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EFFECTS OF CARBARYL AND METHYL PARATHION ON BIMODAL RESPIRATION IN TADPOLES OF *RANA TIGRINA* (DAUD) (COMMON FROG)

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Abstrak: Kesan separa maut carbaryl (CA) dan methyl parathion (MP) ke atas corak respirasi dwimod dalam berudu *Rana tigrina* sebagai fungsi peringkat perkembangan telah dikaji. Kadar respirasi akuatik berudu menurun dengan peningkatan peringkat dari I hingga IV dalam berudu kawalan dan berudu yang didedahkan kepada pestisid. Walau bagaimanapun, respirasi udara menunjukkan trend yang berlawanan dan ini menunjukkan pertukaran mod daripada respirasi akuatik kepada respirasi udara semasa perkembangan berudu. CA menyebabkan penurunan respirasi akuatik dalam peringkat IV manakala MP mengurangkan respirasi akuatik dari peringkat II dan ke atas. Ini mungkin bertujuan untuk mengelakkan dan meminimumkan kerosakan insang oleh air yang dicemari pestisid. Keputusan yang didapati untuk jumlah respirasi adalah hampir sama dengan respirasi akuatik. Perubahan yang bergantung kepada peringkat dalam peratusan respirasi udara/akuatik diperhatikan dalam berudu kawalan dan berudu yang terdedah kepada pestisid. Sebagai contoh, peratusan respirasi akuatik berudu kawalan ialah 96.2% dalam peringkat I dan semakin berkurang kepada 93.8%, 82.6% dan 67.8% masing-masing dalam peringkat II, III dan IV. Respirasi udara menunjukkan trend bertentangan dalam semua berudu kajian. Kepekatan MP tertinggi mengurangkan respirasi udara sebanyak 8% berbanding berudu kawalan dalam peringkat II dan pengurangan ini ialah sebanyak 17% dan 16% masing-masing dalam peringkat III dan IV. Trend serupa juga didapati dalam berudu yang terdedah kepada CA tetapi tahap penurunan respirasi udara adalah kurang daripada berudu yang terdedah kepada MP. Ini menunjukkan bahawa MP adalah lebih toksik daripada CA dan pertukaran respirasi akuatik kepada respirasi udara yang pantas adalah disebabkan oleh tekanan pestisid.

Abstract: Sublethal effects of carbaryl (CA) and methyl parathion (MP) on bimodal pattern of respiration were studied in *Rana tigrina* tadpoles as a function of developmental stages. The rate of aquatic respiration of tadpoles was decreased with an increase in stage from I to IV in the control and pesticide exposed tadpoles; however, the aerial respiration showed the reversed trend and it indicates the shifting of the mode of respiration from aquatic to aerial during the development of tadpoles. CA caused a decline of aquatic respiration in IV stage while MP decreased the aquatic respiration from II stage onwards and it may be due to avoidance and to minimize the gill damage by pesticide contaminated water. The results obtained for total respiration were similar to those of aquatic respiration. A stage dependent change in the percentage of aerial/aquatic respiration was observed in control and pesticide exposed tadpoles. For instance, the aquatic respiration of control tadpoles was 96.2% in I stage and it gradually reduced to 93.8%, 82.6% and 67.8% in II, III and IV stages respectively. The aerial respiration showed the opposite trend in all the experimental tadpoles. The highest concentration of MP reduced the aerial respiration by 8% compared to control tadpoles in II stage and this reduction was 17 and 16% in III and IV stages respectively. Similar trend was also obtained in tadpoles exposed to CA but the level of reduction of aerial respiration was less than those exposed to MP. This suggests

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that, MP is more toxic than CA and shifting of respiration from aquatic to aerial was quick due to the pesticide stress.

Keywords: Carbaryl, Methyl Parathion, *Rana tigrina*

INTRODUCTION

Pesticide pollution poses a constant threat to frog population in India. Ecologists warn that the diminishing frog population can upset the ecological balance, resulting in the abundance of vector and pests (Abdul Ali 1985). *Rana tigrina* is a common frog seen in paddy fields. Water bodies adjacent to paddy fields are directly exposed to the different kinds of pesticides and hence a study on *R. tigrina* tadpole with reference to abundantly used pesticides like carbamates (e.g., carbaryl (CA)) and organophosphates (e.g., methyl parathion (MP)) has become imperative. The present paper reports on the comparative study of toxic effect of CA and MP on the respiratory physiology of *Rana tigrina* tadpoles.

MATERIALS AND METHODS

Rana tigrina tadpoles were produced in the laboratory following the technique of hypophysation (Rugh 1934). Tadpoles were fed with pellet feed (40% protein) and feeding of tadpoles commenced two days after hatching (Pandian & Marian 1986). Ten healthy tadpoles of three days old (15 ± 2 mg) were separately exposed to nine different concentrations of carbaryl (CA: 0, 2, 3, 4, 5, 6, 7, 8 and 9 ppm) and methyl parathion (MP: 0, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 ppm). A control was also maintained in well water. Static, renewable bioassay test was conducted to determine the 96 hours LC50 value of tadpole against CA and MP. The experiment was conducted in circular plastic trough containing 6 liters of test media. Test animals were not fed during the bioassay test. Fresh concentrations were prepared daily to maintain the constancy of the pesticide in the test media. Mortality of the tadpoles was observed for 96 hours and its median lethal concentrations were calculated following the method of Finney (1971). One third of the LC50 was selected as the maximum sublethal concentration for the present study (James *et al.* 1992).

After determining 96 hours LC50 (5.68 ppm for CA and 4.36 ppm for MP), three levels of CA (0.57, 1.14 and 1.70 ppm) and MP (0.44, 0.87 and 1.31 ppm), viz, 10, 20 and 30% of 96 hours LC50 were chosen as the test concentrations. A control was also maintained. Ten tadpoles (15 ± 2 mg) were reared in each concentration and control group until they reached the froglet stage (31 days). The experiment was conducted in circular plastic troughs containing 6 liters of test media and triplicates were maintained for each group. Tadpoles undergo distinct morphological changes during metamorphosis (growth). In the present study, tadpoles were classified into four stages based on their morphological indices following the method of Gosner (1960). They are appearance of hind limb (stage I), disappearance of external gill (stage II),

appearance of fore limb (stage III) and disappearance of tail (stage IV). The experimental tadpoles were fed *ad libitum* with pellet feed and uneaten feed was removed after 2 hours. The experimental medium was changed daily and fresh concentrations were prepared before feeding. The hydrobiological parameters such as Dissolve oxygen (DO), temperature, pH, hardness and salinity of different experimental exposures did not vary much and averaged to 4.40 ml l^{-1}, 26 ± 1ºC, 7.28, 70 mg $CaCO_3^{-1}$ and 0.11 ppt respectively.

Simple respiro manometric technique was followed to estimate aquatic and aerial respirations (bimodal) of tadpoles simultaneously. Tadpoles of different developmental stages (I, II, III and IV) exposed to chosen sublethal concentrations (10, 20 and 30% of 96 hours LC50) of CA (0, 0.57, 1.14 and 1.70 ppm) and MP (0, 0.44, 0.87 and 1.31 ppm) were weighed and taken in animal chamber where the same concentrations were maintained in 350 ml water. The tadpoles were fed *ad libitum* between 8 and 10 hours and the experiment was conducted after 12 hours. The size and number of animals per animal chamber (1–5) were varied to achieve a similar ratio of animal volume for control and experimental animals. Aerial respiration of tadpole was measured by multiplying the constant (calibrated value) with the rise of indicator fluid in the capillary tube of manometer. For the estimation of aquatic respiration, 10 ml of water was drawn every hour through the outlet and the dissolved oxygen content was measured following the unmodified Winkler's method. Oxygen level at the end of each hour was considered as the initial reading for the next hour estimation. After 3 hours, the water was changed. The next set of readings was taken after 1 hours. The reading was taken only after leaving the animals for about 30 minutes to acclimatize in the animal chamber. The difference between the initial and final oxygen contents represented the aquatic respiration of the animals. The oxygen consumption values were calculated as $mlO_2g^{-1}hr^{-1}$. These values were converted into calorific values using oxy-calorific value of 20.1 J $ml^{-1}O_2$ (Elliott & Davidson 1975). The rate of oxygen consumption was calculated using the following equation.

$$\text{Rate of aquatic respiration } (J\ g^{-1}day^{-1}) = \frac{\text{Aquatic respiration of a stage } (J\ day^{-1})}{\text{Weight of the tadpole (g)}}$$

$$\text{Rate of aerial respiration } (J\ g^{-1}day^{-1}) = \frac{\text{Aerial respiration of a stage } (J\ day^{-1})}{\text{Weight of the tadpole (g)}}$$

$$\text{Rate of total respiration } (mlO_2g^{-1}day^{-1}) = \frac{\text{Aquatic + aerial respiration of a stage } (J\ day^{-1})}{\text{Weight of the tadpole (g)}}$$

Two-way ANOVA was applied to determine the significance of interaction between pesticide concentrations and developmental stages of tadpoles on the bimodal respiration. Students 't' test was used to determine the significance of

mean values between control and experimental groups. Correlation and regression were applied following the least square method (Zar 1974).

RESULTS

The 96 hours LC50 values of tadpoles exposed to CA and MP were 5.68 ppm and 4.36 ppm respectively. The rate of aquatic respiration of tadpoles was decreased with an increase from stage I to IV in control and pesticide exposed tadpoles (Table 1).

A significant ($P < 0.05$) negative correlation was obtained between the stages of tadpoles and rate of aquatic respiration. The results obtained for aerial respiration was opposite to that of aquatic respiration and a significant ($P < 0.05$) positive relationship existed against the developmental stages of tadpoles and aerial respiration. Another interesting observation of the present study was an increase in the concentrations of CA showed a significant positive correlation with the aquatic respiration till third stage, followed by a negative correlation ($P < 0.01$) in the IV stage.

Table 1: Effect of sublethal concentrations of CA and MP on aquatic and aerial respiration ($Jg^{-1}day^{-1}$) in different stages of *R. tigrina* tadpoles. Each value represents the average ($\overline{X}$ ± SD) of three observations at 26 ± 1^0C.

	Aquatic	Aerial	Aquatic	Aerial	Aquatic	Aerial	Aquatic	Aerial
	Sublethal levels of CA (ppm)							
Stages	0		0.57		1.14		1.70	
I	580.0 ± 26.46	23.8 ± 4.36	626.7 ± 11.55	28.3 ± 5.51	642.3 ± 21.40	29.0 ± 3.60	663.7 ± 41.30	38.7 ± 4.70
II	491.3 ± 45.40	32.3 ± 4.90	539.7 ± 16.26	49.0 ± 5.00	547.7 ± 15.50	57.3 ± 2.30	585.3 ± 64.30	60.70 ± 5.00
III	443.7 ± 22.50	93.3 ± 6.10	450.0 ± 2.00	94.0 ± 5.30	460.0 ± 43.00	108.7 ± 10.10	530.0 ± 20.00	118.7 ± 4.20
IV	292.3 ± 10.26	138.7 ± 5.10	300.0 ± 20.00	138.7 ± 5.10	243.3 ± 41.60	150.3 ± 12.50	221.3 ± 22.00	163.0 ± 2.00
	Sublethal levels of MP (ppm)							
	0		0.44		0.87		1.31	
I	580.0 ± 26.46	23.0 ± 4.36	637.3 ± 20.60	30.3 ± 3.79	704.7 ± 39.27	32.0 ± 1.00	725.0 ± 93.20	37.3 ± 3.06
II	491.3 ± 45.45	32.3 ± 4.93	539.0 ± 18.19	52.0 ± 5.60	431.7 ± 27.50	61.3 ± 7.50	395.7 ± 22.10	65.0 ± 2.00
III	443.7 ± 22.50	93.3 ± 6.11	389.7 ± 62.63	119.0 ± 3.00	380.0 ± 20.00	136.7 ± 15.30	323.7 ± 7.23	168.0 ± 2.00
IV	292.3 ± 10.26	138.7 ± 5.13	240.0 ± 20.00	148.7 ± 9.50	230.0 ± 10.00	165.0 ± 6.08	200.0 ± 20.00	183.7 ± 2.08

Table 2: Correlation coefficient (r) and regression equations obtained for aquatic and aerial respiration of *R. tigrina* tadpoles exposed to CA and MP.

Parameters	Stages	r	Y = a + bx	r	Y = a + bx
		CA		MP	
Aquatic	I	0.67	Y = 589.0 + 2.75	0.78	Y = 585.7 + 5.10x
	II	0.68	Y = 497.9 + 2.84x	–0.73	Y = 523.6 - 3.94x
	III	0.72	Y = 430.6 + 2.69x	–0.81	Y = 439.7 - 3.70
	IV	–0.77	Y = 304.7 - 2.70x	–0.90	Y = 283.6 - 2.87x
Aerial	I	0.79	Y = 22.61 + 0.50x	–0.86	Y = 24.0 + 0.45x
	II	0.90	Y = 35.8 + 0.93x	0.90	Y = 36.6 + 1.07x
	III	0.84	Y = 90.1 + 0.91x	0.96	Y = 93.0 + 2.42x
	IV	0.81	Y = 135.0 + 0.85x	0.95	Y = 136.3 + 1.51x

MP showed a significant positive relationship with aquatic respiration in first stage, however, it became negative from II stage onwards (Table 2). While considering the aerial respiration in the selected stages of tadpoles exposed to CA and MP, the rate increased significantly ($P < 0.01$) in all the chosen sublethal levels of pesticides. The 'b' value indicated that the impact of MP on respiration was more than that of CA (Table 2). Two-way ANOVA test revealed that aerial respiration was significantly ($P < 0.01$) influenced by both the sublethal concentrations of CA or MP and stages. However, the rate of aquatic respiration was not significantly ($P > 0.05$) influenced by the concentrations of CA or MP but the stage exerted highly significant ($P < 0.01$) influence.

The results obtained for total respiration was similar to those of aquatic respiration. In CA exposed tadpoles, correlation coefficient obtained revealed that there was a significant ($P < 0.05$) positive relationship between the total respiration and concentrations up to third stage; however, the relationship became negative ($r = –0.70$; $P < 0.01$) in IV stage (Table 3). Similarly, in MP exposed tadpoles, correlation showed a positive relationship with total respiration only in I stage ($r = 0.81$; $P < 0.01$) and in other stages the trend was negative (Table 3). A stage dependent change in the percentage of aerial/aquatic respiration was noticed in control and pesticides exposed tadpoles. For instance, the aquatic respiration of control tadpole was 96.2% in first stage and it gradually reduced to 93.8%, 82.6% and 67.8% in II, III and IV stages respectively. The aerial respiration elicited the opposite trend in all the experimental tadpoles (Fig. 1 and 2). MP (1.31 ppm) reduced the aerial respiration by 8% compared to control in II stage and this reduction was 17% and 16% in III and IV stages respectively. Similar trend was also obtained in tadpoles exposed to CA but the level of reduction of aerial respiration was less than those exposed to MP.

Table 3: Effect of different sublethal concentrations of CA and MP on total respiration ($Jg^{-1}day^{-1}$) in different stages of *R. tigrina*. Each value represents the average ($\overline{X}$ ± SD) of three observations at 26 ± 1^0C.

	Sublethal levels of CA (ppm)					
Stage	0	0.57	1.14	1.70	r	Y = a + bx
I	603.0 ± 3.60	648.3 ± 17.62	671.0 ± 23.69	702.0 ± 37.90		
II	523.7 ± 24.50	590.0 ± 20.00	610.00 ± 20.00	644.0 ± 39.43	0.78	Y = 534.8 + 3.81x
III	537.0 ± 26.85	544.0 ± 24.25	568.7 ± 22.55	648.7 ± 17.24	0.77	Y = 520.6 + 3.60x
IV	431.0 ± 6.08	438.7 ± 23.80	393.7 ± 29.94	384.3 ± 23.18	–0.70	Y = 440.0 - 1.85x
	Sublethal levels of MP (ppm)					
	0	0.44	0.87	1.31		
I	603.0 ± 3.61	667.7 ± 24.00	736.7 ± 39.88	762.3 ± 50.47	0.81	Y = 616.7 + 5.20x
II	523.7 ± 24.52	591.0 ± 18.30	493.0 ± 31.40	460.7 ± 22.50	–0.60	Y = 560.0 - 2.87x
III	537.0 ± 26.85	508.1 ± 31.70	516.7 ± 5.77	491.0 ± 9.07	–0.44	Y = 532.7 - 1.28x
IV	431.0 ± 6.08	388.7 ± 17.04	395.0 ± 12.12	383.7 ± 18.01	–0.69	Y = 419.9 - 1.36x

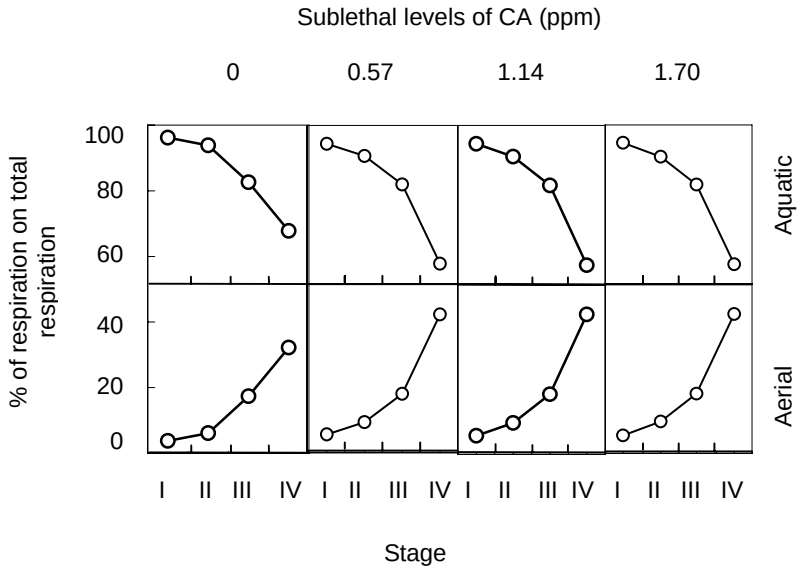

Figure 1: Effect of sublethal levels of CA on the percentage of aquatic and aerial respiration on total respiration (100%) in different stages of *R. tigrina t*adpoles.

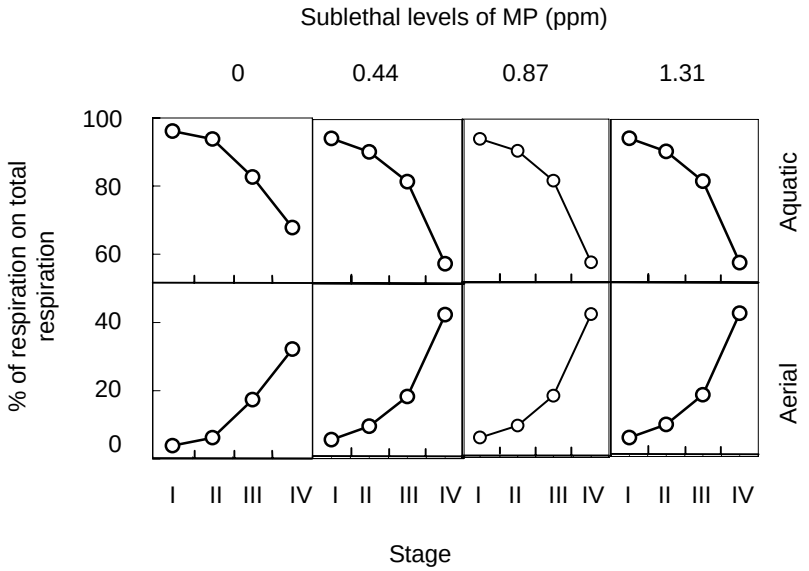

Figure 2: Effect of sublethal levels of MP on the percentage of aquatic and aerial respiration on total respiration (100%) in different stages of *R. tigrina* tadpoles.

DISCUSSION

The present study reveals that the rate of aquatic respiration showed a declining trend while aerial respiration showed an elevating trend as a function of developmental stages of tadpoles. This trend indicates the shifting of respiration from aquatic to aerial during the development of tadpoles which is known as respiratory transition (Feder 1981). A stage and concentration wise change in aquatic respiration was observed in the tadpoles exposed to pesticides. The positive correlation between pesticide concentrations and the aquatic respiration in early stages of tadpoles exposed to both CA (up to III stage) and MP (in I stage) might be due to some behavioural manifestation like hyperactivity (Bhusari *et al.* 1985). In the tadpoles exposed to CA, the oxygen consumption from aquatic medium significantly decreased in IV stage and this decrease occurred from II stage onwards in MP treated tadpoles. The decrease in aquatic respiration might be due to some escape behaviours like avoiding polluted water to minimize gill damage or protective measures like secreting mucous over gills (Bhusari *et al.* 1985). Since both gill and vascularized skin (Strawinski 1956) serve the function of aquatic respiration, there is the possibility of damage to these respiratory surfaces and a consequent decrease in aquatic respiration during long term exposure to pesticides. Another possibility for the reduction in aquatic respiration might be more utilization of aerial respiratory surface to avoid pesticide contaminated water. Reduction in aquatic respiration could be an adaptation to conserve energy as aquatic respiration requires considerable energy due to physical property of water (Holeton 1980) as the oxygen

concentration per unit volume in saturated water is 30 times less than in air (Schmidt-Nielsen 1979).

The present study revealed that *R. tigrina* tadpoles commence aerial respiration from I stage itself. The tadpoles of *Xenopus laevis* started respiring air from 25^{+} stage, the first free swimming larval stage (Feder 1985). Ontogenic observations of aerial respiration in amphibians document, the initiation of air-breathing well before metamorphic climax. Thus anurans could perform, bimodal gas exchange for a substantial portion of the larval period, if not during all free-swimming stages (Feder 1985). The remarkable increase in aerial respiration with regard to the pesticide concentrations in all stages indicated a preference for aerial respiration by tadpoles in pesticide polluted water. To corroborate this view, Costa (1967) and Feder (1983) reported that the tadpoles stay out of water contaminated with toxicants. During stress (e.g., aquatic hypoxia), the anuran larvae resort to aerial respiration, to a far greater extent than aquatic respiration as the former is an adaptation for amphibian vertebrates (Feder 1985). Working on *R. cyanophylictis*, Marian *et al.* (1980) found that the tadpole exposed to low dissolved oxygen (5.8 mg l^{-1}), preferentially used the aerial respiratory surface (above 50%) over aquatic which is suggested as a shift in the mode of respiration due to vascular mechanism (Wassersug & Seibert 1975). In the present study, the proportion of aerial respiration was less (32%) in the control tadpoles (IV stage) compared to MP (48%) treated group (1.31 ppm). This observation indicates that *R. tigrina* tadpoles exposed to 1.31 ppm MP were on for a 'shift' in vascular mechanism.

The tadpoles exposed to CA showed a decreasing trend in total respiration in IV stage; MP, however showed similar trend in II stage itself (Table 1). The reduction in total respiration indicates an alternative response of tadpole to get energy due to stress, which increases the energy demand. Stress caused due to MP exposure increased the energy requirement in *R. cyanophlictis* tadpoles (Naiyara Yasmeen & Nayeemunnisa 1992). Feder (1983) emphasized that the amphibians reduce or interrupt normal respiration as a response to stress which induces anaerobiosis. MP, because of its higher toxicity than CA, perhaps reduced the total respiration leading to anaerobiosis well before metamorphosis (from II stage). Anaerobic metabolism can provide more than half of the energy requirement of an amphibian and it predominates during stress (Hutchison & Miller 1979).

CA altered the percent contribution of aerial respiration in IV stage ($P < 0.05$) when tadpoles were exposed to the highest concentration (1.70 ppm). However, MP caused the alteration in II stage ($P < 0.05$) itself in 0.87 ppm. It indicates that the pesticides not only change the respiratory pattern but also the ratio by which the tadpole respire in water and air. Figures 1 and 2 illustrate a gradual narrowing of the difference in the percentage of aquatic and aerial respiration due to increase in the concentration of pesticides. This suggests a possible shift from one respiratory pattern to the other due to the pesticide stress. It could be assumed that the trend in the percentage change is due to significant decrease in aquatic respiration in that particular stage (CA: $P < 0.05$ at IV stage; MP: $P < 0.01$ at II stage) (see Verma *et al.* 1980). The general implication is that both CA and MP induce change in the ratio of aquatic and aerial respiration due

to irregular alterations in respiratory patterns with the possibility of entering into anaerobiosis beyond certain stress level.

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