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Full Length Research Paper

Study on twenty-five percent pymetrozine-thiamethoxam suspension concentrate as an insecticide against rice planthopper

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A blended pesticide, 25% pymetrozine-thiamethoxam suspension concentrate (SC), was evaluated for its insecticidal activity against rice planthoppers in a greenhouse and field. The results of the seedling-dipping and rice-stem-dipping methods used in the greenhouse indicated the excellent activity of the SC against the brown rice planthoppers, with the lethal concentration causing 50% mortality (LC_{50}) values reaching 3.27 and 3.79 μg/ml, respectively. The co-toxicity coefficient (CTC) value >120 indicated that the obtained SC had a synergistic effect. The results from the field efficacy trials revealed that the controlling effect of the proposed reagent on rice planthoppers was better than (or comparable with) commercial insecticide-25% pymetrozine SC and 2.2% abamectin-imidacloprid emulsifiable concentrate (EC) at comparable doses and differential time intervals. The proposed reagent had low toxicity from the data of acute oral, acute dermal, eye irritation and skin irritation. These characteristics suggested 25% pymetrozine-thiamethoxam SC as a potential candidate insecticide.

Key words: 25% pymetrozine-thiamethoxam suspension concentrate, rice plant hopper, toxicological test, field efficacy.

INTRODUCTION

Rice is one of the major food crops that provide necessary calories and nutrients to humans. Rice is mainly grown in Asian, African, as well as American countries, and covers about 95.0% of the gross cultivated areas. In Asia, the rice fields are mainly located in the Eastern, Southeastern and Southern regions where favourable warm and rainy conditions prevail. Hence, the main cultivators of rice are the countries of China, India, Indonesia, Thailand and Japan, which account for more than 90% of the total global rice production (FAOSTAT, 2006, http://faostat.fao.org/default.aspx).

The rice planthopper has been recognized as a potential threat to rice cultivation in East and Southeast Asia given its adaptability to changes in agricultural structures and climate change. The life cycle of a rice planthoppers is about 20 to 28 days. Several dozens of generations of rice planthoppers are exposed to synthetic insecticides every year (Jahn et al., 2000, 2007). This routine treatment often leads to enhanced pest resistance against synthetic insecticides, even the effective ones (such as imidacloprid, chlorpyrifos and abamectin (Jahn, 1992; Huang et al., 2005; Preap et al., 2006; Sangha et al., 2008). Rice planthoppers can carry the rice streak virus (RSV), rice black-streaked dwarf virus (RBSDV) and

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southern RBSDV (SRBSDV), both of which cause the loss of annual production of rice, maize, wheat, barley or other crops in some countries (Ruan et al., 1984; Zhou et al., 2008; Hoang et al., 2011). In 2009, disasters involved rice planthoppers and the RSV have affected about 29 348 000 and 20 000 ha of croplands in China, respectively. The resulting rice disease has brought about a USD 80 billion loss in rice production (Zhang et al., 2009).

Given the hazards caused by rice planthoppers, several measures for plant are available. Some of the measures include cultivating insect- or disease-resistant crop varieties, as well as using insecticides, insect-proof nets and trap lamps. However, a large area cultivating of the rice variety with high yield characteristic and the modern cultivation module caused an outbreak of planthopper in PR. China (Cheng et al., 2008; Zhai, 2011). Preventive physical measures are also not effective given the large areas of cultivated fields.

As a novel class of insecticides containing the pyridine-azomethine group, pymetrozine compounds have a good selectivity against plant-sucking insects (such as aphids, whiteflies and rice planthoppers). These compounds disable the mouthparts of the insects, eventually causing them to starve and die (Kristinsson, 1994; Harrewijn and Kayser, 1997; Castle et al., 2009). On the other hand, thiamethoxam, a second-generation neonicotinoid insecticide, acts on acetylcholine nicotinic receptors (Maienfisch et al., 2001; Nauen et al., 2003), quickly paralyzing and killing pests.

Some insecticides (such as pymetrozine, thiamethoxam, nitenpyram, isoprocarb and buprofezin) that are effective against rice planthoppers have been recently developed to enhance rice production in China. However, the continuous large-scale application of these insecticides often leads to the enhanced resistance of pests. To resolve this problem, a new blended pesticide containing pymetrozine and thiamethoxam was screened in the present study. The results of greenhouse and field bioassays revealed that the agent possessed excellent pesticidal activity, and was fast acting as well as long lasting. Resistance development was also delayed, and the amount of single insecticide use was decreased. Moreover, the blended insecticide had low levels of toxicity, and had irritation effects within permissible limits.

MATERIALS AND METHODS

Pymetrozine (96%), pymetrozine wet powder (WP; 25%), thiamethoxam (96%) and chlorpyrifos emulsifiable concentrate (EC; 20%) were obtained from the Jiangsu Kesheng Group Co. Ltd., Nanjing Longxin Chemical Company, and Guangxi Tianyuan Biochemistry Co. Ltd., respectively. The brown rice planthoppers (*Nilaparvata lugens*) were provided by the Key Laboratory of Monitoring and Management of Plant Diseases and Insects (Ministry of Agriculture, PR. China), Nanjing Agricultural University.

The rice variety Xiangyou 109 was purchased from the Hunan Keyulong Seed Company.

Assay for formulation screening

The 25% pymetrozine-thiamethoxam suspension concentrate (SC) was mixed in different weight ratios (1:9, 1:3, 1:1, 3:1, 9:1, 11.5:1 and 19:1). The other components were alkane sulfonate (1.5%), fatty alcohol ether (2.5%), a compound dispersant, and an antifoaming agent (5.0%). The final water volume was 100.0%.

The rice stem-dipping method and the rice stem-dipping method were always used to screen the new ingredient with higher insecticidal activity. Compare with the method of residual film, these methods previously described had an advantage in displaying a true insecticidal activity based on the interaction model of planthopper and rice (Wu, 1988). Firstly, the rice seeding-dipping method was used to evaluate the insecticidal activity of the blended pesticides against the brown rice planthoppers, and the method was conducted using a known method described by Ni (2007). The brown rice planthoppers were placed in an artificial climate incubator, which was kept at 25°C and 60.0% humidity. Xiangyou 109 was cultivated for 8 to 12 days at 25°C in an artificial climate incubator. Rice seedlings 5 to 7 cm in height were dipped in the insecticide solution for 10 s, and were placed on a disposable plastic cup after air drying. Fifteen (15) larvae (second or third instars) were placed on the disposable plastic cup, and were bred in an artificial climate incubator at the same conditions per treatment. Mortalities were calculated after 72 h; various ratios of the two active ingredients were also evaluated. Each treatment was performed 4 times.

The mortality and corrected mortality were calculated using Equations 1 and 2, respectively:

$$P_1 = \frac{K}{N} \times 100 \quad (1)$$

$$P_2 = \frac{P_t - P_0}{1 - P_0} \times 100 \quad (2)$$

Where P_1 is the mortality, *K* is the number of dead insects, *N* is the number of treated insects, P_2 is the corrected mortality, P_t is the mortality in the treatment group, and P_0 is the mortality for CK. If the mortality for CK was <5%, the value need not be corrected. If the mortality for CK was 5 to 20%, the value should be corrected by Equation 2. If the mortality of CK was >20%, the experiment should be repeated. The lethal concentration causing 50% mortality (LC_{50}) of the agents was obtained from the regression equation.

The verification experiment for the formulation with a higher insecticidal activity against the brown rice planthoppers on Xiangyou 109 was completed using the rice-stem-dipping method described by Zhuang et al. (1999). A young rice stem (Xiangyou 109) was pulled out, washed, and cut to a height of 10 cm. Subsequently, the rice stem was dipped in the pesticide solution for 30 s. Water was used as the contrast agent. After air drying, one end of the rice stem was wrapped in wet cotton to keep it moist, and was placed in a disposable cup. About 20 larvae (third instars) of the brown rice planthoppers were transferred to the cup. At least 4 replicates were performed for each treatment. The mortality and corrected mortality were calculated using Equations 1 and 2, respectively. LC_{50} was also calculated according to the regression equation.

The co-toxicity coefficient (CTC) was calculated based on the data from the rice-seedling-dipping method; and on Equations 3 to 5 (Sun and Johnson, 1960), as follows:

$$\mathrm{ATI} = \frac{S}{M} \times 100 \quad (3)$$

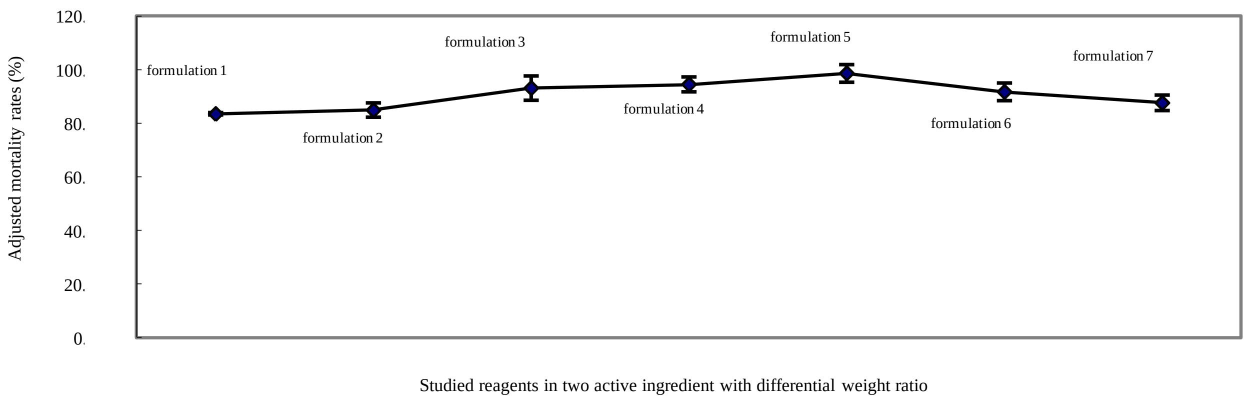

Figure 1. Formulations 1 to 7 represent the weight ratios of pymetrozine and thiamethoxam, which were 1:9, 1:3, 1:1, 3:1, 9:1, 11.5:1, and 19:1, respectively. All data were tested by the homogeneity test of variances, and the results showed heterogeneity of variance. The means were compared by one-way ANOVA (Dunnett's T3) using the SPSS 11.5 software. The *p* value of formulation 5 against formulations 1, 2, 3, 4, 6, and 7 revealed <0.05, >0.05, >0.05, >0.05, >0.05, and <0.05, respectively. *p* values <0.05 indicate significant differences.

$$\mathrm{TTI} = \mathrm{TI_A} \times P_\mathrm{A} + \mathrm{TI_B} \times P_\mathrm{B} \quad (4)$$

$$\mathrm{CTC} = \frac{\mathrm{ATI}}{\mathrm{TTI}} \times 100 \quad (5)$$

In these formulas, *S* is the LC_{50} of standard insecticides (mg/L), *M* is the LC_{50} of the blended pesticide, TI_A or TI_B is the toxicity index of pharmaceutical agent A or B, P_A or P_B is the percentage of pharmaceutical agent A or B in the blended pesticide, *ATI* is the actual toxicity index of the blended pesticide, and *TTI* is theoretical toxicity index.

Field efficacy trials against rice planthoppers

Experimental preparation

The field trials were performed based on the Pesticide Guidelines for Field Efficacy Trials (II) (GB/T17980.4-2000) of the Jiangle county, Sanming city, Fujian province, China. The experiment was divided into 6 treatment groups using non-glutinous rice (Yiyou 99 variety) as subjects. Group 1 was 25% pymetrozine-thiamethoxam SC, 0.02248 ml/m^2; Group 2 was 25% pymetrozine-thiamethoxam SC, 0.03748 mL/m^2; Group 3 was 25% pymetrozine-thiamethoxam SC, 0.05247 mL/m^2; Group 4 was 25% pymetrozine WP, 0.03748 g/m^2; group 5 was 2.2% abamectin-imidacloprid EC, 0.08995 ml/m^2; and Group 6 was the water-treatment group. An area of 7 to 10 m^2 was set as the field plot, which was randomly arranged in the experimental field. A ridge approximately 10 cm long was built to prevent the flow of water to the adjacent treatment groups. The solution containing the insecticide was diluted to 60 g/m^2, and was sprayed on the rice seedlings using handheld aerosol after half a month of sowing. Each treatment was performed in three replicates. After 30 to 40 days of sowing, the seedlings of all treatment groups were transplanted to a rice field, and were using regular methods.

Experimental analyses

The basic and vestigial numbers of rice planthoppers were investigated before the application of pesticides, as well as after 2, 5, 7 and 10 days. There were 5 sites analyzed according to the cater-corner mode per sub-district, and each site was set to 0.33 m^2. The rice planthoppers were collected using a white porcelain basin measuring 33 × 45 cm^2 (length × width). The insecticidal activity was calculated using Equations 6 and 7.

$$\mathrm{CMPFR} = \frac{\text{the pest number before application} - \text{the pest number after application}}{\text{the pest number before application}} \times 100 \quad (6)$$

$$\text{Control effect (\%)} = \frac{\text{CMPFR (treatment group)} - \text{CMPFR (control group)}}{1 - \text{CMPFR (control group)}} \times 100 \quad (7)$$

Where CMFR is the corrected mite population falling rate.

Toxicological tests for 25% pymetrozine-thiamethoxam SC

Toxicological tests for acute oral, acute dermal, eye irritation, and skin irritations were performed according to the Standard Procedure of the People's Republic of China (1995) in the Guiyang Medical College (GB 15193.3-94).

Data analyses

All data were analyzed by using the SPSS 11.5 software. The dose-response data were analyzed using linear regression analyses. The LC_{50} value was calculated using the value of *X* in the regression equation. The means of the different groups were compared by one-way analysis of variance (ANOVA) (Dunnett's T3), and the field efficacy was evaluated by one-way ANOVA (least significant differences).

RESULTS

Formulation screening of 25% pymetrozine-thiamethoxam SC against the brown rice planthoppers

As shown in Figure 1, 25% pymetrozine-thiamethoxam SC with different ratios of active ingredients had good

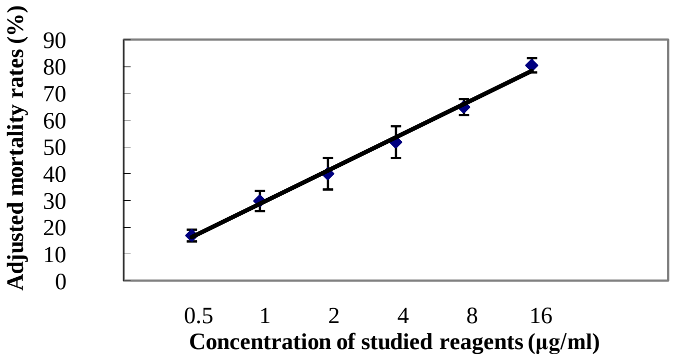

Figure 2. Corrected mortality for the studied reagents in increasing concentration as determined by the rice breeding-dipping method. Data are represented as mean ± SD (n = 4).

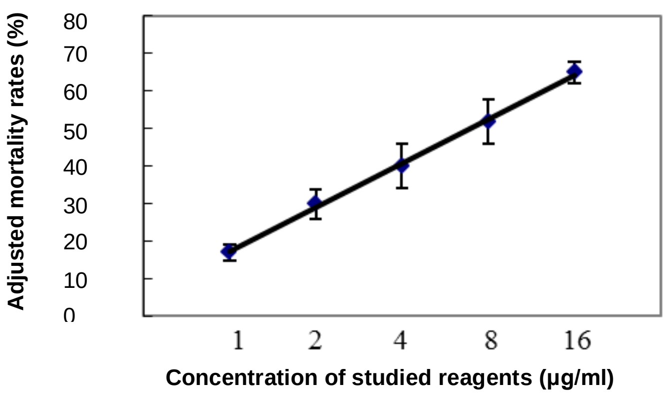

Figure 3. Corrected mortality for the studied reagents in increasing concentration as determined by the rice stem-dipping method. Data are represented as mean ± SD (n = 4).

activities against the brown rice planthoppers at 30.0 µg/ml. The corrected mortality changed from 83.56 to 98.17%, which was higher than that of commercial 20% chlorpyrifos EC (76.25%). Furthermore, at a weight ratio of 9:1 pymetrozine:thiamethoxam, the highest activity of 98.17% was achieved and the LC_{50} value of 3.27 ± 0.12 µg/ml was also calculated and shown in Figure 2; the values were lower than that of 96% pymetrozine (LC_{50} = 6.90 µg/ml) and that of 96% thiamethoxam (LC_{50} = 5.44 µg/ml) in Table 3. Aiming at the different grown stage of rice, the rice planthopper had a different degree of feeding at a period of time, so the rice-stem-dipping method was also employed to determine the insecticidal activity. As shown in Table 3 and Figure 3, the LC_{50} values about pymetrozine, thiamethoxam and their blended pesticides were 8.01 ± 0.17 µg/ml, 7.57 ± 0.14 µg/ml and 4.69 ± 0.10 µg/ml, respectively. The results were also shown that the LC_{50} values of the blended pesticides were lower than that of two single agents. Meanwhile, LC_{50} value of the latter method was higher than that of the former method, the results were caused by the litter content of feeding to the brown planthopper in rice stem, and acquired a litter content of pesticides. Finally, the combined action of the two active ingredients was evaluated according to Equations 3 to 5. The CTC value was 205.46 (≥120), demonstrating a kind of synergistic effect amongst the studied reagents.

Field efficacy of 25% pymetrozine-thiamethoxam SC against rice planthoppers

Table 1 shows the controlling effects of 25% pymetrozine-thiamethoxam SC against rice planthoppers. The values were 87.14, 93.72 and 95.30% at 3 different concentrations after 2 days of application. The results indicated that the dose response and controlling effect of the studied reagents were higher than those of commercial 25% pymetrozine WP, and were comparable with 2.2% abamectin-imidacloprid EC. With the passage of application time, the controlling effect of the reagents reached a peak value 5 days after application, and then decreased until 10 days after application. The controlling effects of the reagents 10 days after application were 77.58, 79.39, 89.40, 88.21 and 80.55%. Compared with the other treatment groups, there was no noticeable reduction in the controlling effect of the reagents. On the other hand, the controlling effect of the reagents was significantly higher than that of commercial 2.2% abamectin-imidacloprid EC at the highest concentration. In addition, the effect of 25% pymetrozine-thiamethoxam SC was rapid and lasted longer, as demonstrated in the field experiment. The pesticides were also environment friendly and are safe to humans.

Toxicological tests for 25% pymetrozine-thiamethoxam SC

The toxicology tests were carried out by known standard procedures in China [toxicological test methods of resticides for registration (GBl5670-1995)-National standards of PR. China]. The results shown in Table 2 indicated that 25% pymetrozine-thiamethoxam SC had low toxicity for the value (LD_{50}) of acute oral and acute dermal over 5000 and 2000 mg/kg•bw, respectively. The result of the irritation test also showed that the agent did not have an irritating effect. Therefore, the blended pesticide is safe to humans, and is environment friendly as well.

DISCUSSION

Pymetrozine is particularly effective against plant-sucking insects. It destroys the mouthparts of insects, causing death by starvation (Harrewijn and Kayser, 1997; Ausborn et al., 2005). To date, the exact action

Table 1. Field efficacy of 25% pymetrozine-thiamethoxam SC against rice planthopper in rice.

Treatment group	Days after application							
	2		5		7		10	
	Control effect (%)	Significant difference	Control effect (%)	Significant difference	Control effect (%)	Significant difference	Control effect (%)	Significant difference
25% Pymetrozine-thiamethoxam SC, 0.02248 ml/m^2	87.14	bB	84.35	bC	81.22	bB	77.58	bB
25% Pymetrozine-thiamethoxam SC, 0.03748 ml/m^2	93.72	aAB	92.40	aAB	91.67	aA	79.39	bB
25% Pymetrozine-thiamethoxam SC, 0.05247 ml/m^2	95.30	aA	96.14	aA	93.36	aA	89.4	aA
25% Pymetrozine WP, 0.03748 g/m^2	76.54	cC	86.02	bBC	89.28	aA	88.21	aA
2.2% Abamectin-imidacloprid EC	98.08	aA	94.44	aA	87.82	aA	80.55	bB

Analyses of the controlling effects for field efficacy were completed by using one-way ANOVA (least significant differences) using the SPSS 11.5 software. a, b, and c were analyzed according to $p < 0.05$. A, B, and C were analyzed according to $p < 0.01$. The same letter between the different treatment groups in the same treatment time indicates no significant difference between the two treatment groups.

Table 2. Toxicology of 25% pymetrozine-thiamethoxam SC.

Acute oral or dermal toxicological assay				
Tested item	**Research object**	**Sex**	**LD_{50} (mg/kg·bw)**	**Toxicological grade**
Acute oral	SD rats	Male/female	>5000	Low toxicity
Acute dermal			>2000	
Eye or skin irritation toxicological assay				
Tested item	**Research object**	**Sex**	**Value**	
Eye irritation	Rabbit	Male	No irritation	
Skin irritation				

Table 3. The data of CTC about 96.0% pymetrozine, 96.0% thiamethoxam and 25% pymetrozine-thiamethoxam SC.

Method	Treatment group	Toxicity regression equations (Y)	Correlation coefficient (R^2)	LC_{50} (95% Confidence interval) (μg/ml)	CTC	LC_{95} (95% Confidence interval) (μg/ml)
The rice-seedling dipping methods	96.0% Pymetrozine	Y = 4.094 + 1.080X	0.999	6.90 (0.02 ~ 19.16)	-	230.09 (104.38 ~ 18484.58)
	96.0% Thiamethoxam	Y = 4.093 + 1.233X	0.993	5.44 (1.52 ~ 13.68)	-	117.41 (58.06 ~ 470.14)
	25% Pymetrozine-thiamethoxam SC	Y = 4.408 + 1.150X	0.993	3.27 (0.09 ~ 8.46)	205.46	88.13 (42.62 ~ 1661.69)
The rice stem dipping methods	96.0% Pymetrozine	Y = 3.698 + 1.441X	0.987	8.01 (0.02 ~ 22.62)	-	110.92 (62.25 ~ 2800.06)
	96.0% Thiamethoxam	Y = 4.100 + 1.024X	0.998	7.57 (0.01 ~ 20.34)	-	305.70 (104.25 ~ 4231.33)
	25% Pymetrozine-thiamethoxam SC	Y = 3.927 + 1.598X	0.996	4.69 (1.14 ~ 8.62)	169.81	50.21 (27.10 ~ 256.07)

mechanism about pymetrozine has not been clear. But some reports indicated it can affect chordotonal mechanoreceptors to cause starvation (Ausborn et al., 2005; He et al., 2010). Pymetrozine also has an inhibiting function against the transmission of viruses from rice planthoppers (Polston and Sherwood, 2003), and against plant-sucking insects in plants (Wyss and Bolsinger, 1997). On the other hand, thiamethoxam is a neonicotinoid insecticide that selectively and quickly acts on nicotinic receptors, causing pest death in a short time (Maienfisch et al., 2001).

The activities of pymetrozine-thiamethoxam with different ratios (1:9, 1:3, 1:1, 3:1, 9:1, 11.5:1 and 19:1) were investigated. The results revealed that the best ratio of pymetrozine to thiamethoxam was 9:1. The activity and EC_{50} were 98.17% and 3.27 μg/ml, respectively. The CTC value indicated that the active ingredients of the blended pesticides had synergistic effects.

The field trials also revealed that 25% pymetrozine-thiamethoxam SC had a better and quicker action against rice planthoppers than commercial 25% pymetrozine WP. The effect of the blended reagent had a longer duration as well than commercial 2.2% abamectin-imidacloprid EC. Most importantly, the blended insecticidal reagent was not harmful to the rice crops and to the environment. No significant toxicity and irritation effect were observed. Therefore, 25% pymetrozine-thiamethoxam SC has both practical and economic benefits, especially in terms of improving rice production. The blended reagents can also be considered as a potential green insecticide.

Conclusion

The indoor bio-assay and field efficacy trial of 25% pymetrozine thiamethoxam SC as an insecticide against rice plant hopper was reported. Field efficacy trials from Jiangle county, Sanming city, Fujian province, PR. China revealed that control effect of the reagents against plant hopper on rice was better than or comparable with commercial insecticide-25% pymetrozine SC and 2.2% abamectin·imidacloprid EC at the comparable dose and at differential time interval. According to the toxicological data, the blend insecticide has low toxicity. The results suggested that 25% pymetrozine·thiamethoxam SC is expected to a potential candidate insecticide.

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