# EFFICANCY OF THE MOLLUSCICIDE NICLOSAMIDE (2-HYDROXYEHYLAMMONIUM SALT OF 2', 5 DICHLORO -4-NITROSALICYLANITIDE) AS A MOSQUITO LARVICIDE

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*(Received 17 - 12 - 1995)*

## INTRODUCTION

Many human beings are victims of parasitic diseases borne and transmitted by snails and mosquitoes. Schistosomiasis and Elphantiasis are among the most dangerous and widespread diseases affecting the Egyptian population. These diseases are aggravated by the irrigation and agricultural development.

The most outstanding and widely used molluscicide during the past 20 years is Niclosamide (El-Bayouki and Bayouni, 1991). Niclosamide is marketed under the trade names of Bayluscide and Mollutox.

Slow moving and stagnant, water bodies which may be treated with molluscicides are sometimes mosquito breeding areas. The use of one compound with molluscicidal and mosquito larvicidal properties will be of great advantage. The present study was designed to evaluate the efficacy of Niclosamide (2- hydroxyehylammonium salt of 2',5 dichloro -4-nitrosalicylanilide against *Culex pipiens* which is the main filaria vector in Egypt (Khalil, *et al.*, 1932), and to discuss the possibility of its use in anti mosquito work.

## MATERIALS AND METHODS

Mosquito larvae *(Culex pipiens)* were collected from natural breeding places in Qaluobiya Governorate and reared for two years under controlled conditions (25 ± 2°C and 65 ± 5% R.H.), using the method described by Ibrahim (1993).

Niclosamide (2-hydroxyehylammonium salt of 2',5 dichloro -4-nitrosalicyla-

nilide), was provided by Dr. M.E. Abdel Badei who prepared it in the laboratories of the Faculty of Science in Benha, Egypt, following the method used by Abdel-Rahman *et al.* (1974).

*Laboratory experiments :*

All tests were conducted in 150 ml, plastic cups containing 100 ml distilled water and using 25 larvae in each cup. Eahc concentration was replicated four times. Mortalities were recorded and corrected by Abbott's formula (1925).

Mosquito larvicidal activity of Niclosamide was monitored by exposing third instar larvae to different concentratons of the compound. Mortalities were recorded after 24 and 48 hours.

When studying the variance in susceptibility of different larval instars to Niclosamide, 2*nd* and 4*th* larval instars were treated with different concentrations. Mortality was recorded after 24 hours.

To study the effect of exposure time on the efficacy of Niclosamide, 3*rd* instar larvae were exposed to serial concentrations of Niclosamide for 1, 4 & 24 hours or continuously (until adult emergence). At the end of each period insects were removed, rinsed with distilled water and transferred to other cups containing distilled water. Larvae were provided with food and observed until adult emergence. Dead and alive larvae or pupae were recorded.

To monitor the possible influences of water types and water pH values on the efficacy of Noclosamide against *Culex pipiens* larvae distilled, tap (chlorinated and dechlorinated), and Nile waters as well as water with different pH values (pH 7.3 to pH 8.7) were treated with a fixed concentration of Niclosamide (30 ppm) and assayed against 3rd instar larvae under laboratory conditions. Dechlorinated tap water was obtained by adding three crystals of sodium thiosulphate to each liter of tap water.

*Simulated field experiments :*

To study the persistence of Niclosamide under simulated field conditions, two concentrations of the compound (1 and 5 ppm) were applied, using one liter hand atomizer, to out-door artificial ponds established in the garden of the Faculty of Science in Benha. Each pond is a stainless steel basin burried in the soil leaving its edge within the ground surface. The basin holds about 500 liters. Each basin was filled with 250 liters of tap water and retained for 48 hours so that the water was dechlorinated and acquired all environmental con-

ditions. The volume of water in relation to depths was calibrated, scaled on the inner side of the basin and kept throughout the time of experiment (30 days). Each concentration (1 or 5 ppm) was applied in two basins and two untreated basins were left as chack. Water samples from the basins were taken daily and bioassayed in the laboratory using, laboratory reared, 3*rd* instar *Culex pipiens* larvae. Mortality readings were taken after the emergence of all surviving adults.

## RESULTS AND DISCUSSION

Comparatively low larval mortalities were recorded after 24 and 48 hours of exposing 3*rd* instar *Culex pipiens* larvae to the tested compound (Fig. 1). A concentration of 75 ppm induced 100% mortality after 48 hours of exposure. But the higher concentration (100 ppm) gave complete mortality during 24 hours. The LC50 values after 24 and 48 hours were 23.4 and 4.95 ppm respectively. In fact, these values are much higher than those needed for snails. Laboratory snail mortality, based on a large number of references in the literature, required concentrations from 0.15 to 0.6 ppm to achieve the 24 hours LC50 value (Cardarelli, 1977).

When mortality readings of the treated larvae were recorded after the emergence of all surviving adults, the least concentration used (0.3 ppm) killed 70% of the larvae treated, whereas a concentration of 7.5 ppm gave complete mortality. Larval mortality increased proportionally by increasing the time of exposure. The LC90s of 3*rd* instar larvae treated with Niclosamide for 1, 4 and 24 hours were 100, 60 and 7.5 ppm respectively (Fig. 2).

Generally, 2*nd* instar larvae were significantly ($p < 0.05$) more susceptible to the tested molluscicide than those of the 4*th* instar (Fig. 3). The LC50 values after 24 hours were 21 and 34.5 ppm for the 2*nd* and 4*th* instars respectively.

The results also show that, larval mortality varied considerably when Niclosamide was bioassayed against 3*rd* instar *culex pipiens* larvae using different water types (Fig. 4). When larvae were treated with a concentration of 50 ppm, the recorded larval mortalities after 48 hours were 92%, 81%, 60% and 57% in distilled water, tap (chlorinated) water, dechlorinated tap water and Nile water respectively. Lower larval mortalities in tap and Nile waters may be due to the presence of organic and non colloidal inorganic materials in these water types. These materials can reduce the toxicity of Niclosamide through irreversible chemi-sorption or physical adsorption. The significant difference ($p < 0.05$) observed in the activity of Niclosamide in the chlorinated and dechlorinated tap water may indicate that the presence

of chlorine ion in water increase the effecacy of this compound against *Culex pipiens* larvae. On the other hand, the lowest activity observed in Nile water may be due to a biological degradation of the compound by certain bacteria.

pH of the tested waters showed no significant effect on the toxicity of Niclosamide against 3*rd* instar *Culex pipiens* larvae. But activity was significantly ($p < 0.05$) decreased with the increase in water acidity (Fig. 5). Acidic water may alter Niclosamide ions to forms that would react with complementary ions and produce insoluble non toxic salts. Low activity of Niclosamide against *Schistosoma mansoni* was also observed at low pH values by Mohammed (1993).

Results of the laboratory assays of field water treated with 1 and 5 ppm of the compound against 3*rd* instar *Culex pipiens* larvae (Fig. 6) revealed no significant reduction in its larvicidal activity for a period of 5 days post treatment, then larval mortalities were sightly and gradually decreased. At the end of our experment (30 days post treatment), waters treated with 1 and 5 ppm. killed only 34 and 75% of the larvae, respectively.

On the basis of the obtained results, we can conclude that Niclosamide is an effective larvicide for *Culex pipiens* larvae. The rates of this molluscicide, in our tests, for effective and complete control of *Culex pipiens* larvae in laboratory and simulated field experiments were higher than the rates generally recommended for snail control programs (1 ppm). But we can expect that a considerable proportion of *Culex pipiens* larvae inhabiting water bodies treated with this molluscicide will be killed. In our simulated field test, this concentration (1 ppm) killed 70% of the treated larvae. This larvicidal effect was extended for at least 30 days (the period of our experiment) where mortality reached 34%. Although, early publications indicated that this compound is not dangerously toxic to man, his domestic animals or his crops (Ritchie, 1969, Ansari, 1971 and Sturrock, 1974), we do not recommend its use in useable water bodies because this compound is highly toxic to fishes (Cardarelli, 1977) and higher concentrations of it may create a serious problem of commulative pollution. But it can be effective in only few specialized habitats such as sewage water collections and swamps.

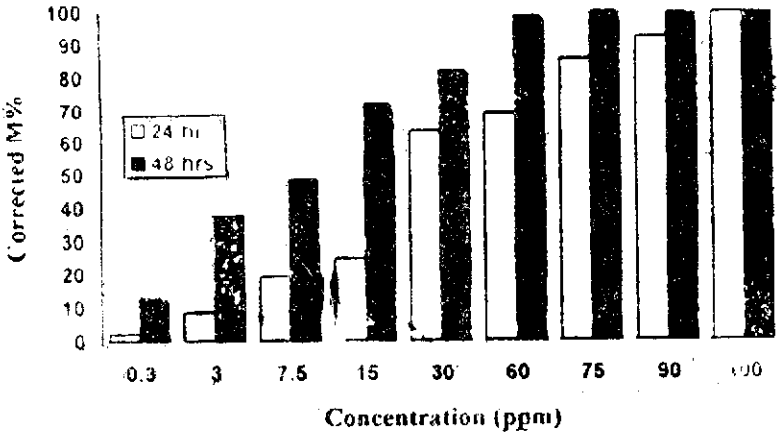


Fig. 1 : Corrected Mortality % among 3*rd* instar *Culex pipiens* larvae treated with the molluscicide Niclosamide

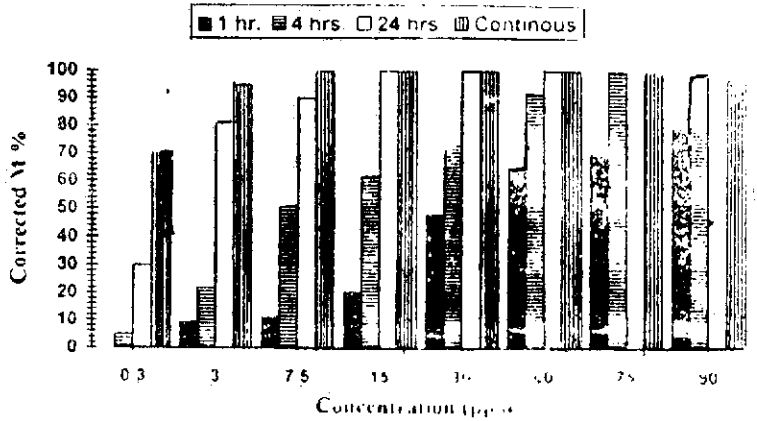


Fig. 2 : Corrected Mortality % among *Culex pipiens* larvae treated for different times with the molluscicide Niclosamide

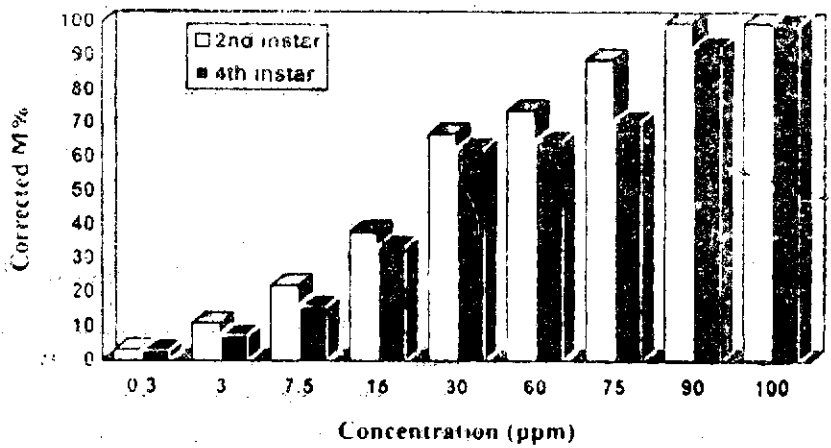


Fig. 3 : Susceptibility of 2*nd* and 4*th* instar larvae of *Culex pipiens* exposed to the molluscicide Niclosamide

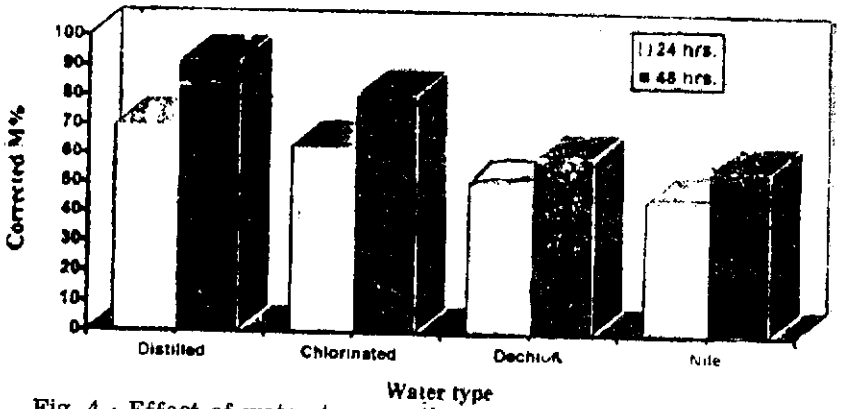


Fig. 4 : Effect of water type on the activity of the molluscicide Niclosamide (30 ppm) against 3*rd* instar *Culex pipiens* larvae.

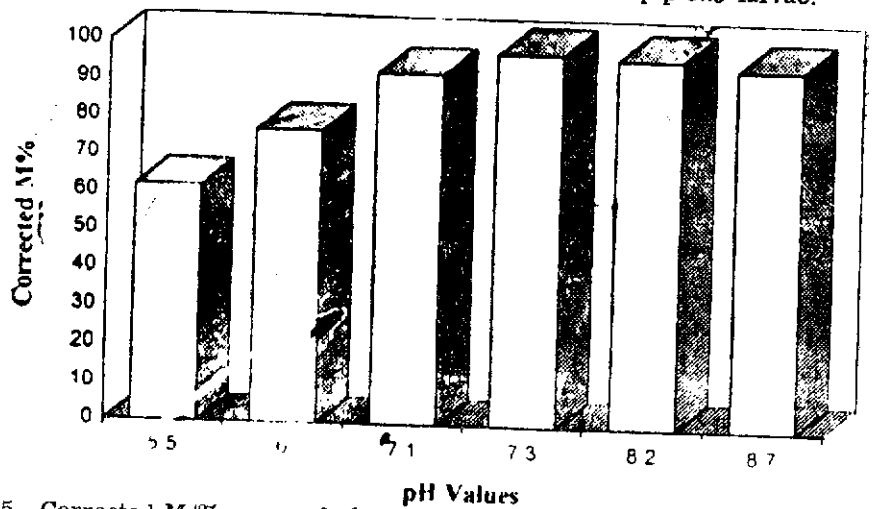


Fig. 5 . Corrected M % among 3*rd* instar *Culex pipiens* larvae treated for 48hrs. with 50 ppm of the molluscicide Niclosamide in water with different pH values.

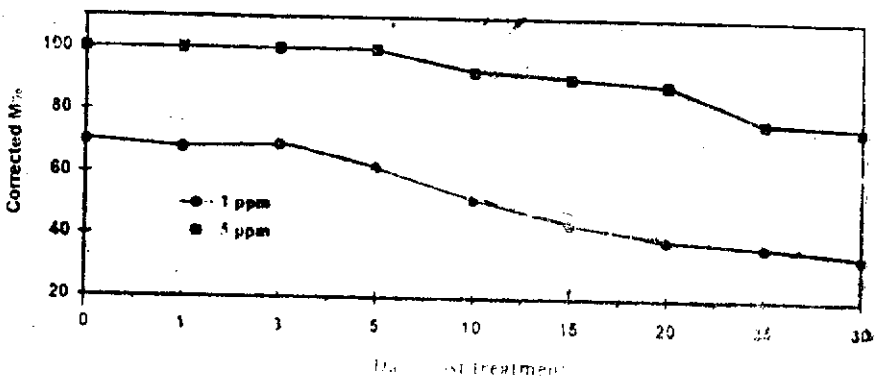


Fig. 6 : Laboratory assays of field water treated with Niclosamide against 3*rd* instar *Culex pipiens* larvae

## SUMMARY

The molluscicide, Niclosamide (2-hydroxyehylammonium salt of 2',5 dichloro -4- nitrosalicylanilide) was prepared and evaluated against *Culex pipiens* larvae under laboratory and simulated field conditions. The concentrations of Niclosamide required for effective control of *Culex pipiens* larvae were much higher than those recommended for snail control. Second larval instar was more susceptible than the 4*th* instar. The highest effectiveness of Niclosamide was observed when using distilled and chlorinater tap water, whereas the lowest activity was found in dechlorinated tap water and Nile water. The activity of the compound was significantly decreased with the increase in water acidity. The possibility of using this molluscicide in anti mosquito work was discussed.


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