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Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms☆

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ABSTRACT

The neonicotinoid insecticide imidacloprid is used in Bangladesh for a variety of crop protection purposes. Imidacloprid may contaminate aquatic ecosystems via spray drift, surface runoff and ground water leaching. The present study aimed at assessing the fate and effects of imidacloprid on structural (phytoplankton, zooplankton, macroinvertebrates and periphyton) and functional (organic matter decomposition) endpoints of freshwater, sub-tropical ecosystems in Bangladesh. Imidacloprid was applied weekly to 16 freshwater microcosms (PVC tanks containing 400 L de-chlorinated tap water) at nominal concentrations of 0, 30, 300, 3000 ng/L over a period of 4 weeks. Results indicated that imidacloprid concentrations from the microcosm water column declined rapidly. Univariate and multivariate analysis showed significant effects of imidacloprid on the zooplankton and macroinvertebrate community, some individual phytoplankton taxa, and water quality variables (i.e. DO, alkalinity, ammonia and nitrate), with *Cloeon* sp., *Diaptomus* sp. and *Keratella* sp. being the most affected species, i.e. showing lower abundance values in all treatments compared to the control. The observed high sensitivity of *Cloeon* sp. and *Diaptomus* sp. was confirmed by the results of single species tests. No significant effects were observed on the species composition of the phytoplankton, periphyton biomass and organic matter decomposition for any of the sampling days. Our study indicates that (sub-)tropical aquatic ecosystems can be much more sensitive to imidacloprid compared to temperate ones.

1. Introduction

The shift from traditional to modern and intensive agricultural practices in developing countries like Bangladesh, has led to an increasing use of pesticides over the last decades (Rahman, 2013). Pesticide use in Bangladesh raised from 7350 metric tons in 1992 to 45,172 metric tons in 2010 (Ali et al., 2017). This was partly due to governments' policy to stimulate chemical control measures against insect pests to increase crop production as well as to prevent pre- and post-harvest crop losses (Shahjahan et al., 2017; Sumon et al., 2016).

Imidacloprid ((*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine; CAS No. 138261-41-3) is a neonicotinoid synthetic insecticide and veterinary substance. It was first introduced in the USA in the 1990s to control insect pests and is now registered in about 120 countries for use in more than 140 crops including rice, maize, cotton, potatoes, tomatoes, sugar beets and various greenhouse-grown plants (Jeschke and Nauen, 2008; Morrissey et al., 2015; Lewis et al., 2016).

Imidacloprid may affect non-target aquatic organisms via exposure due to spray drift (Hilz and Vermeer, 2012) and runoff resulting from its' high solubility in water (Armbrust and Peeler, 2002). After entering into water bodies, the dissipation time 50% (DT50) of imidacloprid merely depends on photolysis, however,

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variation in $DT50_{water}$ was observed between different water bodies. For example, the European Food Safety Authority (EFSA) reported $DT50_{water}$ values ranging from 30 to 150 days for three water-sediment studies performed at 22 °C in laboratory in the dark (EFSA, 2008), indicating a likely long-term exposure of imidacloprid to aquatic ecosystem when light conditions are poor. However, imidacloprid was found to dissipate very rapidly in different studies under UV light due to photolysis (e.g. Lavine et al., 2010). Colombo et al. (2013) recorded a DT50 of 1.2 day from the water column monitored for 28 days in field-based microcosms in Germany, whereas a DT50 of 8.2 day was reported in a pond microcosm in Germany (Posthuma-Doodeman, 2008). A DT50 of 1 day was recorded by Thuyet et al. (2011) for a rice paddy system in autumn in Japan. However, imidacloprid has been detected worldwide in surface waters at concentrations ranging from 0.001 to 320 μg/L, the highest of which was found in Netherlands (Morrissey et al., 2015). Imidacloprid has been found in aquatic ecosystems at 3.29 μg/L in the California's agricultural regions in the USA (Starner and Goh, 2012) and up to 11.9 μg/L in Canadian agricultural areas (CCME, 2007). The field monitoring data on imidacloprid is only available for temperate countries, but the systemic study from sub- (tropical) countries is lacking.

During the past years, a large number of studies focusing on the toxicity of imidacloprid to the aquatic environment have been published, partly also due to the debate on the negative relationship between the use of neonicotinoids and non-target beneficial invertebrates, in particular arthropods (EASAC, 2015; Van Dijk et al., 2013; Vijver and Van den Brink, 2014). Both single species laboratory tests (Alexander et al., 2007; Stoughton et al., 2008; Roessink et al., 2013; Cavallaro et al., 2017; Van den Brink et al., 2016) and model ecosystem studies (Hayasaka et al., 2012a; Mohr et al., 2012; Colombo et al., 2013) using imidacloprid, were all conducted in temperate regions. To date no study seem to have been undertaken to investigate the sensitivity of imidacloprid on the aquatic organisms in the sub-tropics and tropics. Van den Brink et al. (2016) found that a reproducing, summer generations of several arthropods were more sensitive to imidacloprid than their non-reproducing, winter generation. Earlier studies demonstrated that higher temperature also might increase the sensitivity of arthropods (Camp and Buchwalter, 2016; Van den Brink et al., 2016). Hence, a difference in sensitivity between tropical and temperate communities to imidacloprid can be hypothesized. To address this knowledge gap, the present study aimed at assessing fate and effects of imidacloprid on the structural (phytoplankton, zooplankton, macroinvertebrates, and periphyton) and functional (organic matter decomposition) endpoints of freshwater ecosystems located in the sub-tropical country Bangladesh.

2. Materials and methods

Most of the materials and methods used for the microcosm experiment have been described by Rico et al. (2014).

2.1. Design of the microcosm study and acute toxicity tests

The present study was conducted in sixteen freshwater microcosms at the Faculty of Fisheries, Bangladesh Agricultural University (Mymensingh, Bangladesh; 24.7434°N, 90.3984°E). The open experimental area was roofed with transparent plastic slates (Fig. S1). Each microcosm comprised of a PVC tank (diameter: 172 cm; total height: 78 cm) which was coated with non-toxic epoxy paint. Each microcosm was initially filled with 4.5 cm of sediment (collected from nearby ponds of Bangladesh Agricultural University campus) and 400 L of tap water (a layer of 56 cm). Microcosm water was allowed to dissipate the possible chlorine residues for one week. Each system was gently aerated to provide some water movement. The systems were stocked with algae and invertebrates collected from same ponds where sediment was collected. These ponds were selected because they were uncontaminated sources (as agricultural activities were not practised near the Bangladesh Agricultural University campus) and were quite biodiverse in terms of algae and invertebrates. Macroinvertebrates were stocked by distributing an equal numbers of each of the taxa into each microcosm, while equal amounts of concentrated plankton in terms of volume were added into each microcosm. The algae and invertebrate communities were allowed to develop themselves over a pre-treatment period of 6 weeks. During the pre-treatment period, every two weeks about 20% of the water volume was exchanged between the microcosms to promote the uniformity in the structure of the communities between the microcosms. As recommended by Daam and Van den Brink (2011), urea (containing 1.4 mg/L nitrogen) and trisodium phosphate (0.18 mg/L phosphorus) were administered every two weeks to the systems during the experimental period.

For the acute toxicity tests, *Cloeon* sp. and *Diaptomus* sp. were collected from the nearby ponds of Bangladesh Agricultural University campus (see some photos of *Cloeon* sp. and *Diaptomus* sp. in Figs. S2 and S3, respectively). *Cloeon* sp. was transferred in an aerated plastic bucket with a mixture of pond and de-chlorinated test water first and then only in test water to acclimate to the laboratory conditions for at least 3 days at ambient temperature. During the acclimation period, they were fed ad libitum with *Enhydra fluctuans*, *Eichhornia crassipes* and biofilms. *Diaptomus* sp. was stocked in an aerated glass beaker with de-chlorinated test water in the laboratory condition at ambient temperature and fed with algae. After an acclimation period of 3 days, 10 individuals of *Cloeon* sp. were transferred into each of the 21 glass beakers containing 500 mL de-chlorinated tap water (water holding capacity: 750 mL) and 20 individuals of *Diaptomus* sp. were transferred into 21 glass beakers containing 50 mL de-chlorinated tap water (water holding capacity: 100 mL), which were put in the laboratory at ambient temperature and receiving no direct sunlight. An aeration system was introduced in all beakers to provide sufficient oxygen throughout the experimental period of 96 h. Feeding was stopped 24 h before and throughout the exposure period. Both species were exposed to seven different concentrations (0, 3, 10, 30, 100, 300, 3000 ng/L) of imidacloprid including control with triplicate treatment for 96 h separately. Imidacloprid (as Premier with 20% active ingredient, 6% adjuvants and 74% water and produced by the world of Hayleys) was purchased from a local pesticide seller (Mymensingh, Bangladesh). The stock solutions were prepared by dissolving the required weighed amount of imidacloprid in distilled water so a concentration of 200 g/L imidacloprid was achieved. Water quality variables (i.e. dissolved oxygen, temperature, pH and EC) were measured in the lowest and highest treatment, and in the control at 0 h and 96 h of exposure. Mortality and immobility were checked at every 24 h of exposure for *Cloeon* sp. and after 96 h of exposure for *Diaptomus* sp. Individuals were considered immobile when there was no observed movement within 20 s for *Cloeon* sp. and 15 s for *Diaptomus* sp., and dead when there was no observed movement within 3–5 s for both after a tactile stimulation using a Pasteur's capillary pipette (OECD, 2004). Dead individuals were removed immediately from the experimental units. Immobile individuals were kept in the systems because there was a possibility for recovery, and these specimens were used to calculate effect concentration levels based on immobilization. The test was valid when the mortality of the control did not exceed 10% at the end (96 h) of the test (OECD, 2004).