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Efficacy of three formulations of diflubenzuron, an insect growth regulator, against *Culex quinquefasciatus* Say*,* the vector of Bancroftian filariasis in India

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Background & objectives**: Insect growth regulators (IGRs) offer alternatives to conventional chemical larvicides that pose problem of resistance and environmental safety. However, only a limited number of IGRs have been approved for use in mosquito control. In the present study, two new formulations of the IGR diflubenzuron, 2 per cent granular (GR) and 2 per cent tablet (DT) were tested for its efficacy against *Culex quinquefasciatus*, in comparison to its 25 per cent wettable powder (WP) formulation.**

Methods**: The WP, GR and DT formulations were tested in cesspits, street drains and abandoned wells each at four dosages, 25, 50, 75 and 100 g ai/ha. Additionally, the DT formulation was tested at a higher dosage of 1 tablet/m^2 (equal to 400 g ai/ha).**

Results**: The WP and GR formulations yielded >80 per cent inhibition of adult emergence (IE) for 7-10 days in cesspits, 4-7 days in street drains and 7-21 days in abandoned wells at all dosages tested. The DT formulation was effective only at higher dosage 100 g ai/ha and or 1 tablet/m^2 for 7-15 days at all habitats.**

Interpretation & conclusions**: The trial showed that the dosage 25 g ai/ha of 25 per cent WP could be the field dosage for cesspits and wells, and 50 g ai/ha for drains, to be applied at weekly intervals. The dosages 25, 50 and 100 g ai/ha of 2 per cent GR could be the field dosages for application in cesspits at weekly intervals, in abandoned wells every three weeks and in drains at weekly intervals, respectively. Diflubenzuron 25 per cent WP and 2 per cent GR could be used for larval control of *Cx. quinquefasciatus* under integrated vector management programme.**

Key words *Culex quinquefasciatus* - diflubenzuron - insect growth regulator - mosquito control

Insect growth regulators (IGRs) and microbial insecticides are intrinsically non-toxic, biologically specific, and environmentally safe compared to conventional chemical larvicides[1-3]. However, only a limited number of IGRs such as juvenoid methoprene, chitin synthesis inhibitors such as diflubenzuron and triflumuron have been approved for use in mosquito control by WHO Pesticide Evaluation Scheme (WHOPES)[3]. Among these, resistance to methoprene has already been reported in some vector mosquito species[4-6]. Therefore, there is a need to identify and evaluate newer IGR compounds/formulations to be used in vector control programmes.

Diflubenzuron is an IGR of the benzoyl urea family that acts by disturbing chitin synthesis and deposition. Diflubenzuron 25 per cent active ingredient (ai) wettable powder (WP) formulation has been evaluated against mosquito vectors in different ecological settings since 1975[7-16]. Now, 2 per cent granular (GR) and 2 per cent tablet (DT) formulations of diflubenzuron have been developed and made available through WHOPES for field-testing in different ecological settings. Here we present the results of a small-scale field trial carried out to determine the efficacy, residual activity and optimum field application dosages of two newer formulations (2% GR and 2% DT) of the IGR diflubenzuron in comparison to its 25 per cent WP formulation against *Culex quinquefasciatus* Say, the vector of Bancroftian filariasis in India are presented.

Material & Methods

Test materials: Diflubenzuron an acylurea compound (N-[[(4-chlorophenyl) amino] carbonyl]–2, 6-difluorobenzamide) acts as a chitin synthesis inhibitor and is also known as Dimilin®. Three formulations of Dimilin, 25 per cent WP, 2 per cent GR and 2 per cent DT were supplied by WHOPES/WHO, Geneva, Switzerland (manufactured by Crompton Chemical Co., Italy) for field evaluation against *Cx. quinquefasciatus*.

Study area: The trial was carried out in Cuddalore, a town in Tamil Nadu State on the Coromandal coast of peninsular India, which has a population of about 0.6 million. The area has been endemic for lymphatic filariasis transmitted by the vector, *Cx. quinquefasciatus.* Transmission occurs throughout the year[17]. Cesspits, street drains and abandoned wells are the major larval habitats of the vector. Wells were used for domestic water supply in the past and most of these were closed as piped water supply has been provided. However, a few public wells have been left unused and these wells are polluted with floating debris and garbage[18]. The trials with diflubenzuron 25 per cent WP, 2 per cent GR and 2 per cent DT were carried out in *Cx. quinquefasciatus* breeding habitats in the study area following the sampling procedures of WHO Pesticide Evaluation Scheme (WHOPES)[19] from August 2004 to September 2005.

Study design (small scale trial for dosage determination): The WP, GR and DT formulations were tested in cesspits, street drains, and abandoned wells each at four application rates: 25, 50, 75 and 100 g ai/ha. The DT formulation contains 40 mg ai of diflubenzuron per tablet and manufacture's recommended application rate was 40 mg/40-100 liters or 1 tablet/m^2. The minimum surface area of the selected larval habitats was about 1 m^2, holding approximately 100 liters of water. Therefore, additionally, the DT formulation was tested at one more application rate, 1 tablet/m^2 (approximately 400 g ai/ha). A total of 80 cesspits were selected, of which 65 were treated, five at each application rate of WP (four dosages), GR (four dosages) and DT (five dosages) formulations and the remaining 15 were left untreated for comparison. The size of the cesspits ranged from 1.0 to 3 m diameter, with a surface area ranging from 1.0 to 7.1 m^2. Drains (cement lined, U-shaped) that were choked with silt and debris and had no network connection were selected. A total of 16 drains were selected, of which 13 were treated (four each for WP and GR formulations and five for DT formulations) and the remaining three were kept as untreated controls. Depending on the length, these drains were divided into sectors of 10 m length and each sector was considered as a replicate and a minimum of five replicates from each drain was treated for each dosage. For treatments, however, the IGR was applied uniformly to the entire stretch of the drain. Separate drains were selected as controls. A total of 62 abondoned wells were selected, of which 50 were treated (14 for WP, 15 for GR and 21 for DT formulations) and 12 were kept untreated as controls wells. The approximate depth of water in cesspits and street drains was 10 cm and the volume of water was 100 liters/m^2 The volume of water in different wells ranged from 1700 to 5000 liters as these were deeper habitats without any replenishment of water.

Sampling was done using dippers (300 ml capacity) in cesspits and drains or buckets (3-liter capacity) in abandoned wells and larval and pupal counts were made twice a week for 1-2 wk prior to application of IGR. Three dips were taken from each replicate and the larvae dipped were counted according to instars. Adult mosquitoes emerging from these habitats were collected using emergence traps. Traps made of 15 cm cubical metal frames covered with mosquito netting set over a thermocol base were used in cesspits and drains. In wells, emergence trap made of 30 cm cubical metal frames with a 21 cm high pyramidal of metal frames, covered with mosquito netting was used. A sleeve on one side of the cage enabled mosquitoes to be removed using a mechanical aspirator. For each replicate, one trap was used and a minimum of five replicates of each type of habitat were maintained for each dosage. The traps were set afloat on the surface of the water in the evening and the adults trapped were collected the next day morning and counted. For each formulation, habitats of each type were made into five groups with comparable pre-treatment adult emergence counts and pupal densities (statistically tested). The groups were assigned randomly to four treatments/dosages and one control. Prior to treatment, the surface area of each larval habitat was measured. The required dosage for a particular habitat was calculated based on surface area of the selected habitat. To the treatment groups, the required quantity of the WP formulation of diflubenzuron was applied after suitably diluting in water using a hand compression sprayer (2-liter capacity) with a jet nozzle (Foggers India Pvt. Ltd.). The GR formulation was evenly distributed over each habitat manually. The DT formulation was dropped in the larval habitats at uniform distance on to the surface area of the habitats. After treatment, larvae and pupae and emerging adults were sampled at 24 h and subsequently every 2-3 days in the treated and untreated habitats. Sampling was continued until the density of larvae and pupae or adult emergence in the treated habitats reached a level comparable to that in untreated habitats. The *p*H and temperature of the habitat water were recorded. Dissolved oxygen, dissolved and total solids in habitat waters were also determined at two points of time during the trial period.

Data analysis: The mean number of pupae and larvae collected per dip and the mean number of adults emerged per trap were calculated for each sampling site. The number of 1st and 2nd larval instars was grouped into "early instars" and that of 3rd and 4th instars were grouped into "late instars." The reduction of pupal, late and early instar larval densities and inhibition of adult emergence (IE) on post-treatment days was estimated by comparing the pre- and post-treatment densities/emergence in the treated habitats with the corresponding densities/emergence in the untreated habitats using Mulla's formula[20]. The per cent reduction of pupal and larval density or %IE = 100 – (C1/T1) x (T2 x C2) x 100. Where C1 is the number of adults emerged in control habitats before treatment, C2 the number of adults emerged in control habitats after treatment period, T1 the number of adults emerged in treated habitats before treatment and T2 the number of adults emerged in treated habitats after treatment. The differences between the dosages or formulations were compared by two-way analysis of variance with dosage and day as the main factors after transforming percentage reduction/emergence inhibition to arcsine values[21]. The interaction effect of dosage and day was used to compare the effect of treatment over days. Pair-wise comparison of dosages was done using the pos-hoc test based on least significant difference (LSD). The optimum field application dosage was determined based on the effective duration of control by each dosage. The minimum dosages at which maximum effect (immediate as well as residual) was achieved, was selected as the optimum field application dosage for each type of habitat. The SPSS software, version 19.0, USA was used for data analysis.

Results

Impact of diflubenzuron formulations on inhibition of adult emergence (IE)

Cesspits: In cesspits, both 25 per cent WP and 2 per cent GR formulations were equally effective, yielding 80-100 per cent IE for 1 wk (7-10 days) post-treatment at all application rates (25, 50, 75 and 100 g ai/ha). On day 14 post-treatment, the activity declined and the IE was 36-75 per cent (Figs 1a and 2a). In both WP and GR formulations, the percentage of IE did not differ significantly between the dosages over time (Interaction effect: dosage x day; Table I).

The DT formulation did not show good IE activity at 25-75 g ai/ha. Only at 100 g ai/ha and 1 tablet/m^2, the formulation yielded 86-100 per cent IE for 10 days (Fig. 3a) after treatment. The percentage of IE varied significantly (P<0.05) between the dosages of DT formulation, but interaction effect of dosage and day did not differ significantly (Table I). Pair-wise comparison showed that the higher dosages, 1 tablet/m^2 and 100 g ai/ha produced significantly greater effect than the lower dosages (P< 0.05), while there was no significant difference between these two higher dosages.

Street drains: In street drains, the 25 per cent WP formulation yielded >80 per cent IE only on days 4-7 post-treatment at 25 g ai/ha. At higher application rates of 50, 75 and 100 g ai/ha, the IE was >80-100 per cent up to day 7 post-treatment and reached <50 per cent level on day 14 post-treatment (Fig. 1b). The overall percentage of IE differed significantly between the dosages (P<0.05), but interaction effect of dosage and day did not differ significantly (Table I). The higher dosages 50, 75 and 100 g ai/ha produced significantly greater effect than the lower dosage, 25 g ai/ha (P<0.05).

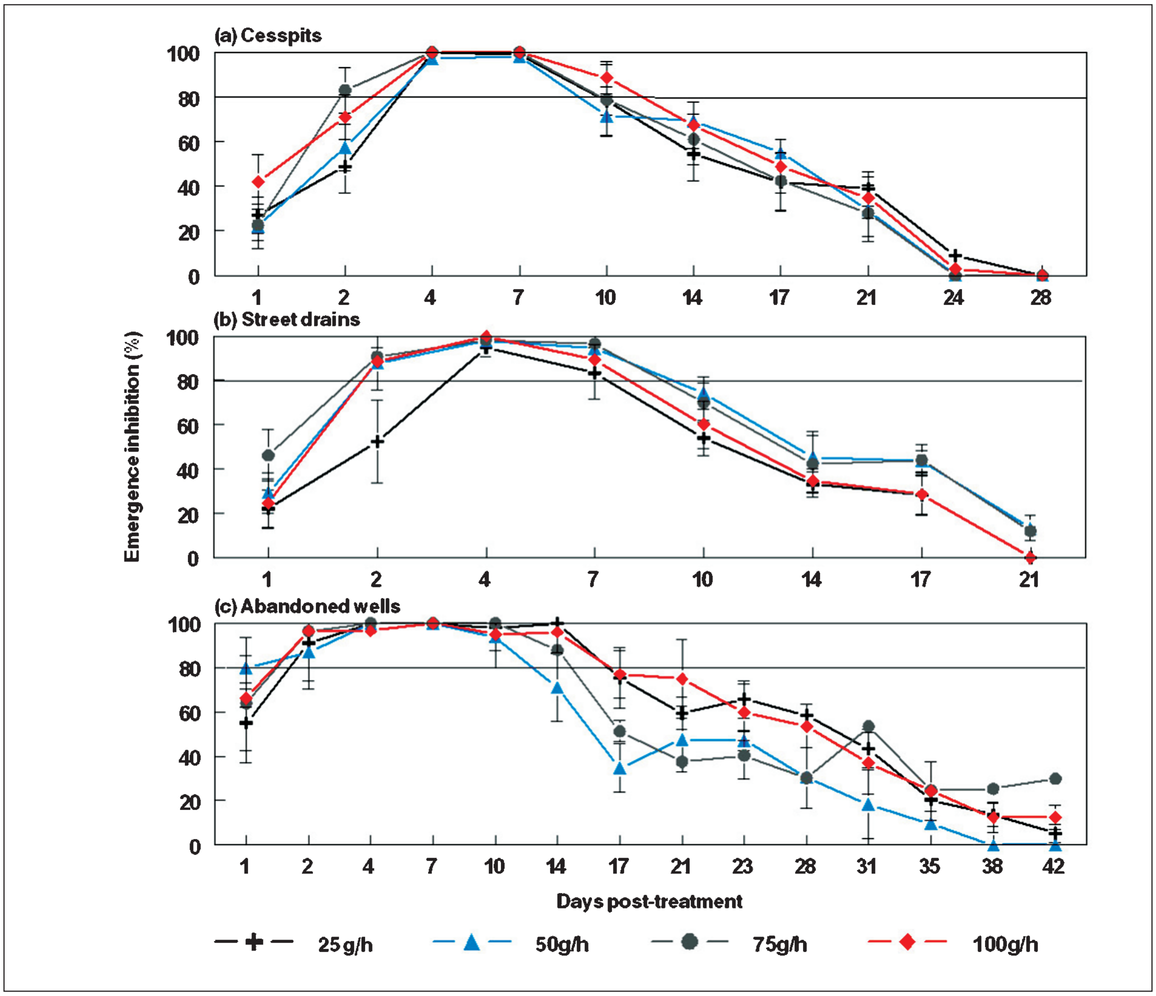

Fig. 1. Per cent emergence inhibition (mean ± SE) of *Culex quinquefasciatus* adults in cesspits **(a)**, street drains **(b)** and abandoned wells **(c)** treated with diflubenzuron 25% WP formulation.

There was, however, no significant difference between the effects at 50, 75 and 100 g ai/ha.

In this habitat, the 2 per cent GR formulation was not effective at 25, 50 and 75 g ai/ha. At these application rates, >80 per cent IE was observed only on day 4 post-treatment and 55-80 per cent on days 7-10 post-treatment. Only at 100 g ai/ha, >80 per cent IE was recorded up to day 7 post-treatment, which declined to <50 per cent level on day 14 post-treatment (Fig. 2b). The per cent IE varied significantly between dosages (P<0.05), but interaction effect of dosage and day did not differ significantly (Table I). The IE activity was significantly (P<0.05) greater at 100 g ai/ha than at the lower dosages.

The 2 per cent DT formulation did not give effective control at the dosages of 25 to 100 g ai/ha. At a higher dosage of 1 tablet/m^2, this formulation produced >90 per cent IE on days 4-14 post-treatment and the IE was <50 per cent after third week post-treatment (Fig. 3b). Overall as well as over time the percentage of IE differed significantly between the dosages (P<0.05; Table I). The dosage, 1 tablet/m^2 gave significantly greater reduction than the lower dosages tested (P<0.05).

Abandoned wells: In abandoned wells, the 25 per cent WP formulation was active for more than a week (10-14 days) post-treatment, yielding >80-100 per cent IE at all dosages tested. On day 21 post-treatment, the IE was in the range of 38-74 per cent at all dosages (Fig. 1c). The percentage of IE did not vary significantly between the dosages and over time (Table I). In this habitat, the 2 per cent GR formulation showed >80 per cent IE up to day 7 post-treatment at 25 g ai/ha, 56 per cent up to day 14 and <50 per cent on day 21 post-treatment. However, at 50, 75 and 100 g ai/ha, >80-100

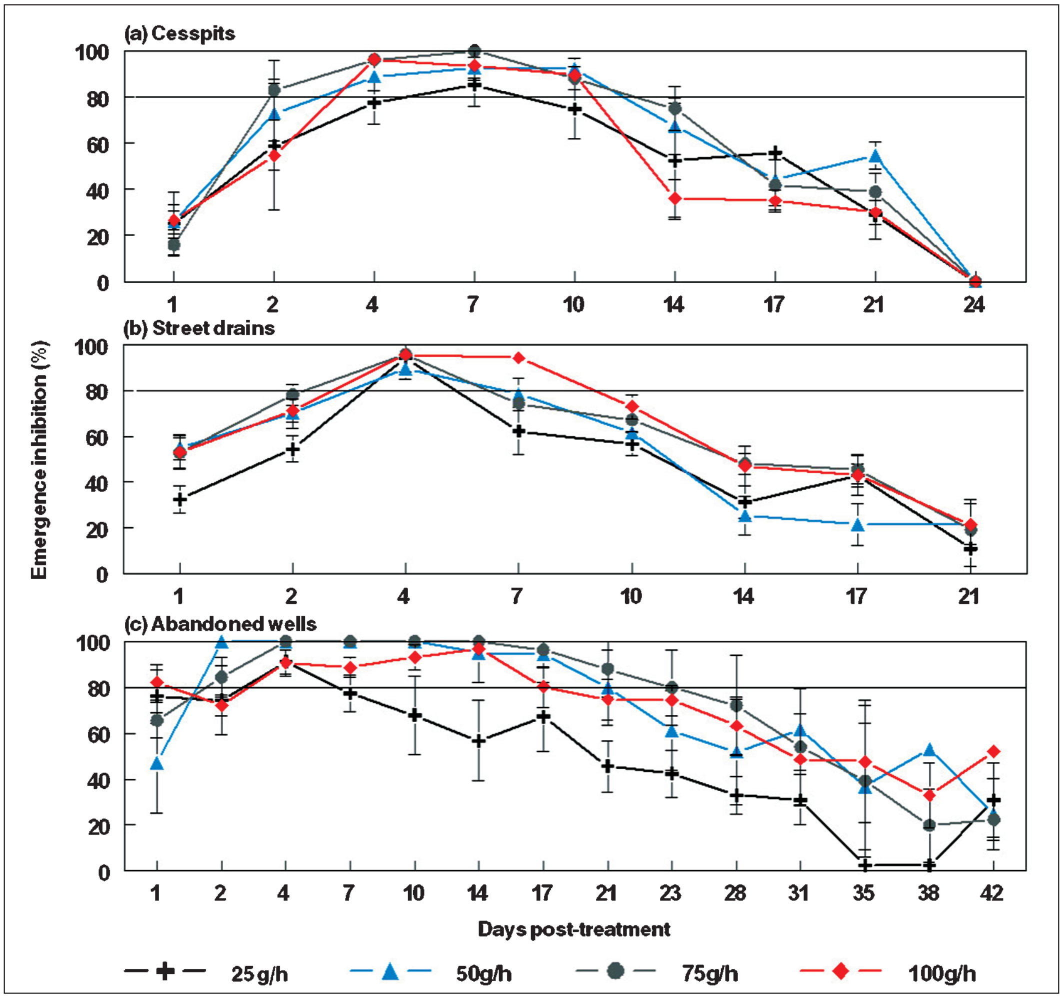

Fig. 2. Per cent emergence inhibition (mean ± SE) of *Culex quinquefasciatus* adults in cesspits **(a)**, street drains **(b)** and abandoned wells **(c)** treated with diflubenzuron 2% GR formulation.

per cent IE was recorded for about three weeks, which declined to <50 per cent on day 35 post-treatment (Fig. 2c). The overall percentage of IE differed significantly between dosages (P<0.05), but interaction effect of dosage and day did not differ significantly (Table I). The percentage of IE at 50, 75 and 100 g ai/ha was similar, but was significantly greater than at 25 g ai/ha (P<0.05). Application of 2 per cent DT formulation in this habitat did not yield >80 per cent IE at 25, 50 and 75 g ai/ha. At 100 g ai/ha, the IE was in the range of 60-80 per cent for 7 days post-treatment and at 1 tablet/m^2 >80 per cent up to day 7 post-treatment (Fig. 3c). The percentage of IE did not differ significantly between the dosages over time (Table I).

Impact of diflubenzuron formulations on pupal and larval density: In cesspits treated with WP, GR and DT formulations, the reduction of pupal density fluctuated between 10 and 100 per cent during post-treatment period (Table II), the maximum reduction (>80%) was observed up to 7 days post-treatment at all dosages of WP and GR formulations. The same level of reduction was observed with DT formulation only at 100 g ai/ha and 1 tablet/m^2.

In street drains treated with WP, GR and DT formulations, the reduction of pupal density fluctuated between 8 and 100 per cent during post-treatment period at all dosages tested (Table II). While WP formulation yielded >80 per cent reduction of pupal density for 7 days post-treatment at 50, 75 and100 g ai/ha, the GR formulation caused only 43-76 per cent reduction of pupal density during post-treatment period at all application rates. The DT formulation yielded >80 per cent reduction of pupal density for a week only at 1 tablet/m^2 (Table II). In abandoned wells treated with

Table I. Results of two-way ANOVA showing main effect (dosage) and interaction effect (dosage x day) values for three formulations of diflubenzuron tested in different habitats

Formulation	Habitat	Main effect (dosage)			Interaction effect (dosage x day)		
		F value	df	*P* value	F value	df	*P* value
25% WP	Cesspit	1.04	3, 109	>0.05	0.56	22, 109	>0.05
	Street drain	5.84	3, 92	<0.05	0.58	19, 92	>0.05
	Abandoned well	2.77	3, 108	>0.05	1.39	39, 108	>0.05
2% GR	Cesspit	2.60	3, 113	>0.05	0.77	23, 113	>0.05
	Street drain	5.73	3, 97	<0.05	1.53	21, 97	>0.05
	Abandoned well	15.44	3, 109	<0.05	0.93	39, 109	>0.05
2% DT	Cesspit	5.23	4, 165	<0.05	0.61	40, 165	>0.05
	Street drain	38.59	4, 138	<0.05	3.73	39, 138	<0.05
	Abandoned well	2.33	4, 135	>0.05	0.47	35, 135	>0.05

WP, wettable powder; GR, granular; DT, diflubenzuron tablet

WP, GR and DT formulations, the reduction of pupal density fluctuated between 7 and 100 per cent during post-treatment period at all dosages (Table II). In this habitat, the WP formulation produced >80 per cent reduction of pupal density for 7 days post-treatment at all application rates and the GR formulation caused >80 per cent reduction of pupal density at 50-100 g ai/ha up to day 7 post-treatment. The DT formulation yielded the same level of reduction of pupal density in this habitat for a week only at 1 tablet/m^2. Treatments with WP, GR and DT formulations reduced the late instar larval density by 0-84 per cent during post-treatment period and reduction was >50-84 per cent on days 4-7 post-treatment at all application rates in all habitats (Table II). The effect of these three formulations on early instar larval density was less marked and was fluctuating between 0-50 per cent in cesspits and street drains during post-treatment period. In abandoned wells, the reduction of early instar larval density fluctuated between 4 and 82 per cent during post-treatment period and the reduction was about 60-82 per cent level at 75 and 100 g ai/ha of WP and GR formulations (Table II).

Physico-chemical characteristics of habitat water: The *p*H of the water in different habitats ranged from 7.0 to 9.0, and water temperature from 25 to 33°C. There was no variation between the habitats or habitat types with respect to *p*H. However, the maximum water temperature recorded in cesspits was higher by 1°C than that recorded in other two types of habitats. The dissolved oxygen ranged from 0.0 to 1.6 mg/litre in cesspits, 0.0 to 1.0 mg/litre in drains and 0.0 to 3.8 mg/litre in wells. The dissolved oxygen recorded in wells was higher compared to cesspit and drains. However, the dissolved solids and total solids ranged from 0.40 to 0.81 g/litre and 0.5 to 3.8 g/litre in cesspits, 1.4 to 3.0 g/litre and 2.6 to 4.2 g/litre in drains and 0.31 to 0.75 g/litre and 0.51 to 3.2 g/litre in wells, respectively indicating that the dissolved and total solids were higher in drains compared to the other two types of habitats.

Discussion

In the present evaluation, the activity of 25 per cent WP, 2 per cent GR and 2 per cent DT formulations of diflubenzuron were evaluated against *Cx. quinquefasciatus* breeding in cesspits, drains and abandoned wells all polluted with sullage, debris and garbage. The results showed that both the WP and GR formulations were equally effective, producing >80 per cent IE of *Cx. quinquefasciatus* for 7-10 days in cesspits, 4-7 days in street drains and 7-21 days in abandoned wells at 25-100 g ai/ha. The DT formulation was effective only at higher dosage 100 g ai/ha and or 1 tablet/m^2 for 7-15 days at all habitats. Efficacy of the formulations varied with the habitats tested. In abandoned wells, the WP and GR formulations provided relatively longer duration of control compared to other two habitats. This might be due to absence of the degradation-conducive factors such as sunlight and

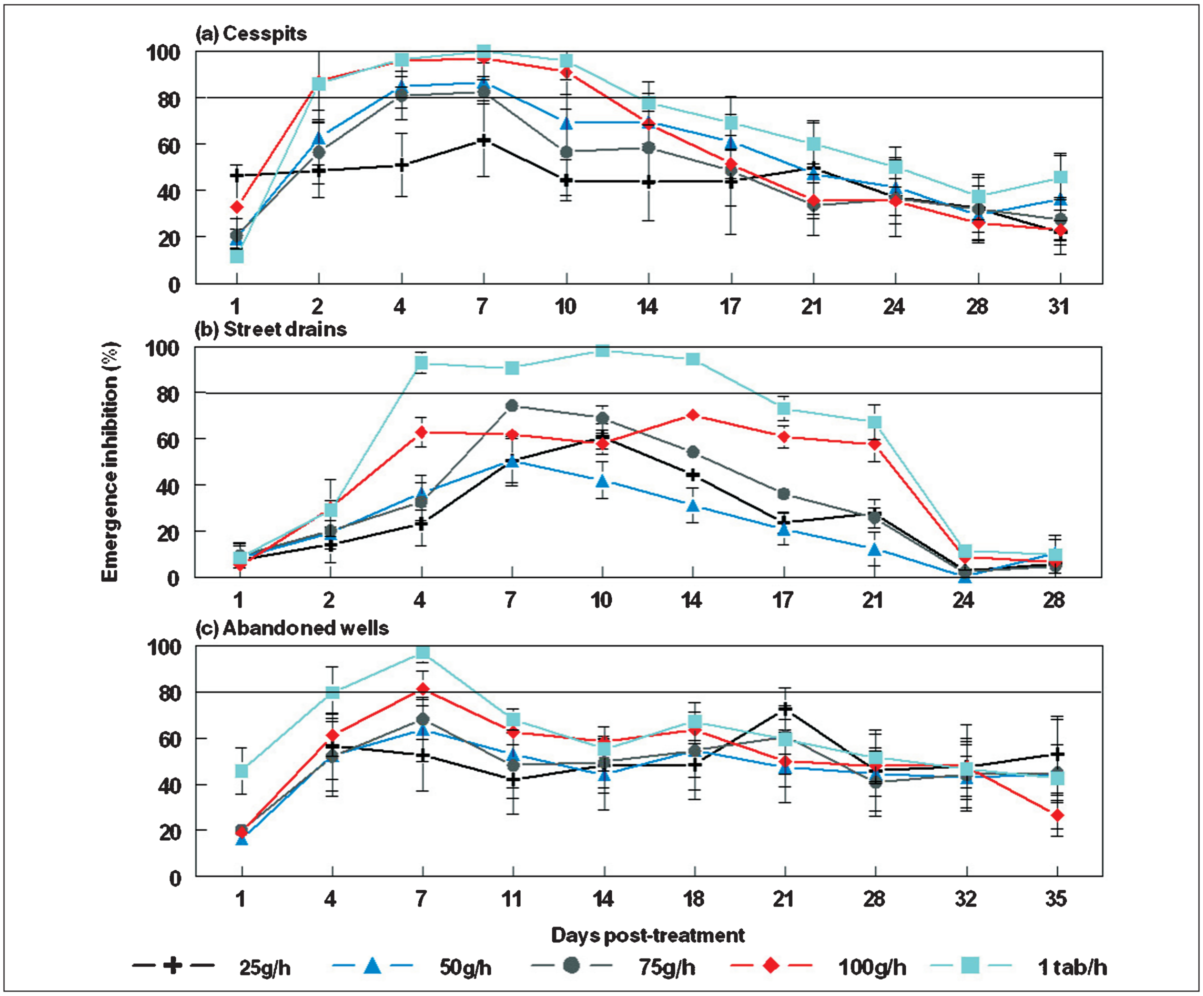

Fig. 3. Per cent emergence inhibition (mean ± SE) of *Culex quinquefasciatus* adults in cesspits **(a)**, street drains **(b)** and abandoned wells **(c)** treated with diflubenzuron 2% DT formulation.

high organic content. The abandoned wells are deeper and shaded water bodies with high dissolved oxygen content and relatively lower dissolved and total solids compared to cesspits and drains.

Except in abandoned wells, the efficacy (% IE and residual activity) of the three formulations of diflubenzuron was not dose dependent in all the habitats. The higher dosages (50-100g ai/ha) did not enhance the effective duration of control considerably compared to the lower dosage (25 g ai/ha). This might be due to low persistence and rapid degradation of diflubenzuron in water with high temperature, alkaline *p*H values and high organic content[22]. The half-life of diflubenzuron was less than 7 days in the environment. In most of the treatments, although the % IE at higher dosages (50-100 g ai/ha) was significantly greater than the lower dosage (25 g ai/ha), the interaction effect of dosage and day was not significant as the trend of %IE was constant on different days of observation between the dosages during the post-treatment period. Further, in all habitats treated with WP and GR formulations, the %IE at the high dosage of 100 g ai/ha was slightly lower on days 14 to 21 post-treatment compared to lower dosage of 25 g ai/ha. These variations were not statistically significant. The slight variations observed in %IE during post-treatment might be due fluctuations in the levels of chemical contents of sewage or sullage in the habitats.

Water quality analysis revealed that the temperature and the alkaline *p*H values recorded in different larval habitats were in the optimum range for the breeding of *Cx. quinquefasciatus*. Further, these habitats were characterized by very low levels of dissolved oxygen. The higher levels of dissolved and total solids indicated

Table II. Per cent reduction of pupal and late and early instar larval densities of *Culex quinquefasciatus* observed during post-treatment in larval habitats treated with diflubenzuron formulations

Habitats	Dose (g/ha)	Percentage reduction								
		25% WP			2% GR			2% DT		
		Pupae	Late	Early	Pupae	Late	Early	Pupae	Late	Early
Cesspits	25	21-99	20-79	15-38	22-95	4-69	6-43	11-59	8-35	7-36
	50	18-94	25-68	13-29	10-93	5-72	11-44	25-79	12-57	7-48
	75	31-98	25-81	15-43	37-100	4-84	13-47	17-71	8-46	13-40
	100	30-98	10-78	11-35	24-97	0-71	0-30	28-88	7-69	15-49
	1 tablet/m^2							24-96	17-92	8-49
Street drains	25	11-67	8-59	5-33	8-61	12-78	10-44	11-53	3-25	6-26
	50	26-100	3-80	2-30	9-63	8-58	10-51	10-62	0-27	3-28
	75	12-100	0-70	0-23	22-62	15-65	7-46	9-70	0-46	0-36
	100	13-94	16-60	4-28	16-76	9-66	11-46	11-63	5-25	2-14
	1 tablet/m^2							7-100	7-74	5-52
Abandoned wells	25	15-100	13-70	7-53	7-79	21-62	8-51	22-79	22-46	5-28
	50	14-100	12-80	10-44	34-96	13-52	21-44	27-58	24-66	14-39
	75	16-100	8-77	4-74	33-100	31-65	18-48	33-67	14-45	13-38
	100	30-100	27-66	8-82	14-99	16-62	9-64	34-73	10-61	13-40
	1 tablet/m^2							32-93	30-71	20-38

that these habitats were rich in organic load. It has been reported that the *Cx. quinquefasciatus* prefers to breed in habitats with high alkalinity, rich organic matter and low level of dissolved oxygen[23].

At the same application rates (100 g ai/ha), both the WP and GR formulations were equally effective, yielding >80-100 per cent IE of *Cx. quinquefasciatus* for 7-21 days in all habitats. Both WP and GR formulations showed superior activity than that of DT formulation in all habitats. Four to eight times higher quantity of DT formulation (1 tablet/m^2, equivalent to 400 g ai/ha) is required to achieve the same level of IE, particularly in abandoned wells and drains. Use of such a higher concentration is not recommended for safety and economical reasons for these two habitats. It has been reported that application of diflubenzuron WP and GR formulations, at >150 g ai/ha had a high adverse effect on a number of non-target aquatic insects[24].

At equal application rates (0.02 kg ai/ha), the efficacy of diflubenzuron was comparable to that of methoprene[14]. Laboratory bioassays have indicated that the activity of WP and GR formulations of diflubenzuron against *Culex* mosquitoes were relatively lower than that of other IGRs, novaluron and pyriproxyfen[25,26]. The present study showed that at equal dosage (100 g ai/ha), the activity of WP and GR formulations of diflubenzuron against of *Cx. quinquefasciatus* in cesspits, drains and abandoned wells were relatively lower when compared to the results of earlier trials carried out in India, with the IGRs pyriproxyfen and novaluron[18,27].

Though handling, transportation and application of DT formulation were easier compared to WP and GR formulations, the DT formulation could not yield desirable level of control against *Culex* mosquitoes breeding in polluted larval habitats. However, the 2 per cent DT formulation was highly effective against *Aedes aegypti* at 0.02-0.25 mg ai/liter for 2-4 months and suitable for direct application for the control of mosquitoes breeding in container habitats[24].

The trial showed that 25 g ai/ha of 25 per cent WP formulation could be the field application dosage for cesspits and abandoned wells, and 50 g ai/ha for street drains, to be applied at weekly intervals. The dosages 25, 50 and 100 g ai/ha of 2 per cent GR could be the optimum field application dosages for application in cesspits at weekly intervals, in abandoned wells every three weeks and in drains at weekly intervals, respectively. Diflubenzuron 25 per cent WP and 2 per cent GR formulations could be considered for the control of *Cx. quinquefasciatus* breeding in heavily polluted larval habitats under Integrated Vector Management Programme particularly in urban situations in India.

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