SENSITIVITY OF ODONATE NYMPHS TO DIFFERENT CLASSES OF AGRICULTURAL INSECTICIDES, FREQUENTLY APPLIED IN SWAT VALLEY PAKISTAN

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Abstract. The sensitivity of blue-tailed damselfly (Ischnura elegans) and crimson marsh glider dragonfly (Trithemis aurora) nymphs to six different insecticides were studied during 48-hour exposure in the laboratory conditions. Lambda cyhalothrin was found to be the most toxic. Chlorpyrifos was found least toxic. The highest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid that caused no mortality of I. elegans were 0.0078, 0.0039, 0.00048, 0.0078, 0.0039 and 0.00195 ppm, respectively. The highest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid that caused no mortality of T. aurora were 0.0039, 0.00195, 0.00048, 0.0156, 0.0078 and 0.000975 ppm, respectively. The lowest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos, and acetamiprid that caused 100% mortality of I. elegans were 0.5, 0.5, 0.0156, 1.0, 0.5 and 0.25 ppm, respectively. The lowest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos, and acetamiprid that caused 100% mortality of T. aurora were 0.25, 0.25, 0.0312, 2, 1 and 0.125 ppm, respectively. Significantly (P<0.05) lowest LC90 values were observed for lambda cyhalothrin (LC90 against I. elegans= 0.01 ppm, LC90 against T. aurora= 0.018 ppm). Next to the lambda cyhalothrin, significantly (P<0.05) lowest LC90 values were observed for acetamiprid (LC90 against I. elegans= 0.122 ppm, LC90 against T. aurora= 0.093 ppm). From the findings of the present study, it was concluded that I. elegans and T. aurora nymphs are highly sensitive to lambda cyhalothrin and acetamiprid.

Keywords: deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos, acetamiprid

Introduction

Damselfly (order Odonata, sub order Zygoptera) and dragonfly (order Odonata, sub order Anisoptera) nymphs are well known predators of mosquito larvae that play important role in the natural regulation of mosquito population (Boyd, 2005; Din et al., 2013). Odonate nymphs are very useful biological control agent against mosquitoes (Mitra, 2006). Odonate nymphs face environmental pressure due to increasing pollutants in their habitats. Aquatic habitats are contaminated with agricultural insecticides as a result of spray drift or runoff (Armbrust and Peeler, 2002; Hilz and Vermeer, 2012). Aquatic insects are very sensitive to insecticides (Mokry and Hoagland, 1990; Mian and Mull, 1992).
Insecticides are the agents applied for the control of insect pests. They are generally called adulticides, ovicides, pupicides and larvicides. Insecticides may be synthetic chemicals or derived from plants. Insecticides which are derived from plants are also called botanical insecticides. Alkaloids, pyrethrins, rotenone, rotenoids and neem are the known botanical insecticides. Some synthetic chemical insecticides have been modeled after natural botanical insecticides. For example nicotinoids or neonicotinoids such as imidacloprid, acetamiprid etc. are the synthetic chemical insecticides modeled on plant nicotine, while pyrethroids such deltamethrin, lambda cyhalothrin, cypermethrin etc. have been modeled on plant pyrethrins (Gullan and Cranston, 2005). Carbamates (e.g. carbofuran, carbaryl etc.), organochlorines (DDT, endosulfan, aldrin, dieldrin etc.) and organophosphates (e.g. dichlorovos, chlorpyrifos, malathion etc.) are the synthetic chemical insecticides which are not modeled on botanical insecticides (Gullan and Cranston, 2005).

Organochlorine insecticides are highly toxic, bioaccumulative and persistent (Jayaraj et al., 2016). The vast application of organochlorines destroy both, pests and non-target organisms (Zacharia, 2011). After the application of organophosphates on target organisms, they can reach to the target pests, or reach surface water bodies and ground water; they can also contaminate the atmosphere or they can be ingested by non-target organisms. The chemical and physical characteristics, application methods and conditions of sites influence the destiny and effect of organophosphates (Lourencetti et al., 2008). Due to the frequent and excessive application of organophosphate pesticides and their slow decomposition rates, they accumulate in the soils and subsequently contaminate surface water bodies (Sirotkina et al., 2012). Chlorpyrifos which is an organophosphate, has been detected in arctic sea water and air (Vorkamp and Rigét, 2014). Some carbamates have also been detected in aquatic habitats (Tien et al., 2013). Herbicides compounds are also persistent in the soil from where they reach to the ground water and surface water bodies (Cai et al., 2004). It has been reported that the degradation of organochlorine herbicide compounds occur in soil due to light effect and microbial action (Fenoll et al., 2014). The presence of some of these herbicide compounds and their breakdown products have been detected in ground water and surface water bodies (Osano et al., 2002). Among the organochlorines, DDT (Dichloro diphenyl trichloroethane) played effective role in eradication of insect pests but on the other hand it has also damaged wild life and human health due to its persistence in the environment and bioaccumulation (Tursosov et al., 2002; Jayaraj et al., 2016). Organophosphates, inhibitors of acetylcholinesterase, rapidly degrade by hydrolysis when exposed to water, soil, air and light, however their small amout have been detected in water and food (Jayaraj et al., 2016). They have been reported for their adverse effect on non-target aquatic organisms (Smolka, 2004; Huynh and Nugegoda, 2012; Rubach et al., 2012). The insecticides of class pyrethroids are very effective against insect pests and they kill insect pests at low dose and are less bioavailable in the natural environment due to their strong absorptive characteristic and low water solubility (Davies, 1985). Pyrethroids inhibit ATPase enzymes that result in the disturbance of ionic balance which is the main toxic effect of pyrethroids (Coats et al., 1989). Pyrethroids are highly toxic for aquatic insects, aquatic crustaceans and fish (Mian and Mulla, 1992; Werner and Moran, 2008). Neonicotinoid cause toxic effects on insects by acting directly on the nicotinic acetylcholine receptors (nAChRs) (Nishiwaki et al., 2003; Casida and Durkin, 2013). There is emerging evidence about the adverse effect of neonicotinoids on non-target organisms (Malev et al., 2012; Anderson et al., 2015; Morrissey et al., 2015).

To the author knowledge, very limited studies have been reported about the insecticides toxicity with odonate nymphs. For example, Beketov (2002) studied the comparative sensitivity of larvae of damselfly, dragonfly, mayfly and Daphnia magna to deltamethrin and...
esfenvalerate. They reported the deltamethrin 48-hour LC50 values of 0.0145 µg/l and 0.0760 µg/l against nymphs of Lestes sponsa (damsel fly) and Cordulia aenea (dragonfly), respectively. The LC50 values of deltamethrin against larvae of two species of mayflies, Cloeon dipterum and Caenis miliaria and larvae of Daphnia were 0.005 µg/l, 0.0091 µg/l and 0.0293 µg/l, respectively.

In Swat valley, Pakistan, insecticides are regularly applied in agricultural fields on peach orchards, vegetables and cereal crops for many years (Nafees et al., 2008; Nafees and Jan, 2009). The watershed of River Swat in Malakand Division, Pakistan, is called Swat Valley, which comprises of Swat, Malakand (Swat Ranizai Tehsil), and Chakdara (Adenzai Tehsil). Keeping in view, the impact of frequent application of insecticides on non-target organisms in the area, a laboratory study was conducted for the assessment of toxicity of agricultural insecticides to non-target aquatic insects, specifically native odonate nymphs, which are the predators of mosquito larvae.

Materials and methods

Selection of insecticides for sensitivity study

During the present study, the farmers who were cultivating vegetables and growing peach orchards in Swat, Pakistan, were interviewed about the type and brand of pesticides they apply on vegetables and fruit trees. Questionnaires were arranged for interview, in which the type, brand and frequency of application of pesticides were asked. The pesticide dealers at Matta bazar, Khwazakhela bazar, Mingora city, Shamozo bazar and Barikot bazar were also interviewed about the type and brand of pesticides they provide to the farmers. Six hundred farmers in Swat, Pakistan, were interviewed. The farmers were applying insecticide spray on tomato crops, three times from seedling to fruit ripening. They were applying insecticide spray on peach orchards, four times from before flowering to fruit ripening. Peaches are grown throughout Swat Valley (Figure 1). According to the information obtained during interview, the main insecticides that the farmers apply are deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid. The details of manufacturer of these insecticides were also inquired. The farmers apply deltamethrin (25% w/w) of HERANBA Industries Limited India, cypermethrin (10% w/v) of M/S Halex (M) SDN (BDH) Malaysia, lambda cyhalothrin (2.5% w/v) of Jiangsu Fengshan Group Co. Ltd China, chlorpyrifos (40% w/v) of M/S Halex (M) SDN (BDH) Malaysia, dichlorvos (100% w/v) of Insecticides India Limited and acetamiprid (20% w/w) of Jiangsu Fengshan Group Co. Ltd China. The reason of choice of insecticides of the above manufacturers by the farmers when inquired was the lower price. Table 1 shows the outcome of questionnaires.

Collection of odonate nymphs

Damsel fly and dragonfly nymphs of 6 to 8 instars were collected from slow moving water on the bank of River swat near the Chirchil picquit at Chakdara, Dir Lower, Khyber Pakhtunkhwa, Pakistan. A rectangular plastic dipper (38 cm length, 28 cm width and 6.5 cm height) was used as dipper during collection. The nymphs were brought to the laboratory in large plastic bottles along with water of collection site to the laboratory at University of Malakand, Khyber Pakhtunkhwa, Pakistan. In laboratory, the nymphs were maintained in small fish aquarium (45 cm length, 40 cm width and 40 cm height) in water of collection site. The laboratory was well ventilated and receiving sunlight through windows. Before starting experiment provided mosquito larvae as food. Proper literature was used the identification of
specimens (Yousuf et al., 1996; Anjum, 1997; Mitra, 2002; Din et al., 2013). One species of blue-tailed damselfly, *Ischnura elegans* (Vander Linden, 1820) and one species of crimson marsh glider dragonfly namely, *Trithemis aurora* (Burmeister, 1839) were found in sufficient number; therefore, experiments were conducted on nymphs of these two species. The sensitivity of nymphs to the three classes of insecticides i.e., pyrethroids, organophosphates and neonicotinoids was studied in different times during May-September 2017. The maximum laboratory temperature was 29-33°C during experiments.

Figure 1. Figure derived from Google map showing watershed of River Swat in Malakand Division, Pakistan, where pesticides are applied on peach orchards. Latitude and Longitude: 34° 49' 19'' North, 72° 29' 20'' East. Blue line represents River Swat

Table 1. Types of insecticides applied on vegetables and peach orchards in Tehsil Matta District Swat

| Insecticides       | Manufacturer                                      | Number of Farmers | Farmers % |
|--------------------|----------------------------------------------------|-------------------|-----------|
| Deltamethrin       | HERANBA Industries Ltd, India                      | 60                | 10        |
| Cypermethrin       | M/S Halex (M) SDN (BDH), Malaysia                 | 90                | 15        |
| Lambda cyhalothrin | Jiangsu Fengshan Group Co. Ltd, China             | 120               | 20        |
| Chlorpyrifos       | M/S Halex (M) SDN (BDH), Malaysia                 | 120               | 20        |
| Dichlorvos         | Insecticides India Limited                        | 90                | 15        |
| Acetamiprid        | Jiangsu Fengshan Group Co. Ltd, China             | 120               | 20        |
| Total              |                                                   | 600               | 100       |

Sensitivity of Odonate nymphs to pyrethroids

The sensitivity of odonate (damselfly and dragonfly) nymphs to pyrethroids i.e., deltamethrin, cypermethrin and lambda cyhalothrin were studied during May-June 2017 (max temperature 29-33°C). Initially range finding bioassay was conducted for finding
concentration range of each pyrethroids for each odonate species, to be used for determining lethal concentrations (LC$_{50}$ and LC$_{90}$ values) in definitive test. The ecological effects test guidelines of Environmental Protection Agency, USA (US EPA, 1996) for aquatic invertebrate acute toxicity test were followed for determining the concentration range. The following are the details.

**Solutions preparation**

Deltamethrin (25% w/w) of HERANBA Industries Limited, India, cypermethrin (10% w/v) of M/S Halex (M) SDN (BDH), Malaysia and lambda cyhalothrin (2.5% w/v) of Jiangsu Fengshan Group Co. Ltd, China, were used. A 500 ml stock solution of 100 ppm of each insecticide was prepared in non-chlorinated tap water. Based on dilution equation (C1V1=C2V2), 600 ml test solution of 2 ppm of each pyrethroid was prepared from the respective stock solution (of 100 ppm). The 2 ppm solution of each pyrethroid was serially diluted by a factor of 2 and thus several dilutions of reducing concentrations were prepared in polyethylene containers (400 ml each). The detail of serial dilution of each pyrethroid was as under: From 600 ml initial solution of 2 ppm, 300 ml solution was taken and put into 1000 ml volumetric flask in which 300 ml tap water was already added to make a 600 ml solution of 1 ppm (1/2 dilution). Again, from this solution, 300 ml was taken and put into 1000 ml volumetric flask in which 300 ml tap water was already added to make a 600 ml solution of 0.5 ppm (1/4 dilution). Again, from this solution, 300 ml was taken and put into 1000 ml volumetric flask in which 300 ml tap water was already added to make a 600 ml solution of 0.25 ppm (1/8 dilution). This serial dilution was continued till obtaining solution of 0.00195 ppm (1/1024-fold). At each step, there was a 2-fold dilution in concentration, however volume of each dilution remained constant i.e. 300 ml. From the last dilution, 300 ml solution was discarded so as to keep 300 ml volume as in the previous dilutions. A total of 11 solutions of each pyrethroid were arranged. The 11 solutions were of the following concentrations: 2 ppm, 1 ppm, 0.5 ppm, 0.25 ppm, 0.125 ppm, 0.0625 ppm, 0.03125 ppm, 0.015625 ppm, 0.0078125, 0.003906 and 0.00195 ppm. The schematic for stock solutions preparation and serial dilution of pyrethroids is shown in *Figure 2*.

![Figure 2. Schematic of stock solutions preparation and serial dilution of pyrethroids for toxicity study in odonate nymphs](image)
Exposure of nymphs to pyrethroids for range finding

The detail of procedure of range finding bioassay for each pyrethroid was as under: Eleven test solutions of 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0315, 0.0156, 0.0078, 0.0039 and 0.00195 ppm concentrations of each pyrethroid were separately tested against nymphs of each odonate species in 400 ml polyethylene containers. To each concentration of each pyrethroid, five nymphs of I. elegans were individually exposed in five polyethylene containers. To avoid cannibalism, nymphs were individually exposed to test solutions. The volume of test solution in each container was 250 ml. In addition, five nymphs were placed in five 400 ml polyethylene control containers, each containing 250 ml non-chlorinated tap water. In short, for testing each pyrethroid against I. elegans, 60 polyethylene cups (55 containers for 11 concentrations and 5 control containers) containing 60 nymphs were arranged. At the same time, the nymphs of T. aurora were also exposed to each pyrethroid. The detail of the exposure was the same as for I. elegans.

The period of exposure was 48 hours. Following standard toxicity protocols, the nymphs were not fed during the 48-hour exposure period (ASTM standard E47, 2008). After 48 hours of exposure period, the number of dead nymphs was noted. The criterion for death was lack of response to prodding. In case of lambda cyhalothrin, mortality was higher therefore further dilutions were made (diluted up to 0.00048 ppm) and tested.

The highest concentration of each pyrethroid that caused no mortality was noted for each odonate species. The concentration of each pyrethroid that caused lowest mortality was determined for each odonate species. Similarly, the lowest concentration of each pyrethroid that caused 100% mortality was also determined for each species. The schematic for range finding bioassay is shown in Figure 3.

Figure 3. Schematic of range finding bioassay for determining the concentration ranges of pyrethroids during toxicity study in odonate nymphs

Definitive test for determination of lethal concentrations of pyrethroids

LC_{50} also called median lethal concentration is a measure of the lethal concentration of a toxin that kill 50% of the test population after exposure for a specified period. Lower LC_{50} values indicate higher toxicity of a toxin. During definitive test, LC_{50} value of each pyrethroid against nymphs of each odonate species was determined. For
determination of LC$_{50}$ values, nymphs of each odonate species were separately exposed to various concentrations of each pyrethroid within the concentration range determined during range finding bioassay. The schematic for definitive bioassay is shown in Figure 4. The following are the details.

**Figure 4. Schematic of experiment for determining LC$_{50}$ and LC$_{90}$ values of three pyrethroids against odonate nymphs**

**Exposure to deltamethrin for determining LC$_{50}$**

The concentrations of deltamethrin used against *I. elegans* were 0.0156, 0.03125, 0.0625, 0.125, 0.25 and 0.5 ppm. Thus seven intact last instar nymphs of *I. elegans* were placed in seven polyethylene containers (six concentrations and one control). Similarly, the concentrations of deltamethrin used against *T. aurora* were 0.0078, 0.0156, 0.03125, 0.0625, 0.125 and 0.25 ppm. In this case, seven intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (six concentrations and one control). The volume of each container was 400 ml and testing solution was 250 ml. The control container was containing only 250 non-chlorinated tap water.

**Exposure to cypermethrin for determining LC$_{50}$**

The concentrations of cypermethrin used against *I. elegans* were 0.0078, 0.0156, 0.03125, 0.0625, 0.125, 0.25 and 0.5 ppm. Thus eight intact last instar nymphs of *I. elegans* were placed in eight polyethylene containers (seven concentrations and one control). The concentrations of cypermethrin used against *T. aurora* were 0.0039, 0.0078, 0.0156, 0.03125, 0.0625, 0.125 and 0.25 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (seven concentrations and one control).

**Exposure to lambda cyhalothrin for determining LC$_{50}$**

The concentrations of lambda cyhalothrin used against *I. elegans* were 0.00097, 0.00195, 0.0039, 0.0078 and 0.0156 ppm. Thus six intact last instar nymphs of *I. elegans* were placed in six polyethylene containers (five concentrations and one control). Similarly, the concentrations of lambda cyhalothrin used against *T. aurora* were 0.0097, 0.00195, 0.0039, 0.0078, 0.0156 and 0.0312 ppm. Thus seven intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (six concentrations and one control).
Sensitivity of odonate nymphs to Organophosphates

The sensitivity of odonate (damselfly and dragonfly) nymphs to organophosphates i.e., chlorpyrifos and dichlorvos was studied during July-August 2017 (max temperature 28-32°C). Chlorpyrifos (40% w/v) of M/S Halex (M) SDN (BDH), Malaysia and dichlorvos (100% w/v) of Insecticides India Limited were used.

Solutions preparation

The detail of preparation of solutions of each organophosphate was the same as described for pyrethroid.

Exposure of nymphs to organophosphates for range finding

The procedure of bioassay for finding concentration ranges of two organophosphates (chlorpyrifos and dichlorvos) against odonate nymphs was the same as described for pyrethroids.

Definitive test for determination of lethal concentrations of organophosphates

During definitive test, LC$_{50}$ and LC$_{90}$ values of each organophosphate against each odonate nymph was determined. For determination of LC$_{50}$ and LC$_{90}$ values, nymphs of each odonate species were separately exposed to various concentrations of each organophosphate within the concentration range determined during range finding bioassay. The following are the details.

Exposure to chlorpyrifos for determining LC$_{50}$

The concentrations of chlorpyrifos used against *I. elegans* were 0.0156, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in eight polyethylene containers (seven concentrations and one control). Similarly, the concentrations of chlorpyrifos used against *T. aurora* were 0.03125, 0.0625, 0.125, 0.25, 0.5, 1 and 2 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in eight polyethylene containers (seven concentrations and one control). The procedure of bioassay was the same as described for pyrethroids.

Exposure to dichlorvos for determining LC$_{50}$

The concentrations of dichlorvos used against *I. elegans* were 0.0078, 0.0156, 0.03125, 0.0625, 0.125, 0.25 and 0.5 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (seven concentrations and one control). Similarly, the concentrations of dichlorvos used against *T. aurora* were 0.0156, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (seven concentrations and one control). The procedure of bioassay was the same as described for pyrethroids.

Sensitivity of odonate nymphs to neonicotinoid

The sensitivity of odonate (damselfly and dragonfly) nymphs to neonicotinoid i.e., acetamiprid was studied during September 2017 (max temperature 29-33°C). Acetamiprid (20% w/w) of Jiangsu Fengshan Group Co. Ltd, China was used.
Solutions preparation

The detail of preparation of solutions of neonicotinoid (acetamiprid) was the same as described for pyrethroid.

Exposure of nymphs to neonicotinoid (acetamiprid) for range finding

The detail of bioassay for finding concentration range of neonicotinoid (acetamiprid) against odonate nymphs was the same as described for pyrethroids.

Definitive test for determination of lethal concentrations of neonicotinoid (acetamiprid)

During definitive test, LC$_{50}$ and LC$_{90}$ values of neonicotinoid (acetamiprid) against each odonate nymph was determined. For determination of LC$_{50}$ and LC$_{90}$ values, nymphs of each odonate species were separately exposed to various concentrations of neonicotinoid (acetamiprid) within the concentration range determined during range finding bioassay. The following are the details:

The concentrations of acetamiprid used against *I. elegans* were 0.0039, 0.0078, 0.0156, 0.03125, 0.0625, 0.125 and 0.25 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (seven concentrations and one control). Similarly, the concentrations of acetamiprid used against *T. aurora* were as 0.00195, 0.0039, 0.0078, 0.0156, 0.03125, 0.0625, and 0.125 ppm. Thus eight intact last instar nymphs of *T. aurora* were placed in seven polyethylene containers (seven concentrations and one control). The procedure of bioassay was the same as described for pyrethroids.

Period of exposure and observations

The period of exposure for each insecticide was 48 hours. Following standard toxicity protocols, the nymphs were not fed during the 48-hour exposure period (ASTM standard E47, 2008). After 48 hours of exposure period, the number of dead nymphs was noted. The criterion for death was lack of response to prodding. Experiment for each insecticide was run in triplicate. Several trips were conducted for collection of nymphs and experiments were repeated continuously till the number of nymphs in each replica of each concentration of each insecticide reached 20. In total 20 independent experiments were conducted for each insecticide (*Figure 5*). There occurred the death of only two nymphs in control containers during the whole experiments, indicating that conditions during each experiment were suitable and the nymphs were healthy.
Analysis of data

The percent mortality of nymphs in each replica of each concentration of each insecticide against each odonate species was calculated from cumulative total of 20 nymphs after 20 independent experiments (single nymph exposed to each concentration during each experiment). The average percent mortality data were subjected to log probit analysis (Finney, 1971) for calculating LC$_{50}$ and LC$_{90}$ values, using SPSS 16 software. The LC$_{50}$ values were compared by 95% confidence limits overlap method of Wheeler et al. (2006).

Results

Sensitivity of damselfly and dragonfly nymphs to pyrethroids

The sensitivity of odonate nymphs of damselfly (I. elegans) and dragonfly (T. aurora) were studied during exposure to three pyrethroids (deltamethrin, cypermethrin and lambdacyhalothrin) for 48 hours in laboratory conditions. Table 2 shows the 48-hour mortality data for three pyrethroids (deltamethrin, cypermethrin and lambdacyhalothrin) against the 7$^{th}$ to 8$^{th}$ instar nymphs of I. elegans. The highest concentrations of deltamethrin, cypermethrin and lambdacyhalothrin that caused no mortality of I. elegans were 0.0078 ppm, 0.0039 ppm and 0.00048 ppm, respectively. The lowest concentrations of deltamethrin, cypermethrin and lambdacyhalothrin that caused 100% mortality of I. elegans were 0.5 ppm, 0.5 ppm and 0.0156 ppm, respectively. Table 3 shows the 48-hour mortality data for three pyrethroids against the 7$^{th}$ to 8$^{th}$ nymphs of T. aurora. The highest concentrations of deltamethrin, cypermethrin and lambdacyhalothrin that caused no mortality of T. aurora were 0.0039 ppm, 0.00195 ppm and 0.00048 ppm, respectively. The lowest concentrations of deltamethrin, cypermethrin and lambda cyhalothrin that caused 100% mortality of T. aurora were 0.25 ppm, 0.25 ppm and 0.0312 ppm, respectively.

Table 2. 48-hour percent mortality data for three pyrethroids against I. elegans nymph

| Pyrethroids      | Concentrations | Mortality (Mean ± SE) % |
|------------------|----------------|-------------------------|
| Deltamethrin     |                |                         |
| 0.0078           | 0              |
| 0.0156           | 3.3±1.6        |
| 0.03125          | 6.6±1.6        |
| 0.0625           | 15±2.8         |
| 0.125            | 50±11.5        |
| 0.25             | 76.6±3.3       |
| 0.5              | 100±0          |
| Cypermethrin     |                |                         |
| 0.0039           | 0              |
| 0.0078           | 3.3±1.6        |
| 0.0156           | 8.3±1.6        |
| 0.03125          | 16.6±3.3       |
| 0.0625           | 30±5.8         |
| 0.125            | 46.6±8.8       |
| 0.25             | 70±5.8         |
| 0.5              | 100±0          |
| Lambdacyhalothrin|                |                         |
| 0.000485         | 0              |
| 0.00097          | 10±2.8         |
| 0.00195          | 23.3±6         |
| 0.0039           | 51.6±4.4       |
| 0.0078           | 75±5.8         |
| 0.0156           | 100±0          |
Table 3. 48-hour percent mortality data for three pyrethroids against T. aurora nymph

| Pyrethroids used | Concentration (ppm) | Mortality (Mean ± SEM) % |
|------------------|---------------------|-------------------------|
| Deltamethrin     |                     |                         |
|                  | 0.0039              | 0                       |
|                  | 0.0078              | 3.3±1.6                 |
|                  | 0.0156              | 6.6±1.6                 |
|                  | 0.03125             | 18.3±6.01               |
|                  | 0.0625              | 46.6±8.8                |
|                  | 0.125               | 70±5.8                  |
|                  | 0.25                | 100±0                   |
| Cypermethrin     |                     |                         |
|                  | 0.00195             | 0                       |
|                  | 0.0039              | 1.6±1.6                 |
|                  | 0.0078              | 6.6±1.6                 |
|                  | 0.0156              | 15±2.8                  |
|                  | 0.03125             | 45±8.6                  |
|                  | 0.0625              | 66.6±8.8                |
|                  | 0.125               | 86.6±3.3                |
|                  | 0.25                | 100±0                   |
| Lambda cyhalothrin |                   |                         |
|                  | 0.000485            | 0                       |
|                  | 0.00097             | 6.6±1.6                 |
|                  | 0.00195             | 15±2.8                  |
|                  | 0.0039              | 35±2.8                  |
|                  | 0.0078              | 61.6±4.4                |
|                  | 0.0156              | 85±2.8                  |
|                  | 0.0312              | 100±0                   |

LC50 values of pyrethroids against damselfly and dragonfly nymphs

Columns of the Table 4 show the comparison of LC50 values of three pyrethroids against nymph of each odonate species. During the study of I. elegans, significantly lower LC50 value (48-hour LC50= 0.004 ppm) was recorded for lambdacyhalothrin when compared with the LC50 values of deltamethrin and cypermethrin. Similarly, during the study of T. aurora, lowest LC50 value was recorded for lambdacyhalothrin (LC50= 0.005 ppm) followed by cypermethrin (LC50= 0.038) and deltamethrin (LC50= 0.064). The LC50 values of all the three pyrethroids against T. aurora were significantly different from each other when compared through 95% confidence overlap method.

Table 4. 48-hour LC50 values of three pyrethroids against I. elegans and T. aurora nymphs

| Pyrethroids     | I. elegans       | T. aurora        |
|-----------------|------------------|------------------|
| Deltamethrin    | 0.112 (0.061-0.22) b | 0.064 (0.045-0.054) c |
| Cypermethrin    | 0.111 (0.073-0.161) b | 0.038 (0.033-0.043) b |
| Lambda cyhalothrin | 0.004 (0.002-0.005) a | 0.005 (0.005-0.006) a |

The alphabetical order in column is according to increasing LC50 values. LC50 values sharing no letter are significantly different at P<0.05 significance level

Susceptibility of damselfly and dragonfly nymphs to organophosphates

Table 5 shows the 48-hour mortality data for two organophosphates i.e., chlorpyrifos and dichlorvos against 7th to 8th instars nymphs of I. elegans. The highest concentrations of chlorpyrifos and dichlorvos that caused no mortality of I. elegans were 0.0078 ppm and 0.0039 ppm, respectively. The lowest concentrations of chlorpyrifos and
dichlorovos that caused 100% mortality of *I. elegans* were 1.0 ppm and 0.5 ppm, respectively. *Table 6* shows the 48-hour mortality data for two organophosphates i.e., chlorpyrifos and dichlorvos against 7th to 8th instars nymphs of *T. aurora*. The highest concentrations of chlorpyrifos and dichlorvos that caused no mortality of *T. aurora* were 0.0156 ppm and 0.0078 ppm, respectively. The lowest concentrations of chlorpyrifos and dichlorvos that caused 100% mortality of *T. aurora* were 2.0 ppm and 1.0 ppm, respectively.

*Table 5. 48-hour percent mortality data for two organophosphates against last instar nymph of *I. elegans**

| Organophosphates | Concentration (ppm) | Mean ± SE % |
|------------------|---------------------|-------------|
| Chlorpyrifos     | 0.0078              | 0           |
|                  | 0.0156              | 6.6±1.6     |
|                  | 0.03125             | 8.3±1.6     |
|                  | 0.0625              | 23.3±6.1    |
|                  | 0.125               | 46.6±8.8    |
|                  | 0.25                | 61.6±10.1   |
|                  | 0.5                 | 83.3±6.6    |
|                  | 1                   | 100±0       |
| Dichlorvos       | 0.0039              | 0           |
|                  | 0.0078              | 3.3±1.6     |
|                  | 0.0156              | 6.6±1.6     |
|                  | 0.03125             | 10±2.8      |
|                  | 0.0625              | 18.3±6.01   |
|                  | 0.125               | 45±5.8      |
|                  | 0.25                | 66.6±8.8    |
|                  | 0.5                 | 100±0       |

*Table 6. 48-hour mortality data for two organophosphates against last instar nymphs of *T. aurora* *

| Organophosphates | Concentration (ppm) | Mean ± SEM % |
|------------------|---------------------|--------------|
| Chlorpyrifos     | 0.0156              | 0            |
|                  | 0.03125             | 3.3±1.6      |
|                  | 0.0625              | 15±2.8       |
|                  | 0.125               | 35±8.6       |
|                  | 0.25                | 46.6±12.01   |
|                  | 0.5                 | 63.3±12.01   |
|                  | 1                   | 81.6±4.4     |
|                  | 2                   | 100±0        |
| Dichlorvos       | 0.0078              | 0            |
|                  | 0.0156              | 1.6±1.6      |
|                  | 0.03125             | 8.3±1.6      |
|                  | 0.0625              | 23.3±8.8     |
|                  | 0.125               | 53.3±14.5    |
|                  | 0.25                | 80±5.8       |
|                  | 0.5                 | 93.3±3.3     |
|                  | 1                   | 100±0        |

**LC₅₀ values of organophosphates against damselfly and dragonfly nymphs**

*Figure 6* shows the LC₅₀ values of chlorpyrifos and dichlorvos against *I. elegans*. The LC₅₀ values of chlorpyrifos and dichlorvos against *I. elegans* were 0.142 ppm.
and 0.125 ppm, respectively. Based on 95% confidence interval overlap method, there was no significant difference in the LC$_{50}$ values of dichlorovos and chlorpyrifos against *I. elegans*. Figure 7 shows the LC$_{50}$ values of chlorpyrifos and dichlorovos against *T. aurora*. The LC$_{50}$ values of chlorpyrifos and dichlorovos against *T. aurora* were 0.257 ppm and 0.119 ppm, respectively. Based on 95% confidence interval overlap method, the LC$_{50}$ value of dichlorovos against *T. aurora* was significantly lower when compared to the LC$_{50}$ value chlorpyrifos against *T. aurora*.

**Figure 6.** Comparison of LC$_{50}$ values of chlorpyrifos and dichlorvos against *I. elegans*. Error bars represent 95% confidence limits. Similar letter represents that LC50 values are not different significantly at P<0.05 significance level.

**Figure 7.** Comparison of LC$_{50}$ values of chlorpyrifos and dichlorvos against *T. aurora*. Error bars represent 95% confidence limits. Different letters represent that LC50 values are significantly different at P<0.05 significance level.

*Figure 8* shows the comparisons of chlorpyrifos LC$_{50}$ values for *I. elegans* and *T. aurora*. The LC$_{50}$ value of chlorpyrifos for *I. elegans* (0.142 ppm) was significantly lower than its LC$_{50}$ value for *T. aurora* (0.3 ppm). *Figure 9* shows the comparison of dichlorvos LC$_{50}$ values for *I. elegans* (0.125 ppm) and *T. aurora* (0.12 ppm). There was no significant difference in the LC$_{50}$ values of dichlorvos for *I. elegans* and *T. aurora*.

**Sensitivity of damselfly and dragonfly nymphs to neonicotinoid**

*Table 7* shows the 48-hour mortality data of acetamiprid against the nymphs (7th to 8th instar) of *I. elegans* and *T. aurora*. The highest concentrations of acetamiprid that caused no mortality of *I. elegans* was 0.00195 ppm. The highest concentration of acetamiprid that caused no mortality of *T. aurora* was 0.000975 ppm. The lowest
concentrations of acetamiprid that caused 100% mortality of *I. elegans* and *T. aurora* were 0.25 ppm and 0.125 ppm, respectively.

**Figure 8.** Comparison of chlorpyrifos LC₅₀ values for *I. elegans* and *T. aurora*. Error bars represent 95% confidence limits. Different letters represent that LC₅₀ values are significantly different at P<0.05 significance level.

**Figure 9.** Comparison of dichlorvos LC₅₀ values for *I. elegans* and *T. aurora*. Error bars represent 95% confidence limits. Similar letter represents that LC₅₀ values are not different significantly at P<0.05 significance level.

**Table 7.** 48-hour percent mortality data for a neonicotinoid, acetamiprid against damselfly and dragonfly nymphs nymphs.

| Odonate species | Concentration (ppm) | Mean ± SEM % |
|-----------------|---------------------|--------------|
| *I. elegans*    |                     |              |
| 0.00195         | 0                   |              |
| 0.0039          | 3.3±1.6             |              |
| 0.0078          | 10±2.8              |              |
| 0.0156          | 18.3±4.4            |              |
| 0.03125         | 46.6±8.8            |              |
| 0.0625          | 56.6±8.8            |              |
| 0.125           | 85±2.8              |              |
| 0.25            | 100±0               |              |
| *T. aurora*     |                     |              |
| 0.000975        | 0                   |              |
| 0.00195         | 3.3±1.6             |              |
| 0.0039          | 6.6±1.6             |              |
| 0.0078          | 16.6±7.3            |              |
| 0.0156          | 30±11.5             |              |
| 0.03125         | 56.6±8.8            |              |
| 0.0625          | 80±5.8              |              |
| 0.125           | 100±0               |              |
**LC₅₀ values of neonicotinoid against damselfly and dragonfly nymphs**

Figure 10 shows the LC₅₀ values of a neonicotinoid, acetamiprid against predator of mosquito larvae i.e., *I. elegans* and *T. aurora*. The LC₅₀ values of acetamiprid against *I. elegans* and *T. aurora* were 0.038 ppm and 0.023 ppm, respectively. Based on 95% confidence interval overlap method, the LC₅₀ value of acetamiprid for *T. aurora* was significantly lower than its LC₅₀ value for *I. elegans*.

![Figure 10. LC₅₀ values of acetamiprid against *I. elegans* and *T. aurora*. Error bars represent 95% confidence limits. Different letters represent that LC₅₀ values are significantly different at P<0.05 significance level](image)

**LC₉₀ values of six insecticides against damselfly and dragonfly nymphs**

Table 8 shows the LC₉₀ values of six insecticides for *I. elegans*. Minimum LC₉₀ values was observed for lambda cyhalothrin (0.01 ppm). The LC₉₀ value of lambda cyhalothrin was significantly different from the LC₉₀ values of the remaining insecticides. Next to the lambda cyhalothrin, acetamiprid showed lowest LC₉₀ value (0.122 ppm) which was significantly different from the LC₉₀ values of the remaining insecticides. Maximum LC₉₀ value was observed for chlorpyrifos (0.655 ppm) and dichlorvos (0.535 ppm). The difference in LC₉₀ values against *I. elegans* among chlorpyrifos, dichlorvos, cypermethrin and deltamethrin were not significant.

**Table 8. 48-hour LC₉₀ values of six insecticides against *I. elegans* nymph**

| Insecticides      | LC₉₀ (95% confidence limits) |
|-------------------|------------------------------|
| Deltamethrin      | 0.351 (0.188-2.19) c         |
| Cypermethrin      | 0.504 (0.291-1.35) c         |
| Lambda cyhalothrin| 0.01 (0.007-0.027) a         |
| Chlorpyrifos      | 0.655 (0.436-1.214) c        |
| Dichlorvos        | 0.535 (0.283-2.108) c        |
| Acetamiprid       | 0.122 (0.086-0.204) b        |

The alphabetical order in columns is according to increasing LC₉₀ values. LC₉₀ values sharing no letter are significantly different.
Table 9 shows the LC₉₀ values of six insecticides for *T. aurora*. Minimum LC₉₀ value was observed for lambdacyhalothrin (0.018 n ppm). The LC₉₀ value of Lambdacyhalothrin was significantly different from the LC₉₀ values of the remaining insecticides. The second most toxic insecticide was acetamiprid which showed LC₉₀ value of 0.093 ppm. The LC₉₀ value of Lambdacyhalothrin was significantly different from the LC₉₀ values of the remaining insecticides. The LC₉₀ value of dichlorvos was insignificantly higher than the LC₉₀ value of deltamethrin however it was significantly higher than the LC₉₀ value of cypermethrin.

**Table 9. 48-hour LC₅₀ values of six insecticides against of T. aurora nymph**

| Insecticides       | LC₉₀ (95 % confidence limits) |
|--------------------|-------------------------------|
| Deltamethrin       | 0.205 (0.13-0.49) d           |
| Cypermethrin       | 0.132 (0.11-0.165) c          |
| Lambda cyhalothrin | 0.018 (0.015-0.023) a         |
| Chlorpyrifos       | 1.306 (0.852-2.526) e         |
| Dichlorvos         | 0.404 (0.34-0.498) d          |
| Acetamiprid        | 0.093 (0.062-0.174) b         |

The alphabetical order in columns is according to increasing LC₉₀ values. LC₉₀ values sharing no letter are significantly different

**Discussion**

During the study of susceptibility of predators of mosquito larvae (*I. elegans* and *T. aurora*) to pyrethroids, the predators were found more susceptible to lambdacyhalothrin than to deltamethrin and cypermethrin (*Tables 2-3*). The 48-hour LC₅₀ values of lambdacyhalothrin against nymphs of *I. elegans* and *T. aurora* were significantly lower when compared to the 48-hour LC₅₀ values of deltamethrin and cypermethrin against these nymphs (*Table 4*). Aquatic insects appear highly susceptible to synthetic pyrethroids, even very low concentration (< 1 µg/L) of pyrethroid can create toxic effects (Mian and Mulla, 1992). The high sensitivity of aquatic insects to synthetic pyrethroid may be attributed to disruption of ionic balance in aquatic insects (Coats et al., 1989). During the present study, *I. elegans* showed similar sensitivity to both, deltamethrin and cypermethrin (LC₅₀ of deltamethrin = 0.112 ppm, LC₅₀ of cypermethrin = 0.111 ppm) (*Table 4*). Such trend has been also observed in the reported work of Beketov (2004), where *Daphnia magna* showed similar susceptibility to two different synthetic pyrethroids, deltamethrin and esfenvalerate. Their 96-hour LC₅₀ values of deltamethrin and esfenvalerate were 0.00003 ppm and 0.00003 ppm, respectively. During the present study, *T. aurora* appeared more susceptible to cypermethrin (LC₅₀= 0.038 ppm) than deltamethrin (LC₅₀= 0.064 ppm) (*Table 4*). It has been reported that a given insect species may not be equally susceptible to different insecticides of the same class (Boiteau and Noronha, 2007; Nielsen et al., 2008).

During the present study, the susceptibility of nymphs of *I. elegans* and *T. aurora* to a particular pyrethroids was not the same. During lambdacyhalothrin toxicity study, nymphs of *I. elegans* were more susceptible than nymphs of *T. aurora*. On the other hand, during deltamethrin and cypermethrin toxicity study, nymphs of *T. aurora* were found more susceptible than nymphs of *I. elegans* (*Table 4*). It has been shown that different insect species may respond differently to an insecticide (Banks et al., 2017).
The differential tolerance of different insect species to an insecticide is due to differences in size, behavior, insecticide penetration, target sensitivity, excretion and metabolism (Wen et al., 2011). The enzymes P450 catalyse the oxidation of toxic compounds in the presence of NADPH cytochrome P450-reductase and or cytochrome b5 which are obligatory electron donor (Murataliev et al., 2008). The resistance of insects to insecticides is due to the presence of P450 enzymes (Karunker et al., 2009). Deltamethrin, cypermethrin and lambda-cyhalothrin that were studied for their toxicity with odonate nymphs during the present research, belong to type II pyrethroids. These pyrethroids cause hyperactivity, incoordination, convulsions and writhing. These type II pyrethroids produce stimulus-dependent nerve depolarization and blockage (Ecobichon, 1996).

The toxicity of deltamethrin with aquatic invertebrates has been reported. For example, Beketov (2002), studied the comparative sensitivity of larvae of damselfly, dragonfly, mayfly and Daphnia magna to deltamethrin and esfenvalerate. They reported the deltamethrin 48-hour LC50 values of 0.0000145 ppm and 0.000076 ppm against nymphs of one damselfly species, Lestes sponsa and one dragonfly species such as Cordulia aenea, respectively. During the present research, the LC50 values of deltamethrin against odonate nymphs were well above than the LC50 values of deltamethrin reported by Beketov (2002). This difference might be explained by differences in species, brand of insecticides and experimental design. Deltamethrin toxicity with other invertebrates has also been reported. For example, de Castro et al. (2013) exposed the major lepidopteran pest of soybean, Anticarsia gemmatalis and its predators Podisus nigrispinus and Supputius cincticeps (Heteroptera: Pentatomidae) to deltamethrin, methamidophos, spinosad and chlorantraniliprole. The LC50 values of deltamethrin against Anticarsia gemmatalis, Podisus nigrispinus and Supputius cincticeps were 2.76 ppm, 1.83 ppm and 1.83 ppm, respectively.

Lambdacyhalothrin toxicity to aquatic invertebrates has been reported. For example, in laboratory tests, the 48-hour EC50 value of lambdacyhalothrin against nymph of I. elegans was 0.00013 ppm (Maund et al., 1998). Schroer et al. (2004) studied the toxicity of the pyrethroid insecticide λ-cyhalothrin to freshwater invertebrates in laboratory, in situ bioassays and in field microcosms. In laboratory tests, the 48-hour LC50 value against Erythromma viridulum (Zygoptera, dragonflies) was 0.001583 ppm, Asellus aquaticus (Crustacea, Isopoda) 0.00014 ppm, Gammarus pulex (Crustacea, Amphipoda) 0.000314 ppm, Cloeon dipterum (Ephemeroptera, mayflies) 0.000122 ppm, Sigara striata (Hemiptera, true bugs) 0.0000492 ppm and Daphnia galeata (Crustacea, Cladocera) was 0.397 ppm. The 48-hour LC50 values of lambdacyhalothrin against I. elegans and T. aurora observed during the present research are far below than the lambdacyhalothrin 48-hour LC50 values reported by Maund et al. (1998) and Schroer et al. (2004).

Organophosphate insecticides cause toxicity through inhibition of acetylcholinesterase, which is responsible for the degradation of the excitatory neurotransmitter, acetylcholine, thereby terminating transmission of nerve impulses at cholinergic synapses (Fukuto, 1990). Inhibition of this enzyme prolongs the residence time of acetylcholine at synapses resulting in hyper-excitation and eventual death (Fukuto, 1990). During the present study, I. elegans was found more susceptible to dichlororvos than to chlorpyrifos (Table 5). The lowest concentration of dichlororvos that caused 100% mortality of I. elegans nymphs was lower than the lowest concentration of chlorpyrifos that caused 100% mortality of I. elegans nymphs. The 48-hour LC50 value
of dichlorvos for *I. elegans* nymphs was significantly lower than the 48-hour LC value of chlorpyrifos for *I. elegans* nymphs (Figure 6). Similar trend was observed during the study of susceptibility of *T. aurora* to chlorpyrifos and dichlorvos (Table 6, Figure 7). The findings of the present research showed that nymphs of *I. elegans* and *T. aurora* are more susceptible to dichlorvos than to chlorpyrifos. Similar trend was also reported by Ahmed and Irfanullah (2004). They studied the toxicity of dichlorvos and chlorpyrifos against house fly, *Musca domestica*. They reported dichlorvos as the most effective insecticide chlorpyrifos. The 48-hour LC50 values for the nymphs of *I. elegans* and *T. aurora* were 0.142 ppm and 0.3 ppm, respectively (Figure 8). These LC50 values are far above than the LC50 values reported by and Rubach et al. (2010) for chlorpyrifos against aquatic stages of Odonates or other insect species. To the author knowledge there are no reports about the toxicity of dichlorvos with the nymphs of *I. elegans* and *T. aurora*. However, there are reports about dichlorvos toxicity with fish (Kumar and Gautam, 2014; Patar et al., 2015), birds (Ezeji and Onwurah, 2017), mammals (WHO, 1989) and other aquatic organisms (McHenery et al., 1996). US EPA Ecotox database was searched for the LC50 values of dichlorvos against damselfly and dragonfly nymphs. In the database, 24-hour LC50 values of dichlorvos against the damselfly nymphs of *Agriocnemis sp.*, *Copera sp.* and *Ceriagrion sp.* were 4.57 ppm, 0.91 ppm and 0.63 ppm, respectively. The experimental medium was fresh water and the experiments were conducted in laboratory. The 24-hour LC50 value of dichlorvos for the nymphs of *Agriocnemis sp.* is far above than the 48-hour dichlorvos LC50 values for the *I. elegans* and *T. aurora* nymphs during the present research. The 24-hour LC50 value of dichlorvos for the nymphs of *Copera sp.* and *Ceriagrion sp.* were closer to the 48-hour LC50 value of dichlorvos against *I. elegans* and *T. aurora* during the present study.

The nymphs of *I. elegans* and *T. aurora*, showed different susceptibility against chlorpyrifos. For example, during exposure to chlorpyrifos, *I. elegans* was found more susceptible than *T. aurora* (Figure 8). All insecticides are not equally toxic to a given insect species, neither is a given insecticide equally effective against all insect species (Boiteau and Noronha, 2007; Nielsen et al., 2008). It has been shown that different insect species may respond differently to different insecticides (Banks et al., 2017). Differences among insect species in their capacity for P450–mediated detoxification of insecticides are an important factor responsible for differential tolerance among insect species to insecticides (Wen et al., 2011). During the present research, the susceptibility of *I. elegans* and *T. aurora* to acetamiprid (a neonicotinoid) were studied (Table 7). The nymphs of *T. aurora* was more susceptible than *I. elegans* nymphs. The 48-hour LC50 value of acetamiprid for *T. aurora* (LC50= 0.023 ppm) was significantly lower than its LC50 value for *I. elegans* (LC50= 0.038 ppm) (Figure 10). Thus *T. aurora* was found more sensitive to acetamiprid than *I. elegans*. It has been already reported that different insect species respond differently to an insecticide (Banks et al., 2017). Neonicotinoids act as agonists on nicotinic acetylcholine receptors (nAChRs) opening cation channels (Casida and Durkin, 2013). At the same time, voltage-gated calcium channels are also
involved (Jepson et al., 2006). This agonistic action results in continuous excitation of neuronal membrane, production of discharge that lead to paralysis and exhaustion of cell energy that leads to the death of insects. Thus, the channel opening of nAChRs induced by the binding of neonicotinoids to receptors leads to insecticidal activity (Nishiwaki et al., 2003). To the author knowledge, there are no reports about the LC<sub>50</sub> values of acetamiprid with dragonflies or damselflies nymphs. However, acetamiprid toxicity with other terrestrial and aquatic invertebrates has been reported. For example, the acetamiprid 48 hours LC<sub>50</sub> value of 49.8 ppm against <i>Daphnia magna</i> in static fresh water condition has been reported (Mineau and Palmer, 2013). Wang et al. (2012) studied the comparative acute toxicity of 24 insecticides against earthworm, <i>Eisenia fetida</i>. The LC<sub>50</sub> values of 0.0088 µg/cm<sup>2</sup> and 1.52 mg/kg were observed for acetamiprid against <i>Eisenia fetida</i> during contact filter paper test and contact artificial soil test, respectively.

During the present study, the LC<sub>90</sub> values of different insecticides for the nymphs of <i>I. elegans</i> and <i>T. aurora</i> were compared (Tables 8-9). Based on the comparison of LC<sub>90</sub> values, <i>I. elegans</i> and <i>T. aurora</i> were found more susceptible to lambdacyhalothrin and acetamiprid. They were found least susceptible to chlorpyrifos and dichlorovos. These results are in accordance with the early reports about the toxicity of different classes of insecticides to the aquatic insects and other invertebrates. According to Poirier and Surgeoner (1988), organophosphates insecticides and carbamates insecticides are less toxic to insect larvae but very toxic to some species. According to Anderson (1989), the most toxic group of insecticides is the pyrethroids which have broad spectrum of activity and kill most species.

**Conclusion and recommendation**

From the findings of the present study, it was concluded that damselfly i.e., <i>Ischnura elegans</i> and dragonfly i.e., <i>Trithemis aurora</i> nymphs are highly sensitive to a pyrethroid, lambda cyhalothrin and a neonicotinoid, acetamiprid. Synthetic chemical insecticides are highly toxic to damselfly and dragonfly nymphs even at very low concentrations. The application of synthetic chemical insecticides should be minimized and safe application in the areas adjacent to aquatic habitats must be ensured. Application of other methods of insect pest control such as integrated pest management should be encouraged. Further research is recommended for determining the effect of environmentally realistic high concentrations of agricultural insecticides in water bodies of agricultural areas on the predator avoidance behavior, development and predatory ability of odonate nymphs.

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