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Field evaluation of the biolarvicide, spinosad 20 per cent emulsifiable concentrate in comparison to its 12 per cent suspension concentrate formulation against *Culex quinquefasciatus*, the vector of bancroftian filariasis in India

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***Background & objectives*: Biolarvicides may offer alternatives to chemical larvicides as these are known to be safe to environment and selective against the target species. However, only a limited number of biolarvicides have been approved for mosquito larval control. In the current study, a new formulation of spinosad, 20 per cent emulsifiable concentrate (EC) was tested for its efficacy against *Culex quinquefasciatus*, in comparison to its 12 per cent suspension concentrate (SC).**

***Methods*: Spinosad 20 per cent EC was tested against *Cx. quinquefasciatus* immature at 25, 50, 100 and 150 mg active ingredient (ai)/m^2 in cesspits, drains and abandoned wells in comparison with spinosad 12 per cent SC at the optimum field application dosage of 50 mg ai/m^2.**

***Results*: The 20 per cent EC caused 90-100 per cent reduction of pupal density for 7-14 days in cesspits, 10-17 days in drains and 14-30 days in abandoned wells at all dosages tested. At lower dosages of 25 and 50 mg ai/m^2, >90 per cent reduction of pupal density was observed for one week in cesspits and street drains and for two weeks in abandoned wells. The effective duration of control provided by the higher dosages, 100 and 150 mg ai/m^2 was 1.4 to 2 times greater than the lower dosages, 25 and 50 mg ai/m^2.**

***Interpretation & conclusions*: The findings showed that the spinosad 20 per cent EC can be used for larval control against *Cx. quinquefasciatus*, at the dosage of 25 mg ai/m^2 at weekly interval in cesspits and drains and at fortnightly interval in abandoned wells. Spinosad 20 per cent EC could be one of the options to be considered for larval control under integrated vector management.**

Key words Biolarvicide - *Culex quinquefasciatus* - filariasis - India - mosquito control - spinosad

Biolarvicides of bacterial origin may offer alternative to chemical larvicides that pose problems of resistance and environmental safety, as these are known to be relatively safer to environment, selective against target species and suitable for community use[1]. Various commercial formulations of *Bacillus thuringiensis* and *B. sphaericus* have been developed and evaluated for mosquito larval control. However, most of these formulations have short residual activity in the field[2]. Further, the efficacy and residual activity of the biolarvicides vary with the type of formulations used. Therefore, there is a need to develop new larvicides/formulations with long residual activity, unique modes of action and favourable mammalian and environmental safety profiles for the use in vector control programme.

Spinosad is a natural product produced by fermentation technology employing the soil bacterium, *Saccharopolyspora spinosa* (Actinomycetales). Spinosad, a tetracyclic macrolide compound, is a mixture of two metabolites such as spinosyn A ($C_{41}H_{65}NO_{10}$) and spinosyn D ($C_{42}H_{67}NO_{10}$). Spinosins are neurotoxins that activate post-synaptic nicotinic acetylcholine and gamma-aminobutyric acid receptors and cause excitation of insect nervous system[3,4]. Spinosad 0.5 per cent granular (GR) and 12 per cent suspension concentrate (SC) formulations have been reported to be effective against the larvae of *Aedes*, *Anopheles* and *Culex* species under laboratory and field conditions[5-7]. An emulsifiable concentrate (EC) formulation of spinosad with 20 per cent active ingredient (ai) was made available through the WHO Pesticide Evaluation Scheme (WHOPES) for testing against mosquito vectors under different ecological settings. The current study was undertaken to test the efficacy of the 20 per cent EC formulation of the spinosad in comparison with its 12 per cent SC formulation against *Cx. quinquefasciatus* Say, the vector of bancroftian filariasis.

Material & Methods

The field evaluation was conducted in Cuddalore, a town in Tamil Nadu State on the Coromandel coast of peninsular India, having a population of about 0.6 million. The area has been endemic for lymphatic filariasis with perennial transmission by *Cx. quinquefasciatus*[8], which was abundant throughout the year; January to March being the most favourable period. Cesspits, street drains and abandoned wells were the major larval habitats of the vector. Although single-dose mass drug administration of diethylcarbamazine citrate is being done annually in the area as a part of the filariasis elimination programme, vector control measures (mainly larvicidal) are continued under National Filaria Control Programme to reduce vector density and thereby transmission. Despite the routine vector control operations, the vector breeding was high. The evaluation of spinosad 20 per cent EC and 12 per cent SC was carried out following the WHOPES protocol[9] from September 2009 to June 2010.

Small-scale trial for dose determination: The 20 per cent EC formulation of spinosad was tested in cesspits, drains and abandoned wells at four dosages, namely, 25, 50, 100 and 150 mg ai/m^2. For comparison, 12 per cent SC formulation of spinosad was tested at the optimum application dosage of 50 mg ai/m^2 in the three types of habitats[7]. Five replicates of cesspits, abandoned wells and drains were selected for each dosage. An equal number of replicates of each type were kept as controls (untreated). Drains (cement lined 'U' shaped) choked with silt and debris and had no connection with other drains were selected. Every segment of 10 m length of the drain was considered as a replicate. However, while applying the formulations, the entire length of the drain was treated. Separate drains were selected for each dosage/formulation and for control.

For the trial, habitats of each type were made into six groups with comparable pre-treatment larval and pupal densities (statistically tested) and assigned randomly to five treatments/dosages (four for spinosad 20% EC and one for spinosad 12% SC) and one control. To the treatment groups, the formulations were applied after suitably diluting in water, using a hand compression sprayer (2 l capacity) with a jet nozzle (Foggers India Pvt. Ltd., Pune) at the selected dosages.

Before application, the larval and pupal densities were monitored twice a week for 1-2 weeks. While dipper (300 ml capacity) was used for sampling in cesspits and drains, bucket (3 l capacity) was used for wells. Three dips were taken from each replicate and the larvae sampled were counted instar wise. Post-treatment monitoring was done at 24 h and subsequently every 2/3 days. Sampling was continued until the density of larvae and pupae in the treated habitats reached a level comparable to that in the untreated habitats. Temperature and *p*H of the habitat water were recorded on each day of sampling. Dissolved oxygen, dissolved solids and total solids in the habitats were determined on two occasions during the trial period.

Statistical analysis: The mean number of pupae and larvae collected per dip was calculated for each day of sampling and for each replicate. The 1st and 2nd larval instar larvae were clubbed together as 'early instars' and similarly the 3rd and 4th as 'late instars'. The reduction of larval and pupal densities on post-treatment days was estimated by comparing the pre- and post-treatment densities in the treated habitats with the corresponding densities in the untreated habitats using Mulla's formula[10]. The differences between the dosages were compared using two-way ANOVA test with dosage and day as the main factors after arcsine transformation of percentage values[11]. 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 *post hoc* test based on the least significant difference. The mean arcsine values were back-transformed to per cent values and presented in the text and tables. The optimum field application dosage was determined on the basis of effective duration for each dosage. The effective duration is the day post-treatment up to which the lower limit of the 95 per cent confidence interval for the mean per cent reduction of density will be ≥80 per cent.

Results

Efficacy of spinosad 20 per cent emulsifiable concentrate (EC)

Cesspits: Spinosad 20 per cent EC reduced the pupal density by 60-80 per cent in cesspits on day 1 post-treatment at all dosages (Fig. 1A and Table I). At 25 and 50 mg ai/m^2, the reduction was 99-100 per cent on day 7, about 70-80 per cent on day 14 and it declined to 20 per cent on day 21 post-treatment. At 100 and 150 mg ai/m^2, the reduction was 68 per cent on day 1, 100 per cent on day 7 and 95-100 per cent up to day 14 post-treatment. On day 21, the reduction declined to 72-78 per cent, and on day 28, post-treatment, the reduction was only 30 per cent.

The late instar larval density was reduced by around 100 per cent on day 1 post-treatment at all dosages. At 25 and 50 mg ai/m^2, the reduction ranged from 97 to 100 per cent for one week post-treatment and thereafter the reduction was <80 per cent. The reduction at 100 and 150 mg ai/m^2 was 97-100 per cent on day 7, 65-92 per cent on day 14 and thereafter it declined, 57-65 per cent on day 21 and 30 per cent on day 28 post-treatment (Fig. 1B and Table I).

At 25 and 50 mg ai/m^2, the early instar density was reduced by 100 per cent on day 1 post-treatment (Fig. 1C and Table I), around 79 per cent on day 7 and only <50 per cent on day 14 post-treatment. At 100 and 150 mg ai/m^2, the reduction was >95-100 per cent for one week, 60 per cent on day 14 and thereafter gradually declined reaching <50 per cent on day 21 post-treatment.

ANOVA (two-way) showed significant difference in per cent reduction of pupal and late and early

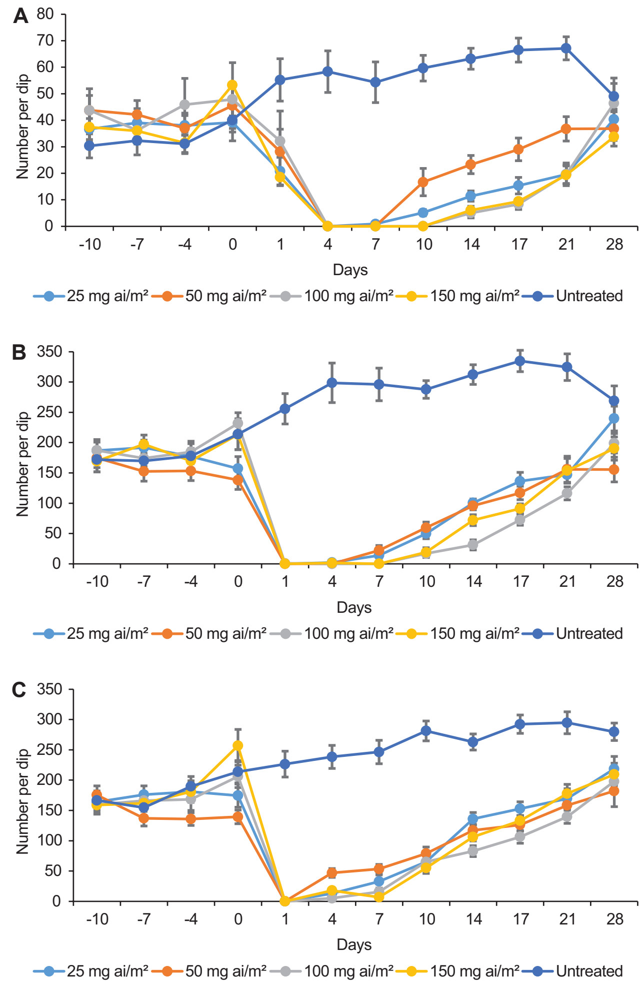

Fig. 1. Mean number of *Culex quinquefasciatus* pupae, (**A**) late instar (**B**) and early instar (**C**) larvae per dip in cesspits treated with spinosad 20 per cent emulsifiable concentrate.

instar densities between the dosages ($P<0.05$) and not over time (Table II). Comparison of means indicated significantly greater effect at 100 and 150 mg ai/m^2 than 25 and 50 mg ai/ m^2.

Drains: In street drains, on day 1 post-treatment, pupal density was suppressed by >85-90 per cent at all application rates. At 25 and 50 mg ai/m^2, the reduction was around 100 per cent on day 7, 79-88 per cent on day 14 and thereafter the effect of the formulation declined drastically as the reduction was only 6 per cent on day 21 post-treatment. At 100 and 150 mg ai/m^2, 100 per cent suppression was observed on day 7 post-treatment. On day 14, the reduction was 94-100 per cent, and on day 21, it was 70-82 per cent. Afterwards, the reduction was only 40-55 per cent (Fig. 2A and Table I).

The late and early instar densities were reduced by 100 per cent on day 1 post-treatment at all dosages. At

Table I. Effective duration (days) for spinosad 20% emulsifiable concentrate and 12% suspension concentrate formulations in different larval habitats (>80% reduction density per dip)

Habitats	Dosages (mg ai/m^2)	Pupal density		Late instars		Early instars	
		20% EC	12% SC	20% EC	12% SC	20% EC	12% SC
Cesspits	25	7	-	7	-	4	-
	50	10	10	7	7	4	7
	100	14	-	10	-	7	-
	150	14	-	10	-	7	-
Street drains	25	10	-	7	-	1	-
	50	10	10	10	10	3	3
	100	14	-	10	-	7	-
	150	17	-	14	-	7	-
Abandoned wells	25	14	-	11	-	4	-
	50	17	17	17	11	7	11
	100	30	-	24	-	17	-
	150	30	-	28	-	28	

EC, emulsifiable concentrate; SC, suspension concentrate; ai, active ingredient

Table II. Results of two-way ANOVA showing main effects (dosage) and interaction effect (dosage × day) values for 20% emulsifiable concentrate formulation of spinosad tested in different habitats

Habitat	Stage	Main effects (dosage)			Interaction effects (dosage × day)		
		F	df	*P*	F	df	*P*
Cesspits	Pupa	7.15	3, 160	<0.05	0.74	21, 160	>0.05
	Late instar	14.33	3, 160	<0.05	1.32	21, 160	>0.05
	Early instar	7.12	3, 160	<0.05	0.56	21, 160	>0.05
Street drains	Pupa	179.63	3, 180	<0.05	18.77	24, 180	<0.05
	Late instar	18.83	3, 180	<0.05	3.79	24, 180	<0.05
	Early instar	3.86	3, 180	<0.05	1.78	24, 180	<0.05
Abandoned wells	Pupa	22.06	3, 266	<0.05	1.10	45, 266	>0.05
	Late instar	26.42	3, 249	<0.05	1.84	42, 249	<0.05
	Early instar	40.60	3, 255	<0.05	2.02	45, 255	<0.05

25 mg ai/m^2, the reduction of late instar density was 95-100 per cent for one week after treatment (Fig. 2B and Table I) and on day 14 post-treatment, it was only <50 per cent. At 50 and 100 mg ai/m^2, the reduction was 98-100 per cent up to day 7, and on day 14 post-treatment, it declined to 50 per cent. At 150 mg ai/m^2, the formulation caused >90-100 per cent reduction up to 14 days after treatment, and thereafter the reduction was not considerable, only 35 per cent on day 21 post-treatment.

At 25 and 50 mg ai/m^2, the reduction of early instar was 70-79 per cent on day 7, and on day 14 post-treatment, it declined to <50 per cent (Fig. 2C and Table I). At 100 and 150 mg ai/m^2, >90-100 per cent reduction was observed for one week, and afterwards, it was <50 per cent on day 14 post-treatment.

Overall, as well as over time, the reduction of pupal and larval densities differed significantly between the dosages (P<0.05) (Table II). Significantly greater effect was obtained at 150 mg ai/m^2 than at 25, 50 and 100 mg ai/m^2.

Abandoned wells: At all dosages, >90-100 per cent reduction of pupal density was recorded on day 1 post-treatment (Fig. 3A and Table I). At 25 and 50 mg ai/m^2, the reduction was 95-100 per cent up to two weeks post-treatment, thereafter the effect of

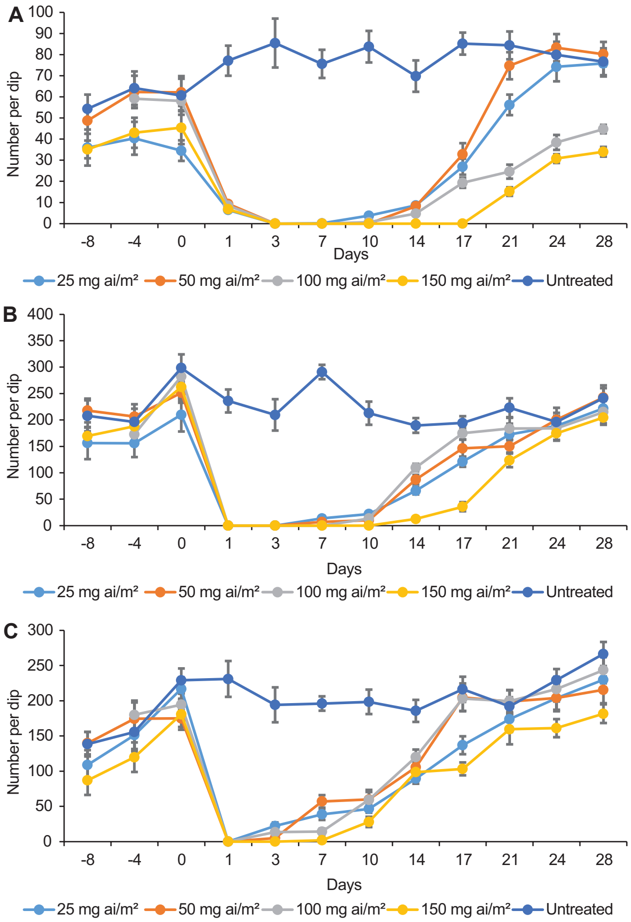

Fig. 2. Mean number of *Culex quinquefasciatus* pupae (**A**), late instar (**B**) and early instar (**C**) larvae per dip in street drains treated with spinosad 20 per cent emulsifiable concentrate.

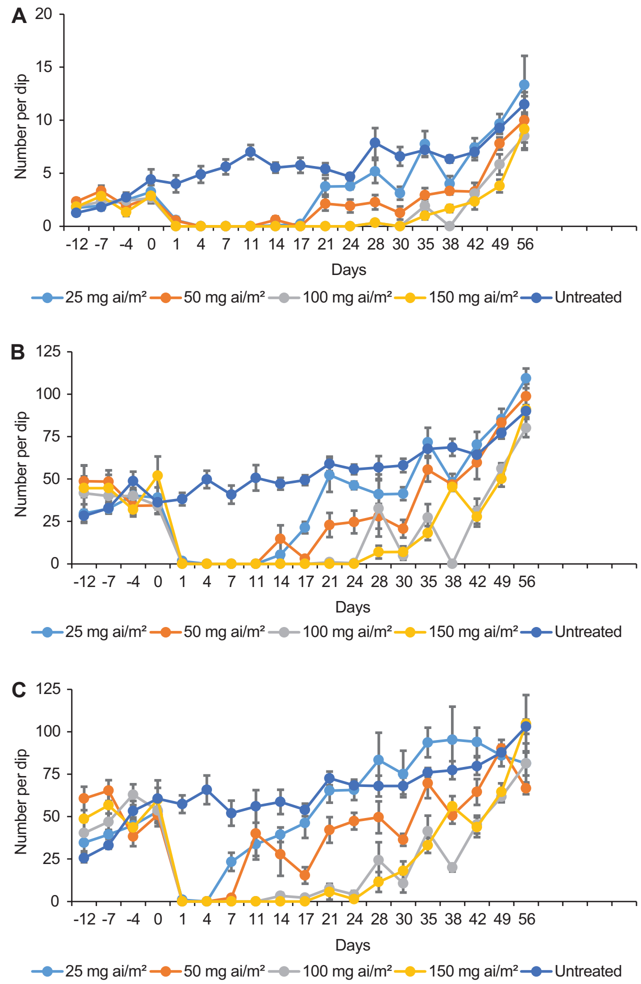

Fig. 3. Mean number of *Culex quinquefasciatus* pupae (**A**), late instar (**B**) and early instar (**C**) larvae per dip in abandoned wells treated with spinosad 20 per cent emulsifiable concentrate.

the formulation at 25 mg ai/m^2 declined. At 100 and 150 mg ai/m^2, the reduction was 98-100 per cent up to 28 day post-treatment, and beyond this period, the effect declined reaching a reduction of <50 per cent on days 42-49 post-treatment at 50, 100 and 150 mg ai/m^2.

On day 1 post-treatment, the late instar density was reduced by 88-100 per cent at all dosages. Application at 25 and 50 mg ai/m^2, caused 100 per cent reduction for seven days after treatment, and on day 14 post-treatment, the reduction was around 90 per cent, whereas, at 100 and 150 mg ai/m^2, the same level of effect was obtained for 21-28 days (Fig. 3B and Table I). Further, at 25 and 50 mg ai/m^2, the reduction declined to <50 per cent on days 21-28 post-treatment, while it was on day 49 at 100 and 150 mg ai/m^2.

The reduction of early instar density was by 60-100 per cent for seven days after treatment at 25 mg ai/m^2, and on day 14, the reduction was only 30 per cent (Fig. 3C and Table I). At 50 mg ai/m^2, the reduction was 95-100 per cent for seven days. The same level of reduction was observed for 14 and 28 days at 100 and 150 mg ai/m^2, respectively. The level of reduction declined to <50 per cent on day 21 at 50 mg ai/m^2, but at 100 and 150 mg ai/m^2, it was on day 56 post-treatment.

ANOVA showed a significant difference in per cent reduction of pupal density between the dosages (P<0.05) but not overtime (Table II). In the case of late and early instar densities, the per cent reduction varied significantly between dosages and also over time (P<0.05). As expected, the higher dosages, 100 and 150 mg ai/m^2, produced significantly greater effect than the lower dosages, 25 and 50 mg ai/m^2 (P<0.05).

Efficacy of spinosad 12 per cent suspension concentrate (SC)

Cesspits: Treatment of cesspits with spinosad

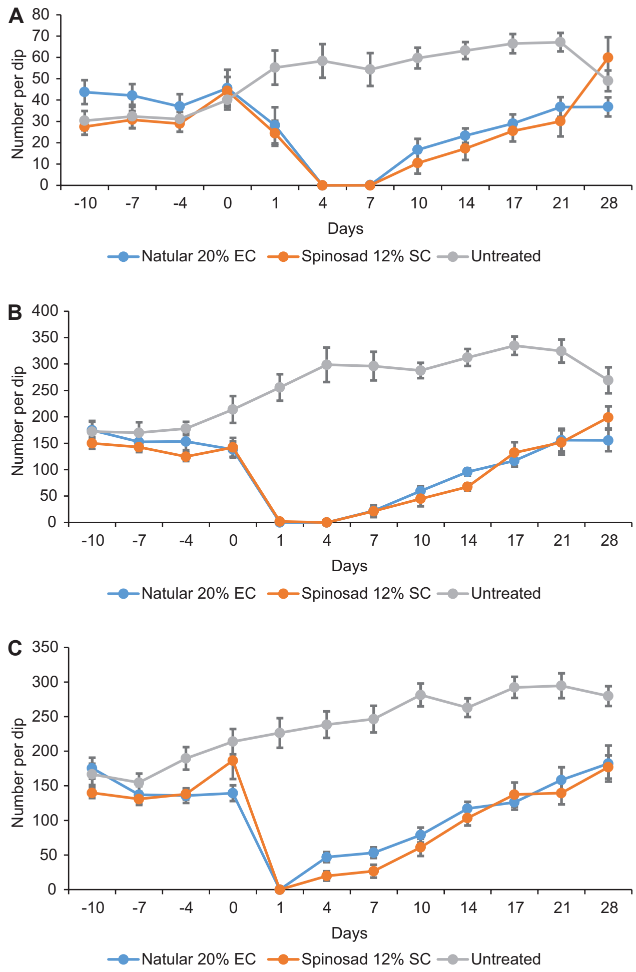

Fig. 4. Mean number of *Culex quinquefasciatus* pupae (**A**), late instar (**B**) and early instar (**C**) larvae per dip in cesspits treated with spinosad 20 per cent emulsifiable concentrate and spinosad 12 per cent suspension concentrate at 50 mg ai/m^2.

12 per cent SC at 50 mg ai/m2 resulted in 58 per cent reduction of pupal density on day 1 and 100 per cent on day 7 post-treatment. The effect gradually declined reaching 80 per cent on day 14 and 50 per cent on day 21 post-treatment (Fig. 4A and Table I). Reduction of late instar density was 97-100 per cent up to day 7 post-treatment (Fig. 4B), thereafter declined to 70 per cent on day 14 and 38 per cent on day 21 post-treatment. The early instar density was reduced by 95-100 per cent up to day 7 (Fig. 4C), and afterwards, the effect declined causing only <50 per cent reduction on day 14 post-treatment.

<u>Drains:</u> At 50 mg ai/m2, the reduction of pupal density was 89 per cent on day 1 and 100 per cent reduction was attained on day 7 post-treatment. Thereafter, the effect was on a declining trend, 70 per cent on day 14 and <20 per cent on day 21 post-treatment (Fig. 5A and Table I). The reduction of late instar density was >90-100 per cent up to day 7, and thereafter the effect of the formulation declined drastically, reaching as low as <20 per cent on day 14 post-treatment (Fig. 5B). The early instar density was reduced by 100 per cent on day 1, around 70 per cent on day 7 and only 14 per cent on day 14 post-treatment (Fig. 5C).

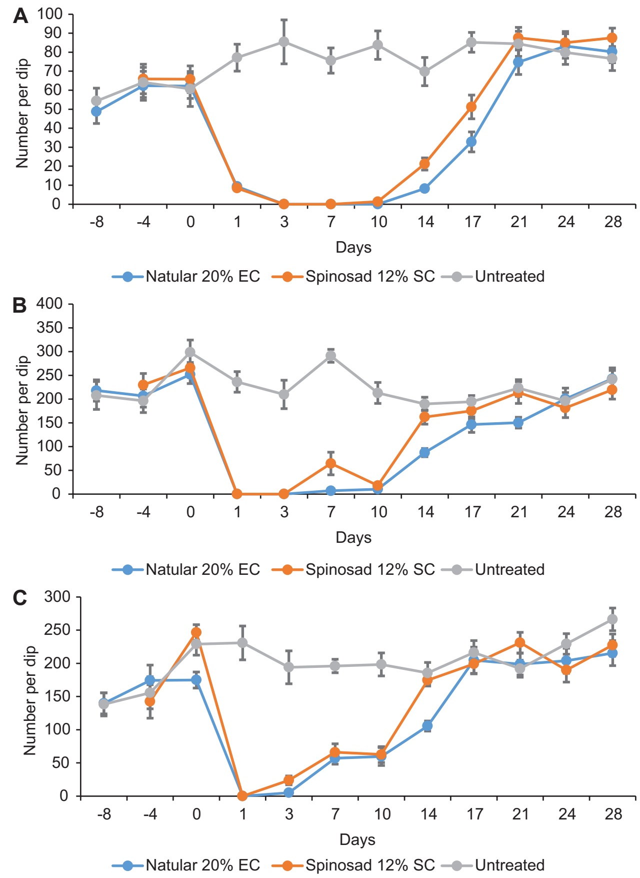

Fig. 5. Mean number of *Culex quinquefasciatus* pupae (**A**), late instar (**B**) and early instar (**C**) larvae per dip in street drains treated with spinosad 20 per cent emulsifiable concentrate and spinosad 12 per cent suspension concentrate at 50 mg ai/m^2.

<u>Abandoned wells:</u> There was 100 per cent reduction of pupal density in abandoned wells up to day 14 post-treatment, and beyond this period, the effect was not considerable, reduction reaching 63 per cent on day 21 and <50 per cent on day 28 post-treatment (Fig. 6A and Table I). Density of both late and early instar larvae was suppressed by 100 per cent up to day 7 post-treatment. Thereafter, the effect of the formulation declined causing only <50 per cent on day 21 post-treatment (Fig. 6B and C).

Physicochemical characteristics of habitat water: The *p*H and temperature of the water in the habitats ranged

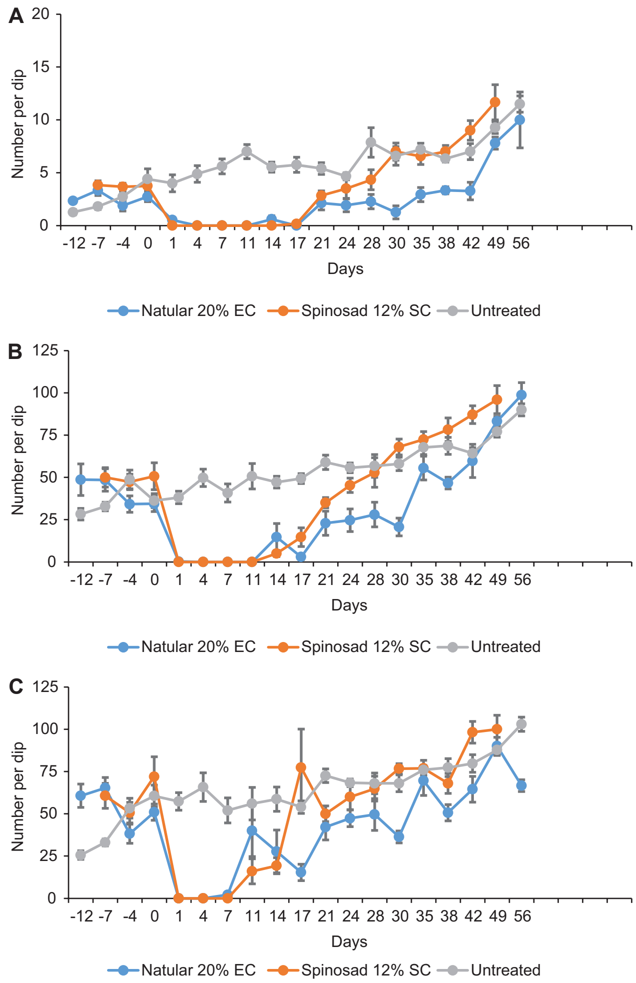

Fig. 6. Mean number of *Culex quinquefasciatus* pupae (**A**), late instar (**B**) and early instar (**C**) larvae per dip in abandoned wells treated with spinosad 20 per cent emulsifiable concentrate and spinosad 12 per cent suspension concentrate at 50 mg ai/m^2.

from 6.1 to 8.2 and 26.2 to 33.0°C, respectively. There was no variation between the habitats or habitat types with respect to *p*H. However, the mean maximum water temperature recorded in wells was lesser by 1 and 2°C than that recorded in drains and cesspits, respectively. The dissolved oxygen in abandoned wells (0.6 to 4.8 mg/l) was higher compared to drains (0.0 to 2.0 mg/l) and cesspits (0.0 to 1.6 mg/l), whereas dissolved and total solids were higher in cesspits (0.20 to 3.35 and 0.32 to 4.45 g/l) and drains (0.67 to 2.25 and 0.80 to 2.48 g/l) compared to that in abandoned wells (0.16 to 1.9 and 0.22 to 2.14 g/l).

Discussion

Spinosad, a compound of biological origin, has low mammalian toxicity and a favourable environmental profile including low persistence and no toxicity to fish and wildlife at mosquito larvicidal rate[6,12,13]. Spinosad GR and SC formulations have previously been evaluated against mosquito vectors by various workers. In Mexico, application of spinosad 48 per cent SC at 1 and 10 ppm in plastic containers yielded complete (100%) control of *Aedes aegypti* breeding for 13 and 22 wk and *Culex* breeding for 17 and 22 wk, respectively[5]. In Turkey, spinosad SC formulation at the dosage of 100 g ai/ha in septic tanks produced 80-100 per cent reduction of *Culex pipiens* for 7-14 days[14]. In Thailand, application of spinosad 12 per cent SC at 0.89 mg/l in earthen jars yielded >90 reduction of *Ae. aegypti* larvae for 104 days, whereas spinosad 0.5 per cent GR at 1.0 mg/l caused the same level of reduction for 111 days[6]. In Iran, application of spinosad 12 per cent SC and 0.5 per cent GR formulations at 2 mg/m^2 in experimental ponds, resulted in >80 per cent control of *Anopheles* and *Culex* breeding for 7-9 days. At 4 mg/m^2, these formulations showed the same level of control of *Anopheles* and *Culex* species for 9-11 days[6]. In an earlier trial in Puducherry, India, at a concentration of 50 mg ai/m^2, both 12 per cent SC and 0.5 per cent GR formulations were found equally effective, causing 80-100 per cent reduction of *Cx. quinquefasciatus* breeding for a week in cesspits and drains and for a month in abandoned wells[7].

In the current study, the efficacy of spinosad 20 per cent EC formulation was tested against *Cx. quinquefasciatus* in cesspits, drains and abandoned wells in comparison with spinosad 12 per cent SC formulation. The results showed that, at the application rates of 25, 50, 100 and 150 mg ai/m^2, the 20 per cent EC formulation suppressed the density of pupae by 90-100 per cent for 7-14 days in cesspits, 10-17 days in drains and 14-30 days in abandoned wells. The residual effect was longer in abandoned wells compared to drains and cesspits. The abandoned wells are closed habitats without any replenishment of water. Further, they are deeper and shaded habitats with relatively higher dissolved oxygen and relatively lower dissolved and total solids. The absence of the degradation-conducive factors such as sunlight and high dissolved organic content has resulted in longer residual activity in this habitat[15].

The efficacy increased with higher dosages in the three habitats tested. At the low dosage of 25 mg ai/m^2, >90 per cent reduction of pupal density was observed for one week in cesspits and street drains and for two weeks in abandoned wells. When the dosage was increased to 50 mg ai/m^2, no enhancement in the residual activity was observed. At the higher dosages of 100 and 150 mg ai/m^2, the same level of efficacy (>80% reduction) was observed for two weeks in cesspits and street drains and for one month in abandoned wells. The effective duration of control with the higher dosages (100 and 150 mg ai/m^2) was 1.4 to 2 times greater than the lower dosages, 25 and 50 mg ai/m^2. To achieve the 1.4 to 2 times greater control, about four times higher quantity of the formulation would be required. Use of such a higher concentration may not be a justifiable option for economical and safety reasons. Therefore, the dosage 25 mg ai/m^2 could be selected as the field application dosage for cesspits, street drains and abandoned wells.

At the application rate of 50 mg ai/m^2, both 12 per cent SC and 20 per cent EC formulations of spinosad were equally effective, suppressing larval and pupal populations of *Cx. quinquefasciatus* in cesspits, drains and abandoned wells for 10-17 days. At the low dosage of 25 mg ai/m^2, spinosad 20 per cent EC produced >90 per cent reduction of pupal density for seven days in cesspits, 10 days in street drains and 14 days in abandoned wells. Application of spinosad 12 per cent SC at the same dosage (25 mg ai/m^2) did not produce >80 per cent reduction of pupal and larval densities in the three habitats tested. When compared to 12 per cent SC formulation, the use of 20 per cent EC formulation would be cost-effective (two times) and safer to non-target organisms as it is effective even at the low dosage of 25 mg ai/m^2.

Temephos, an organophosphorus compound, is used for larval control in urban areas by the National Programme in India. At the dosage of 100 mg ai/m^2, the spinosad 20 per cent EC provided longer duration of control of *Cx. quinquefasciatus* (14 days in cesspits and drains and 30 days in abandoned wells) compared to temephos 1 per cent G (seven days in cesspits, 14 days in drains and 21 days in abandoned wells)[7]. The cost of spinosad-based formulations is higher compared to temephos-based formulations. However, the improved stability and longer residual activity of spinosad 20 per cent EC formulation in these habitats could reduce the frequency of application, thereby compensating the higher cost by minimizing operational cost. Further, development of resistance to temephos has already been reported in the larval population of *Cx. quinquefasciatus*[16,17]. Under these circumstances, the spinosad compound with a novel mode of action as a nicotinic agonist has shown no cross-resistance with existing insecticides and can be rotated with other classes of currently used mosquito larvicides for resistance management.

In conclusion, the spinosad 20 per cent EC formulation can be used for larval control against *Cx. quinquefasciatus,* at the lower dosage of 25 mg ai/m^2 at a weekly interval in cesspits and street drains and at fortnightly interval in abandoned wells. Spinosad 20 per cent EC formulation could be one of the options to be considered for larval control under integrated vector management.

Acknowledgment

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***Conflicts of Interest*:** None.

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