

REPELLENCY OF PLANT ESSENTIAL OILS TO KEY COLEOPTERAN STORED GRAIN INSECTS OF RICE

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ABSTRACT

Laboratory studies were conducted to assess the repellant effects of three essential oils from plants viz., orange, eucalyptus and cinnamon oils against four major coleopteran stored grain insect pests of rice viz., *Sitophilus oryzae, Oryzaephilus surinamensis, Rhyzopertha dominica,* and *Tribolium castaneum*. The % repellency (PR) and index of repellency (RI) were observed to range from 10 to 100% and 0.00 to 0.90, respectively. Eucalyptus oil @ 5% showed maximum repellent action against *Tribolium castaneum*, registering PR and RI values of 93.33 (F=0.921), 100 (F=1.66), 100 (F=3.772) and 0.07, 0.00 and 0.00, respectively at 3, 6 and 12 hrs after treatment and were found significantly superior over rest of the treatments. Chemical profiling of tested oils through GCMS showed presence of 2- 3 chemical constituents amounting to >90 % of total composition of oil. The results highlight the repellency effects of the essential oils and indicate that these can be ecofriendly ones for the post-harvest protection of rice.

Key words: Orange, eucalyptus, cinnamon oils, *Sitophilus oryzae, Oryzaephilus surinamensis, Rhyzopertha dominica, Tribolium castaneum*, rice, index of repellency, % repellency, GCMS

In India, stored product insect pests in cereals, pulses and oilseeds cause severe post-harvest losses in the range of 3.9-6.0%, 4.3-6.1% and 2.8-10.1%, respectively (Dhingra, 2016). Amongst these pests, the most important and common are the coleopterans attacking stored rice and their products, viz., Sitophilus oryzae L., Oryzaephilus surinamensis (L.), Rhyzopertha dominica (F.) and Tribolium castaneum (Herbst). Management of these depends mostly on the use of fumigants and persistent insecticides. Although effective fumigants like phosphine or methyl bromide (only quarantine treatment) are available to manage stored grain insect pests, but substantial increase in awareness of their ill effects viz., toxicity to nontargets, pesticide residues and environmental pollution is noticed in recent days (Benhalima et al., 2004; Collins et al., 2005; Haririmoghadam et al., 2011). Aromatic essential oils of plant origin are promising alternative to insecticides in protecting the post-harvest produce and are traditionally been used to kill or repel stored-grain insects (Isman, 2006). These are better alternative to conventional insecticides due to their low mammalian toxicity and high volatility (Shaaya et al., 1997; Li and Zou, 2001). Basically, these are volatile in nature; their secondary metabolites are characterized by a strong aroma and having density lower than water (Bakkali et al., 2008). It has been

well established that products of biological origin are reported to have useful insecticidal compounds against insect pests (Arthur, 1996). Recently, these essential oils have been recognised as pesticides (Isman et al., 2011). Essential oils are considered insecticides because they are selectively bioactive, have little or no harmful effects on non-target organisms and environment (Dong et al., 2004; Kestenholz et al., 2007; Regnault et al., 2012). In this study, repellent activity of three plant essential oils viz., orange (*Citrus sinensis* L.), eucalyptus (*Eucalyptus obliqua* L'Her) and cinnamon (*Cinnamomum verum*) oils are evaluated against stored grain insect pests of rice viz., *S. oryzae*, *T. castaneum*, *O. surinamensis* and *R. dominica* under laboratory conditions.

MATERIALS AND METHODS

The samples of *S. oryzae*, *T. castaneum R. dominica* and *O. surinamensis* were collected from rice storage godown of ICAR- National Rice Research Institute (NRRI), Cuttack and were maintained in the Grain Entomology laboratory $(28\pm 2^{\circ}C; 65\pm 5\%$ RH). Initially, 50 pairs of freshly emerged adults were placed in a jar containing rice grains (0.5 kg). The open end of jars were covered with muslin cloth and allowed for 7 days for mating and oviposition. Then parental stocks were removed and the left over content of each jar (freshly laid eggs and rice grains) were allowed for further

multiplication and insects were collected and reused for subculturing. The subsequent progenies (adults) were used for bioassays. For the bioassay, commercially available eucalyptus, orange and cinnamon oils were obtained from commercial suppliers (NICE Chemicals Private Limited, India). For chemical characterization, the tested plant essential oils (2 μ l) were dissolved in Hexane (HPLC grade) and were analyzed using GC-MS (Jeol GC mate) system following Thanigaivel et al. (2017). The molecular weight/ formula and structure of the compounds of test materials were ascertained by interpretation on mass spectrum of GC-MS using the database of the National Institute of Standards and Technology (NIST).

'Area preference method' in a completely randomized design was used to assess the repellent effects of essential oils (Obeng- Ofori et al., 1998) during 2017-2018. Preparation of test solutions (@ 1, 2 and 5 %) was done by dissolving essential oils in acetone (AR grade). Half cut filter paper (diameter 9.0 cm, Whatman No.1) was dipped in 1.0 ml of the respective test oil while, remaining half was dipped in 1.0 ml acetone which served as control. Both halves were allowed for solvent evaporation. All treatment and control halves were attached together on 9 cm glass petridishes using adhesive tape from bottom of filter paper. Three replicates were maintained for each concentration of oil. Test insects (20 No) were released at the centre of each filter paper disc and were then covered and sealed using para films. Petridishes were kept under dark at 26° C and $65 \pm 5\%$ RH. The number of adults on the treated and untreated sides were counted at 3, 6, 12 and 24 hr after treatment, with the experiment repeated twice. Effectiveness of plant essential oil was evaluated by Percent Repellency (PR) and Repellency Index (RI) using the formula PR (%) = [(Nc - Nt)/(Nc)]+ Nt)]x 100 (where, Nc- no. of insects in untreated side, Nt- no. of insects in treated side as per Nerio et al., 2009). Ranges of PR values and their categories used are: 0-0.1%: Class 0; 0.1-20%: Class I; 20.1-40%: Class II; 40.1-60%: Class III; 60.1-80%: Class IV; 80.1-100%: Class V (Tapondjou et al., 2005). Repellency index was calculated by using RI= 2G/(G+P) as per Mazzonetto (2002), wherein G= number of insects in treated side and P= number of insects in untreated side. RI values ranges from 0 to 1 and inversely related with PR values.

RESULTS AND DISCUSSION

The constituents detected in the study from

eucalyptus, cinnamon and orange oils are shown in Table 1. These reveal that the major compounds of eucalyptus oil were eucalyptol (64.80%), α -pinene (11.17%), β-pinene (8.19%), γ-terpinene (5.91%), α -phellandrene (3.88%), terpinen-4-ol (0.72%), α -terpineol (1.01) and 4-carene (0.51%); beside these, carvacrol, α -terpinene, 1-epi-alpha-gurjunene, aromandendrene, alloaromadendrene, α -farnesene, γ -gurjunene, γ -eudesmol and β -eudesmol were observed. Cinnamon oil resulted in eugenol (82.68%) as the major constituent; in addition, caryophyllene (4.60%), safrole (2.19%), trans-isoeugenol (1.82%), caryophyllene oxide (0.88%), humulene (0.56%), linalool (0.51%) were the other major constituents. Orange oil had D-limonene (83.35%) as the major constituent and cis-limonene oxide (3.31%), trans-2caren-4-ol (2.08%), tricyclo [4.1.0.0(2,7)] heptanes (0.84%), chrysanthenone (0.73%) and β -myrcene (0.59%) were the other constituents.

The present study reveals that the repellent activity of the essential oils depends on the insect pest and time after treatment, with concentration dependent repellant activity noticed with all the four pests evaluated. Toxicity of eucalyptus oil to coleopteran stored product pests had been attributed to metabolic compounds such as terpenoids and phenolic compounds (Lee et al., 2004; Tapondjou et al., 2005), and its toxicity to lepidopteran agricultural pests is known (Isman, 2006). Numerous plant species had been reported to have repellency properties, contact and fumigant toxicity (Golob et al., 1999). Important constituents of Cymbopogon spp., Ocimum spp. and Eucalyptus spp., and repellent activity of essential oils on insects had been reviewed by Nerio et al. (2010). Essential oils and many other plant extracts are known to possess repellent, insecticidal and ovicidal activities against various stored product insects (Ahmed et al. 1980; Hill and Schoonhoven, 1981; Jilani and Saxena, 1990; Desmarchelier, 1994; Papachristos and Stamopoulos, 2002) and was due to presence of monoterpenoids (Tong and Coats, 2010, Waliwitiya et al., 2005). Due to the high volatile nature of oils these are known to possess repellent and fumigant activities that have pest management significance (Koul, 2004; Konstantopoulu et al., 1992). The present results of chemical profiling of essential oils through GC MS showed presence of 2-3 chemical constituents amounting to >90 % of total composition of oil which is responsible for repellent activity.

Bioassay against stored grain insect pests revealed increased repellency with concentration of evalauted

Cinna	mon oil		Eucalyp	tus oil		Orange	oil	
Compound	Retention	%	Compound	Retention	%	Compound	Retention	%
	time	area		time	area		time	area
Benzaldehyde	3.29	0.03	α-Pinene	3.08	11.17	α-Pinene	3.05	0.33
o-Cymene	3.95	0.15	β-Pinene	3.51	8.19	β-Myrcene	3.54	0.59
Linalool	4.81	0.51	α-Phellandrene	3.76	3.88	D-Limonene	4.08	83.35
4-Carene	6.03	0.24	Eucalyptol	4.11	64.80	α-Ocimene	4.83	0.27
cis-p-Mentha-2,8-	6.20	0.01	γ-Terpinene	4.43	5.91	cis-Limonene oxide	5.33	3.31
dien-1-ol								
3-Phenylpropanol	6.53	0.06	4-Carene	4.73	0.51	1,1,2-trimethyl-	5.86	0.29
						Cyclopropane		
Chavicol	6.79	0.10	Terpinen-4-ol	5.89	0.72	Tricyclo[4.1.0.0(2,7)]	6.17	0.84
						heptane		
Safrole	7.33	2.19	α-Terpineol	6.06	1.01	trans-2-Caren-4-ol	6.42	2.08
Cinnamyl alcohol	7.81	0.06	Carvacrol	7.41	0.06	(-)-Carvone	7.12	0.20
Eugenol	8.48	82.68	2,4-dimethyl-1,3-	7.68	0.21	5-ethylidene-1-	7.54	0.66
			Cyclopentanedione			methyl-Cycloheptene		
Caryophyllene	9.18	4.60	α-Terpinene	8.09	0.30	2,5,5-trimethyl-1,3,6-	7.72	0.50
						Heptatriene		
Humulene	9.57	0.56	1-Epi-alpha-	8.94	0.50	Chrysanthenone	8.25	0.73
			gurjunene					
β-Patchoulene	10.03	0.11	Aromandendrene	9.33	0.43	8-oxo-cis-Ocimene	8.45	0.35
trans-Isoeugenol	10.33	1.82	Alloaromadendrene	9.59	0.17	Caryophyllene oxide	11.09	0.06
Caryophyllene	11.13	0.88	α-Farnesene	10.71	0.08	Santolina triene	12.27	0.08
oxide								
α-Farnesene	11.91	0.24	γ-Gurjunene	11.09	0.23	Eicosane	17.99	0.06
Coniferol	12.73	0.15	γ-eudesmol	11.61	0.22			
Benzyl Benzoate	13.11	3.78	β-eudesmol	11.85	0.48			
Total	-	98.17	Total	-	98.87	Total	-	93.7

Table 1. Chemical constituents of oils used and their relative composition

Concentration (%): % of concentrations based on peak area integration.

oils. Repellency Index and percent repellency values ranged from 0.00 to 0.90 and 10 to 100 (Table 2). Against S. oryzae, eucalyptus oil @ 5% concentration exhibited significantly higher PR 83.33% (F=4.282) and RI value of 0.17 when tested at 3 hr after treatment; cinnamon oil @ 5% concentration found on par with eucalyptus oil @ 5% with same PR and RI values; at 6, 12 and 24 hrs after treatment, cinnamon oil @ 5% exhibited significantly higher repellency with PR values of 96.67 (F=10.68), 90.00 (F=3.857), and 86.67 (F=3.25) and RI of 0.03, 0.10 and 0.13, respectively. A repellent action of eucalyptus oil against stored grain pests is known earlier. Extracts of eucalyptus leaf prepared in different solvents are known to possess repellent action against S. oryzae adults (Lee et al., 2004); similarly repellent potential of leaves of six vegetables species against adults of S. zeamais was observed by Procópio et al. (2003) and they reported only leaves of E. citriodora (PI = -0.81) and Capsicum frutescens (PI=-0.17) have shown the repellency based on Preference Index (PI).

Similarly, against T. castaneum eucalyptus oil @

5% showed maximum repellent action with PR and RI values of 93.33 (F=0.921), 100 (F=1.66), 100 (F=3.772) and 0.07, 0.00 and 0.00 at 3, 6 and 12 hr after treatment, and were found significantly superior; at 12 hr after treatment orange oil @ 5% had shown repellant action on par with eucalyptus oil @ 5% with PR value of 98.33 and RI value of 0.03; and after 24 hr after treatment orange oil @ 5% exhibited significantly superior repellency (F=20.26) with PR and RI values 100 and 0.00, respectively. Against R. dominica, at all time intervals, eucalyptus oil @ 5% has shown maximum repellency; at 3 and 6 hr after treatment, the said oil exhibited repellent action with PR value of 86.67 (F=3.87), 83.33 (F=2.60) and RI value of 0.13, 0.17 and found significantly superior; at 12 hr after treatment, eucalyptus oil @ 2% having PR value of 80.00 (F=10.99) and RI value of 0.20 was found on par with eucalyptus oil @ 5% having PR value of 83.33 RI value of 0.17; same eucalyptus oil @ 2% having PR value of 76.67 (F=12.56) and RI value of 0.23 was found on par with eucalyptus oil @ 5% with PR value of 83.33 (F=12.56) and RI value of 0.17 and orange oil @ 5% having PR value of 83.33 (F=12.56)

No.	Treatments	3 H Adult individuals	3 HAI iduals	r .	RI	6 F Adult individuals	6 HAT /iduals	r .	RI	12 F Adult individuals	12 HAT viduals		RI	24 F Adult individuals	24 HAT viduals	T PR	RI
		(%) UT	L	(%)		(%) UT	L	(%)		(%) UT	Н	(%)		(%) UT	L	(%)	
	Eurodinatus cil @ 1.02	70.22	71 67	56 67de	0.42	בא אר	Sitophilus	0		70 22	71 67	369 22	0.42	71 67	10 22	12 22d	73.0
	Eucalyptus oil @ 2 %	85.00	15.00	70.00^{10}	0.30	83.33	16.67	66.67 ^d	0.33	81.67	18.33	50.07 63.33°	0.37	78.33	21.67	56.67°	0.43
	@ @ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	91.67 80.00	8.33	83.33 ^a	0.17	91.67 86.67	8.33 12 22	83.33 ^b 72.23 ^c	0.17	90.00 88 23	10.00	80.00 ^b 76.67 ^b	0.20	90.00 81.67	10.00 18 22	80.00 ^{ab} 62-23c	0.20
	- 2	88.33	11 67	76.67 ^{ab}	0.73	93.33	667	86.67 ^b	0.13	01.67	8 33	A3 33 ^{ab}	0 17	88 33	11 67	4L9.91	0.23
	Cinnamon oil @ 5%	91.67	8.33	83.33ª	0.17	98.33	1.67	96.67ª	0.03	95.00	5.00	90.00ª	0.10	93.33	6.67	86.67 ^a	0.13
	ange oil @ 1%	65.00	35.00	30.00^{f}	0.70	66.67	33.33	33.33	0.67	65.00	35.00	30.00°	0.70	73.33	26.67	46.67 ^d	0.53
8 0 0 0	Orange oil @ 2% Orange oil @ 5%	76.67 81.67	23.33 18.33	$53.33^{\rm cd}$ $63.33^{\rm cd}$	0.47 0.37	73.33 76.67	26.67 23.33	46.67^{1} 53.33°	$0.53 \\ 0.47$	71.67 88.33	28.33 11.67	43.33 ^d 76.67 ^b	$0.57 \\ 0.23$	80.00 91.67	20.00 8.33	60.00 ^c 83.33 ^{ab}	$0.40 \\ 0.17$
SEm± CD= 0.01		4.08 16.62		8.16 33.24		3.19 12.99		6.38 25.98		5.09 20.73	6.00	10.18 41.45		4.44 18.09		8.89 36.18	I I
							bolium	castaneum									
	_	86.67	13.33	73.33 ^{bc}	0.27			76.67 ^{de}	0.23	88.33	11.67	76.67°	0.23	86.67	13.33	73.33 ^d	0.27
	\sim	90.00	10.00	80.00°	0.20	93.33	6.67	86.67^{bc}	0.13	91.67	8.33	83.33 ^b	0.17	93.33	6.67	86.67°	0.13
	Eucalyptus oil @ 5 %	96.67	3.33	93.33ª	0.07	100.00	0.00	100.00^{a}	0.00	100.00	0.00	100.00^{a}	0.00	98.33	1.67	96.67 ^{ab}	0.03
	Cinnamon oil @ 1 %	/0.0/	25.33	53.33 ⁴	0.4/	80.07	15.33	15.33°	0.27	88.33	11.6/	/0.0/	0.23	90.07	5.55	93.33°	0.0/
	Cinnamon oil @ 2%	81.0/ 22/23	18.33	03.33°	0.57	00.06	10.00	80.00 ^{cm}	07.0	93.33	0.0/	80.07	0.13	98.33 100.00	1.0/	90.0/"	0.03
	nnamon oil @ 5%	80.07 78 22	15.55	15.33 ⁰⁰ 56 67ef	17.0	91.07	8.33 10 22	83.33 ⁵⁵	0.17	90.00 00.00	1.0/		0.05	100.00	0.00	100.00"	0.00
	ange oil @ 2%	81.67	18 33	53 33de	0.37	03.33	667	86.67 ^{bc}	0.13	91.67	8.33	83 33 ^b	0.17	96.67	0.0	93.33	0.07
00 00	Orange oil @ 5%	85.00	15.00	70.00 ^{cd}	0.30	96.67	3.33	93.33 ^{ab}	0.07	98.33	1.67	96.67ª	0.03	100.00	0.00	100.00^{a}	0.00
SEm ± CD= 0.01		6.41 26.08		12.81 52.16	1 1	4.23		8.46 34.44		3.24 13.19	⁴ 0	6.48 2.6.37		2.94 11 97		5.88 23.93	1 1
						RI	vzopertha	t dominica									
	@ 1	81.67	18.33	63.33°	0.37	86.67	13.33	73.33 ^b		81.67	18.33	63.33 ^b	0.37	76.67	23.33	53.33°	0.47
	Eucalyptus oil @ 2 %	88.33	11.67	76.67 ^b	0.23	88.33	11.67	76.67 ^{ab}		90.00	10.00	80.00 ^a	0.20	88.33	11.67	76.67 ^a	0.23
	Eucalyptus oil @ 5 %	93.33 72 22	19.9 19.90	80.6/" 16 67de	0.13	91.0/ 79.22	8.33 71 67	83.33° 56 67°	0.17	91.6/	8.33	83.33 ^a	0.17	91.0/	8.33 11 67	83.33" 16 67e	0.17
	nnamon oil @ 2%	75,00	25.00	50.00 ^{de}	0.50	80.00	20.00	50.00°	040	63.33	36.67	26.67°	0.73	60.00	40.00	20.00	0.80
	Cinnamon oil @ 5%	76.67	23.33	53.33 ^d	0.47	86.67	13.33	73.33 ^b	0.27	66.67	33.33	33.33 ^d	0.67	68.33	31.67	36.67 ^d	0.63
	ange oil @ 1%	71.67	28.33	43.33°	0.57	68.33	31.67	36.67°	0.63	75.00	25.00	50.00°	0.50	76.67	23.33	53.33°	0.47
Öċ ∞∘	Orange oil @ 2%	80.00 86.67	20.00 12 22	60.00°	0.40	73.33	26.67 11 67	46.67 ^d 76 67 ^{ab}	0.53	81.67	18.33	63.33 ^b 76 67a	0.37	85.00 01.67	15.00	70.00 ^b	0.30
Em ±		3.77	00.01	7.54		4.87	10.11	9.75		3.60	-	7.20		3.64		02.C0 07.29	- 1.0
CD= 0.01		15.34		30.67		19.84	-	39.69		14.66	9	29.31		14.83		29.66	ı
						Oryz	Ś	surinamensis	sis								
_ ,	8 0 0	66.67	33.33	33.33 ^d	0.67	63.33	36.67	26.67 ^{de}	0.73	61.67	38.33	23.33 ^{cd}	0.77	56.67	43.33	13.33^{d}	0.87
2 6 1 H	Eucalyptus oil @ 2 % Encalymtus oil @ 5 %	80.00 80.00	28.33	43.33°	0.57	66.67 68 33	33.33	33.33 ^{0cd} 36.67 ^{bc}	0.67	68.33 71.67	31.67	$36.6/^{30}$	0.63	65.00 68.33	31.67	30.00 ⁰⁰ 36.67 ^{ab}	0/.0
	Cinnamon oil @ 1%	63.33	36.67	26.67^{d}	0.73	61.67	38.33	23.33°	0.77	58.33	41.67	16.67^{de}	0.83	61.67	38.33	23.33°	0.77
	Cinnamon oil @ 2%	65.00	35.00	30.00^{d}	0.70	70.00	30.00	40.00°	0.60	65.00	35.00	30.00^{bc}	0.70	65.00	35.00	30.00^{bc}	0.70
	nnamon oil @ 5%	71.67	28.33	43.33°	0.57	81.67	18.33	63.33 ^a	0.37	70.00	30.00	40.00ª	0.60	70.00	30.00	40.00ª	0.60
	Orange oil @ 1%	18.33	21.67	50.07°	0.43	00.00	35.00	30.00 ^{ue}	0/.0	20.00	45.00	16.67de	0.90	20.00/ 61.67	43.33	13.33° 72.22°	18.0
	Orange oil @ 5%	01.07 86.67	13.33	73.33ª	0.27	85.00	15.00	70.00^{a}	0.30	60.00 61.67	41.0/ 38.33	23.33 ^{cd}	0.77 0.77	01.0/ 68.33	31.67	36.67 ^{ab}	0.63
SEm ± CD= 0.01		5.30		10.60 43 15		5.24 21 33		10.48 42.67		5.44 22.16	-+ v	10.89 44.32		6.74 27 42		13.47 54.84	
		10.12							1	1.11	5	77.77		71.17	4	10.10	

and RI value of 0.17. All the evaluated oils at different concentrations against *O. surinamensis* did not exhibit significant differences.

Thus, the present results reveal the excellent repellency exhibited by eucalyptus oil @ 5% against T. castaneum and R. dominica. These results are in line with those of earlier ones- viz., Olivero-Verbal et al. (2010) with T. castaneum observed that Eucalyptus citriodora is a good repellent. Essential oils of E. kingsmillii and E. salmonophloia tested at four concentrations against female adults of Tetranychus urticae Koch indicated that repellency index was neutral at 9 and 17%, whereas, at 23 and 29% it stands as repellent (Haririmoghadam et al., 2011). The essential oil of E. dundasii and E. astringens against O. surinamensis show a more repellent effect corroborating the present results (Khemira et al., 2012). Study of Salvadores et al. (2007) indicated that clove oil was the best repellent against R. dominica, S. oryzae and T. castaneum. Strong repellent and deterrent activity against T. castaneum was observed with the leaf extract of Ocimum viride (Owsu, 2001); fumigant and repellent effects of oil of Ocimum gratissimum and its constituents as a better alternatives to synthetic fumigants against T. cactaneum, S. oryzae, R. dominica, O. surinamensis and Callosobruchus chinensis (Ogendo et al., 2008). Insecticidal and repellent properties of C. citrinus against C. maculatus were described by Zandi-Sohani et al. (2013) and repellent effects of essential oils of E. citriodora, Lippia origanoides, Tagetes lucida against S. zeamais are known earlier (Nerio et al., 2009); also, repellency against T. castaneum with Piper retrofractum oil had been documented (Tripathi et al., 2000). Wild species of 21 botanical families screened by Pascual-Villalobos (1999) revealed that the family Compositae reveals maximum repellency against T. castaneum; likewise, antifeedant and repellent properties of Cyperus articulatus against T. castaneum are known (Abubakar et al., 2000).

Thus, the current study revealed repellent activity of the essential oils against key stored grain insect pests. Traditionally plant based products have been used to kill or repel stored grain insects since time immemorial, hence the evaluated essential oils could be better alternative to conventional insecticides.

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