T                                     |                                                            |                                                              | Ameliorative potency of <i>S. auriculata</i> against Cl T         |                                                                    |                                                                     |
|-------------|----------------------------------------------------------|------------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------|---------------------------------------------------------------------|
|             | Diet I (control) vs. Diet I (Cl T higher) of the table 4 | Diet II (control) vs. Diet II (Cl T higher) of the table 4 | Diet III (control) vs. Diet III (Cl T higher) of the table 4 | Diet I + Cl T (higher) vs. Diet II + Cl T (higher) of the table 4 | Diet I + Cl T (higher) vs. Diet III + Cl T (higher) of the table 4 | Diet II + Cl T (higher) vs. Diet III + Cl T (higher) of the table 4 |
| Weight gain | ↓S                                                       | ↓NS                                                        | ↓NS                                                          | ↑S                                                                | ↑S                                                                 | ↑NS                                                                 |
| Survival    | ↓S                                                       | ↓NS                                                        | ↓NS                                                          | ↑S                                                                | ↑S                                                                 | ↑NS                                                                 |

Cl T, chloramine T

↑NS, elevation not significant; ↑S, elevation significant.

↓ NS, decrease not significant; ↓S, decrease not significant.

In this study, under the diet type I, a dose dependent significant decrease in growth and survival of *C. carpio* fry was recorded against Cl-T toxicity (Table 4). Chloramine-T is inadvertently overdosed or overexposed on fry, fingerling, and juvenile during therapeutic treatment, 20 mg/L in a static or flow-through bath for 60 min/d on three alternate or consecutive days was safely used to control mortality in freshwater reared salmonids and rainbow trouts due to bacterial gill disease caused by *Flavobacterium branchiophilum* (Bowker *et al.* 2011) [12]. But, Absolute growth rates were suppressed significantly in 20 mg/L chloramine-T treated rainbow trout (Powell *et al.* 1994; Tkachenko *et al.* 2013). According to Altinok, 2004) [4, 70, 91], when the concentration of chloramine-T was increased from 15 to 20 or 25 mg/l, survival of fish decreased while treating the columnaris disease in *C. auratus*. Chloramine-T had significant growth suppression due to significant reduction of feed conversion efficiency in rainbow trout, *Oncorhynchus mykiss* (Sanchez *et al.* 1996) [80]. But, intermittent exposure to chloramine-T at 10 mg/L does not elicit a primary (blood cortisol level was not affected) or secondary (hematocrit, plasma glucose, sodium, and chloride levels were not affected) stress responses in rainbow trout and that stress is not the mechanism responsible for growth reduction through endocrine mediation (Sanchez *et al.* 1997) [79].

Chloramine is a water disinfectant rather than a systemic toxin like pesticides. Therefore, ameliorative potency of *S. auriculata* or any other plant against chloramine toxicity is limited. However, the non-enzymatic antioxidants (flavonoids, carotenoids, polyphenols, and vitamins) of plants must be supplemented through the diet (e.g. garlic, ginger, turmeric, moringa, olive, *Artemisia*, *Catharanthus*, *Tephrosia*, spinach, stinkvine, beetroot, pepper etc.) offer the free radicals scavenging ability against reactive oxygen

species generated by xenobiotics (Khan *et al.* 2025; Chandimali *et al.* 2025; Egwu *et al.* 2026) [23, 26, 43]. Enzymatic antioxidants such as SOD, CAT and GPx are normally produced in cells against toxic stress. Actually supplementation of non-enzymatic antioxidants may improve the efficiency of enzymatic antioxidants. Since, *S. auriculata* possesses flavonoids and polyphenolics (Pushpalatha *et al.* 2025) [73], might have induced the enzymatic antioxidant defence in the fish to maintain the cellular homeostasis when they were fed formulated feed in which *S. auriculata* leaf, and flower as an ingredient. Therefore, xenobiotics induced oxidative stress can be mitigated by supplemented with antioxidants. Many secondary metabolites are actual bioactive compounds (ascorbic acid, naringenin, curcumin, resveratrol, quercetin, withanolides of ashwagandha, moringa, catechins of cocoa, berries, dark chocolate, and tea, and thymol of thyme, oregano, basil, marjoram etc.) are powerful antioxidants. The bioactive compounds of plants scavenge free radicals, and chelate metal ions and pesticides as well (Kumar *et al.* 2014; Dua *et al.* 2015; Ibrahim *et al.* 2019; Highab *et al.* 2020; Kowalczyk *et al.* 2020; Zwolak, 2021; Arslan *et al.* 2022; Jabłonska–Trypuc and Wiater, 2022; Cheng *et al.* 2023; Melebari and Elnaggar, 2023; Khalid *et al.* 2024; Kol *et al.* 2025) [2, 7, 18, 22, 37, 38, 39, 44, 45, 46, 55, 98].

## Conclusion

In this study, *S. auriculata* leaf and flower increased the growth and survival performance of *C. carpio* fry, and also reduced the toxic effect of chloramine T. Among the two plant parts flower has more effect in both growth promotion and amelioration of chloramine T than that of the leaf. When compared with control, the leaf also performed well. Therefore, *S. auriculata* can be taken as an ingredient/ feed supplement to promote sustainable fish culture.

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