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Efficacy of IGR compound Starycide 480 SC (Triflumuron) against mosquito larvae in clear and polluted water

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Background & objectives: An environmental friendly formulation Starycide 480 SC (Triflumuron–OMS-2015), a new insect growth regulator with chitin synthesis inhibitor type mode of action was evaluated against mosquito larvae in laboratory and small-scale field trials carried out in and around Delhi.

Methods: The formulation was tested in laboratory for its bio-efficacy against late III instar mosquito larvae of different species using WHO bioassay procedure. In the field formulation was sprayed at doses of 0.3, 0.5 and 1 ppm (g/m^3) in the natural breeding habitats of *Anopheles* and *Culex* mosquitoes. The impact was assessed by monitoring densities of larvae by dipper and observing the reduction in larval density and inhibition of adult emergence.

Results: In the laboratory, formulation was more effective against larvae of *Anopheles stephensi* and *Aedes aegypti* than *Culex quinquefasciatus*, but it produced 100% inhibition of adult emergence for all mosquito species at a concentration of 0.02 ppm. In the field trials, formulation did not produce 100% reduction in the density of late stage larvae even at 1 ppm (g/m^3), the highest dose tested, but it resulted in 100% inhibition of pupal formation of both *Anopheles* and *Culex* spp in different types of habitats for 3–7 weeks even at a lower dose of 0.5 ppm.

Interpretation & conclusion : Application of triflumuron in the natural breeding habitats in both clean and polluted water @ 0.5 ppm (g/m^3) resulted in complete inhibition of adult emergence of both *Anopheles* and *Culex* spp for 3–7 weeks. This formulation may be tested in large-scale field trials for further use in the vector control programme.

Key words Efficacy – IGR – mosquito larvae – triflumuron

In the control of vector borne diseases, the use of larvicides is one of the vector control options, particularly in urban areas where indoor spraying of insecticides is not feasible and vector control operations mainly relies upon antilarval methods. During the past two decades, considerable progress has been made in the development of natural and synthetic compounds, which are capable of interfering with the process of growth, development and metamorphosis of the target mosquito species, which are known as insect growth regulators (IGRs). Two types of IGRs are available, one which inhibit the growth of larvae due to juvenile hormone like action and known as JH mimics or analogues and the other type of IGR compound which interfere with chitin production leading to moulting disturbances, resulting in death of the insect. IGRs differ widely from the commonly used insecticides as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the

endocrine system. Compared to the conventional larvicides, the IGRs are known to be safer and selective in action[1,2]. Though, a number of IGR compounds have been evaluated against mosquitoes[3–5], only two IGR compounds — methoprene, a juvenoid (JH mimic) and diflubenzuron, an ecdysoid (chitin synthesis inhibitor) are available for use in public health. Therefore, evaluation of more IGR compounds against mosquito vectors is essential in order to provide new tool to vector control programme. A new IGR product, starycide 480 SC, a suspension concentrate formulation, which contains the active ingredient, triflumuron, a chitin synthesis inhibitor, was made available by M/s. Bayer (India) Ltd., Mumbai for its evaluation against insects of public health importance. Triflumuron is safe to mammals and other non-target animals at the doses required against insects.

Anopheles stephensi and *An. culicifacies* (Diptera: Culicidae) are the established vectors of malaria in India and have wide geographic distribution in the Indian subcontinent. These species breed in natural and man-made clear water habitats. *Culex quinquefasciatus*, which breeds in polluted water habitats, is the vector of lymphatic filariasis in several parts of India. In view of this a small-scale trial was carried out to evaluate triflumuron (IGR) against larvae of *Anopheles* and *Culex* spp in their natural larval habitats and results of study are reported in this paper.

Material & Methods

Starycide 480 SC, (Triflumuron-OMS-2015) a suspension concentrate formulation of the IGR compound, supplied by M/s. Bayer (India) Ltd., Mumbai was used in this study.

Laboratory evaluation: Laboratory evaluation was carried out against larvae of laboratory colonised strains of *An. stephensi*, *Cx. quinquefasciatus* and *Aedes aegypti* as per WHO procedure[6]. The tests were performed in 500 ml glass beaker, in 250 ml distilled water to assess the efficacy (growth inhibiting activity) of triflumuron on late III instar larvae. A total of 25 larvae were placed in each beaker for each concentration and four replicates were used for each concentration and control. Thus a total of 100 larvae were exposed to each concentration.

Third instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were obtained from mosquito colony being maintained at the Malaria Research Centre. A stock solution was prepared and 0.02, 0.004, 0.0008, 0.00016, 0.000032, 0.0000064 ppm doses were used in bioassays against three vector species. All larvae were exposed till pupation and emergence of adult mosquitoes. During this period larvae were provided with larval food and mortality recorded at 24 h interval. Dead larvae, pupae or partly emerged adults were regularly removed and counted. Live pupae were observed till emergence. Percent inhibition of adult emergence was calculated in laboratory against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and EC_{50} and EC_{90} values (effective concentration required for 50 and 90% emergence inhibition) were calculated.

Field evaluation: The trial was carried out in rice fields, pools and cemented tanks in locality Jagdishpur under the community health centre (CHC), Badkhalsa of District Sonepat, Haryana state against anopheline breeding, mainly *An. culicifacies* and around the areas of Delhi in stagnated water near locality of Mukundpur in north Delhi against *Cx. quinquefasciatus* breeding. For field trials breeding sites were selected which were unlikely to be used for drinking water by animals.

Triflumuron (starycide 480 SC) was tested at three application doses of 0.25, 0.5 and 1 ppm (g a.i./m^3) in pools, paddy-fields, drains and cemented tanks. Application doses were achieved by spraying of precalculated amount of triflumuron with the help of a hand compression sprayer. Prior to spraying, density of immatures was estimated by dipper sampling method using a standard dipper of 9 cm dia with 300 ml

water capacity. Density per dip (larvae and pupae) was monitored in control and treated habitats daily at 24 h intervals up to three days and later at weekly intervals. Each day samplings of late instars and pupae were also collected and percentage of adult emerged from the treated field habitats was recorded in laboratory. The pH and temperature of water was also recorded. The data obtained at different days of observation were pooled together to get weekly means and the data collected in different periods and replicates were pooled together. The percent reduction in larval and pupal density was calculated by using Mulla *et al*[7] formula given below:

$$\% \text{ reduction} = 100 - \{(C_1 \times T_2)/(C_2 \times T_1)\} \times 100$$

Where, C_1 = Pre-treatment immature density in control sites; C_2 = Post-treatment immature density in control sites; T_1 = Pre-treatment immature density in treated sites; and T_2 = Post-treatment immature density in treated sites.

Efficacy and residual activity of the larvicide were determined from the post-treatment counts of larvae and pupae in treated and control sites as compared to the pre-treatment populations. Emergence inhibition (EI) was calculated using the following formula:

$$\% \text{ inhibition of adult emergence} = 100 - \frac{\text{No. of pupae emerged into adults}}{\text{Total no. of pupae}} \times 100$$

Results

Laboratory evaluation: Efficacy of triflumuron against late III instar larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti* under laboratory conditions is given in Table 1. At a dose of 0.02 ppm, triflumuron produced 100% mortality against larvae of all the three mosquito species. The EC_{50} against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* were 0.0001, 0.0002 and 0.0003 ppm respectively and EC_{90} were 0.0024, 0.0026 and 0.0102 ppm respectively. These results show higher efficacy of triflumuron against larvae of *An. stephensi* and *Ae. aegypti* than *Cx. quinquefasciatus*.

Field evaluation: The efficacy of triflumuron against *Anopheles* spp was evaluated in pools and paddy-fields. A total of six pools and six paddy-fields were treated with different doses of triflumuron and two

Table 1. Efficacy of triflumuron against mosquito larvae (III instar) in laboratory

Dose (ppm)	% mortality (Inhibition of adult emergence)		
	Ae. aegypti	*Cx. quinquefasciatus*	*An. stephensi*
0.02	100	100	100
0.004	100	88	88
0.0008	68	48	92
0.00016	36	36	44
0.000032	26	28	32
0.0000064	–	16	16
EC_{50} (95% CL)	0.0002 (0.00013–0.00034)	0.0003 (0.00023–0.00059)	0.0001 (0.00011–0.00025)
EC_{90}	0.0026	0.0102	0.0024

pools, and two paddy-fields were left untreated as control for comparison. *An. culicifacies* and *An. subpictus* were the predominant anopheline species found in these habitats. Table 2 shows the density per dip (larvae and pupae) of immatures of *An. culicifacies* and *An. subpictus*; and percent reduction in treated habitats before and after treatment with IGR compound at three doses. At 0.25 ppm (g/m^3)application rate no reduction in density of late instars larvae in pools up to one week post-application period was noted, however, between second and fifth week of post-application, the reduction was ranging between 28 and 55%. In pools treated at 0.5 ppm, the reduction in late instar larval density of anopheline species started on third day but it remained only between 45 and 62% from first to third week period and thereafter no reduction was noticed. At the dose of 1 ppm, the reduction in larval density of late instars ranged from 45 to 94.5% up to fifth week. However, reduction in pupal density was high at all the three doses and reached up to 100% between first to fourth week. In paddy-fields, the effect was slightly better than in pools as the reduction of late instars ranged between 58 and 85% between third day and fifth week. Pupal reduction was 100% from third day up to fifth week at 1 ppm and from first week onwards at 0.25 and 0.5 ppm application rates.

The percent inhibition of adult emergence (EI) of *An. culicifacies* and *An. subpictus* in pools after the application is presented in Fig.1. Inhibition of adult mosquitoes was 80% in the samples collected one week post-application at a dose of 0.25 ppm, but 100% inhibition of emergence remained up to five weeks at the dose of 1 ppm. In paddy-field, 100 percent inhibition of adult emergence was obtained at all the three

Table 2. Field evaluation of triflumuron against immatures of *Anopheles* spp in pools and paddy-fields

Duration after treatment	Mean no. of *An. culicifacies* and *An. subpictus* immature per 10 dips											
	Pools						Paddy-fields					
	0.25 ppm		0.5 ppm		1 ppm		0.25 ppm		0.5 ppm		1 ppm	
	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P
Day 0	4.6	2	1.2	0.6	2	1	7.2	4.1	9.1	1.2	7	1.1
Day 3	4.2 (0)	0.8 (0)	0.6 (45.2)	0.2 (58.33)	1 (45)	0.2 (75)	6.1 (26.17)	2 (51.2)	3 (71.27)	0.1 (91.66)	0.8 (90)	0 (100)
1 week	4 (0)	0.4 (80)	0.4 (61.66)	0 (100)	0.2 (88.21)	0 (100)	6 (2.24)	0 (100)	1.1 (85.81)	0 (100)	0.6 (89.94)	0 (100)
2 weeks	2 (55.16)	0.2 (90)	0.2 (61.66)	0 (100)	0.2 (89.68)	0 (100)	4 (17.34)	0 (100)	1 (83.65)	0 (100)	0 (100)	0 (100)
3 weeks	2 (28.26)	0 (100)	0.2 (61.66)	0 (100)	0.1 (91.75)	0 (100)	4 (0)	0.1 (87.8)	2 (58.1)	0 (100)	0.1 (97.27)	0 (100)
4 weeks	1 (55.16)	0 (100)	0.8 (0)	0.1 (72.2)	0.2 (79.3)	0 (100)	3 (0)	0.1 (87.8)	1.2 (63.43)	0.1 (58.33)	0.1 (96)	0.1 (54.54)
5 weeks	3 (28.26)	1 (50)	1 (8.33)	0.8 (0)	0.1 (94.5)	0 (100)	1.2 (66.1)	0 (100)	1.6 (64.24)	0.4 (16.16)	0.6 (82.57)	0.4 (90.9)
6 weeks	–	–	–	–	–	–	1.1 (6.8)	0.1 (75.6)	1.3 (12.8)	0.4 (0)	0.8 (30.28)	0.1 (9.09)

Figures in parentheses indicate percent reduction based on control; III + IV — Larvae; P — Pupae.

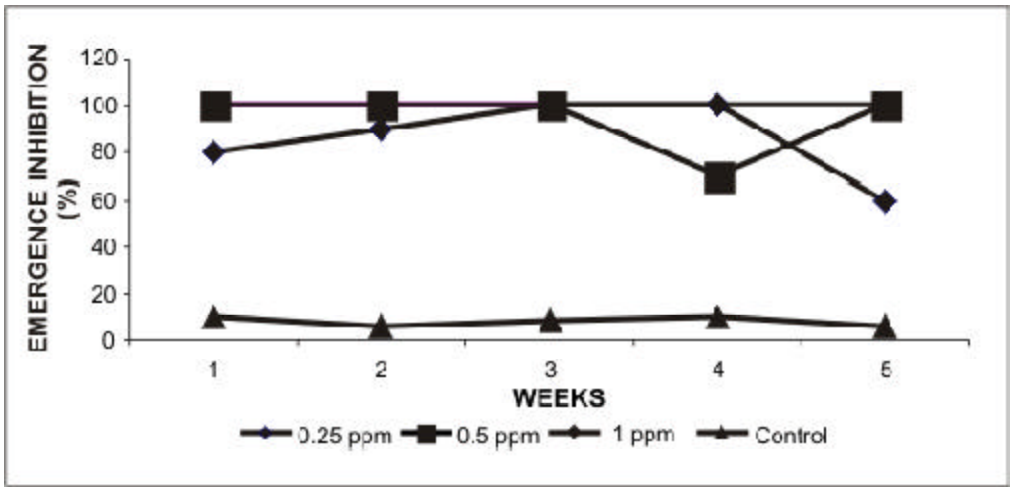

Fig. 1: Percentage emergence inhibition (EI) *An. culicifacies* and *An. subpictus* in pools

doses up to three weeks, but at 1 ppm, cent percent inhibition of adult emergence was obtained up to fourth week (Fig. 2).

The efficacy of triflumuron against *Culex* spp in field conditions was evaluated at two doses of 0.5 and 1 ppm in three different breeding habitats—waste-water pools, drains and cement tanks. Table 3 shows the average density and percent reduction of late instar larvae and pupae in three different habitats. In drains, the density of late larval instars showed decline trend from Day 3 at both the test doses. At 0.5 ppm, the reduction of late instar larvae ranged between 55 and 94% up to sixth week and then declined to 29.3% in seventh week. The pupal reduction was 100% up to fifth week and 93.3% in sixth week. At the dose of 1 ppm, 100% pupal reduction remained up to sixth week. In pools, the reduction of late instar ranged from 61.7 to 95% up to seventh week at both the doses. In tanks the effect of IGR was slightly better than pools, where 100% reduction of late instars was observed after three weeks at 0.5 ppm and two weeks at 1 ppm . However, reduction in pupal density was high at both the doses and reached up to 100% between third day to sixth week in different habitats.

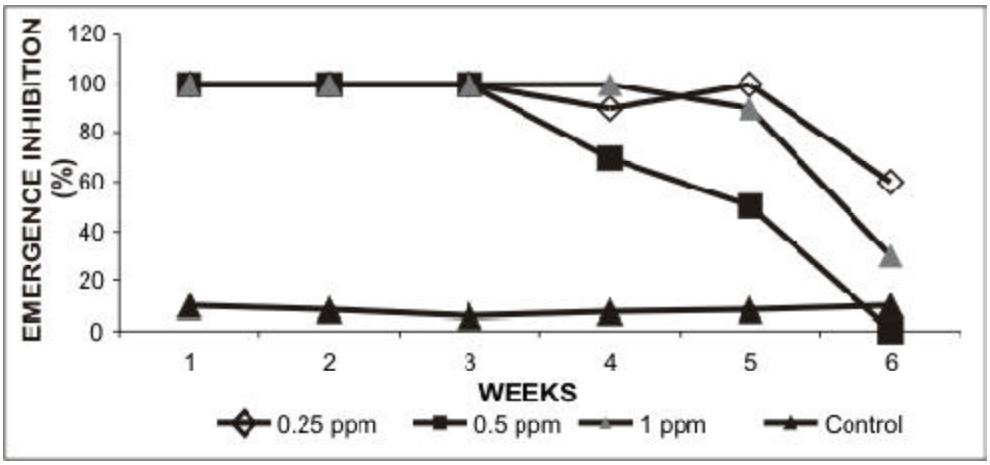

Fig. 2: Percentage emergence inhibition (EI) *An. culicifacies* and *An. subpictus* in paddy field

Table 3. Field evaluation of triflumuron against immatures of *Cx. quinquefasciatus*

Duration after treatment	Mean no. of *Cx. quinquefasciatus* immatures per 10 dips											
	Drains				Pools				Tanks			
	0.5 ppm		1 ppm		0.5 ppm		1 ppm		0.5 ppm		1 ppm	
	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P	III+IV	P
Day 0	37	1.5	50	5	12	3	7	1.2	60 (79.76)	1.3 (98.7)	19.3	4
Day 3	12 (28.21)	0 (100)	19.1 (15.45)	1 (0)	6 (61.7)	1 (63.3)	2 (78.11)	0.2 (81.66)	85 (75.63)	1.3 (98.96)	9 (60.67)	1 (87.5)
1 week	10 (55.13)	0 (100)	0 (100)	0 (100)	3 (90.5)	0.2 (97.5)	4.4 (76.18)	0 (100)	65 (94.02)	1 (98)	2.3 (91.45)	0.3 (97)
2 weeks	6.6 (72.06)	0 (100)	8 (79.94)	0 (100)	3 (91)	0.2 (97)	1 (94.85)	0 (100)	22 (96.77)	0 (100)	0 (100)	0 (100)
3 weeks	2 (86.4)	0 (100)	12 (39.6)	0 (100)	2 (89)	0 (100)	2 (81.46)	0.2 (91.26)	0 (100)	0 (100)	0 (100)	0 (100)
4 weeks	1 (94)	0 (100)	8 (64.5)	0 (100)	1 (94.05)	0 (100)	3 (69.44)	0.2 (91.05)	3.3 (99.25)	0 (100)	0.3 (98.87)	0 (100)
5 weeks	3 (73.34)	0 (100)	12 (21.1)	1 (71.4)	2 (92)	0.1 (98.77)	4 (72.7)	0.1 (96.94)	1.3 (99.25)	0 (100)	1 (97.08)	0 (100))
6 weeks	3 (70.7)	0.1 (93.3)	2 (85.56)	0 (100)	3.3 (80.58)	0.2 (95.5)	3 (69.74)	0.2 (88.88)	1 (98.98)	0.3 (99.7)	0.6 (95.6)	0 (100)
7 weeks	6.3 (29.33)	0.4 (20)	6 (50.2)	1 (40)	4.5 (77.8)	6.3 (75.5)	2.2 (81.45)	0.1 (93.88)	250	50	1 (86.89)	0.3 (96.5)

Figures in parentheses indicate percent reduction based on control; III + IV — Larvae; P — Pupae.

Figs. 3, 4 and 5 show the percentage of emergence inhibition (EI) of *Cx. quinquefasciatus* in drains, pools and tanks respectively. In drains at both the doses of 0.5 and 1 ppm 100% EI was observed up to sixth week period, while in pools 100% EI was observed up to seventh week.

Discussion

IGR compounds such as methoprene, diflubenzuron, pyriproxyfen and triflumuron have been recommended by WHO and diflubenzuron has already passed WHOPES for use against mosquito immature[8]. However, none of these compounds is in use for vector control in India. In general, IGR compounds do not produce immediate mortality among the larvae at the recommended doses and are, therefore, difficult to assess in most situations. Sharma *et al*[9] however, showed effective control (80–100%) of *Culex pipiens fatigans* breeding in polluted drains by using dimilin (Diflubenzuron) at doses of 0.5 to 1 ppm. The residual effect of this larvicide in field application was, however, approximately for four days. The results of present study show the delayed efficacy of triflumuron against breeding of *An. culicifacies*, *An. subpictus* and *Cx. quinquefasciatus* in all habitats tested. The occurrence of delayed mortality in larvae indicated the effective developmental inhibition potential of this IGR compound. About 90 to 100% EI against malaria vector *An. culicifacies* was observed in pools and

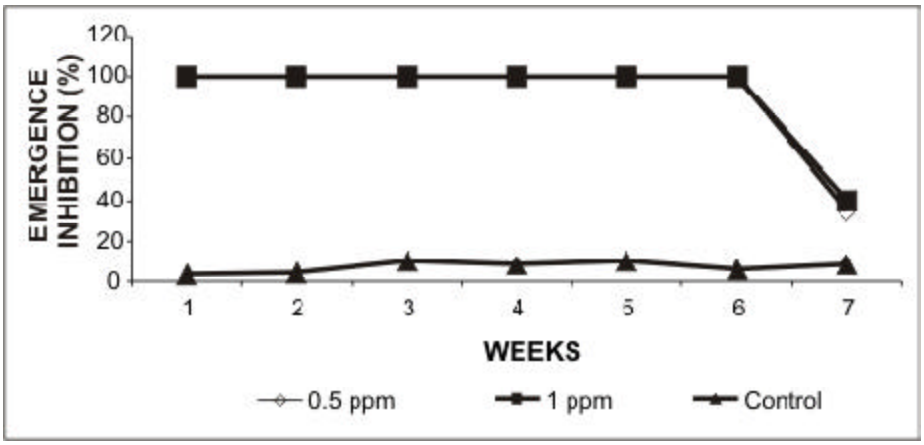

Fig. 3: Percentage emergence inhibition (EI) *of Cx. quinquefasciatus* in drains

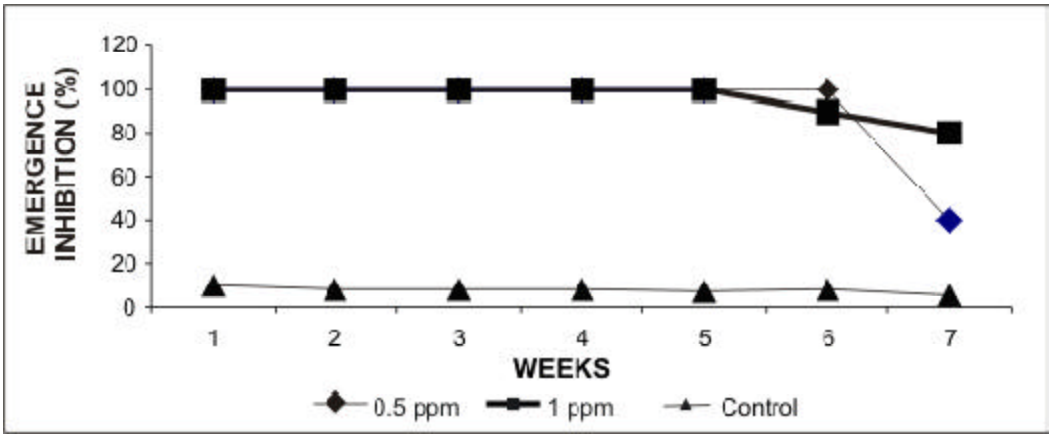

Fig. 4: Percentage emergence inhibition (EI) *of Cx. quinquefasciatus* in pools

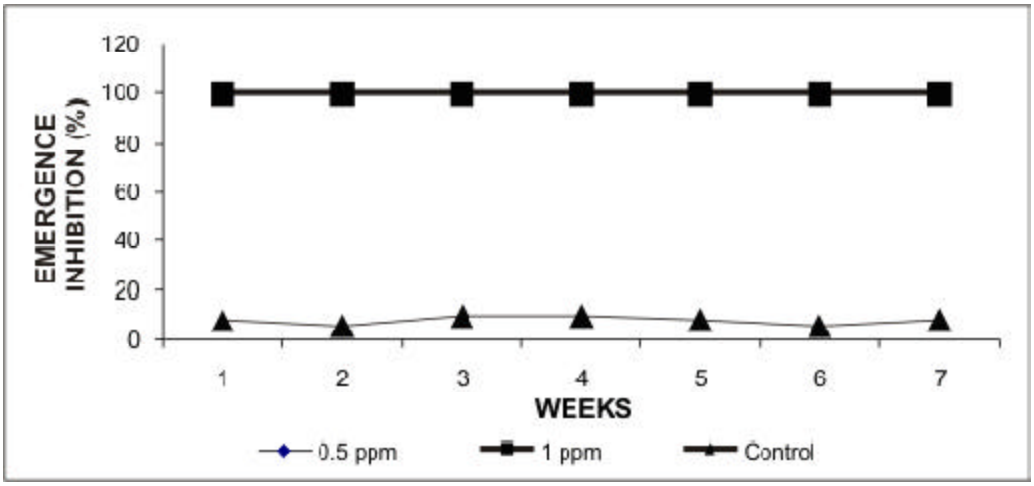

Fig. 3: Percentage emergence inhibition (EI) of *Cx. quinquefasciatus* in tanks

paddy-fields for five weeks post-application when treated @ 1 ppm and therefore, triflumuron can be applied at monthly intervals. As EI was 100% for six weeks in drains against *Cx. quinquefasciatus* @ 1 ppm and also seven weeks in cemented tanks @ 0.5 ppm, IGR could be applied ones in six weeks at the rate of 1 ppm in drains and 0.5 ppm in tanks.

In an earlier study, in cesspits, triflumuron was, however, reported to be effective only for one week against *Cx. quinquefasciatus* larvae[10]. The results of our study clearly show that triflumuron can produce longer duration of control of mosquito larvae in polluted water, where conventional larvicides are effective only for a shorter duration. As the IGR has relatively longer residual effect, the frequency of application would be lesser compared to other larvicides. Therefore, the operational cost can be reduced and IGR could be used as one of the additional tool in the National Vector Borne Disease Control Programme.

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