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INSECTICIDAL AND RESIDUAL EFFECTS OF PLANT EXTRACTS ON F_1 PROGENY AND POTENTIALITY AS GRAIN PROTECTANT OF RICE WEEVIL, *Sitophilous oryzae* L.

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ABSTRACT

Rice weevil (*Sitophilus oryzae* L.) is one of the major pests of stored commodities. The adult weevils feed on rice and the females lay eggs inside rice kernels. In the present study, n-Hexane, dichloromethane (DCM) and methanol extracts from karanja, *Pongamia pinnata* (L.); mahogany, *Swietenia mahogani* Jacq.; neem, *Azadirachta indica* and urmoi, *Sapium indicum* Willd. were tested for their insecticidal and residual effects against this pest under laboratory conditions. Response varied with plant species. Firstly for the activity of toxicitiy, urmoi fruit extract showed the highest toxic effect (mortality, 51.53%) and mahogany leaf extract was lowest toxic effect (mortality, 25.78%) at 72 hours after treatment (HAT). Secondly for the residual activity, grains treated with urmoi extracts showed the lowest number of adult emergence (4.86), grain infestation rate (1.53%) and inhibition rate (53.48%) at 5 days after treatment (DAT). Between neem seed and urmoi fruit extracts, the lowest number of F_1 adult emerged (17.33), the least grain infestation rate (4.38%), and the highest inhibition rate (40.65%) was found in urmoi extract on rice grains at 20 days after treatment. These results increased with increasing time from 5 to 20 days after treatment (DAT). On the other hand, inhibition rate decreased with increasing of time. Results indicated that urmoi extracts were more effective than other plant extracts against adult insects. Urmoi plant extracts can be used for the protection of stored rice from infestations of rice weevil.

Key words: Plant extract, insecticidal effect, residual effect, grain protectant, rice weevil

Introduction

From the very early time, plant materials have been using as a kind of natural protectant to protect the stored grains. Worldwide reports showed that when mixed with stored-grains, leaf, bark, seed powder, or oil extracts of plants reduce oviposition rate and suppress adult emergence of stored-product insects, and also reduced seed damage rates (Tapondjou *et al.*, 2002; Talukder *et al.*, 2004). Talukder (2006) has listed 43 plant species as insect repellents, 21 plants as insect feeding deterrents, 47 plants as insect toxicants, 37 plants as grain protectants, 27 plants as insect reproduction inhibitors, and 7 plants as insect growth and development inhibitors. Eighteen species showed insecticidal potential, and anti-ovipositional properties against *Sitophilus oryzae* (Devi and Devi, 2011). The increasing attempts to replace synthetic insecticides with less expensive and locally available pest control means have been undertaken especially in the tropics (Jermy, 1990). Pesticidal plants are utilized in two main ways: first, the active compounds are isolated, identified, and chemically synthesized. If feasible, these compounds or their active analogues are synthesized and marketed by the chemical industry. The second approach is suitable for farmers in developing countries and for organic farming. Plant tissues or crude products of the plant, such as aqueous or organic solvent extracts, are used directly. These practices are labour intensive, but are often economically and ecologically sound, and do not require sophisticated technology (Yang and Tang, 1988). Research carried out worldwide during last three decades has significantly extended our knowledge on botanical pesticides. While much work has been done on laboratory and field evaluation of botanicals, very little work has been done on isolation and identification of the active principles. Most of the work has been done in South Asian Countries, especially India. The same and further research is necessary to develop botanical insecticides for stored grain insect pest in Bangladesh. The aim of this study was to evaluate the insecticidal and residual effects of n-Hexane, dichloromethane (DCM) and methanol extracts (karanja, *Pongamia pinnata* (L.); mahogany, *Swietenia mahogani* Jacq.; neem, *Azadirachta indica* and urmoi, *Sapium indicum* Willd.) on the impact on progeny production and potentiality as grain protectant of rice weevil based on the development of botanical insecticides.

Materials and Methods

The study was conducted to know the most effective repellent plant extract for management of rice weevil in the laboratory of the Entomology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Insect rearing: The rice weevil was collected from the stock culture of the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh-2202. The rice weevil was reared in round plastic jars (12 x 23 x 6.5cm in size) with rice grains (13 to 14% moisture) in growth chamber at 28±5°C temperature and R.H. 75±5% in the Entomology laboratory of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

Collection and preparation of plant sample: Fresh leaves and fruits of karanja *(Pongamia pinnata* L.); mahogany, *Swietenia mahogani* Jacq. and neem, *A. indica* were collected from BAU. Leaves and fruits of urmoi, *Sapium indicum* Willd. were collected from Chalna under the District of Khulna. After collection, all fresh leaves of the test plants were washed with water and kept in the shade up to 15 days for air-drying. Mature seeds were collected from fresh fruits of karanja, mahogany, neem and mature fruits were collected from urmoi. The dried plant materials were then ground separately with electrical grinder and sieving through 60 micron diameter sieve to obtain fine powder. The powder was preserved into plastic pot at low temperature (4˚C) till their use in extract preparation.

Extraction of plant materials: Prepared leaf, seed and fruit powder were used for preparation of plant extract following the method of Tikum *et al.* (2008). The dried plant powders (50 g) were taken into a 400 ml beaker. The powder of leaves, seeds and fruits were extracted with n-hexane, dichloromethane (DCM) and methanol, respectively. The extract was collected after 24 hours, filtered by fine cloth and concentrated by a rotary vacuum evaporator. The residual solvent was removed by high vacuum pump. Each of the extract was stored in a freezer until use.

Preparation of concentration: Stock solutions (20% w/v) of plant extracts were prepared by diluting the condensed extracts with respective solvent. Different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) of each category of plant extracts were prepared by dissolving them in the same solvent prior to insect bioassay.

Toxicant effect on rice weevil: The experiment was conducted according to the standard method number 1 described by McDonald *et al.* (1970) and modified by Talukder and Howse (1995). The adult insects were chilled for a period of 10 minutes. Then the immobilized insects were picked up individually by using a camel hair brush. One μl solutions of different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) of different extracts were applied to the dorsal surface of the thorax of each adult weevil using micro-pipette**.** Thirty adult weevils (1-2 weeks old) in three replicates of 10 insects (5 ♂ + 5 ♀) each, was treated at each dose. In addition, the same numbers were treated with solvent only as control. After treatment, the 10 weevils were transferred into 9 cm diameter Petri dish containing food. Adult weevils were examined daily and those that did not move or respond to gentle touch were considered as dead. Rice weevil adult mortalities were recorded at 24, 48 and 72 hours after treatment (HAT).
The observed mortality was corrected by Abbott's formula (1987):
Corrected mortality (%) = (Observed mortality–Control mortality/100 - Control mortality) x 100.

Statistics analysis: The data were analysed by ANOVA with CRD 4 Factor and significant mean values were compared with Duncan Multiple Range Test (DMRT). The per cent mortality data were transformed into arcsine values before ANOVA.

Residual effect on rice weevil: The experiment was conducted according to the method of Rahman and Talukder (2006) with certain modification. One ml plant extracts of different concentrations (2.0, 4.0, 6.0, 8.0 and 10.0% w/v) were used for treating each 10 g rice grains. Then each 10 g treated grains were placed into different Petri dish. The control Petri dish contains only solvent treated rice grain. Five pairs of newly emerged adult rice weevil (5 male and 5 female) were released into each Petri dish separately at 5, 10, 15 and 20 days after treatment (DAT) to oviposit. The adult weevils from each Petri dish were removed after 7 days of release.

In this experiment, the following observations were recorded:

i) Number of F_1 adult progeny from each Petri dish (from 30 days up to 43 days of insect release).

ii) The inhibition rates (IR%)

$$IR\% = \frac{Cn - Tn}{Cn} \times 100$$

Where, C_n = Number of insects on control dish and

T_n = Number of insects on treated dish

iii) Number of damaged grain from random sample of 50 rice grains at the end of the experiment.

Grain infestation rate was also calculated by in terms of the grain infestation rate (GIR) = (No. of damaged grain/ No. of total grain) ×100.

The data were statistically analyzed by ANOVA and significant mean values were compared with DMRT (Duncan, 1951).

Results and Discussion

The results of the insecticidal effect of different solvent extracts of leaves and seeds/fruits of karanja, mahogany, neem and urmoi against rice weevil have been presented in Table 1 and figure1.The interaction effect of plant, plant part and time on rice weevil was significant at 24, 72 HAT. The urmoi fruit extract showed the highest mortality (51.53%), whereas mahogany leaf extract possessed least mortality (25.78%) at 72 HAT (Table 1). The interaction effect of plant part and time was significant. The effects of seed/fruit extracts were higher than that of leaf extracts i.e. mortality percentage of rice weevil treated with seed/fruit extract was 47.89% and that of leaf extract was 34.72% at 72 HAT (Fig. 1).

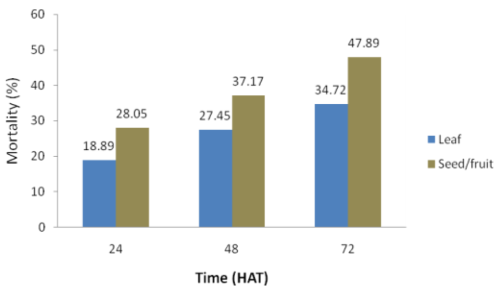

Fig. 1. Mean mortality percentage of rice weevil, *S. oryzae* treated with extracts of different plant parts by topical application method at different HAT).

The residual toxicity effect of two plant extracts were evaluated against rice weevil under laboratory condition. The efficacy of n-hexane, dichloromethane, and methanol urmoi fruit and neem seed extracts as protectants for rice grains against rice weevil were evaluated by comparing the number of F_1 progeny emerging, grain infestation rate, and inhibition rate. Five pairs (5 ♀ + 5 ♂) of adults were released at 5, 10, 15, and 20 days after treatment of 10 g rice grains with one mL plant extracts of different concentrations. The residual effect of neem and urmoi plant extracts on number of F_1 adult emergence was statistically significant at 1% level of significance (Table 2). The emergence of F_1 progeny gradually increased, because toxicity of the extract decreased with the increase of days after treatment. The F_1 progeny from treated grains after 20 DAT was higher than those after 5 DAT. It was observed that urmoi fruit extract had more inhibition effect on F_1 adult emergence than neem seed extract. The grain infestation rate at different DAT was found statistically significant at 1% level (Table 2).

Table 1. Mean mortality percentage of rice weevil, *S. oryzae* treated with plant part extracts of different plant by topical application method at different hours after treatment (HAT)

Name of the plant extracts	Name of the plant part extracts	Mortality percentage		
		24 HAT	48HAT	72HAT
Karanja	Leaf	18.22 cd (20.20)	23.11(26.21)	28.89 d (31.44)
	Seed	23.56 bc (27.38)	35.33(35.75)	48.00 ab (43.72)
Mahogany	Leaf	16.45 d (19.41)	20.89 (24.37)	25.78 d (29.41)
	Seed	24.89 b (28.85)	35.11(35.91)	45.33 bc (42.14)
Neem	Leaf	17.56 cd (23.21)	31.11(33.23)	41.56 c (39.85)
	Seed	35.33 a (34.62)	40.89 (39.19)	46.89 a-c (43.04)
Urmoi	Leaf	23.33 bc (26.27)	34.67 (35.41)	42.67 bc (40.52)
	Fruit	28.44 b (30.95)	41.33 (39.67)	51.53 a (46.76)
	$S\bar{x}$	1.6254	1.3811	1.4551
	Probability level	0.01	NS	0.01

Table 2. Residual effect of two plant extracts on F_1 progeny of rice weevil, *S. oryzae* and their potentiality as grain protectants at different DAT

Name of the plant extracts	Adult released after DAT	Mean No. of F_1 adults emerged	Grain infestation rate (%)	Inhibition rate (%)
Urmoi fruit	5 DAT	4.86 d	1.53 f	53.48
	10 DAT	8.00 d	1.98 f	45.58
	15 DAT	15.00 c	3.26 de	41.53
	20 DAT	17.33 c	4.38 c	40.65
Neem seed	5 DAT	21.93 b	2.78 e	45.79
	10 DAT	24.53 ab	3.81 cd	45.63
	15 DAT	27.93 a	5.64 b	39.73
	20 DAT	25.63 ab	7.16 a	36.50
	$S\bar{x}$	1.0406	0.1912	2.1690
	Probability level	0.01	0.01	NS

NS= Not significant. DAT= Days after treatment.
Within column values followed by different letters(s) are significantly different by DMRT.

Between two plant extracts urmoi fruit extract showed least number of F_1 progeny (17.33) at 20 DAT, whereas neem seed extract had the highest number of F_1 progeny (25.63) at 20 DAT. The urmoi fruit extract showed least grain infestation (1.53%) at 5 DAT, whereas higher grain infestation (4.38%) was observed at 20 DAT. The grain infestation rate gradually increased, because toxicity of the extract decreased with the increase of DAT. The infestation rate from treated grains after 20 DAT was higher than those after 5 DAT. Urmoi fruit extract had more persistent than neem seed extract. The highest inhibition rates of rice weevil were recorded from rice grains when treated with urmoi fruit extracts than neem seed extracts in all days after treatment. Inhibition rate of the insect decreased, because toxicity of the extract decreased with the increase of days after treatment. When rice weevil were released at 5, 10, 15 and 20 days after treatment with 2, 4, 6, 8, 10% extract, then F_1 adult weevil emerged, grain infestation rate and IR% were insignificant. Number of F_1 adults, grain infestation rate was inversely and inhibition rate was directly proportional with doses (Table 3). Between two plant extracts, urmoi fruit extracts was more effective and persistent.

Table 3. Residual toxicity effect of different plant extracts at different dose level on F_1 progeny of rice weevil, *S. oryzae* and their potentiality as grain protectant at different DAT.

Name of the plant extracts	Adult released after	Doses (%)	Mean No. of F_1 adults emerged	Grain infestation rates (%)	Inhibition rate (%)
Urmoi fruit	5 Days	2.0	7.44	2.21	19.86
		4.0	5.77	1.86	37.37
		6.0	4.44	1.47	61.81
		8.0	3.66	1.01	71.75
		10.00	3.00	1.09	76.19
	10 Days	2.0	11.00	2.74	24.44
		4.0	9.11	2.38	38.91
		6.0	7.88	2.83	47.35
		8.0	6.44	1.61	53.95
		10.00	5.55	1.36	63.22
	15 Days	2.0	20.77	4.55	21.90
		4.0	16.33	3.75	37.10
		6.0	14.22	3.12	43.70
		8.0	12.77	2.7	48.32
		10.00	10.88	2.49	56.62
	20 Days	2.0	24.22	5.51	15.39
		4.0	19.55	4.93	32.41
		6.0	16.66	4.27	42.47
		8.0	14.00	3.80	53.07
		10.00	12.22	3.36	59.85
Neem seed	5 Days	2.0	31.00	3.79	26.72
		4.0	26.77	3.14	35.53
		6.0	21.00	2.61	48.62
		8.0	17.33	2.14	55.49
		10.00	13.55	2.22	62.58
	10 Days	2.0	34.55	4.58	24.49
		4.0	27.33	4.70	39.30
		6.0	24.44	3.85	45.90
		8.0	19.77	3.20	55.90
		10.00	16.55	2.71	62.55
	15 Days	2.0	36.55	7.41	21.54
		4.0	31.44	6.57	31.91
		6.0	26.66	5.45	41.78
		8.0	24.00	4.73	48.27
		10.00	21.00	4.03	55.15
	20 Days	2.0	34.37	9.34	16.94
		4.0	29.11	8.11	27.23
		6.0	25.22	7.03	36.72
		8.0	21.11	6.11	47.43
		10.00	18.33	5.19	54.17
$S\bar{X}$			2.3269	0.4276	4.8501
Probability level			NS	NS	NS

NS= Not significant.

From the above findings, the two plant extracts reduced F_1 adult emergence, grain infestation rate and gave higher inhibition rate. The results of the present investigations were quite in agreement with the above mentioned previous findings. All of them were found that botanical plant materials reduced the adult emergence, grain infestation rate and increased inhibition rate which were similar with this study. Figures 2-4 showed the interaction effect of plants and solvents. From the figures it was found that n-hexane extract of urmoi have more toxic effect. The lowest number of F_1 progeny (7.36), least grain infestation (1.81%) was observed when rice grain were treated with urmoi fruit n-hexane extract and the highest inhibition rate (51.41%) were found when rice grains were treated with methanol neem seed extract. The interaction effect of plant and solvent extracts on adult emergence, grain infestation and inhibition rate was found statistically significant at 1% level. Figures 5-7 showed the interaction effect of solvents and time. From the figures, it is found that methanol extract have more toxic effect. When adults were released at 20 DAT, the lowest number of rice weevil F_1 progeny emerged (16.04) in case of rice grain treated with dichloromethane solvent extract. The least grain infestation (5.30%) was observed when rice grains were treated with dichloromethane extract and the highest inhibition rate (40.61%) were found when rice grains were treated with methanol extract in case of adult released at 20 DAT. The interaction effect of time and solvent extracts on adult emergence, grain infestation and inhibition rate was found statistically significant at 1% level. Figures 8-10 showed the interaction effect of plant extracts and doses. Between the plant extracts, urmoi fruit extract was more effective. The residual effect of different plant extracts at different dose level on number of F_1 adult emergence was statistically significant at 1% level. The lowest number of F_1 progeny emerged from the rice grain treated with 10% urmoi fruit (7.91%) and neem seed (17.36) extract.

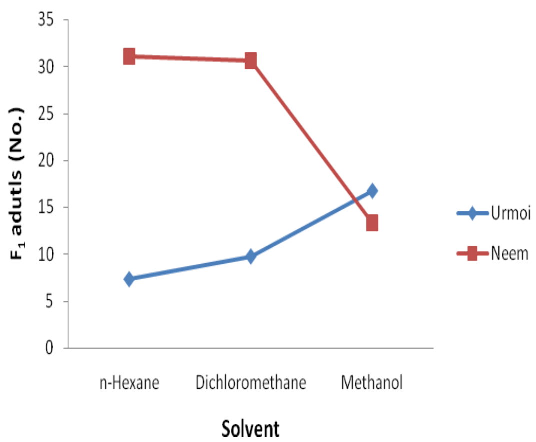

Fig. 2. F_1 progeny of rice weevil adults emerged after treatment with different plant extracts prepared by different solvents.

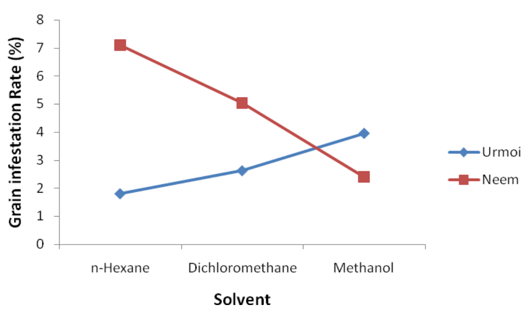

Fig. 3. Rice grain infestation due to rice weevil after treatment with different plant extracts prepared by different solvents.

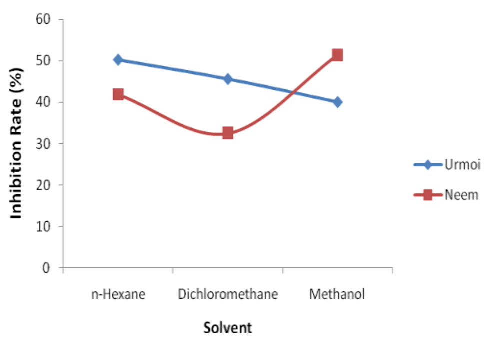

Fig. 4. Inhibition rate of rice weevil after treatment with different plant extracts prepared by different solvents).

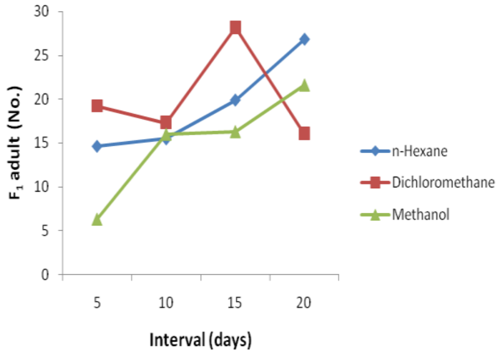

Fig. 5. Number of F_1 progeny of rice weevil adults emerged from rice grain where adults released at different periods after plant extract treatment.

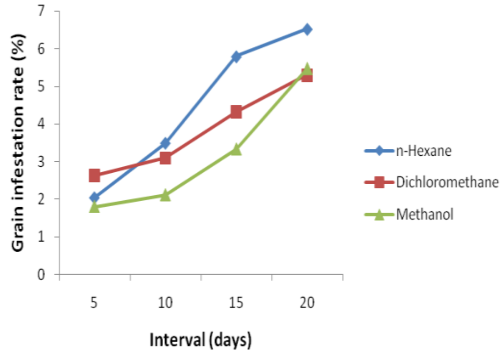

Fig. 6. Rice grain infestation by rice weevil after treatment with different plant extracts where adults released at different periods after treatment.

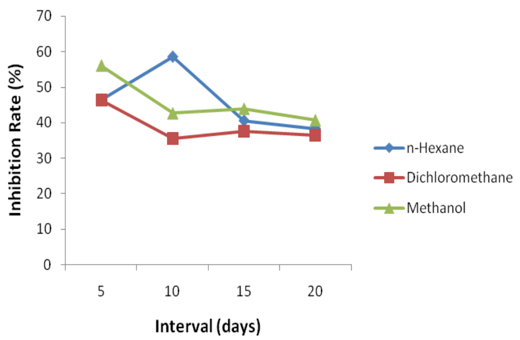

Fig. 7. Inhibition rate of rice weevil from different plant extracts prepared by different solvents where adults released at different periods after treatment.

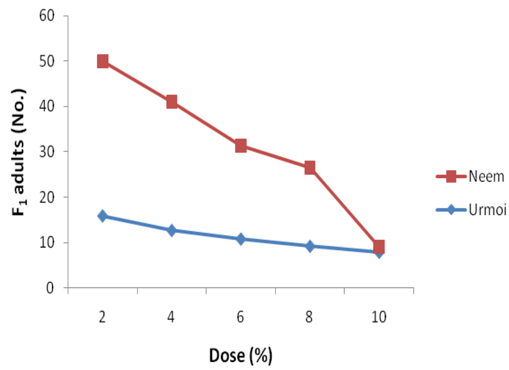

Fig. 8. Progeny of rice weevil F_1 adults emerged after treatment with different plant extracts at different dose level.

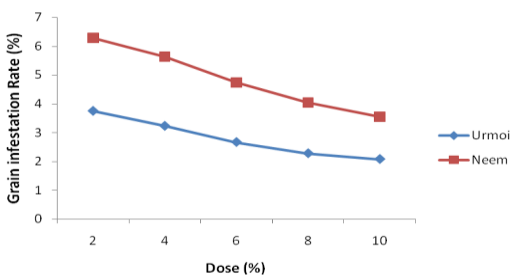

Fig. 9. Rice grain infestations by rice weevil after treatment with different plant extracts at different dose level.

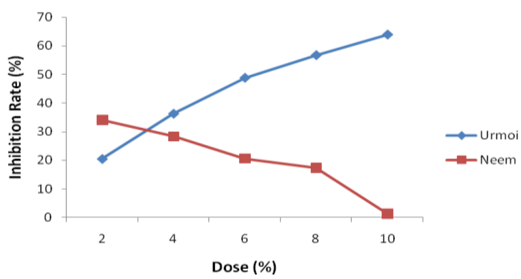

Fig. 10. Inhibition rate of rice weevil after treatment with different plant extracts at different dose level (Interaction of plant and dose).

The emergence of F_1 adult decreased with the increasing level of doses. Kamruzzaman *et al.* (2008) studied the acetone and water extract of leaf and seed of castor, *Ricinus communis* and urmoi, *Sapium indicum* for their direct toxic and residual effects on the pulse beetle, *Callosobruchus chinensis.* The lowest number of F_1 adult emerged, the least seed damage rate, the lowest weight loss and the highest inhibition rate were found in pulses treated with urmoi plant extract. Rayhan *et al.* (2014) observed that neem and mahogany extracts protected seed from damage and weight loss caused by the rice weevil, *S. oryzae.*

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