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RESEARCH ARTICLE :

Screening the populations of *Mallada boninensis* for cross resistance to newer molecules of insecticides

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SUMMARY : Positive resistance ratios were recorded when the population of *M. boninensis* was subjected to imidacloprid for four generations suggesting that the grubs showed resistance to the insecticide. Resistance ratio of 1.12 fold was recorded in the second generation and resistance ratio of 1.15 fold was recorded in the third generation. In the fourth generation grubs of *M. boninensis* when, treated with imidacloprid recorded resistance ratio of 1.25 fold in the fourth generation. Cross resistance ratio of 1.25 fold was recorded in the fifth generation to acetamiprid. Cross resistance ratio of 2.75 fold was recorded in the fifth generation to thiamethoxam. Cross resistance ratio of 2.09 fold was recorded in the fifth generation to buprofezin. Cross resistance ratio of 1.68 fold was recorded in the sixth generation to acetamiprid. Cross resistance ratio of 2.68 fold was recorded in the sixth generation to thiamethoxam. Cross resistance ratio of 1.93 fold was recorded in the sixth generation to buprofezin. Cross resistance ratio of 1.62 folds was recorded in the seventh generation to acetamiprid. Cross resistance ratio of 2.53 folds was recorded in the seventh generation to thiamethoxam and cross resistance ratio of 1.81 folds was recorded to buprofezin.

KEY WORDS :
M. boninensis, Resistance, Cross resistance, Imidacloprid, Acetamiprid, Thiamethoxam, Buprofezin

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BACKGROUND AND OBJECTIVES

Biological control is, "the action of parasitoids, predators and pathogens in maintaining other organisms' density at a lower average level than would occur in their absence" (DeBach, 1965). The ability of natural enemies to reproduce rapidly, to search out their hosts and survive at relatively low host densities makes outstanding advantages possible (Stelzel and Devetak, 1999; Saini and Salto, 1999 and Singh and Manoj, 2000). The cost of developing and maintaining good quality natural enemies is a small price to pay for consistent and satisfactory performance in the field (Larock and Ellington, 1996). The procedures necessary will vary with the entomophagous species and the intended usage (Penny *et al.*, 2000 and Florkin and Jeuniaux, 1974).

Among complex network of bioagents, Chrysopids or green lacewings are known to be the most effective predators, they belongs to order 'Neuroptera'. This order consists of a group of insects with soft bodies, biting mouthparts and two pairs of very similar membranous wings, which are usually held roof-like along the abdomen at rest. Their agricultural importance lies in their carnivorous habits. Green lacewings are considered to be one of the most effective generalist predators used in biological control. The larvae feed on pest aphids, scales, caterpillars, spider mites etc. infesting a variety of plants (McEwen *et al.*, 2001). The green lacewing, *Mallada boninensis* (Okamoto) is an important predator or sucking insects like mealybugs, syrphids and psyllids. Grubs of *M. boninensis* use discarded prey items and environmental debris ('trash'), carried on the dorsal abdominal segments, as camouflage. Larvae that carry trash were confirmed experimentally to experience lower rates of cannibalism, an effect attributed to the camouflage conferred by the package. Adults are generally not predatory and feed on nectar, pollen or honeydew, while a few of them are predatory (Coppel and Mertins, 1977). For the effective use of lacewing *Mallada boninensis* in integrated management of insect and mite pests of different crops, the information on toxicities of insecticide on the predatory lacewing is needed. Hence the present study was formulated to check the cross resistance of different insecticides to *M. boninensis*.

RESOURCES AND METHODS

Laboratory investigations on the predator *Mallada boninensis* were carried out during the year 2014 in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. The grubs of *M. boninensis* collected from field were reared in biocontrol laboratory, Department of Agricultural Entomology, TNAU, Coimbatore.

***Mallada boninensis* on *C. cephalonica* eggs :**

Grubs of *M. boninensis* were reared on *C. cephalonica* eggs kept inside separate small plastic bottles (3 cm diameter) closed with lid. Fresh eggs were given till the pupation of the grubs. Pupa were collected and transferred to G.I. round troughs for adult emergence. The adults were collected daily and transferred to pneumatic glass troughs or G.I. round troughs (30 cm x 12 cm). Before allowing the adults, the rearing troughs were wrapped inside with brown sheets, which act as egg receiving card. About 250 adults (60% females) were allowed into each trough and covered with georgette cloth secured by rubber band. On the cloth outside three bits of foam sponge (2 sq.inch) dripped in water is kept. Besides an artificial protein rich diet was provided in semisolid paste form in three spots on the cloth outside. This diet consisted of equal parts of yeast, fructose, honey, Proteinex R and water. The adults lay eggs on the brown sheet. The adults were collected daily and allowed into fresh rearing troughs with fresh food. From the old troughs, the brown paper sheets along with *Mallada* eggs were removed. Emerged grubs were collected and rearing was continued for getting a steady supply of grubs for different experiments. Two to three days old grubs were used for various experiments.

Effect of sublethal doses of imidacloprid to the predator *Mallada boninensis* :

Screening the populations of Mallada boninensis for cross resistance to newer molecules of insecticides :

Exposure unit :

Pesticidal solution was prepared using acetone as solvent. Following the dry film method the pesticide film was coated using the camelin hair brush inside the individual cell wells and allowed to dry. After drying 2 to 3 days old grubs were placed in each cell well along with *Corcyra* eggs as diet and surface was covered with a plate, which prevents the grubs from escaping. Larval mortality was registered every day.

The formulated insecticides *viz.*, imidacloprid 200 SL, acetamiprid 20%SP, thiamethoxam 25 WG and buprofezin 25% SC were used. Upto G4 imidacloprid selected population was assessed for resistance to imidacloprid. In G4 the imidacloprid-selected population was taken and subjected to the above mentioned insecticides to evaluate the cross resistance pattern to different insecticides. In G5 and G6 imidacloprid selection was stopped and assessed for cross resistance to the above mentioned insecticides. Two to three day old grubs were used for bioassays. Each insecticide was tested with six concentrations to determine the LC_{50} value. Mortality was assessed upto 72 hours after exposure to insecticides and mortality data were recorded and corrected using Abbott's formula (Abbott, 1925). Grubs were considered dead if they fail to make coordinated movement when probed with a probe. The degree of cross resistance acquired by *M. boninensis* was calculated by dividing LC_{50} value of F_n th generation with the LC_{50} value of F_1 generation for each insecticide and thus the relative degree of cross resistance was assessed by using the formula suggested by Ramasubramanian and Regupathy (2004).

Cross resistance (CR) = LC_{50} of F_n (selected) / LC_{50} of F_1 (unselected)

If CR = >1 (Positive)

CR = <1 (Negative)

Evaluation of stability of resistance in imidacloprid-selected populations :

The imidacloprid-selected population for four generations, was cultured in the absence of selection pressure at G5 and G6 in the laboratory. A decline or increase in resistance to imidacloprid in the population was measured by calculating an R (response per generation) value. The R-value was estimated by using the formulae: R= [log (Final LC_{50}) – log (Initial LC_{50})/ n].

Statistical analysis :

Completely randomised design (CRD) for laboratory experiments as described by Panse and Sukhatme (1981). The data were transformed to square root and arc sine wherever required as per the method described by Poisson for statistical analysis (Snedecor and Cochran, 1967) and the data was analysed using AGRES soft ware.

OBSERVATIONS AND ANALYSIS

The results obtained from the present study as well as discussions have been summarized under following heads:

Screening the populations for cross and stability of resistance to newer molecules of insecticides :

Resistance to imidacloprid in the grubs of Mallada boninensis by dry film method:

Grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0033, 0.0032, 0.0030 and LC_{90} values of 0.013, 0.013, 0.012 at 24,48 and 72 HAT in the first generation, respectively. In the second generation grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0037, 0.0036, 0.0034 and LC_{90} values of 0.016, 0.016, 0.016 respectively at 24, 48 and 72 HAT. Resistance ratio of 1.12 fold was recorded in the second generation. Grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0038, 0.0037, 0.0036 and LC_{90} values of 0.017, 0.017, 0.017 at 24,48 and 72 HAT in the third generation, respectively. Resistance ratio of 1.15 fold was recorded in the third generation. In the fourth generation grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0042, 0.0040, 0.0039 and LC_{90} values of 0.020, 0.019, 0.018 respectively at 24,48 and 72 HAT. Resistance ratio of 1.25 fold was recorded in the fourth generation (Table 1).

Positive resistance ratios were recorded when the

Table 1 : Resistance to imidacloprid to the grubs of *Mallada boninensis* by dry film method

Generation	Time (HAT)	LC_{50}	Fiducial limits		LC_{95}	Fiducial limits		Heterogeneity $\chi 2$	Slope ± SE	Regression equation y=a+bx	R
			LL	UL		LL	UL				
First	24	0.0033	0.003	0.0037	0.013	0.009	0.018	15.97	2.65 ± 0.56	y=11.56+2.65x	---
	48	0.0032	0.002	0.0035	0.013	0.009	0.018	17.02	2.59 ± 0.52	y=11.49+2.59x	
	72	0.0030	0.002	0.0033	0.012	0.009	0.017	16.27	2.63 ± 0.51	y=11.65+2.63x	
Second	24	0.0037	0.003	0.0042	0.016	0.011	0.025	11.44	2.36 ± 0.42	y=10.73+2.36x	1.125
	48	0.0036	0.003	0.0040	0.016	0.011	0.025	13.08	2.36 ± 0.41	y=10.77+2.36x	
	72	0.0034	0.003	0.0039	0.016	0.010	0.024	12.42	2.35 ± 0.43	y=10.80+2.35x	
Third	24	0.0038	0.003	0.0044	0.017	0.011	0.026	10.09	2.35 ± 0.44	y=10.66+2.35x	1.156
	48	0.0037	0.003	0.0042	0.017	0.011	0.026	10.03	2.32 ± 0.41	y=10.62+2.32x	
	72	0.0036	0.003	0.0041	0.017	0.011	0.027	10.39	2.29 ± 0.39	y=10.58+2.29x	
Fourth	24	0.0042	0.003	0.0049	0.020	0.012	0.032	9.55	2.25 ± 0.37	y=10.32+2.25x	1.250
	48	0.0040	0.003	0.0046	0.019	0.012	0.031	10.54	2.25 ± 0.31	y=10.37+2.25x	
	72	0.0039	0.003	0.0044	0.018	0.011	0.028	8.91	2.31 ± 0.39	y=10.55+2.31x	

HAT : Hours after treatment. UL: Upper limit. LL: Lower limit. CR: Cross resistance

population of *M. boninensis* was subjected to imidacloprid for four generations suggesting that the grubs showed resistance to the insecticide similarly field collected population of *S. exigua* developed 345.4-fold resistance to spinosad (as compared to susceptible strain) when selected with spinosad for five generations under laboratory conditions (Wang and Nordland, 2006).

Response of imidacloprid selected population of *Mallada boninensis* in fifth generation to different insecticides after four generations of selection in the laboratory

Grubs of *M. boninensis* when, treated with imidacloprid for four generations and when subjected to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin recorded the following LC_{50} and LC_{90} values in the fifth generation. Grubs of *M. boninensis* when, treated with imidacloprid for four generations and when subjected to acetamiprid recorded LC_{50} values of 0.0059, 0.0056, 0.0054 and LC_{90} values of 0.018, 0.017, 0.016 at 24,48 and 72 HAT, respectively. Cross resistance ratio of 1.25 fold was recorded in the fifth generation to acetamiprid. Grubs of *M. boninensis* when, treated with imidacloprid for four generations and when subjected to thiamethoxam recorded LC_{50} values of 0.0090, 0.0088, 0.0085 and LC_{90} values of 0.024, 0.024, 0.023 at 24,48 and 72 HAT, respectively. Cross resistance ratio of 2.75 fold was recorded in the fifth generation to thiamethoxam. Grubs of *M. boninensis* when, treated with imidacloprid for four generations and when subjected to buprofezin recorded LC_{50} values of 0.0069, 0.0067, 0.0063 and LC_{90} values of 0.058, 0.061, 0.052 at 24,48 and 72 HAT, respectively. Cross resistance ratio of 2.09 fold was recorded in the fifth generation to buprofezin (Table 2).

Positive cross resistance ratios were recorded to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin. It can be inferred that if grubs exposed *a priori* to imidacloprid, will still remain resistant to acetamiprid, thiamethoxam and buprofezin treatments. Hence, the above chemicals may be used in rotation successfully in the field. Similarly Shad *et al*. (2010) observed the instability of resistance and lack of cross-resistance to emamectin in *Spodoptera litura* to other insecticides, so insecticides with different modes of action may be recommended to reduce emamectin selection pressure.

Response of imidacloprid selected population of *Mallada boninensis* to different insecticides without selection in the laboratory in fifth generation :

Grubs of *M. boninensis* with imidacloprid selection for four generations and unselected in the fifth generation recorded the following LC_{50} and LC_{90} values in the sixth generation when, subjected to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin. In the sixth generation grubs of *M. boninensis* when treated with acetamiprid recorded LC_{50} values of 0.0057, 0.0054, 0.0052 and LC_{90} values of 0.017, 0.017, 0.017 at 24, 48 and 72 HAT, respectively. Cross resistance ratio of 1.68 fold was recorded in the sixth generation to acetamiprid. In the sixth generation grubs of *M. boninensis* when treated with thiamethoxam recorded LC_{50} values of 0.0087, 0.0086, 0.0083 and LC_{90} values of 0.025, 0.026, and 0.025 at 24, 48 and 72 HAT, respectively. Cross resistance ratio of 2.68 fold was recorded in the sixth generation to thiamethoxam. In the sixth generation grubs

Table 2 : Response of imidacloprid selected population of *Mallada boninensis* to different insecticides after four generations of selection in the laboratory

Insecticides	Time (HAT)	LC_{50}	Fiducial limits		LC_{95}	Fiducial limits		Heterogeneity $\chi2$	Slope ± SE	Regression equation y=a+bx	CR
			LL	UL		LL	UL				
Acetamiprid	24	0.0059	0.005	0.006	0.0180	0.012	0.026	1.14	3.33 ± 0.62	y=12.43+3.33x	1.750
	48	0.0056	0.005	0.006	0.0174	0.012	0.025	1.64	3.33 ± 0.61	y=12.47+3.33x	
	72	0.0054	0.005	0.005	0.0167	0.011	0.023	1.56	3.37 ± 0.58	y=12.62+3.37x	
Thiamethoxam	24	0.0090	0.007	0.010	0.0247	0.015	0.040	0.11	3.75 ± 0.68	y=12.68+3.75x	2.75
	48	0.0088	0.007	0.010	0.0248	0.015	0.040	0.24	3.68 ± 0.60	y=12.56+3.68x	
	72	0.0085	0.007	0.009	0.0232	0.014	0.036	0.11	3.81± 0.71	y=12.90+3.81x	
Buprofezin	24	0.0069	0.0050	0.009	0.058	0.021	0.157	1.58	1.69 ± 0.41	y=8.62+1.69x	2.09
	48	0.0067	0.0048	0.009	0.061	0.022	0.171	1.29	1.64 ± 0.40	y=8.54+1.64x	
	72	0.0063	0.0047	0.008	0.052	0.020	0.133	1.41	1.71 ± 0.46	y=8.75+1.71x	

HAT : Hours after treatment. UL: Upper limit. LL: Lower limit. CR: Cross resistance

of *M. boninensis* when treated with buprofezin recorded LC_{50} values of 0.0065, 0.0062, 0.0058 and LC_{90} values of 0.061, 0.068 and 0.064 at 24, 48 and 72 HAT, respectively. Cross resistance ratio of 1.93 fold was recorded in the sixth generation to buprofezin (Table 3).

Positive cross resistance ratios were recorded to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin. From which it can be inferred that grubs exposed *a priori* to imidacloprid will still remain resistant to acetamiprid, thiamethoxam and buprofezin treatments similarly diamondback moth (*Plutella xylostella*) strain NO-QA, tobacco budworm (*Heliothis virescens*) strain YHD2 selected with Cry1Ac exhibited high levels of cross-resistance to Cry1F and little or no cross-resistance to Cry1C and Cry2A (Bruce *et al*., 1996).

Response of imidacloprid selected population of *Mallada boninensis* to different insecticides without selection in the laboratory in sixth generation :

Grubs of *M. boninensis* with imidacloprid selection for four generations and unselected in the sixth generation recorded the following LC_{50} and LC_{90} values in the seventh generation when, subjected to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin. Grubs of *M. boninensis* when treated with acetamiprid recorded LC_{50} values of 0.0055, 0.0052, 0.0049 and LC_{90} values of 0.018, 0.017 and 0.016 at 24, 48 and 72 HAT, respectively in the seventh generation and cross resistance ratio of 1.62 folds was recorded in the seventh generation to acetamiprid. Grubs of *M. boninensis* when treated with thiamethoxam recorded LC_{50} values of 0.0085, 0.0081, 0.0077 and LC_{90} values of 0.025, 0.025, and 0.024 at 24, 48 and 72 HAT, respectively in the seventh generation and cross resistance ratio of 2.53 folds was recorded in the seventh generation to thiamethoxam. Grubs of *M. boninensis*

Table 3 : Response of imidacloprid selected population of *Mallada boninensis* to different insecticides without selection in the laboratory in fifth generation

Insecticides	Time (HAT)	LC_{50}	Fiducial limits		LC_{95}	Fiducial limits		Heterogeneity χ2	Slope ± SE	Regression equation y=a+bx	CR
			LL	UL		LL	UL				
Acetamiprid	24	0.0057	0.005	0.006	0.0178	0.012	0.026	1.37	3.27 ± 0.61	y=12.34+3.27x	1.68
	48	0.0054	0.004	0.005	0.0176	0.012	0.025	1.92	3.20 ± 0.60	y=12.26+3.20x	
	72	0.0052	0.004	0.005	0.0170	0.011	0.024	2.10	3.17 ± 0.58	y=12.25+3.17x	
Thiamethoxam	24	0.0087	0.007	0.010	0.0253	0.015	0.041	0.22	3.59 ± 0.68	y=12.39+3.59x	2.68
	48	0.0086	0.007	0.010	0.0263	0.015	0.044	0.15	3.39 ± 0.60	y=12.00+3.39x	
	72	0.0083	0.007	0.009	0.0257	0.015	0.043	0.55	3.32± 0.71	y=11.89+3.32x	
Buprofezin	24	0.0065	0.004	0.008	0.0615	0.021	0.174	2.12	1.61 ± 0.41	y=8.50+1.61x	1.93
	48	0.0062	0.004	0.008	0.068	0.022	0.206	2.31	1.53 ± 0.40	y=8.36+1.53x	
	72	0.0058	0.004	0.007	0.064	0.022	0.189	2.76	1.52 ± 0.46	y=8.38+1.52x	

HAT : Hours after treatment. UL: Upper limit. LL: Lower limit. CR: Cross resistance

Table 4 : Response of imidacloprid selected population of *Mallada boninensis* to different insecticides without selection in the laboratory in sixth generation

Insecticides	Time (HAT)	LC_{50}	Fiducial limits		LC_{95}	Fiducial limits		Heterogeneity χ2	Slope ± SE	Regression equation y=a+bx	CR
			LL	UL		LL	UL				
Acetamiprid	24	0.0055	0.005	0.006	0.0182	0.012	0.027	3.34	3.14 ± 0.61	y=12.10+3.14x	1.62
	48	0.0052	0.004	0.005	0.0170	0.011	0.024	3.53	3.18 ± 0.60	y=12.26+3.18x	
	72	0.0049	0.004	0.005	0.0162	0.011	0.023	2.83	3.18 ± 0.58	y=12.33+3.18x	
Thiamethoxam	24	0.0085	0.007	0.009	0.0258	0.015	0.043	0.24	3.43 ± 0.68	y=12.09+3.43x	2.53
	48	0.0081	0.007	0.009	0.0250	0.015	0.041	0.29	3.35 ± 0.60	y=12.01+3.35x	
	72	0.0077	0.006	0.008	0.0240	0.014	0.039	0.31	3.31± 0.71	y=11.98+3.31x	
Buprofezin	24	0.0063	0.004	0.008	0.0752	0.023	0.245	3.19	1.48 ± 0.41	y=8.23+1.48x	1.81
	48	0.0058	0.004	0.007	0.0731	0.022	0.230	3.72	1.44 ± 0.40	y=8.21+1.44x	
	72	0.0051	0.003	0.006	0.0660	0.021	0.201	3.78	1.44 ± 0.46	y=8.29+1.44x	

HAT : Hours after treatment. UL: Upper limit. LL: Lower limit. CR: Cross resistance

Table 5 : Response of imidacloprid selected population of *Mallada boninensis* to imidacloprid without selection in the laboratory in fifth and sixth generations

Generation	Time (HAT)	LC_{50}	Fiducial limits LL	Fiducial limits UL	LC_{95}	Fiducial limits LL	Fiducial limits UL	Heterogeneity $\chi 2$	Slope ± SE	Regression equation y=a+bx	R
Sixth	24	0.0041	0.003	0.004	0.0203	0.012	0.032	9.80	2.22 ± 0.61	y=10.29+2.22x	-1.90
	48	0.0039	0.003	0.004	0.0198	0.012	0.031	10.96	2.21 ± 0.60	y=10.31+2.21x	
	72	0.0038	0.003	0.004	0.0202	0.012	0.032	10.64	2.17 ± 0.58	y=10.24+2.17x	
Seventh	24	0.0039	0.003	0.004	0.0206	0.012	0.033	9.01	2.18 ± 0.68	y=10.23+2.18x	-2.00
	48	0.0038	0.003	0.004	0.0200	0.012	0.032	10.03	2.17 ± 0.60	y=10.26+2.17x	
	72	0.0037	0.003	0.004	0.0201	0.012	0.032	10.50	2.15± 0.71	y=10.22+2.15x	

HAT : Hours after treatment. UL: Upper limit. LL: Lower limit. CR: Cross resistan

when treated with buprofezin recorded LC_{50} values of 0.0063, 0.0058, 0.0051 and LC_{90} values of 0.075, 0.073 and 0.066 at 24, 48 and 72 HAT, respectively in the seventh generation and cross resistance ratio of 1.81 folds was recorded in the seventh generation to buprofezin (Table 4).

Positive cross resistance ratios were recorded to different insecticides *viz*., acetamiprid, thiamethoxam and buprofezin. From which it can be inferred that grubs exposed *a priori* to imidacloprid will still remain resistant to acetamiprid, thiamethoxam and buprofezin treatments. *Spodoptera exigua* is able to evolve cross-resistance to highly active Cry proteins when exposed to a protein with marginal toxicity to this species. It is important to take this into account in areas where *S. exigua* is a secondary pest and *B. thuringiensis* Cry1A toxins are used to control other pests (Patricia *et al*., 2009)

Response of imidacloprid selected population of *Mallada boninensis* to imidacloprid without selection in the laboratory in fifth and sixth generations :

In the sixth generation grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0041, 0.0039, 0.0038 and LC_{90} values of 0.020, 0.019, 0.020 respectively at 24,48 and 72 HAT. Resistance ratio of -1.90 folds was recorded in the sixth generation.

In the seventh generation grubs of *M. boninensis* when, treated with imidacloprid recorded LC_{50} values of 0.0039, 0.0038, 0.0037 and LC_{90} values of 0.020, 0.020, 0.020 respectively at 24,48 and 72 HAT (Table 5). Resistance ratio of -2.0 folds was recorded in the seventh generation. In sixth and seventh generation, resistance ratio was negative indicating that there was decline in resistance if the population was left unselected for two or three generations. Similar kinds of results were found when a field population of *S. litura* collected from Dunyapur, Punjab, Pakistan was reared without any selection pressure under the laboratory conditions (Rehan *et al*., 2011).

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