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Effects of imidacloprid on viability and hatchability of embryos of the common carp (*Cyprinus carpio* L.)

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Abstract
Imidacloprid is a neonicotinoid pesticide used worldwide to control insect pest in rural, urban and agricultural area, where it is contaminating the aquatic ecosystems as a pollutant. The present study was conducted to analyze the toxic effects of imidacloprid on viability, hatchability and survival of embryos and larvae of common carp, *Cyprinus carpio* under laboratory condition. Eggs of *C. carpio* were exposed to different sublethel concentrations of imidacloprid. The calculated 48 h LC_{50} was 78 ppm for the fertilized eggs of *C. carpio*. Four different sublethal concentration of imidacloprid were selected for experiment {10% (T_1), 20% (T_2), 30% (T_3) and 40% (T_4) of LC_{50})} along with control. The results provide clear evidence that viability of embryos in eggs decreased from 92.33 percent (control) to 61.33 percent (T_4). Thus, imidacloprid is a serious water pollutant that may have adverse effect on aquatic ecosystem including reproduction and development of fishes.

Keywords: *Cyprinus carpio,* pesticide imidacloprid, viability, hatchability

1. Introduction
The use of pesticides has increased several folds in India and is likely to increase in the coming years [1]. These Pesticides applied into agriculture fields easily get washed away and enter into the aquatic system in large quantities and imbalance the ecosystem and induce both physiological and as well as biochemical effects on aquatic organism.
Imidacloprid is one of the most effective neonicotinoid insecticides used in the large volume against sucking insect pests [2]. The mode of action of this neurotoxic pesticide is similar to that of nicotine and competes with acetylcholine for receptor site [3]. It's binding over stimulates the acetylcholine (Ach) receptors on the postsynaptic membranes of neurons [4-6]. Imidacloprid is very systemic, rapidly absorbed and stored in plant tissues. When an insect feed on such plant, it ingests the insecticide and dies. It is very active insecticide and, its insecticidal property lasts for about 3 months after its application in field [7]. Hence protect the plant from early pests infection. Because of its systemic nature, imidacloprid moves easily between plant tissues, and also from the roots to the soil and water in the field [8]. In this way, imidacloprid is gradually released into the aquatic ecosystem [9], where it is stable to hydrolysis [10]. It is a serious matter of concern as the surface water concentration of imidacloprid may reach upto 14 µg/l [11].

Table 1: Some physico-chemical properties of imidacloprid and its half-life in several media.

Property	Values
Active Ingredient	Imidacloprid
Appearance	Colourless crystal
Chemical Family	Chloronicotinyl (neonicotinoid)
Chemical Formula	$C_9H_{10}ClN_5O_2$
Molecular weight	255.7
Water solubility (mgL^{-1}, 20 ◦C)	610
Vapour pressure (mPa, 20 ◦C)	4×10^{-7}
Half-life	1.2 h[12], 126 min[13]
Aqueous photolysis	4 days[14]
Freshwater (sunlight)	10–24 weeks[10]
(Dark)	66 days[14] (anaerobic)
Water and sediment	50–70 days[15] (aerobic)

It is extremely toxic to nontarget insects, such as bees, *Apis mellifera* [16] and some of the aquatic species including the freshwater crustacean *Hyalella azteca* and the estuary crustacean *Mysidopsis bahia* [2]. According to Liu and Casida [17], margin of safety level of imidacloprid for humans is higher as it binds much more readily invertebrate acetylcholine receptor than it does to vertebrates. Because of its low mammalian toxicity and high insecticidal effectiveness, imidacloprid is being used worldwide for insect control [17]. However, a number of studies revealed its moderate toxicity to fish [4, 5], mammals and birds [18]. It has been reported that imidacloprid causes loss of ability to move, and slight tremors in lab animals, thinning of eggshell, decreased weight, reduced egg production and hatching success in birds [19, 20]. Recent findings indicated that in mammals, imidacloprid enhance the production of reactive oxygen species (ROS), which produce multiple types of toxic responses such as oxidative stress and various tissue damage i.e. liver, kidney and brain [21-23].

Very little information is available regarding neonicotinoid toxicity for fish. However, according to a report [19], imidacloprid is acutely toxic to mature fish at relatively high concentrations (over 80 ppm) with juvenile fish being considerably more susceptible. At the lowest concentration (1.2 ppm) it reduces the survival of rainbow trout fry as well as their weight. The present study was conducted to evaluate the acute toxic effects of imidacloprid on viability, hatchability and survival of eggs and fry of fresh water fish, *Cyprinus carpio*.

2. Materials and Methods

The experiment was conducted in March 2014, at Fish and Fisheries Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra, Haryana, India during March 2013, according to the OECD guidelines [24, 25].

2.1 Test chemical: Imidacloprid (17.8%SL) was purchased from local market of Kurukshetra. Fertilized eggs of *C. carpio* were procured from National Fish Seed Farm, Jyotisar and Fish Seed Farm Mandheri (District Kurukshetra).

2.2 Calculation of LC_{50}: LC_{50} of imidacloprid for fertilized eggs of *C. carpio* was calculated by semi-static method. For this several groups of eggs were exposed to different concentration of imidacloprid with the initial dose starting from 5 ppm to higher concentration. The experiment was conducted continuously with increasing dose of test chemical to determine the pesticide concentration causing 50% of death of eggs. Mortality in each concentration was recorded and LC_{50} was calculated as per probit analysis with the help of IBM SPSS Statics Version 20 for Windows 8. The calculated LC_{50} of imidacloprid for 48 hours (25 °C) for fertilized eggs of *C. carpio* was found to be 78 ppm.

2.3 Experiment lay out: Four different sublethel concentrations of imidacloprid as per Table-2, along with control were selected in triplicate for each treatment. Temperature of all the experimental media ranged between 27-28 ºC and pH was maintained between 7.3-7.8.

Approximately 1500 fertilized eggs of *C. carpio* were exposed to selected concentrations of imidacloprid (Table 2) and were reared in small happa fitted in 50 L plastic tubs filled with 30 liter dechlorinated water.

Table 2. Concentrations of imidacloprid used in different treatment groups

Treatment	Concentration
Control (C)	0.0 ppm
T_1	7.8 ppm (10% of LC_{50})
T_2	15.6 ppm (20% of LC_{50})
T_3	23.4 ppm (30% of LC_{50})
T_4	31.2 ppm (40% of LC_{50})

Proper aeration was maintained with the help of aerators. Water was kept circulating with the help of low pressure pump. Eggs were under regular observation and dead eggs were counted, recorded and removed immediately until the end of test. The dead embryos become white and opaque due to coagulation and precipitation of proteins [26]. Newly hatched larvae were also counted and inspected. The hatchability rate (%) was calculated as a number of hatched larvae per initial number of incubated eggs.

2.4 Statistical analysis: Significant differences among treatment groups were tested by Analysis of variance (ANOVA), followed by Duncan's multiple range tests for the experiments. Statistical significance was settled at a probability value of $P<0.05$. All statistics were performed using IBM SPSS Statics Version 20 for Windows 8.

3. Results

3.1 Viability of eggs: Throughout the study period the observed percent viability of the eggs significantly decreased with increase in imidacloprid concentration and duration of exposure period (Table 3). Viability after 12 hours was 96.66±0.66 percent in control group and it was 94.00±0.57, 90.66±0.88, 86.66±1.20 and 87.66±0.66 percent for T_1, T_2, T_3 and T_4 respectively. After 24 hours of exposure, the observed viable eggs in the control was 94.00±1.15 percent. It was 93.67±0.67 percent in T_1, 87.00±1.00 percent in T_2, 82.33±1.45 percent in T_3 and 83.33±0.88 percent in T_4. Similarly the percent viability of eggs after 36 hours of exposure duration was again significantly less in the treated groups as compared to control group. After 36 hours of exposure the percent viability in the control was 92.33±1.20 percent and in experimental groups T_1, T_2, T_3 and T_4 viability of eggs was respectively found to be 91.00±0.58, 83.00±0.57, 78.67±0.67, 61.33±0.67 percent (Table 3).

3.2 Hatching success

The exposure of eggs to imidacloprid resulted in decrease in hatching success. The hatching was maximum in control group and minimum in treated group having highest concentration of imidacloprid. The values of hatching success for different imidacloprid concentrations i.e. Control, T_1, T_2, T_3 and T_4 were determined as 90.33±0.88 percent, 89.67±0.67 percent, 79.88±0.88 percent, 76.00±1.00 percent and 55.33±1.45 percent respectively (Table 3). The difference in hatching success was significant at different concentration. Although the difference in hatching success in Control and T_1 was insignificant, T_2 onwards survival of eggs and their hatching decreased significantly. Only 55.33 percent of the embryos hatched out in treatment having the highest concentration of the pesticide.

Table 3: Viability and hatching success of eggs of *C. carpio* exposed to different sublethal concentrations of imidacloprid.

Sr. No	Concentration	Exposure duration (h)			Hatching Success
		12	24	36	
1	Control	96.66 ± 0.66^{Aa}	94.00 ± 1.15^{Aba}	92.33 ± 1.20^{Ba}	90.33 ± 0.88^{a}
2	T_1	94.00 ± 0.57^{Ab}	93.67 ± 0.67^{Aa}	91.00 ± 0.58^{Ba}	89.33 ± 0.67^{a}
3	T_2	90.66 ± 0.88^{Ac}	87.00 ± 1.00^{Bb}	83.00 ± 0.57^{Cb}	79.00 ± 0.88^{b}
4	T_3	86.66 ± 1.20^{Ad}	82.33 ± 1.45^{Bc}	78.67 ± 0.67^{Cc}	76.00 ± 1.00^{c}
5	T_4	87.66 ± 0.66^{Ad}	83.33 ± 0.88^{Bc}	61.33 ± 0.67^{Cd}	55.33 ± 1.45^{d}

N = 100

All values are Mean ± S.E of mean

Values with different upper case alphabets superscripts differ significantly ($P<0.05$) with in the same row

Values with different lower case alphabets superscripts differ significantly ($P<0.05$) with in the same column

(Data were analyzed by Duncan's Multiple Range test).

3.3 Hatching period

Besides mortality of eggs, variation in incubation period was also reported in different treatment groups. Hatching of the eggs in pesticide treated groups initiated 3-5 hours in advance as compared to control (Table 4). In control group the hatching of the eggs started after 42 hours and completed by 46 hours of fertilization. The hatching was preponed by 1 hour in T_1 and started after 41 hours of fertilization. In T_2, incubation period was 42 hours as in control but advanced by one hour and completed after 47 hours of fertilization. In T_3 hatching was preponed by 2 hours and it started after 40 hours of fertilization. In T_1, hatching was further preponed by 5 hours and occurred after 37 hours of fertilization. The difference in hatching period may be due to faster development of embryos exposed to higher concentration of test chemical.

Table 4: Effect of imidacloprid on hatching time

Treatment	Beginning of Hatching (Hours)	Completion of Hatching (Hours)
Control	42	46
T_1	41	46
T_2	42	47
T_3	40	46
T_4	37	43

4. Discussion

Imidacloprid significantly affects viability and hatchability of eggs of *C. carpio* in a dose dependent fashion. Acute toxicity tests of adult fish using pesticide have shown that pesticides have adverse effect on all the stages of all fish species [27-29], but early life stages of fishes are the most sensitive to their toxic effects. In the present study, the LC_{50} value for 48 hours of imidacloprid for *C. carpio* embryos was found to be 78 ppm. In a previous study [30], the reported LC_{50} for adult *C. carpio* is 280 mg/l. This shows that imidacloprid is more toxic to fish in early developmental stages. Acute toxicity of imidacloprid varies in different species, for rainbow trout LC_{50} for 96 hours is 211 mg/L [31]; 280 mg/L for carp [30] and 161 mg/L for sheepshead minnow [32]. It has been observed that the number of dead embryos significantly increased in response to imidacloprid concentrations in different treatment. This increase in mortality with increase in concentration may be due to rapid absorption of pesticide and rapid onset of action [33]. It has been reported by the other workers that chorion of fish provides no protection to the developing embryo exposed to various pesticides [34, 35]; hence, exposure to certain chemicals can cause significant increase in mortality. Malone and Blayloc [36] reported that at concentrations of 5-10 ppm, almost all insecticides causes significant mortality of embryos.

It has been also reported that increase in imidacloprid concentration has a significantly effect of hatching success and hatching period. In control group, hatching was maximum (90.33 percent) and it goes on decreased from T_1 to T_4 with minimum hatching 55.33 percent at T_4 group. Similar observations have been reported by Aydin and koprucu [37] and also by Hamm and Hinton [38] in case of diazinon toxicity on early embryonic stages and hatching success of medaka fish. The hypothesis behind this decreased hatching success is that higher concentration of pesticide affects the activity of hatching enzymes [39].

The hatching period was also affected by various sublethal concentrations of imidacloprid (Table 4). In pesticide treated group (T_2 and T_4), hatching was preponed by 2-5 hours as compared to control. This time difference may be due to faster development of embryos in imidacloprid unclear because hatching is a combined result of the activity of the hatching enzyme chorinase, muscle contraction, active water uptake by the embryo and increased perivitelline pressure [40].

However, it may be due to the fact that pesticide triggers the embryonic development or due to weakening of the egg shell under pesticide exposure which may result in immature hatching. Similar difference in hatching period was also observed by Kohler and Scheil [41], in Nickel Chloride, Chlorpyrifos, and imidacloprid toxicity in combination with different temperatures on the embryogenesis of the Zebrafish. Other workers also reported that exposure of embryos to neurotransmitter, whether agonists or antagonists may alter the hatching period. For example, Schoots *et al*. [42] reported that dopaminergic agonists increase hatching period and antagonists cause a decrease. Similarly, DiMichele and Taylor [43] reported that epinephrine decreased average time to hatch.

5. Conclusion

In the present study adverse effects imidacloprid on viability and hatchability of embryos of *C. carpio* were observed. Even its lowest concentration (10% of LC_{50}) had significant effect on viability and hatchability. The adverse effects also depend upon concentration and duration of exposure. Thus, it is concluded that imidacloprid is hazardous for aquatic ecosystem.

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