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Efficacy of IGR compound Lufenuron against mosquito larvae in storage tanks and polluted water

P Rajasekar[1*], N Soundarya[1], Dr. M Meera Maideen[2], Dr. M Salahudeen[2]
[1] Assistant Professor, Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India
[2] Research Scholar, Department of Zoology, Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamilnadu, India

Abstract
Mosquito-borne diseases such as dengue fever, malaria, yellow fever and encephalitis cause significant mortality in human and livestock in various parts of the world. In the present work, the larvicidal activity of Lufenuron, an IGR compound evaluated using laboratory experiments and field trials in the areas in and around Tiruchirappalli. The bio efficacy of the Lufenuron formulation tested against late fourth instar larvae of different mosquito species using the bioassay recommended by WHO. The formulation of Lufenuron was applied in the field at doses of 0.02ppm, 0.002 ppm, 0.0002 ppm, 0.00002 ppm and 0.000002 ppm (g/m³) in the breeding habitats of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes.
The impact of was assessed by monitoring larval density using a dipper method and reduction in density of larvae and inhibition of adult emergence were also observed. The EI_{50} values of Lufenuron were found to be 0.02, 0.002, 0.0002, 0.00002 and 0.000002 ppm for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. This study reveals that Lufenuron is highly effective for controlling mosquito vectors.

Keywords: Lufenuron, IGR, *Aedes*, *Culex*, ei_{50}, vector control

Introduction

Vector-borne diseases were major public health challenges in the developing countries. Controlling mosquitoes at the larva stage play a major role in the elimination of mosquito before the transmission of the diseases. Every year millions of people were died because of vector-borne diseases such as dengue, malaria, encephalitis, Zika fever, filariasis and chikungunya (WHO, 2014; 2015) [1, 2].

Mosquitoes are the most well-known disease vectors, and it is estimated that more than 700 million people are infected annually worldwide by various mosquito-borne pathogens (arboviruses, parasitic worms, and protozoa), including in Asia (El-Bahnasawy *et al*., 2013) [3]. *Ae. aegypti* is one of the important vectors involved in the transmission of viruses that causes various illnesses such as chikungunya, dengue and yellow fever to human beings and animals (Gubler, 1998) [4].

Worldwide, dengue fever is recognized as the most significant viral disease caused by arthropods. Nearly 50% of the world population are living in areas which are more prone to dengue prevalence (Murray *et al*., 2013) [5]. In countries in Asia and India, *Anopheles stephensi* is another important vector of malaria transmission and there is need of newer methods of vector control are mandatory for the management of human population (Kleinschmidt *et al*., 2000) [6]. Malaria is the most important disease transmitted by insects and causes major health problems in all the developing countries (Nathan *et al*., 2006) [7].

Nearly 120 million people in worldwide were affected by tropical disease such as filariasis transmitted by *Cx. quinquefasciatus* (Bernhard *et al*., 2003) [8]. India contributes a significant proportion of the global filariasis burden, accounting for an annual economic loss of about Rs720 crores (Hotez *et al*., 2004) [9]. Vector-borne diseases play a major role in the loss of national economy and disruption of society due to high mortality rates in countries like India (Madhumathy *et al*., 2007) [10].

Control of mosquito populations involves prevention of stagnation of water near ponds, stagnation in ditches for longer duration and by ensuring proper irrigation of surface water drains within this period (Lawler and Lanzaro, 2005) [11]. Mosquito larvae can also be controlled by the release of larvivorous fishes into the water bodies directly (Chandra *et al*., 2013) [12]. Compared to chemical agents, biological agent was used in the control of vectors which were more expensive and also affects some non-target organisms (Gillette, 1988) [13].

Numerous insecticides and pesticides were used for many decades in controlling population of mosquitoes' species and the most commonly used were organophosphates, organochlorines, and synthetic pyrethroids. Among these, pyrethroid-based formulations are the most widely used, followed by certain organophosphate and insecticides like carbamate. However, the common use of chemical insecticides like pyrethroids had developed resistance among mosquitoes (Grieco *et al*., 2007; Roberts *et al*., 1997) [14, 15] which in turn become a major challenge in the control programs against mosquitoes.

The present study is designed to evaluate the efficacy of the insect growth regulator (IGR) compound Lufenuron against mosquito larvae in storage tanks and polluted water.

Material and Methods

Laboratory evaluation was carried out against larvae of laboratory-colonized strains of *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* as per WHO procedure (WHO, 1981) [16]. The tests were performed in 500 ml glass beakers containing 250 ml of distilled water to assess the efficacy (growth-inhibiting activity) of Lufenuron on late second instar larvae. A total of 25 larvae were placed in each beaker for every concentration, and four replicates were used for each concentration and control. Thus, a total of 100 larvae were exposed to each concentration.

The eggs of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* were collected from ICMR–VCRC, Madurai. The

laboratory colony was maintained at 75–85% relative humidity, 27 ± 2ºC temperature, and a 14:10 light–dark photoperiod cycle. The larvae were fed with a powdered mixture of dog biscuits and yeast tablets in a 3:1 ratio.

The IGR compound, namely Lufenuron, chemically known as 1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-3-(2,6-difluorobenzoyl) urea, was received gratis as a 50% EC formulation from Joshi Agrochem Pharma Private Limited, Mumbai. A stock solution was prepared, and doses of 0.02, 0.002, 0.0002, 0.00002, and 0.000002 ppm were used in bioassays against the three-vector species.

All the larvae were exposed to Lufenuron formulation up to pupation stage and adult emergence stage. During the experimental condition, larvae were fed with larval food and mortality of larvae at every 24hr interval was recorded. The number of dead larvae, dead pupae and the number of partly emerged adults were regularly counted and removed from the field. The number of live pupae was observed until adult emergence stage. The inhibition percentage of adult emergence was calculated for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, and the EC_{50} and EC_{90} values (effective concentrations required for 50% and 90% emergence inhibition, respectively) was determined.

Field Evaluation

The field trial was conducted in cemented tanks and stagnant water bodies located in Ariyamangalam and Ponmalai, Tiruchirappalli District. The fields used by the animals for water drinking were selected as a breeding site to conduct the field trials.

Lufenuron was tested at four application doses: 0.02, 0.002, 0.0002, and 0.00002 ppm, in cemented tanks and stagnant water. Application doses were achieved by spraying pre-calculated amounts of Lufenuron using a hand compression sprayer.

Before spraying on the field, the density of immature stages was calculated using the samples collected by dipper method using a standard dipper of 300ml capacity with 9 cm diameter. Density of larvae and density of pupae per dip was measured in control and treated fields regularly at 24hr intervals for three days and then on weekly intervals in the later stage. Every day, from the samples collected from the fields, the number of late instars and pupae were counted and the percentage of adults emerged from the Lufenuron treated fields were recorded.

Data collected on different observation days were grouped to estimate mean of weekly observation and data collected on different periods and replicates were combined.

The reduction percentage of larvae density and pupae density was calculated using the formula given by Mulla *et al.* (1971) [17].

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

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

T_2 = Post-treatment immature density in treated sites

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

$$\% \text{ of inhibition of adult emergence} = 100 - \frac{No\ of\ pupae\ emerged\ into\ adults}{Total\ no\ of\ Pupae} X100$$

Larval culture and collection

Results

The Larvicidal efficacy of Lufenuron formulation against late fourth instar larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* under laboratory conditions is presented in Table 1. The larvae of *Cx. quinquefasciatus* were found to be more susceptible, followed by *Ae. aegypti* and *An. stephensi*, to the insect growth regulator (IGR) compound Lufenuron. The EI_{50} values indicated that the larvae of *Cx. quinquefasciatus* (EI_{50}: 0.012, 0.025, 0.035, and 0.045) were more susceptible than *Ae. aegypti* (EI_{50}: 0.026, 0.032, 0.037, and 0.047) and *An. stephensi* (EI_{90}: 0.028, 0.034, 0.042, and 0.051) at the concentrations of 0.02, 0.002, 0.0002, and 0.00002 ppm, respectively.

Table 1: Emergence inhibition activity of Lufenuron, an insect growth regulator (IGR), against three vector mosquitoes

Species	EI_{50} of emergence of mosquito larvae			
	0.02 ppm	0.002 ppm	0.0002 ppm	0.00002 ppm
Ae. Aegypti	0.026	0.032	0.037	0.047
Cx. quinquefasciatus	0.012	0.025	0.035	0.045
An. Stephensi	0.028	0.034	0.042	0.051

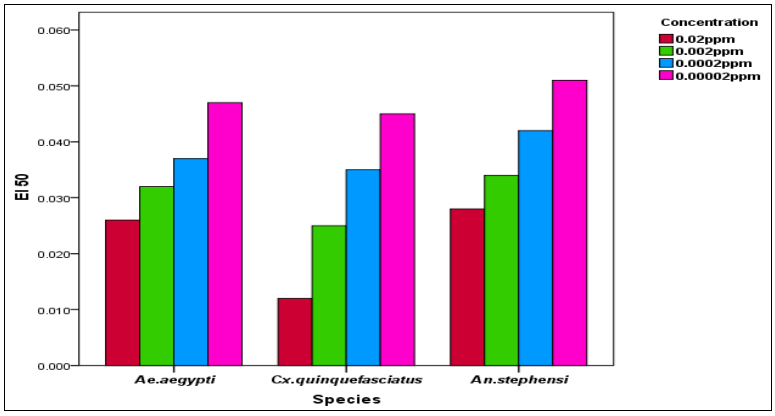

Fig 1: Emergence inhibition activity of Lufenuron, an insect growth regulator (IGR), against three vector mosquitoes

Table 2: Field evaluation of the efficacy of Lufenuron on adult emergence against *Aedes aegypti* was evaluated at 0.02 and 0.002, in two different breeding habitats

Days	Mean no of *Aedes aegypti* immature in 10 dips (% reduction)							
	Cement tank				Stagnant water			
	0.02 ppm		0.002 ppm		0.02 ppm		0.002 ppm	
	II-IV	P	II-IV	P	II-IV	P	II-IV	P
1st day	6.8	1.5	9.1	5.8	7.8	3.1	11.2	4.3
	(72.8)	(94)	(63.6)	(76.8)	(68.8)	(87.6)	(55.2)	(82.8)
5th day	4.5	1.1	6.8	3.9	5.1	2.9	5.8	3.1
	(82)	(95.6)	(72.8)	(84.4)	(79.6)	(88.4)	(76.8)	(87.6)
10th day	3.2	1	5.3	3	4.4	2.7	4.5	2.3
	(87.2)	(96)	(78.8)	(88)	(82.4)	(89.2)	(82)	(89.2)
15th day	2.7	1	3.2	2.8	1.8	1.6	3	1.8
	(89.2)	(96)	(87.2)	(88.8)	(92.8)	(93.6)	(88)	(92.8)
20th day	1.1	1	2.9	1.7	1.2	1	2.3	1.2
	(95.6)	(96)	(88.4)	(93.2)	(95.2)	(96)	(90.8)	(95.2)
25th day	0	0	1.2	1	0	0.8	1	1
	(100)	(100)	(95.2)	(96)	(100)	(96.8)	(96)	(96)
30th day	0	0	0	0	0	0.6	0	0.8
	(100)	(100)	(100)	(100)	(100)	(97.6)	(100)	(96.8)

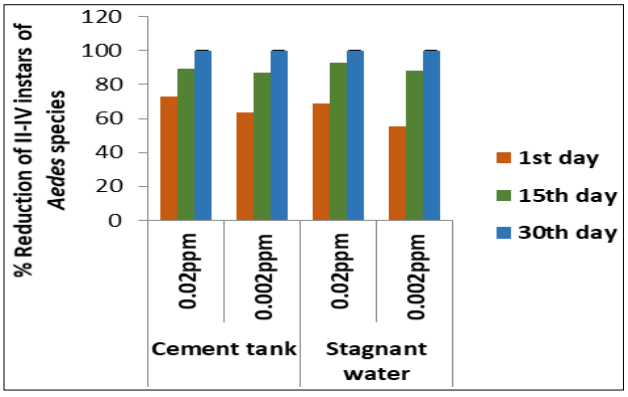

Fig 2: Reduction % of II-IV instars of *Aedes aegypti*

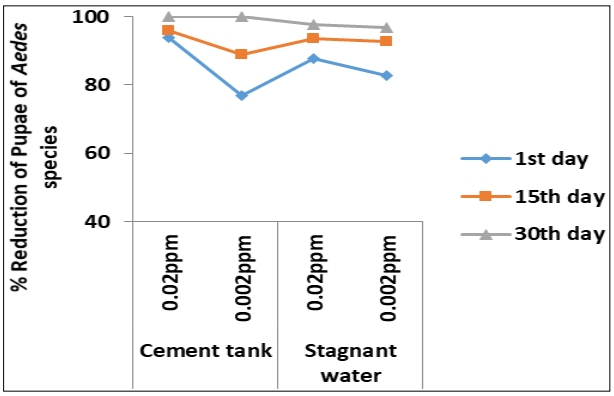

Fig 3: Reduction % of pupae of *Aedes aegypti*

Table 2 shows the mean number and reduction percentage of immature stages of *Ae. aegypti* in cement tanks and stagnant water. At the dose of 0.02 ppm, the mean number of instars was greatly reduced from day 0 to the 30th day in the cement tank. On day 0, a 72.8% reduction was observed, which gradually increased to 82%, 87.2%, and 89.2% on the 5th, 10th, and 15th days, respectively. Nearly 95.6% reduction of instars was recorded on the 20th day, and 100% reduction was achieved on the 30th day of treatment.

A 94% reduction in pupae was observed on the 1st day at 0.02 ppm of Lufenuron treatment, and complete (100%) reduction occurred by the 25th day post-treatment.

At the dose of 0.002 ppm, a 63.6% reduction of instars was recorded during the initial period of treatment, which gradually increased to 78.8% on the 10th day, 95.2% on the 25th day, and finally reached 100% reduction on the 30th day of treatment. The reduction in pupae was 76.8% at the initial stage, which increased to 88.8% on the 15th day and reached 100% reduction by the 30th day of treatment.

In stagnant water, at the dose of 0.02 ppm, the reduction in instar density ranged from 68.8% to 100% from the initial period up to the 25th day of treatment. The density of pupae decreased gradually from 87.6% to 97.6% within 30 days of treatment.

At the dose of 0.002 ppm, 55.2% reduction in instars was recorded during the initial period, which increased to 90.8% by the 20th day, and 100% reduction was observed on the 30th day (Figure 2). The pupal density reduced by 82.8% initially and reached 96.8% reduction after 30 days of treatment with 0.002 ppm Lufenuron.

When compared to stagnant water, the density of instars and pupae in cement tanks was completely reduced by the 25th day at 0.02 ppm (Figure 3). However, in stagnant water, 100% reduction was observed at 0.02 ppm on the 25th day after treatment, while complete reduction of instars at 0.002 ppm was achieved only after 30 days of treatment.

Table 3: Field evaluation of the efficacy of Lufenuron on adult emergence against *Culex quinquefasciatus* was evaluated at 0.02 and 0.002, in two different breeding habitats.

Days	Mean no of *Culex quinquefasciatus* species immature in 10 dips (% reduction)							
	Cement tank				Stagnant water			
	0.02 ppm		0.002 ppm		0.02 ppm		0.002 ppm	
	II-IV	P	II-IV	P	II-IV	P	II-IV	P
1st day	5.6	4.5	8.9	3.5	7.2	3.8	7.4	4.1
	(77.6)	(82)	(64.4)	(86)	(71.2)	(84.8)	(70.4)	(83.6)
5th day	4.7	3.7	5.5	2.8	4.5	3.1	5.2	3.9
	(81.2)	(85.2)	(78)	(88.8)	(82)	(87.6)	(79.2)	(84.4)
10th day	3.9	3.1	4.8	2	4.3	2.7	3.4	3.4
	(84.4)	(87.6)	(80.8)	(92)	(82.8)	(89.2)	(86.4)	(86.4)
15th day	2.6	2.7	2.8	1	1.4	1.6	2.6	2
	(89.6)	(89.2)	(88.8)	(96)	(94.4)	(93.6)	(89.6)	(92)
20th day	1.1	1.6	1.2	1	0.4	1	1.4	2
	(95.6)	(93.6)	(95.2)	(96)	(98.4)	(96)	(94.4)	(93)
25th day	0.8	1	1	0	0	0	0.5	1
	(96.8)	(96)	(96)	(100)	(100)	(100)	(98)	(96)
30th day	0	0	0	0	0	0	0	0.8
	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(96.8)

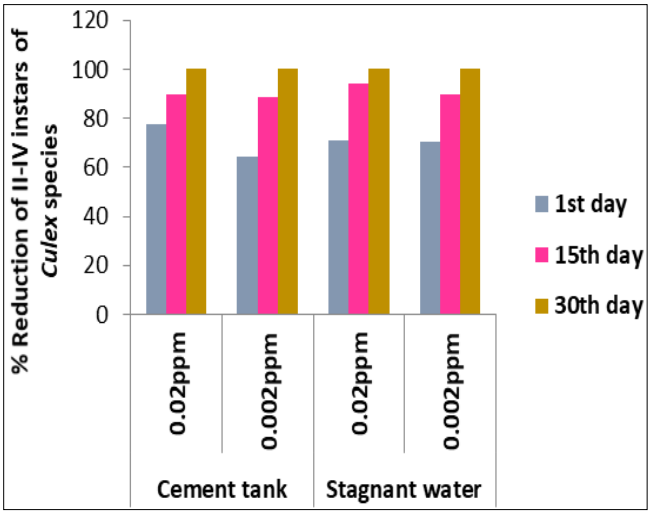

Fig 4: Reduction % of II-IV instars of *Culex quinquefasciatus*

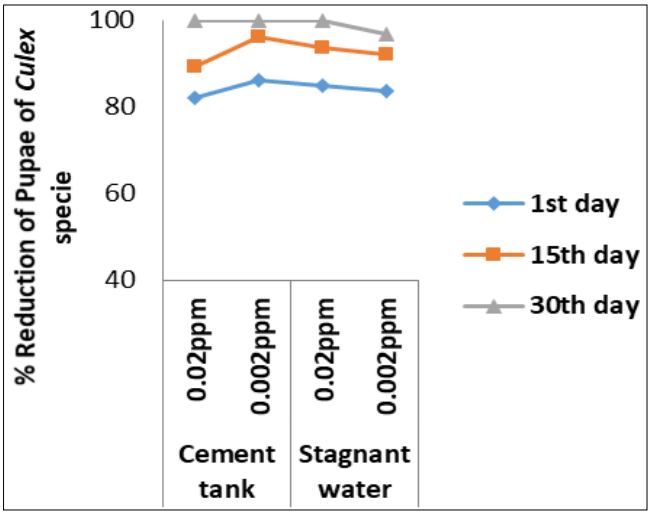

Fig 5: Reduction % of pupae of *Culex quinquefasciatus*

Table 3 showed the mean number and percent reduction of immature stages of *Cx. quinquefasciatus* in cement tank and stagnant water. In the cement tank, at a dose of 0.02 ppm, 77.6% reduction in instar density was observed during the initial period, which gradually increased to 81.2%, 84.4%, 89.6%, and 95.6% on the 5th, 10th, 15th, and 20th days of treatment, respectively. The 95.6% reduction observed on the 20th day increased to 96.8% on the 25th day, and a complete (100%) reduction in instar density was recorded on the 30th day of treatment.

The pupal density reduction was 82% during the initial period, which increased markedly to 96% on the 25th day and reached 100% on the 30th day of treatment. At a lower dose of 0.002 ppm, initially only 64.4% of instars were reduced, which gradually ranged from 78% to 96% between the 5th and 25th days, and a complete (100%) reduction was observed on the 30th day of treatment (Figure 4). Regarding pupal density, the reduction was 86% during the initial period, followed by a range of 88.8% to 96% between the 5th and 15th days, and complete reduction was achieved on the 30th day of treatment (Figure 5).

In stagnant water, at a dose of 0.02 ppm, 71.2% of *Cx. quinquefasciatus* instars were reduced during the initial observation period, which gradually increased to 82%, 82.8%, 94.4%, 98.4%, and finally 100% between the 5th and 25th days of treatment. Pupal density reduction was 84.8% initially, which increased from 87.6% to 100% within 30 days of treatment. At a dose of 0.002 ppm, the reduction of immature instars was 70.4% during the initial period, which increased from 79.2% to 94.4% within 5–20 days, and complete reduction was observed after 30 days of treatment. At this concentration, pupal density reduction was 83.6% initially, gradually increasing from 84.4% on the 5th day to 96.8% on the 30th day after treatment.

In the cement tank, 100% reduction of both instar and pupal densities was observed at both concentrations. In stagnant water, complete (100%) reduction of instars was observed at

both concentrations, while 100% pupal reduction occurred at 0.02 ppm and 96.8% at 0.002 ppm.

Overall, the results indicate that in both cement tank and stagnant water, reduction of instars, pupae of *Ae. aegypti* and *Cx. quinquefasciatus* occurred at a concentration of 0.02 ppm of Lufenuron, and the compound was more effective in reducing the density of instars and pupae of *Culex quinquefasciatus* compared to *Aedes aegypti*.

Discussion and Conclusion

Insect Growth Regulator (IGR) compounds such as triflumuron, diflubenzuron, methoprene and pyriproxyfen were recognized by WHO and diflubenzuron had been recommended by WHOPES evaluation for the controll of immature stages of mosquitoes (Chavasse and Yap, 1997) [18]. However, none of these compounds are currently in use for vector control in India.

IGR compound doesn't cause mortality among larvae at the doses recommended and causes difficulty in assessment in experimental situations. Sharma *et al.* (1979) [19] reported effective control (80–100%) of *Cx. pipiens fatigans* using Dimilin (diflubenzuron) at doses of 0.5 to 1 ppm in polluted drains. The residual larvicidal effect of Dimilin was lasted in field up to four days. The results proved efficacy of triflumuron against the breeding of *Ae. culicifacies*, *An. subpictus*, and *Cx. quinquefasciatus* in all the tested habitats were delayed. The delayed mortality of larvae indicated the strong developmental inhibition by IGR compound. About 90–100% emergence inhibition (EI) against the malaria vector *An. culicifacies* was observed in pools, fields of paddy for five weeks when treated with 1ppm of triflumuron. Therefore, triflumuron can be used at monthly intervals. Since EI was 100% for six weeks in drains against *Cx. quinquefasciatus* at 1 ppm and in cemented tanks at 0.5 ppm for seven weeks, the IGR could be applied once in every six weeks at a rate of 1 ppm in drains and 0.5 ppm in tanks.

In an earlier field report, effect of triflumuron was lasted for only one week against the larvae of *Cx. quinquefasciatus* in cesspits (Amalraj *et al.*, 1988) [20]. The study proved that triflumuron can be used for control of mosquito larvae in polluted water for longer duration where as conventional larvicide were ineffective which can last for shorter period. As the IGR shows a relatively long residual effect, the application frequency would be lower compared to conventional larvicide.

With reference to previous studies, the present results demonstrate that Lufenuron also exhibits significant larvicidal activity and can be used as an IGR to reduce the population of larval instars and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* at a concentration of 0.02 ppm. This, in turn, contributes to vector control more effectively than conventional chemical measures. Therefore, IGRs could be used as an additional tool in the National Vector Borne Disease Control Programme, ultimately reducing operational costs associated with vector control.

Conflict of Interest

There is no conflict of interest

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