"Quantitative and qualitative evaluation of effect of Dimethoate on amylase activity in Silkworm *Bombyx mori* Linnaeus."

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ABSTRACT

Two races of silkworm namely PM X CSR_2 and CSR_2 were selected for the present investigation. The digestive juice and haemolymph were collected from 2nd, 4th and 6th day larvae of fifth instar fed with Dimethoate treated leaves in 10, 20 and 30 ppm. The samples were subjected to spectrophotometric analysis for amylase activity and the recorded O.D. values (at 525 nm) were converted into maltose released.

Accordingly, the results clearly indicate that the amylase activity gets enhanced with the increase in number of feedings along with the advancement of day wise in 5th instar larva. PM X CSR_2 performed better for amylase activity in haemolymph with respect of dimethoate treated mulberry leaf varieties in 30ppm concentration at 12h after treatment, compared to CSR_2 and control batch and was also true in 10ppm, 20ppm. But in gut juice of silkworm $CSR_{2\ has}$ shown better performance better at 30ppm, at12h after treatment than PM X $CSR_{2\ and}$ also in other concentrations used for treatment. Amylase activity was found to be higher in gut juice than haemolymph of silkworm in all the batches understudy.

Key words: *Bombyx mori* L. Amylase, Digestive juice, Haemolymph, Spectrophotometer.

INTRODUCTION

The *Bombyx mori* is an important economic sericigenous insect which feed mainly on mulberry leaf and convert protein into silk protein (Babu *et. al*. 2009). In silkworm majority of the characters that contribute to the yield of silk are under the control of polygenes (Kanata *et. al*., 2011).

Enzymes play a vital role in the metabolism of dietary food in the body of an organism. Amylase is an important enzyme involved in the metabolism of carbohydrates in the silkworm. Amylase performs the

function of catalysis by way of hydrolysis of starch into sugars. The two forms of hydrolyses, alpha-amylase and alpha-glucosidase are key enzymes involved in starch breakdown and absorption, respectively. It is now believed that inhibition of these enzymes involved in the digestion and uptake of carbohydrates can significantly alter the total carbohydrate level in the mid-gut and haemolymph. **Dimethoate** is a widely used organophosphate used to kill insects on contact. It was patented and introduced in the 1950s by American Cyanamid. Like other organophosphates, Dimethoate is an acetyl cholinesterase inhibitor which disables cholinesterase, an enzyme essential for the function of the central nervous system. The present study was carried out to understand the effect of sub-lethal dose of Dimethoate on important economic traits and physiology of the treated batch of silkworm *Bombyx mori* and correlate the same with untreated batch.

Methods and Materials: In the present study the disease free layings of multi-bi hybrid $PMXCSR_2$ and bivoltine hybrid CSR_2 were procured from Silkworm Seed Production Centre (SSPC), Central Silk Board, Mysuru a unit of National Silkworm Seed Organization were reared following the standard rearing methodology (Dandin and Giridhar, 2010*)*. During the rearing S_{36} variety leaves were fed to young age larvae, while V_1 variety mulberry leaves were fed to late-age larvae thrice a day. Both the varieties were cultivated in the irrigated garden of Department of Sericulture Science, Manasa Gangothri, Mysuru. The larvae hybrid $PMXCSR_2$, and CSR_2 after 4^{th} moult were divided into four batches of 100 larvae each. Batch 1 to 3 was sprayed with three different concentrations of Dimethoate and the batch 4 was control batch without any treatment and all batches were maintained in three replicates. The performances of various economic traits were recorded during silkworm rearing for evaluation and correlation with the results of the biochemical studies. The selected traits are matured larval weight, total larval duration; cocoon yield, single cocoon weight, single shell weight, shell ratio, filament length, reelability, renditta, fecundity and hatching percentage were recorded. The temperature ranged between 26 - 30^0C and the relative humidity was in the range of 55 – 70 % during the conduct of rearing.

Dimethoate (10, 20 & 30ppm) were prepared in 100 ml distilled water, mulberry leaves were dipped in all three concentrations of Dimethoate solutions and were surface dried in shade and these leaves were then fed to the 2^{nd}, 4^{th} and 6^{th} day larvae of 5^{th} instar. Samples were collected from 2^{nd}, 4th and 6^{th} day of 5th instar dimethoate treated larvae into precooled tubes (5°C). Larvae were exposed to chloroform vapours for collecting the digestive juice and abdominal legs were cut for haemolymph collection. Collected samples

was then centrifuged at 10,000rpm for 10 minutes to remove undigested leaf particles add pinch of thiourea and stored at -20°C until further use.

Estimation of Amylase activity (Noelting and Bernfeld, 1948)

2ml of 0.2% starch and 20 micro liters of samples were added to each test tubes were incubated at 30°c for 30 minutes on hot water bath. Then 2ml of 3-5 DNS (Dinitrosalicylic acid **)** was added and boiled on water bath at 50-60°c for 5 minutes and cooled immediately under running water and take the OD values of the end products by using spectrophotometer at 525nm.

Results:

The present investigations were undertaken in the digestive (gut) juice and haemolymph (day 2, 4 and 6) of 5th instar treated and untreated larvae from multi-bi hybrid namely PMXCSR$_2$ along with CSR$_2$ to understand the diversity of amylase activity by quantitative estimation using spectrophotometric analysis. The amylase activity in different samples and treatments is described below. The mean values of amylase activity with standard error are expressed in µm of maltose per ml of sample/30 minutes

Amylase activity in digestive/gut juice:

Among the batches selected for the present study, the amylase activity in digestive juice was highest in CSR$_2$ (202 µm/ml of sample) followed by PM X CSR$_2$ (95µm/ml) where similar results were seen in 10 ppm concentration with 128 µm/ml of sample being highest and lowest was 62 µm/ml of sample in PM X CSR$_2$ while 71 µm/ml of sample and 128 µm/ml of sample for CSR$_2$. Similarly, the trend of amylase activity was highest with 185 µm/ml and lowest value of 44 µm/ml of sample in CSR2 on 6th day after 12 and 24 hour treatment was observed for 20 ppm. While in 30 ppm, a lowest of 26 µm/ml of sample and highest of 192 µm/ml of sample was recorded.

Amylase activity in Haemolymph:

Among the batches selected for the present study, the amylase content in haemolymph was found higher in PMXCSR$_2$. Amylase activity was lowest in PMXCSR2 with 89 µm/ml of sample in 2nd day and CSR$_2$ recorded highest of 179 µm/ml of sample in 4th day for control. In 10 ppm concentration, lowest is 62 µm/ml of sample and 128 µm/ml of sample both in PMXCSR$_2$ was observed. In 20 ppm concentration,

31 µm/ml of sample in CSR_2, in 6th day of treatment and 128 µm/ml of sample was highest in $PMXCSR_2$ in 4th day was recorded. In 30 ppm, lowest was 26 µm/ml of sample for CSR_2, while 210 µm/ml of sample µm/ml of sample for $PMXCSR_2$ in 6thday of treatment was observed.

The mean values with standard error of all the three batches selected for the study is presented in the tabular form and the results in the form of graphs are presented below.

Fifth instars fourth day, the amylase activity of Haemolymph 12 hours with respect to treated leaves fed, PM X CSR_2 (210 µm) 30ppm 6th day and CSR_2 (174µm) 30ppm followed by CSR_2,20ppm(166µm) and PM X CSR_2 20ppm(140 µm) showed higher activity followed by 4th day 30ppm PM X CSR_2 (192µm) and CSR_2 (178µm) followed by 4th day 20ppm PM X CSR_2 (168µm) showed higher activity .In 2nd day decreased the Amylase activity(Fig:3).

Fifth instars fourth day, the amylase activity of Haemolymph 24 hours with respect to treated leaves fed, CSR_2 10ppm(116µm) and PM X CSR_2 (107µm) 10ppm followed by PM X CSR_2,20ppm(100µm) and CSR_2 (90µm) 20ppm showed higher activity In 6thday decreased the Amylase activity. In 2nd day showed minimum activity in 10 and 20ppm (Fig:4).

*After 12 hours with respect to treated leaves fed showed higher amylase activity

Compare to after 24 hours. Control CSR_2 showed higher activity compare to control PM X CSR_2 and other ppm Treated worms in all days.

* Fifth instars fourth day, the amylase activity of Haemolymph 12 hours with respect to treated leaves fed, PM X CSR_2 (210µm) 30ppm showed higher activity than other ppm and 24h and second, fourth day sample.

* In 2nd day showed minimum activity in 10 and 20ppm.

Discussion and conclusion:

Profound biochemical changes occur in the insect body particularly by secretion of hormones and enzymes, which undergo variations during growth and development of the organism. There are many reports that show gradual increase in amylase activity during feeding stages throughout the larval stage of *Bombyx mori*. The increase in the levels of amylase may be due to ingestion and digestion of mulberry

leaves consumed which perhaps creates a suitable environment for absorption and its utilization during the growth and developmental process.

Amylase activity gradually increases with the advancement of age of the larvae during 5^{th} instar from day 1 to 6 and number of feedings in the control NB_4D_2, and larval mutants viz., Zebra, Knobbed and Ursa (Umakanth and Devamani, 2016). The amylase content is higher in the digestive juice than haemolymph in the order of merit, PMXCSR2 show highest activity followed by CSR2. As the concentration of Dimethoate increases, amylase activity also increased substantially which is due to the larvae getting more active physiologically. However, the role of haemolymph amylase is not yet known, it may participate in the degradation of glycogen in haemolymph according to Wyatt (1967). Chatterjee *et al* (1973) reported the importance of digestive amylase activity for the survival of the silkworm by way of disease resistance.

The activity is lower in 10 ppm treatment than control batch while 20 and 30 ppm show higher activity than control. There is higher activity in 4^{th} day of treatment followed by 6^{th} day and 2^{nd} day of treatment in both 12 h and 24 h after treatment in the amylase content in all the batches of the silkworm under study, as the larvae mature and leaf consumption gets reduced as they approach the spinning process.

- In all days of treated worms showed result as below mentioned
- Untreated worms showed significant correlation than treated worms and also in other economic traits.
- CSR_2 control showed higher shell weight than other races ,renditta low in control CSR_2
- Larval weight and cocoon weight higher in control PM X CSR_2.

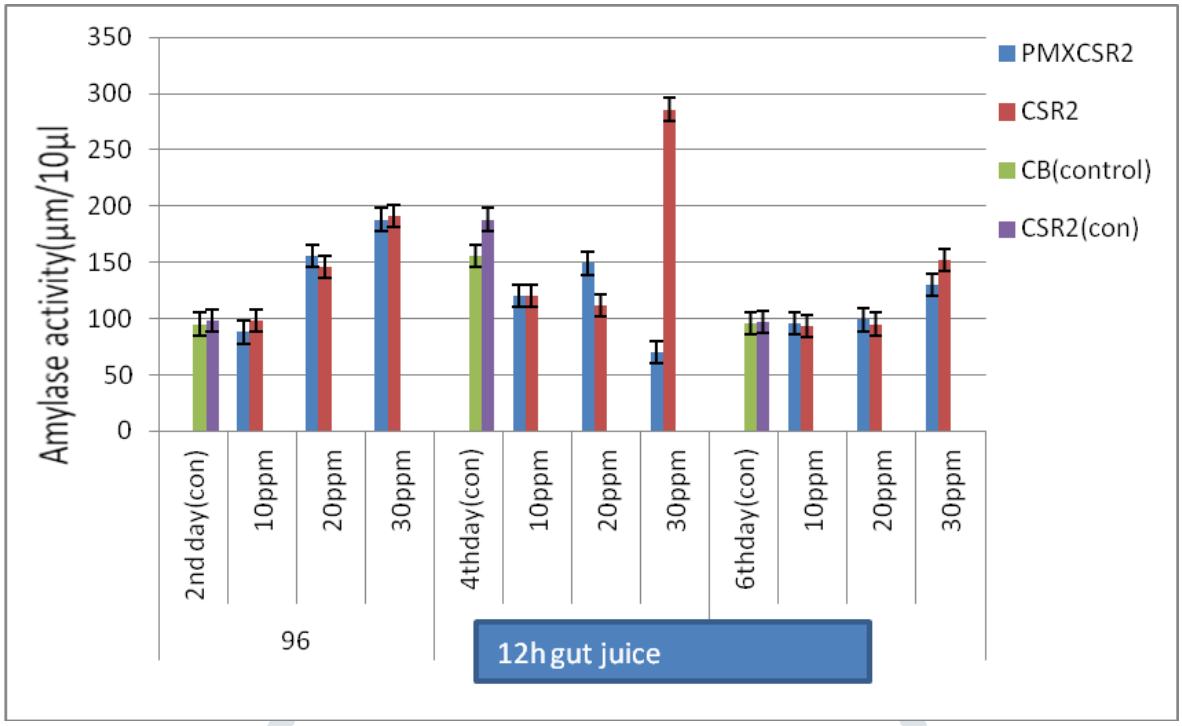

Fig : 1 Amylase activity in silk worm gut juice (after 12h of treatment)

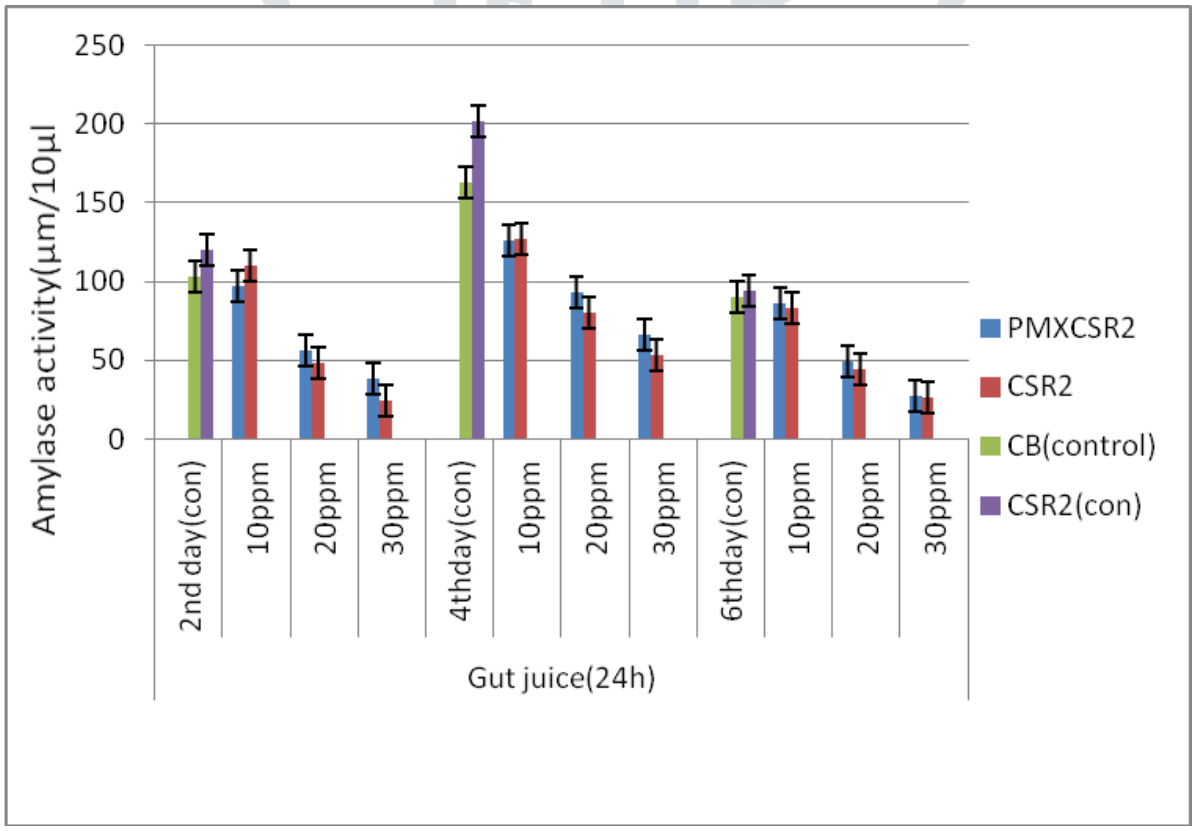

Fig : 2 Amylase activity in silk worm gut juice (after 24h of treatment)

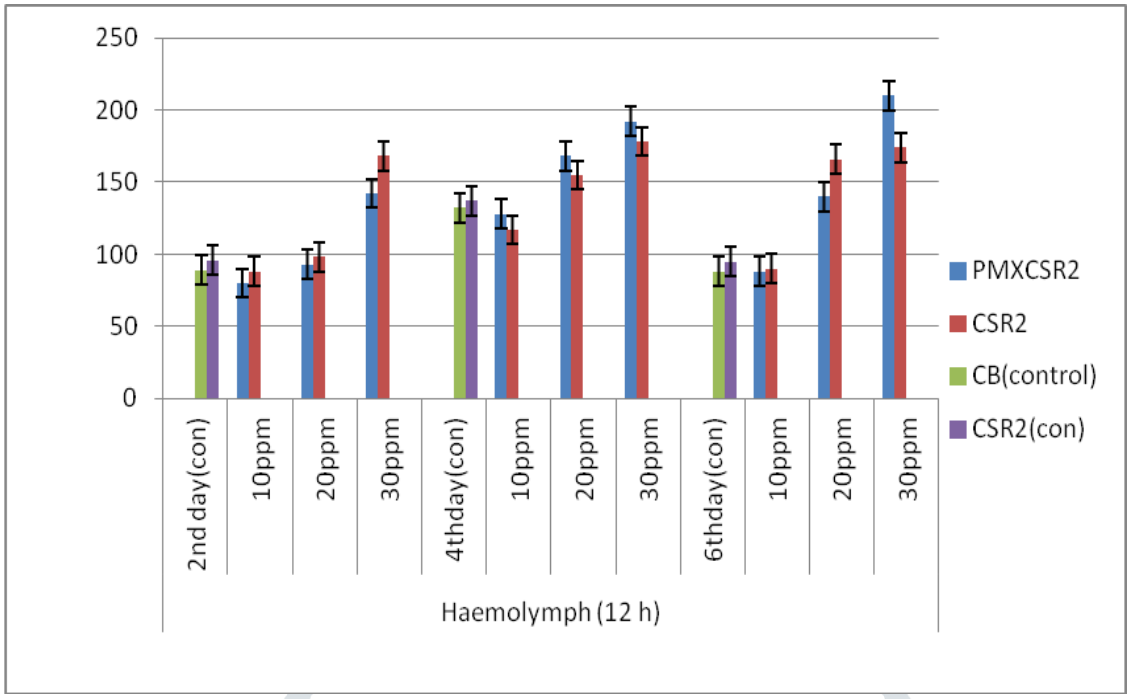

Fig : 3 Amylase activity in silk worm Haemolymph (after 12h of treatment)

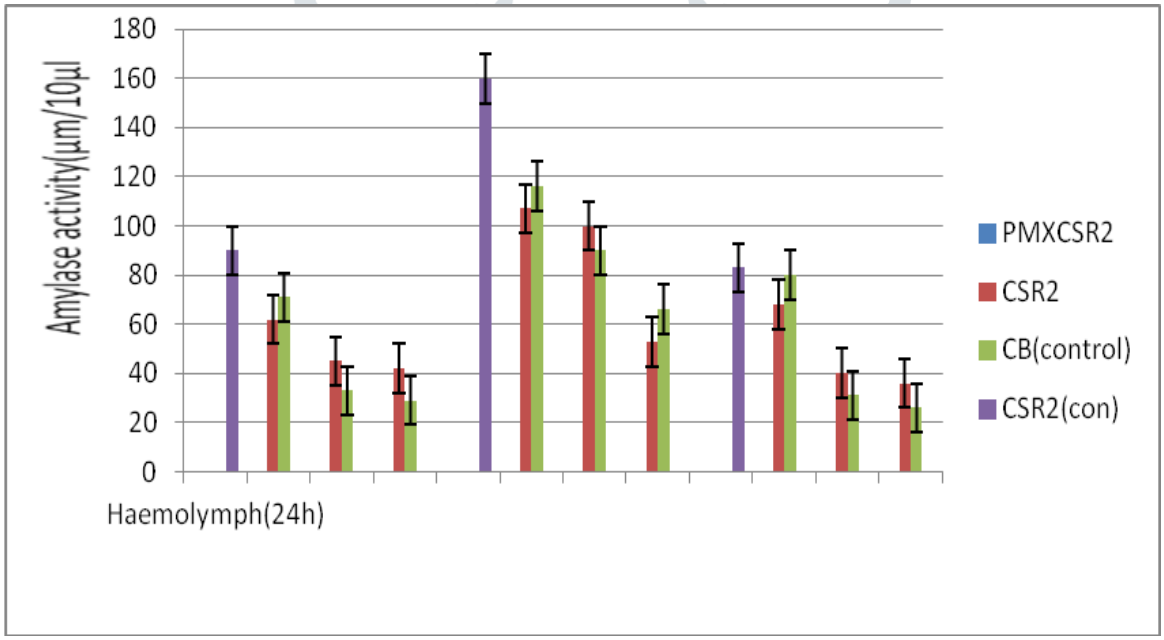

Fig : 4 Amylase activity in silk worm Haemolymph (after 24h of treatment)

Table: 1 showing the economic traits of Dimethoate 2nd day treated silkworm, *B. mori L.*

Race	Ppm	Hatching Percentage (%)	Larval Duration (hrs) V-instar	Larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	Filament weight (g)	Filament length (m)	Denier (d)	Renditta (kg)
PM× CSR_2	10	94	152	2.29	1.1	0.979	0.154	14	0.149	477.75	2	7.04
	20			2.49	0.856	0.808	0.119	13.9	0.129	368.37	1.77	7.63
	30			2.59	0.832	0.629	0.102	12.25	0.082	234.5	1.41	14.7
CSR_2	10	86	152	2.34	0.962	0.781	0.141	14.65	0.122	506.25	0.97	7.88
	20			2.60	0.89	0.418	0.121	13.59	0.107	351.37	0.92	8.46
	30			2.64	0.82	0.207	0.106	12.92	0.083	224.5	0.63	14.9
Control PM× CSR_2			160	2.78	1.227	1.045	0.189	15.54	0.152	789.72	2	7.9
Control CSR_2			160	2.68	1.104	0.898	0.24	21.73	0.171	830.02	1.8	6.9

Table: 2 showing the economic traits of Dimethoate 4th day treated silkworm, *B. mori* L

Race	Ppm	Hatching Percentage (%)	Larval Duration (hrs)	Larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	Filament weight (g)	Filament length (m)	Denier (d)	Renditta (kg)
PM X CSR_2	10	94	152	2.44	0.98	1	0.142	14.48	0.114	566	1	10.76
	20			2.62	0.94	0.964	0.129	13.72	0.108	383	0.93	11.01
	30			2.80	0.99	0.699	0.118	11.91	0.086	247.7	0.74	14.83
CSR_2	10	86	152	2.44	0.982	0.707	0.182	18.53	0.134	563.5	1.72	7.32
	20			2.65	0.87	0.733	0.154	17.7	0.109	430.2	1.42	8.5
	30			2.83	0.985	0.303	0.143	14.51	0.81	214.5	1.01	13.3
Control PM X CSR_2			160	2.85	1.232	1.04	0.192	15.58	0.149	789.75	1.92	8.3
Control CSR_2			160	2.60	1.118	0.898	0.22	19.67	0.169	831.37	1.77	7.1

Table: 3 showing the economic traits of Dimethoate 6th day treated silkworm, *B. mori* L

Race	Ppm	Hatching (%)	Larval Duration (hrs)	Larval weight (g)	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)	Filament weight (g)	Filament length (m)	Denier (d)	Renditta (kg)
PM X CSR_2	10	94	152	2.44	1	1	0.159	15.9	0.134	511	1.69	9.4
	20			2.69	0.92	0.781	0.136	14.78	0.073	350.37	0.88	11.1
	30			2.87	0.86	0.402	0.121	14.06	0.045	325.62	0.55	14.2
CSR_2	10	86	152	2.44	0.97	0.7	0.175	18.04	0.134	648.37	1.27	9.3

	20			2.70	0.9	0.423	0.158	17.55	0.045	490.37	0.48	13.8
	30			2.88	0.81	0.301	0.141	17.4	0.083	245.87	1	15.6
Control PM X CSR_2			160	2.85	1.227	1.045	0.198	16.13	0.148	789.75	1.91	8.3
Control CSR_2			160	2.60	1.108	0.898	0.204	18.41	0.168	831.37	1.77	7.1

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