Effect of Selected Agrochemicals on Protozoans and Algae Isolated from Mosquito Larval Habitats in Bamenda

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Abstract
The present study establishes the in-vitro effect of some selected agrochemicals(Glycot, Lamida and Pencozeb) on protozoans and some algae isolated from mosquito larval habitats in Bamenda urban zone, Cameroon. A convenience sampling technique was used in which water samples were randomly collected from 125 mosquito larval habitats from Bamenda urban zone. Out of the 123 samples tested, 41(33.33%) samples were found to be positive. Dinoflagellates predorminated with a frequency of 20(16.26%). One genera of protozoa(chillodenella) pathogenic to mosquito larvae was seen. Hook worm, Faciola, Paramecium and Trichomonas were found to be of medical importance while Euglena, Diatoms and Dinoflagellates were found to be of agricultural importance. All the protozoans and algae tested were susceptible to Lamida and Penncozeb, but resistant to glycot at all concentrations tested. Conclusively, the susceptibility of Chillodenella spp to Lamida and Penncozeb suggest their interference with natural biological control for mosquito population and also distortion of the ecosystem.
Keywords: Agrochemicals, protozoans and algae , mosquito larval habitats

1. Introduction

Mosquitoes act as vectors for most of the life threatening diseases like malaria, Japanese encephalitis(JE), yellow fever, dengue fever, Chikungunya fever, filariasis, West Nile Virus infection, etc. Extensive use of synthetic chemical insecticides such as organochlorine and organophosphate compounds against vector mosquitoes to control malaria and other mosquito borne diseases for about four decades has not been very successful(Das et al., 2016)

Sustained and prolonged use of chemical insecticides has led to the development of insecticide resistance in the target mosquito population, severe suppression of non-target organisms and general pollution of the environment. Additional and innovative vector control methods are therefore required either alone or in combination with traditional approaches, to decrease the transmission of the disease (Rojas et al., 1987).

Natural population of mosquitoes is kept under check by the activities of parasites and predators. Several species of viruses, bacteria, fungi, protozoa and nematodes are known to cause infection in mosquito larvae. Endoparasitic ciliated protozoa have been known to infect mosquito larvae since 1921, when Lamborn first reported the occurrence of *Lambornella stegomyiae* infection in the larvae of *Aedes albopictus* in a sample collected from an earthen pot in Kuala Lumpur1.

Ch. Uncinata is a free living facultative, ciliated, protozoan parasite with many attributes of a good microbial pathogen, widely distributed in typical JE vector breeding habitats in and around Delhi. It causes low to very high (25–100) per cent mortalities in mosquito larvae, particularly Cx. tritaeniorhynchus and Cx. pseudovishnui, important JE vectors in India. Like a pathogen it is highly virulent, desiccation resistant, can be cultured in vitro and has a high reproductive potential with facility to disperse in the environment by way of trans-ovarian transmission through its mosquito hosts.

Pathogenically, a number of them simultaneously attack one single larva at different points (head, thorax, abdomen, siphon, anal fin, antennae, etc.) and enter body cavity of the host larva by drilling through the host cuticle. Thereafter, it multiplies inside the host body at the expense of the internal viscera of the host larva and releases numerous minute motile spores, ultimately killing the larva and finally escaping the cadaver of larva (*recycle in the environment*) to attack fresh larvae to continue the cycle. The organism is so virulent that even a few of them can cause chronic infection leading to death in susceptible host larvae (Das, 2004).

Human activities are contaminating surface waters with chemicals, including pesticides, which enter water bodies as runoff from agriculture, and pharmaceuticals, which are discharged in industrial and sewage treatment wastewater. These pollutants can harm the structure and function of aquatic ecosystems, including biofilms. Biofilms are typically composed of algae, bacteria, fungi and protozoa and they coat surfaces, such as those at the bottom of rivers. They are good indicators of the environmental pressures on river systems, as they are part of the food web and natural cycles of river ecosystems and respond quickly to changing conditions (Proia et al., 2013).

The present study establishes the in-vivo effect of some selected agrochemicals(Glycot, Lamida and

Pencozeb) on protozoans and some algae isolated from mosquito larval habitats in Bamenda urban zone, Cameroon.

2. Materials and Methods

A convenience sampling technique was used in which water samples were randomly collected in a sterile 350 ml urine containers from 125 mosquito larval habitats from Bamenda urban zone and transported at room temperature to Phytobiotech Reseach Foundation laboratory for analysis.

2.1 microscopical examination

10 ml of water samples from mosquito larval habitats were dispensed into centrifuge tubes. They were centrifuged at 1500 rpm for 5 minutes. The supernatants were discarded and the deposits examined microscopically using X10 and X40 power objectives respectively.

Standard atlas (Shiba et al., 1996; Jianzhong and Hengsheng, 2010) were used to established the identity of the parasites and algae

2.2 Susceptibility testing of Protozoans and Algae

Agrochemicals were diluted following manufacturers instruction for use as described above. This initial concentration was diluted with water from mosquito larval habitats that were positive for protozoa and algae to give a ½, ¼, 1/8 dilutions respectively. Water from each larval habitat was centrifuged at 3000 rpm for 10 minutes, and the supernatants examined to role out the presence of algae or protozoans. The supernatant water was used to dilute the agrochemicals as decribed above, and this served as a negative controle. For positive controle, the supernatant water was used to dilute the water samples from larval habitats to give a ½, ¼, 1/8 dilutions respectively. All the dilutions were centrifuged at 1500 rpm for 5 minutes, after which the supernatants were discarded and the sediments vortexed at 500 rpm for 3 seconds and examined microscopically using X10 and X 40 power objectives. Presence of protozoa, algae was an indication of resistance while their absence indicated sensitivity. The number of protozoans or algae per high field was counted for up to 10 fields within five minutes. This was done three times for each sample and average values taken. The percentage inhibition for each dilution was established using the following formula:

$$\% \text{ inhibition} = \frac{\text{Average count of test}}{\text{Average count of sample}} \times 100$$

Results

Out of the 123 samples tested, 41(33.33%) samples were found to be positive. Dinoflagellates predorminated with a frequency of 20(16.26%). One genera of protozoa(chillodenella) pathogenic to mosquito larvae was seen. Hook worm, Faciola, Paramecium and Trichomonas were found to be of medical importance while Euglena, Diatoms and Dinoflagellates were found to be of agricultural importance. Figure 1.

All the protozoans and algae tested were susceptible to Lamida(Table 2) and Penncozeb(Table 3), but resistant to glycot(Table1) at all concentrations tested.

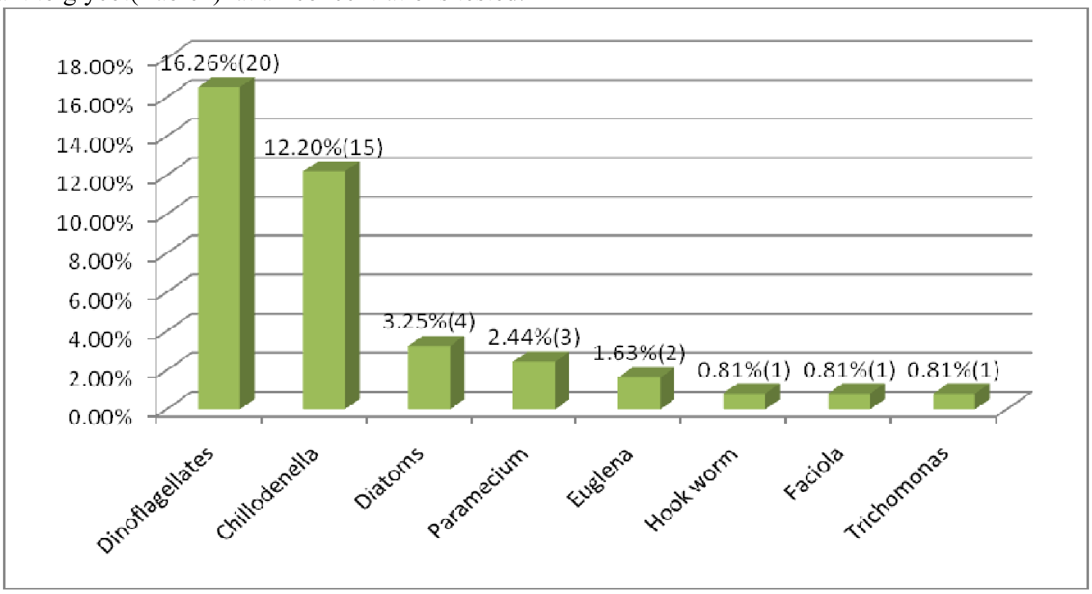

Figure 1. Protozoans, algae and helminths isolated from larval habitats in Bamenda

Table 1: Susceptibility of Protozoans and algae isolated from Bamenda City Council area to Lamida

Agro agrochemicals	Dilution factor	% Inhibition of Protozoans and algae				
		Chillodenella	**Euglena**	**Paramecium**	**Dinoflagellates**	**Diatoms**
Glycot (7.29/L)	1	00.00	00.00	00.00	00.00	00.00
	2	00.00	00.00	00.00	00.00	00.00
	4	00.00	00.00	00.00	00.00	00.00
	8	00.00	00.00	00.00	00.00	00.00

Lamida = imidaclopride + lambdacyhalothrine

Table 2: Susceptibility of Protozoans and algae isolated from Bamenda City Council area to Glycot.

Agro agrochemicals	Dilution factor	% Inhibition of Protozoans and algae				
		Chillodenella	**Euglena**	**Paramecium**	**Dinoflagellates**	**Diatoms**
Lamida (0.18g/L)	1	100	100	100	100	100
	2	100	100	100	100	100
	4	100	100	100	100	100
	8	100	100	100	100	100

Glycot = Glyophosphate

Table 3: Susceptibility of Protozoans and algae isolated from Bamenda City Council area to Penncozeb.

Agro agrochemicals	Dilution factor	% Inhibition of Protozoans and algae				
		Chillodenella	**Euglena**	**Paramecium**	**Dinoflagellates**	**Diatoms**
Penncozeb (6.25 g/L)	1	100	100	100	100	100
	2	100	100	100	100	100
	4	100	100	100	100	100
	8	100	100	100	100	100

Penncozeb = Mancozeb

Discussion and conclusion.

Results obtained reveal complete susceptibility of all protozoans and algae to Lamida and Penncozeb at all concentrations. Chillodenella spp, the second in terms of prevalence in this work (12.20%) is a mosquito larval pathogen, and serves as a natural biological control for mosquito population and mosquito-borne diseases. Its inhibition at even very low concentrations by Penncozeb and Lamida is a call for concern. This suggest the interference of these agrochemicals with natural and environmentally friendly control measures for mosquito populations, as these agrochemicals are routinely use by gardeners within the city of Bamenda.

Cryptococcus neoformans is a free-living pathogenic fungus that primarily inhabits soil contaminated with decaying organic matter and bird excreta (Casadevall and Perfect 1998; Steenbergen and Casadevall, 2003). *C. neoformans* is unusual among the pathogenic fungi in that it has several well characterized virulence factors such as a polysaccha-ride capsule, degradative enzymes, and melanin pigment production (Ma and May, 2009). *C. neoformans* is a non-specific pathogen that has been reported to infect and/or cause disease in a myriad of taxonomically diverse organisms from mammals to birds to such invertebrates as insects and worms (Goodchild et al., 1996).

Euglena, Paramecium, Dinoflagellates and Diatoms inhibited in this work are of public health and ecological importance. Shalom et al.(2010) studied the interaction of *C. neoformans* with three *Paramecium* spp., and reveal that some *Paramecium* spp. rapidly ingested *C. neoformans* and killed the fungus. Their study established another type of protist-fungal interaction supporting the notion that animal-pathogenic fungi in the environment are under constant selection by predation.

The role of *Euglena gracilis* as a good source of nutritionally and medically important substances has called for more research on how to maximize its production. The main importance of *E. gracilis* is that it contains high amounts of proteins, vitamins and polyunsaturated fatty acids (PUFAs). Consequently, it is used as health food and animal feed. Antioxidant vitamins such as a-tocopherol, L-ascorbic acid and b-carotene, which function to prevent diseases caused by oxidative damages among other functions, are accumulated in significant amounts by *E. gracilis* (Takeyama et al., 1997). Although many higher plants are known to produce these vitamins, their production in photosynthetic microorganisms is much higher owing to higher growth rates and absence of seasonal influences, which make it possible for the cells to be cultivated all year round.

Polyunsaturated fatty acids (PUFAs), especially linoleic and a-linolenic acids, which have a number of physiolo-gical functions, have also been reported to be produced in significant amounts by the microorganism (Hayashi et al., 1993). PUFAs are important in the body for synthesis of membrane lipids, reduction of blood cholesterol levels, reduction of risk of heart attack, normal development of brain and retina and for synthesis of other physiologically important substances like prostaglandins (Taylor et al., 1998). It is known that fishes, the major sources of PUFAs, obtain these fatty acids from photosynthetic cells in a food chain.

E. gracilis is among the many photosynthetic microorganisms employed for production of single cell protein (SCP), a new food source, used as an alternative source of proteins in developed countries like Europe, America and Asia. Protein remains the most costly food component in countries like Nigeria, where SCP technology has either not been adopted or developed to supplement the conventional sources, such as meat, eggs, fishes and legumes. SCP from photosynthetic cells (algae) is considered better than those from bacteria or fungi. This is because, algae including *E. gracilis*, accumulate less amounts of nucleic acids than bacteria and fungi. High levels of nucleic acids increase the risk of occurrence of a disease condition known as gout(Afiukwa and Ogbona, 2007).

Conclusively, the present work brings out substaintial evidence on the interference of agrochemicals in the natural biological control measures for mosquito population. Also their inhibition of other microbes that are not mosquito larval pathogens, may result in the distortion of the natural ecology, which calls for a need for continuous monotoring and regulation of the use of these agrochemicals.

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