OPEN ACCESS
Freely Available Online

INTERNATIONAL JOURNAL OF LIMNOLOGY

ISSN NO: Coming Soon

Research

DOI: 10.14302/issn.2691-3208.ijli-20-3268

Impact if Chlorpyrifos on the Second Instar Mosquito Larvae as Bioindicator in El-Beheira Governorate, Egypt

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Abstract

Pesticides are the major source of concern as water pollutants. Chlorpyrifos (CPY) (*O,O*-diethyl-O-(3,5, 6-trichloro-2-pyridinyl) phosphorothioate; CAS No. 2921-88-2). CPY is a widely used organophosphate insecticide. The aim of current study was to determine the effects of CPY on the second instar larvae of *Culex* mosquito as a bio-indicator of water pollution. Levels of CPY in stream water was evaluated. Toxicity of CPY was estimated on mosquito. Along with the evaluation of effects of water polluted with CPY on mosquito to predict the water pollution levels. Results showed that LC_{95} of CPY was 6331.30 mg/kg after 24hr and increased to 230506.4 ppm after 48hr of exposure. It was noted that the activity of CPY is concentration and time dependent. The 0.09 ppm concentration of CPY (the amount that was found in the stream water) had no effect on the second instar *Culex* larvae similar to the control (tap water). There is no effect after 72,96h of exposure of the population to the detected insecticide. It could be concluded that mosquito is not a bioindicator of CPY pollution at the detected level in stream water.

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Keywords: mosquito larvae- Chlorpyrifos- pollution-second instar.

Received: Mar 13, 2020 **Accepted:** Mar 24, 2020 **Published:** Mar 27, 2020

Editor: Bushra Allah Rakha, Department of Wildlife Management, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Introduction

Persistent organochlorines can accumulate in food chains. This bioaccumulation has been well documented with the pesticide dichlorodiphenyltrichloroethane (DDT) [1, 2, 3]. Organochlorine pesticides are washed into the aquatic ecosystem by water runoff and soil erosion. Pesticides can also drift during application and contaminate aquatic systems samples [4, 5]. Wild birds and mammals are damaged by pesticides and these animals are bio indicator species [6, 7, 8].

Organophosphate pesticides have been the insecticides most commonly used by professional pest control bodies [9]. Chlorpyrifos (O,O -diethyl O -(3,5,6-trichloro-2-pyridinyl)phosphorothioate; CAS No. 2921-88-2; CPY). CPY is a widely used organophosphorus insecticide that is available in a granular formulation for treatment in soil [10]. Pesticides are used to control wide range of pests including Mosquitoes. Mosquito borne diseases infect over 7000000 people every year globally, being prevalent in more than 100 countries across the world [11, 12-14].

WHO has declared mosquitoes as "public enemy number one". Worldwide, malaria causes one to two million deaths annually. Lymphatic filariasis has been reported to affect at million people in 73 countries including Africa and Pacific Islands [15]. Mosquitoes serve as vectors of life threatening diseases such as malaria [16, 12, 5]. The current study aimed to monitor water pollutants (persistent organic, minerals and pesticides) and to assess the potential adverse effect of polluted water on the bio indicator insects; mosquitoes. The aim of the current study was to determine the adverse effects of some the detected-pesticides (Chlorpyrifos) on the larvae of second instar Culex mosquito larvae as a bio-indicator of aquatic pollution. Water requirements of different sectors increase rapidly with time due to rapid population increase, ambitious agricultural expansion [17].

Quality of Nile water worsened dramatically in the past few years [18, 2, 3]. It is anticipated that the dilution capacity of the River Nile system will diminish as the program to expand irrigated agriculture moves forward and the growth in industrial capacity increases the volume of pollutants discharged into the Nile [19, 12, 5]. The major pollution sources of Nile and main canals are effluents from agricultural drains and treated or partially treated industrial and municipal wastewaters [20, 13, 14].

There are 76 drains discharging drainage water into Nile system with annual volume of about the half of the total drainage water [21]. Impact of this drainage water on Nile quality has been reported by several authors [18]. Statistics indicate that over one billion of the world population lack access to safe water, and nearly two billion lack safe sanitation worldwide [22, 7, 8]. A growing number of water related diseases such as diarrhea and lymphatic filariasis are responsible for the major health problems in the majority of rural and urban residents [23, 13, 14].

The quantities and quality of wastewater from agricultural lands are highly variable. The most important pollutants found in runoff from agricultural areas are sediments, animal wastes, plant nutrients in addition to domestic wastes [2, 3]. Water pollution sources, has become of public interest. Natural events and anthropogenic influences can affect the aquatic environment in many ways [12, 5, 19].

Water pollution occurs when a body of water is adversely affected due to it is unfitting for its intended use [24, 3, 7, 8]. The aquatic environment is subjected to various types of pollutants which enter water bodies [25, 2]. Among the various pollutants, heavy metals are the most toxic, persistent and abundant pollutants that can accumulate in aquatic habitats [26, 13, 14]. Trace metals such as Zn, Cu and Fe play biochemical role in the life processes of all aquatic plants and animals. In the Egyptian irrigation system, the main source of Cu and Pb are industrial wastes, while that of Cd is the phosphatic fertilizers [27, 2, 3]. The most anthropogenic sources of metals are industrial sources as paints and petroleum contamination [28, 12, 5]. The agricultural drainage water contains pesticides [2, 3, 13, 14].

There are more than half a million tons of unused in several developing and transitional countries [29]. Obsolete pesticides have accumulated in almost every developing country or economy in transition over the past several decades [30]. The FAO is recording the inventories of Latin America [31, 2, 3]. It is difficult to estimate the exact quantities of obsolete pesticides because many of the products are very old and documentation is often lacking [32, 7, 8].

Chlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) were routinely used in large quantities for agricultural and industrial purposes [33, 2, 3]. Insecticides overuse led to several ecological drawbacks over the past years [34]. Mosquitoes of family Culicidae, are vectors for a number of mosquito borne infectious diseases [35] that are maintained in nature through the biological transmission by blood feeding mosquitoes to susceptible vertebrate hosts causing malaria and filariasis [36].

Mosquitoes are a major public health threat as they play a vital role in transmitting serious human diseases to million people annually [37]. *Culex pipiens* is a worldwide mosquito transmitting many dangerous diseases as filarial worms and avian malaria [38]. With the emergence of *C pipiens* resistance to many insecticides, control is becoming more difficult [39].

The control of mosquito is becoming challenging because climate change and global trade favor the spread of invasive mosquito species [40], and strongly increase the associated risk of vector borne diseases [41]. Most strategies for mosquito control are based on the use of insecticides [42] and developing resistance [43]. Treated populations can recover after application of the insecticide. Vector control is by far the most successful method for reducing incidences of mosquito borne diseases [6]. The discovery of the subsequent development of organochlorines, organophosphates and pyrethroids suppressed natural product research, as the problem for insect control were thought be solved [44, 2, 3, 78]. The aim of the current study was to determine the effects of CPY on the second instar larvae of *Culex* mosquito as a bio-indicator of water pollution.

Materials and Methods

Insecticide

The percentage of 48% EC Chlorpyrifos (devagro kimya tarim san vetic Torkey) was used to determine the lethal concentration LC_{25}, LC_{50} and LC_{95} against the second instar *Culex larvae*.

Mosquito's Culture and Rearing

Mosquitoes culture brought from Alexandria University Faculty of Agriculture and accommodate for (2) weeks in Damanhour University, Faculty of Science laboratory.

Bioassay of Detected Pollutant in Water

The second instar mosquito larvae were exposed to a wide range of tested concentrations to find out the activity range of the materials under test. After determining the mortality of larvae in this wide range of concentrations, a range of 5 concentrations, yielding between 10% and 95% mortality in 24 h or 48 h is used to determine LC_{50} and LC_{95} values. Batches of 20 insects at the second instar larvae were transferred by means of droppers to Petri dish each containing 20 ml of distilled water. Small, unhealthy or damaged larvae were removed. The appropriate volume of dilution is added (20 ml) water to Petri dish to obtain the desired target dosage, starting with the 100, 10, 1, 0.1, 0.09 ppm concentration. Five replicates were set up for each concentration and an equal number of controls (5 replicates) are set up simultaneously with tap water. After 24 h exposure, larval mortality was recorded. For slow acting insecticides, 48 h reading was required. Moribund larvae are counted and added to dead larvae for percentage mortality. Dead larvae are those that cannot be induced to move when they probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed. The results are recorded to detect the LC_{25}, LC_{50} and LC_{95} values.

Statistical Analysis

Analysis examined the lethal concentration LC_{25}, LC_{50}, LC_{95} and X^2 of Chlorpyrifos insecticide on Culex larvae.

Results

The Side Effects on the Second Instar Mosquito Larvae

The present study had been undertaken in order to determine the adverse effects of detected-pesticides (Chlorpyrifos) on the larvae of *Culex* mosquito larvae as bio-indicator, with a serial number of

Chloropyrifos concentration (100ppm, 10ppm, 1 ppm, 0.1ppm and 0.09 ppm corresponding to determine the lethal dose concentration LC_{25} ,LC_{50} and LC_{95} of Chlorpyrifos insecticide on *Culex* larvae. The treatment occurred by a serial concentration of Cholorpyrifos; 0, 0.09, 0.1,1,10,100 ppm applied on the mosquitoes larvae. After 24h, 48h mortality percentage was recorded as; at 24h, Cholorpyrifos killed 50% of the mosquito larvae population at 24.52 ppm. While at a longer time 48h, the 50% of the mosquito larvae population were killed by 755.65 ppm. After 24h, the detected concentration of Cholorpyrifos on 25% population mortality was 2.51 ppm, although it was 72.37 ppm after 48h of Cholorpyrifos exposure to 25% population of mosquitoes. It is detected that after 24h, the Cholorpyrifos killed 95% of the mosquito larvae population at 6331.30 ppm, while after 48h, the 95% of the mosquitoes larva population were killed by 230506.4 ppm.

In the present study, bioassays were carried out to evaluate the insecticidal concentration of chlorpyrifos on the second instar *Culex* larvae. Surveys in Egypt date back to 1903. According to these surveys eighteen Culicine and eleven Anopheline species have been encountered in the different parts of Egypt. *Culex pipiens*, the main Filariasis vector in Egypt. Published field and laboratory studies with mosquito control pesticides have concentrated on differential effects with mosquito larvae [84]. The exposure time has an important effect on the values of LC_{50} in this study. In most cases, the LC_{50} values had synergistic interactions with time; thus, it increased after 48h of exposure when compared to 24 h of exposure (Table 1). Very high concentrations of the Chloropyrifos led to high mortality rates. The LC_{50} of Chloropyrifos insecticide in the case of *Culex pipnes* was 24.52 ppm after 24h, and increased to 755.65 ppm after 48h. The lower value was 14.78 ppm after 24h which also increased to 258.10 ppm after 48 h., the higher value of LC_{50} was 45.6576 ppm after 24h and the same value became 5485.96 ppm after 48h. The LC_{25} of Chloropyrifos insecticide was detected as 2.51 ppm after the first 24h and measured at 72.37 ppm after the second 48h.

In El Beheira Governorate, pesticides are used along a large scale. Organochlorine and organophosphate are persistent pesticides which leave residues in drinking water that remain for days to many years .Organochlorine pesticides, prohibited since the early 1980s, are still detectable in the environment. Organophosphates are found in high rate in the stream, Chloropyrifos is an Organophosphate pesticides found at concentration of 0.09 m/l in the stream water. Effect of the exposure time of Chloropyrifos insecticide on the LC_{50}, LC_{25} and LC_{95} values had a synergistic interaction with time as it increased after 48h of exposure when compared to 24 h of exposure. The 0.09 ppm concentration of Chloropyrifos had no effect on the second instar *Culex* larvae, as there is no mortality. Also there is no effect on mosquito mortality after 72h and 96h of exposure to the detected concentration of Chloropyrifos insecticide.

Discussion

The concentration of 0.09 ppm had no mortality on mosquito larvae in the second 48 h to 1h. The control also not had any mortality population on mosquito larvae. It was noted that after 72 h and 96 h

Table 1. Lethal concentrations of Chlorpyrifos

Time	LC_{50}	Confidence Limits of LC_{50}		LC_{25}	LC_{95}	X^2
		Lower	Higher			
24	24.52	14.78	45.65	2.51	6331.3	1.41
48	755.65	258.1	5485.96	72.37	230506.4	1.43

Mortality percentage were calculated using LDP line software (Ehab soft, Egypt) according to Finney 1951 there was no effect on mosquitoes larvae, as the equal number of inserted larvae were constant at the end of the experiment.

As water temperature increases, the rate of chemical reactions increases. The temperature affects the rate of growth and life cycles of most aquatic organisms. It is known to influence the pH, alkalinity, and DO concentration in the water. The turbidity is derived from silt, clay, and sand particles, while organic turbidity is composed of planktonic organisms and detritus. The increasing of turbidity values is referred to increasing of suspended materials will reduce light penetration and restrict plant growth and hence food resources and habitat for organisms [45, 46].

The total dissolved salts along the El Mahmodia stream were less than 450 mg/l and there was no restriction on using it for some susceptible crops [47]. The water stream receives fluxes of elements through natural processes by weathering of bed rocks. The basalts contain weak olivine and pyroxene minerals that are enriched in some elements such as Na, Li, Fe, Mn and Mg in addition to Si. These elements transport with water to increase the TDS of the streams. The TDS of Lake Tana, source of the Blue Nile, varies from 50 to 138 mg/l with an average of 103 mg/l [48]. The major ions represented by TDS have been also significantly increased by anthropogenic contaminations. The average salinity of the Nile River at Cairo ranges from 175 to 680 mg/l with an average of 261 mg/l [49]. The pH is an important limiting chemical factor for aquatic life which may affect the aquatic organisms' biochemical reactions. The severe changes of pH of the water may cause a harmful or even lethal effect on aquatic organisms and consequently affect the animal and human health. Water streams have pH ranging, between 6 and 9, and any changes in this range in pH can affect life forms in aquatic systems [50]. The increase of pH values at the streams water is a result of photosynthesis [2, 3, 12, 5].

The unpolluted streams normally show a near neutral or slightly alkaline pH. The decrease of DO may be attributed to the consumption of DO by respiration of phytoplankton, aquatic plants, and fish, and decay of the aerobic bacteria [51]. Dissolved oxygen value was greatly affected by pollution load where the lowest DO is recorded at all sites, and the excessive effluent discharge of pollution with high load of organic matter into the two stream leads to deoxygenating of water. Waste discharges that are characterized by high inorganic matter and nutrients can lead to decreases in DO concentrations as a result of the increased microbial activity (respiration) occurring during the degradation of the organic matter [52, 2, 3, 53].

It is worth mentioning that unpolluted waters typically have BOD values of 2 mg/l or less, whereas those receiving wastewater may have values up to 10 mg/l or more, particularly near to the point of wastewater discharge [2]. The values of BOD exceeded the desirable limits of (Egyptian Law 48/1982) [54] which was<6-10 mg/l. The high BOD values indicate excessive export of biodegradable organic matter increasing the de-oxygenation of water to the level where fish and other aquatic life cannot survive [55, 12, 5, 13, 14]. The COD is widely used as a measure of the susceptibility to oxidation of the organic and inorganic materials present in water bodies and in the effluents resulting from sewage and industrial plants [56, 5, 12, 7, 8]. The COD high values indicate excessive export of biodegradable organic matter increasing the de-oxygenation of water to the level where fish and other aquatic life cannot survive [55]. Fecal pollution is a major concern for many rivers where it can originate from human sources and nonhuman sources. Fecal coliform can be used as indicator for water pollution and hence for water quality measure [57]. Natural sources of nitrate in surface waters are the interaction with igneous rocks, land drainage, plant and animal debris [47, 2, 3, 62]. Phosphorus is an essential nutrient element for living organisms and exists in water bodies as both dissolved and particulate forms. Natural sources of phosphorus are mainly derived from weathering processes of phosphorus bearing rocks and the decomposition of organic matter [2, 3, 53]. Phosphorus concentration in stream water was 0.01 mg/l. Ca is the major cation of the Nile water, which probably comes mainly from the rocks [58].

The term "heavy metal" refers to any metal and metalloid element that has a relatively high density ranging from 3.5 to 7 g/cm^3 and is toxic or poisonous at low concentrations [59, 13, 14, 7, 8]. They include mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), zinc (Zn), nickel (Ni), copper (Cu), and lead (Pb). Heavy metals are natural constituents of the earth's crust [60]. In developing countries, where environmental protection laws have not been enforced, industrial and domestic wastes are dumped randomly into water bodies [61, 12, 5]. The low concentration values of the heavy metals in the stream water are due to their deposition with sediments on the stream's bottom [62].

The major sources for manganese in water are iron and steel manufacturing and the burning of diesel fuel in the motor cars [2, 3, 13, 14]. The high levels of Cu in water can be attributed to industrial and agricultural discharge [47]. The copper concentration was attributed to the huge amounts of raw sewage, agricultural and industrial wastewater discharged into the stream [63].

Most of the organic pollutants detected were identified as endocrine disrupting phthalate esters, fatty acids, phenolic acids, carcinogens, and aquatic toxicants, plasticisers, which are classified as "priority pollutants" due to their severe toxicity in living being [64, 2, 3, 53]. Phthalates such as Phthalic acid, butyl tetradecyl ester, Phthalic acid, octadecyl ester Phthalic acid, butyl 2-ethylbutyl ester, Phthalic acid, di (2-propylpentyl) discharged along with industrial wastewaters cause water pollution and disturb the ecology of the receiving water bodies by creating serious toxicity to aquatic organisms, such as fishes, as result of bioaccumulation and thus cause toxic effects [65]. Phthalates also are reported to cause endocrine disruption in humans and animals upon long term exposure [64]. Phthalic acid is used in industry has been reported to cause mutagenicity, developmental toxicity, and reproductive toxicity in animals [66].

Dihydroxybenzoic acid might be raised in El Mahmodia stream water as a key metabolite of biodegradation of polyaromatic hydrocarbons (PAHs) during wastewater treatment [67]. 2, 6-Dihydroxybenzoic has been reported as using in blending and formulating a variety of personal care products including shampoos, and deodorants and as a solvent in commercial dry cleaning products and industrial cleaning fluids [68]. Aquatic toxicants reduce the algal growth in the aquatic ecosystem and thereby reduce photosynthesis and ultimately disturb the ecological functioning of receiving water bodies [69, 13, 14]. Fatty acids (n-Hexadecanoic acid, Hexnedioic acid, trans-9-octadecanoic acid) might have originated in during the industry. Benzeneacetonitrile, α-[4-(dimethylamino)-2,5dimethoxyphenyl]methylene]-4-nitro-,1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester, Benzeneacetonitrile, α-[4-(dimethylamino)-2,5-dimethoxyphenyl]methylene]-4-nitro-, from other Benzyl compounds are considered to be moderately aquatic toxicant and poses moderate to low toxicity to aquatic animals, such as fishes, and also is listed as a Group 2A carcinogen [70, 18].

The major pollution sources of Nile and main canals are effluents from agricultural drains and treated or partially treated industrial and municipal waste waters [71, 7, 8]. The drainage water contains dissolved salts which washed from agricultural lands as well as residues of pesticides and fertilizers, at the end these pesticides collected in El Mahmodia stream water, causing severe damage to it. Impact of the drainage water on Nile quality has been reported by Abdel-Dayem *et al.* [18]; Radwan *et al.* [7, 8]. In El Mahmodia stream drainage water mixed with drinking water due to human activities along the stream, there is a large amount of organochlorine pesticides detected in the stream water samples such as Dieldrin [72, 73]. There is no access waste water treatment in Abo Homes rural areas, 20% of Egyptian villages have inadequate potable water [74]. In Egypt, water supply and sewage services are not implemented simultaneously. In the rural areas, where half of the population lives, 90% of the people have no access to waste water treatment facilities [75, 76, 13, 14]. The aquatic environment is subjected to various types of pollutants which enter water bodies [77, 12, 5].

Industrial waste water is considered the second of the main sources of Nile water pollution. There are about 129 factories discharging their waste water into the River Nile system. Effluent wastewater is often partially treated [78, 79]. Major pollutants in agricultural drains are salts, nutrients, pesticide residues, toxic organic and inorganic pollutants [80]. The persistence of the organochlorine compounds and their metabolites, which are often more toxic than the original compound, is dependent on environmental conditions [81, 82]. Toxic substances such as heavy metals and organic micro pollutants occur due to the mixing of domestic with industrial and commercial activities [80]. Organochlorines (OCs) are a generic term for pesticides containing chlorine; however, the term is commonly used to refer to the older persistent materials, including aldrin, chlordane, dieldrin, heptachlor, toxaphene [83].

Recommendations

The Ministry of the Environment in Egypt is observing the enforcement of the legislation regarding the treatment of industrial and domestic wastewater. It is also advocating organic farming and limiting the use of chemical fertilizers and pesticides to reduce water pollution. Improving the quality of drainage water especially in the main drains.

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