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Contact and repellent effects of essential oils of *Chromolaena odorata* (L.) and *Uvaria chamae* (P. Beauv) against *Macrotermes bellicosus* (Smeathman)

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Abstract

Macrotermes bellicosus, though a beneficial termite species causes serious damage to wood and wood products, fabrics and many agricultural crops and ornamental trees. This study was conducted to assess the potentials of essential oils of the leaves of *Chromolaena odorata* and roots of *Uvaria chamae* in the control of *M. bellicosus*. The repellency, knockdown and insecticidal effects of the oils to *M. bellicosus* were tested using the filter paper and cotton ball impregnation technique. Each test oil (0.05, 0.075 and 0.10 ml) was separately used in the repellency assay, for an exposure period of 30 and 60 minutes. For the knockdown and toxicity test, termites were exposed to 0.05, 0.075, 0.10, 0.125 and 0.15 ml of each oil for an exposure period of 60 minutes and 6 hours, respectively. Each test and control (untreated) group had three replicates and in each experiment twenty active workers of *M. bellicosus* were exposed. Repellency results were somewhat irregular and oil volume and exposure, time independent. Knockdown effect of 56.67 and 86.67 % were observed for *C. odorata* and *U. chamae* oils (0.15ml), respectively at the 60th minute. Contact toxicity test with 0.15 ml of the oils resulted in 100 % and 86.67 % mortalities for *U. chamae* and *C. odorata*, respectively. No knockdown or mortalities were observed in the controls. The difference between the repellency of both oils at 30 and 60 minutes was not significant ($p > 0.05$; $p = 0.842$ and 0.212 respectively), whereas for knockdown and insecticidal effects of both oils the difference was significant ^{Q3}($p < 0.05$; $p = 0.000$ and 0.001 respectively).. Essential oils of *C. odorata* and *U. chamae* are potential agents for the control of *M. bellicosus*.

Keywords: Termites, Essential oils, Repellency, Knockdown, Mortality.

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INTRODUCTION

Macrotermes bellicosus (Isoptera: Termitidae) is a species of termite that is commonly referred to as harvester termite or war-like termite or African termite. This species is mainly found in Africa, the Middle East and Asia (Oyedokun *et al.*, 2011). *Macrotermes bellicosus* is relished by people living the traditional lifestyle especially children who snack on it when roasted or fried. It is regarded as a traditional delicacy in Southern as well as Northern Nigeria (Igwe *et al.*, 2011). Adepoju and Omotayo (2014) reported that this *M. bellicosus* species is a good source of protein, calcium, phosphorus, iron, zinc, and antioxidants for its Nigerian consumers. They also reported the presence of vitamins A and E in their nutritional analysis of the termite species. *Macrotermes bellicosus* also plays an important role in ecosystem balance. It is a major agent of decomposition, thereby improving the fertility of the soil and also known to play an important role in nutrient and carbon fixation (Ajao *et al.*, 2018). Besides the beneficial roles played by this species it is also known to cause great damage to wooden structures in buildings as a result of its wood diet. It has the ability to remain hidden until after extensive damage has been done (Okweche *et al.*, 2015; Omoya and Kelly, 2019). It is also known to damage paper, cloth, carpets, and other cellulose materials. Its feeding activity is aided by symbiotic microorganisms such as bacteria and fungi which are capable of helping it to digest cellulose (Ohkuma, 2008, Ohkuma and Brume, 2011; Peterson *et al.*, 2015). Owing to damages caused by *M. bellicosus*, control of this species becomes imperative. Efforts at control have been directed towards the chemical method. An example is the use of Thiamethoxam (Oyedokun *et al.*, 2011), Solignum and used engine oil as preventive measure (Ugbomeh and Diboyesuku, 2019). The use of synthetic insecticides against termites' infestations has its drawbacks. These insecticides are often lethal to non-target and beneficial organisms. Runoffs from sites where these insecticides have been used can contaminate ground water sources. Another drawback is the development of resistance to these artificial insecticides by the target insects (Silva *et al.*, 2012). The search for effective alternatives has been directed extensively to the diverse flora of the tropics. Oyedokun *et al.* (2011), reported on the efficacy of leaf extracts of *Phyllanthus amarus*, *Acassia albida* and

Tithonia diversifolia against workers of *M. bellicosus*, *in vitro*. Similarly, Okweche *et al.*, 2015 reported the comparative efficacy of some insecticidal plant materials against the dry wood termite *Cryptotermes cavifrons*. Okweche and Nnah (2018) also reported on the anti-termitic properties of *Jatropha curcas* against *M. bellicosus*. Omoya and Kelly (2019), assessed the efficacy of fungal entomopathogens against *M. bellicosus*. Their study revealed the lethal activity of the fungal species *Metarhizium anisopliae* on *M. bellicosus*.

Chromolaena odorata (Asteraceae) is a perennial shrub, native to North America, but has been introduced to tropical Asia, West Africa and parts of Australia (Chakravorty *et al.*, 2011). In Nigeria, it is known as independence leaf, because it was discovered shortly after Nigeria's independence from colonial rule in 1960 (Stanley *et al.*, 2018). *Chromolaena odorata* has a life span of approximately ten years (Zahara, 2019). Vaisakh and Pandey (2011) reported the anti-diarrheal, astringent, antiplasmodic, anti-hypertensive and anti-inflammatory activities of *C. odorata*. Besides, the insecticidal properties of *C. odorata* have been reported against *Periplanata americana*, *Callosobruchus maculatus* and *Callosobruchus subinnotus* (Udebuani *et al.*, 2015; Nyamador *et al.*, 2017). *Uvaria chamae* (Annonaceae), known as finger root or bush banana is an evergreen edible plant. The root of *U. chamae* is used for the treatment of nose-bleeding, heart diseases, blood in urine, pile, and fever (Etukudo, 2003). Also, the insecticidal properties of the powdered stem bark and ethanol extract of the root of *U. chamae* have been reported against *Callosobruchus maculatus*, *Rhizopertha dominica* and *Sitophilus zeamais* (Negbenebor *et al.*, 2018).

Copping, 2009 described bio-pesticides as pesticides derived from natural sources such as plants, fungi, nematodes, bacteria, and others. These pesticides are known to be target specific and are often regarded as important components of integrated pest management (IPM) programmes. The use of botanicals in the control of insect pests have been an age long tradition, especially in the African continent. Plants such as *Gmelina arborea*, *Aframomum melegueta*, *Moringa oleifera*, *Zingiber officinale*, *Morinda lucida* and *Jatropha curcas* have been effectively used to control termites

(Okweche *et al.*, 2015; Okweche and Nnah, 2018).

The objective of this research was to determine the major chemical components of essential oils of *C. odorata* and *U. chamae* and evaluate the efficacy of these oils in the management of *M. bellicosus*.

MATERIALS AND METHODS

Collection of Plant Materials

The experimental plants *Chromolaena odorata* and *Uvaria chamae* were harvested fresh from Uyo, Nigeria. The identities were confirmed by a plant taxonomist in the Department of Botany and Ecological Studies; University of Uyo, Nigeria. Voucher specimens were deposited in the herbarium of the same department for future reference.

Extraction of Volatile/Essential Oils

Essential oils from the leaves of *C. odorata* and the roots of *U. chamae* were extracted by hydro-distillation (5 h), using a Clevenger apparatus, as described in the British Pharmacopoeia (2018). Extraction process was carried out in the laboratory of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. The oils were separately stored in labeled glass bottles for the experiments.

Chemical Analysis of the Oils

Each essential oil was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) on an Agilent System. This was carried out at Hussain Ebrahim Jamal (H. E. J) Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Pakistan. The components of each oil were identified by comparison of their mass spectra with library data of the GC-MS system and by comparison of their retention indices (RI) with relevant data provided by Adams, 2007.

Collection and Identification of Test Termite Species (*M. bellicosus*)

Worker termites were collected from a termitarium within the main campus of the University of Uyo, Nigeria, done by excavation of the termitarium. They were transported in a

small plastic cage (14.4 x 28cm), to the Laboratory of the Department of Animal and Environmental Biology, University of Uyo. Identification of *M. bellicosus* was done using taxonomic keys of Constantino, 1999 and Engel *et al.*, 2009. Dr. S. Okweche, an entomologist in the Department of Forestry and Wildlife Resources Management, University of Calabar, Nigeria, assisted in identification.

Test termites were kept in aerated plastic cages and maintained under laboratory conditions of temperature (27 ± 2 °C) and relative humidity of (75 ± 5 %). They were fed with dry wooden materials and dry leaves. Distilled water was sprinkled on filter paper within the cage to provide moisture. Termites were kept away from direct sunlight as described by Alavijeh *et al.* (2014) and Ojianwuna *et al.* (2016).

Test termites were allowed to adjust to laboratory conditions for 24 hours before commencement of experiments reported in this study.

Experimental Procedures

Repellency and contact toxicity tests were conducted in the laboratory of the Department of Animal and Environmental Biology, University of Uyo, Nigeria.

Repellency Test

The procedure of Kadir *et al.* (2014), was adopted for the test where Whatman filter paper (9.1cm diameter) was cut into two equal halves. One half of each was separately impregnated with 0.05, 0.075 and 0.10 ml of the pure essential oils of *C. odorata* and *U. chamae*, using a micropipette arranged in a completely randomized design with three replications. The second half of each filter paper was left untreated and served as the control. The treated/ impregnated half was allowed to dry. In each case, the treated and untreated halves of the filter paper were placed in a test plate with a space of 1.5cm between them. Twenty active worker termites were released into the middle with the aid of camel hair brush as described by Addisu *et al.* (2014) and Alavijeh *et al.*, 2014 and covered with untreated nets with mesh sizes large enough for ventilation, yet too small to allow for escape of test termites. Observations for repellency was made at 30 and 60 minutes per treatment.

This was done by counting the number of termites on the treated and untreated portions of each filter paper.

Contact Toxicity Test

Cotton ball impregnation technique was adopted for the contact toxicity tests. Five volumes (0.05, 0.075, 0.10, 0.125 and 0.15 ml) of the oils of the two experimental plants were separately tested on the termites (worker caste of *M. bellicosus*). These constituted the treatments which were replicated three times. Cotton balls of 1 cm diameter each were separately impregnated with the different concentrations of each oil and introduced into the test plates. Control plates which were also replicated thrice had untreated cotton balls. Both treated and untreated (control) plates contained termite nutrient made of chips of dry wooden materials. Contact toxicity was evaluated by exposing 20 active worker termites to each test concentration within the test plate. Observation for mortality was made after an exposure period of 6 hours. A termite was confirmed dead when it no longer exhibited movement after being prodded by with a small camel hairbrush.

Data Analysis

Percentage repellency was calculated according to the formula given by McDonald *et al.*, 1970:

$$\% \text{ Repellency} = \frac{(NC - NT) \times 100}{(NC + NT)}$$

Where NC is the number of termites in the control/untreated portions and NT, the number of termites on the treated portions.

Percentage mortality

$$= \frac{\text{No. of dead termites}}{\text{Total No. of termites exposed}} \times 100$$

Percentage knockdown is equal to:

$$\frac{\text{No of knockdown insects}}{\text{Total No of insects exposed}} \times 100$$

Results were also presented as mean and standard error of the mean.

Data were analysed using two-way Analysis of Variance (ANOVA), to determine the significant differences within a group or between different test concentrations. The significant means were separated using Turkey's mean separation procedure. Student t-test was used to determine the more potent of the two oils. All analysis was done using SPSS version 21 (2013).

RESULTS

Chemical Constituents of Test Oils

The essential oil of the leaves of *C. odorata* was obtained as light green oil. Gas Chromatography-Mass Spectrometry analysis detected the presence of sesquiterpene and monoterpene hydrocarbons (78.3 and 10.8 % respectively) and oxygenated sesquiterpenes (9.0 %). Other components identified in the oil were α -pinene (4.7 %), β -pinene (3.0 %), α -copaene (3.2 %), β -caryophyllene (11.1 %), germacrene (20.0 %), δ -cadinene (10.6 %), geigerene (2.7 %) and pregeijerene (15.8 %).

The oil obtained from the root of *U. chamae* was dominated by oxygenated monoterpenes (37 %), followed by non-terpene derivatives (23.3 %) and oxygenated sesquiterpenes (15.3 %). Other components detected were benzyl benzoate (23.3 %), dimethyl- P-cymene (14.2 %), T-cadino (12.1 %), methyl thymol (8.7 %), bornyl acetate (6.6 %), cyperne (6.1 %) and Isothymolmethyl ether (5.2 %). (Table 1).

Table 1: Chemical Composition of *C. odorata* and *U. chamae*

Constituent	C.O (%)	U. C (%)
Monoterpene hydrocarbons	10.8	8.8
Oxygenated monoterpenes	-	37.0
Sesquiterpene hydrocarbons	78.3	12.9
Oxygenated sesquiterpenes	9.0	15.3
Non-terpene derivatives	-	23.3
Total Identified	98.1	97.3

Key: C.O = *Chromolaena odorata*; U.C = *Uvaria chamae*.

Susceptibility of *Macrotermes bellicosus* to the test oils

General Observations

It was observed that on exposure of the test termites to both oils, there were aggressive attempts to escape from the test plates. The mandibles of the termites were also observed to be extended. Reduction in termite activities was also observed, prolonged exposure made the termites weak and sluggish. One of the reactions of the termites to the test oils was their secretion of brownish opaque substance. This was observed in both the repellency and toxicity tests of both oils. Knockdown of some of the termites was observed in all tests using both oils. The number of knocked down termites increased with increase in concentration for all tests. More termites were knocked down on exposure to *U. chamae* oil than *C. odorata* oil. Worthy of mention is the case of relatively agile

termites making attempts to revive knocked down termites.

Repellent Effect of test oils on *M. bellicosus*

The test oils used demonstrated repellent effect on the test termites. There was a decrease in repellency with increase in time of exposure of the termites to the various volumes of the oil of *C. odorata*. *Uvaria chamae* oil increased percentage repellency of the termites for 0.05 ml and 0.10 ml volumes from 86.70 to 93.30 % and 90.00 to 100 % respectively. At 0.075 ml volume of *U. chamae* oil, there was a decrease in repellency from 100% at 30 minutes to 90.00 % at 60 minutes exposure period. There was no significant difference ($p > 0.05$) in the repellency of both oils at the various volumes tested as revealed by t-test analysis. When repellency of both oils was compared at 30- and 60-minutes p-value of 0.842 and 0.212 ($p > 0.05$) respectively were obtained. The results obtained for repellency are presented in Table 2.

Table 2: Repellency of Oils of *C. odorata* and *U. chamae* to *M. bellicosus*

Test Oil/Vol. (ml)	30 minutes Exposure Time			60 Minutes Exposure Time		
	C(n=20)	T(n=20)	% Repellency	C(n=20)	T(n=20)	% Repellency
<i>C. odorata</i>						
0.05	19.33±0.67	0.67±0.67	93.30	18.67±1.33	1.33±1.33	86.70
0.075	18.67±0.67	1.33±0.67	93.10	18.67±0.67	1.33±0.67	86.70
0.10	20.00±0.00	0.00±0.00	100.00	18.33±0.88	1.67±0.88	83.30
Total	19.33±0.33	0.67±0.33		18.56±0.50	1.44±0.50	
p-value		0.296 ns			0.964 ns	
<i>U. chamae</i>						
0.05	18.67±0.67	1.33±0.67	86.70	19.33±0.67	0.67±0.67	93.30
0.075	20.00±0.00	0.00±0.00	100.00	19.00±1.00	1.00±1.00	90.00
0.10	19.00±1.00	1.00±1.00	90.00	20.00±0.00	0.00±0.00	100.00
Total	19.22±0.40	0.78±0.40		19.44±0.38	0.56±0.38	
p-value		0.422 ns			0.609 ns	

Values are the means of three (3) replicates, C = control, T = test group, ns = not significant. Differential repellency of the test oils at 30 and 60 minutes = 0.842 and 0.212 respectively ($p > 0.05$)

Table 3: Knockdown and Contact toxicity of *C. odorata* and *U. chamae* on *M. bellicosus*

Test Oil (ml)	Mean No. Exposed	Knockdown Effect (60 minutes)				Toxicity Effect (6 hours)			
		<i>C. odorata</i> Mean value	% knockdown	<i>U. chamae</i> Mean value	% Knockdown	<i>C. odorata</i> Mean value	% mortality	<i>U. chamae</i> Mean value	% mortality
0.05	20	3.33±0.67	16.65	8.00±1.15	40.00	0.67±0.67	3.35	2.67±1.33	13.35
0.075	20	4.67±1.76	23.35	11.33±0.67	56.65	3.33±1.33	16.65	4.67±0.67	23.35
0.10	20	8.67±0.67	43.35	12.00±3.06	60.00	8.00±1.15	40.00	10.00±1.15	50.00
0.125	20	10.67±0.67	53.35	16.00±2.00	80.00	13.33±1.33	66.65	16.00±1.00	80.00
0.15	20	11.33±0.88	56.65	17.33±0.67	86.65	17.33±1.45	86.65	20.00±0.00	100.00
Control	20	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	
Total		6.44±1.05		10.78±1.49		7.11±1.61		8.89±1.77	
p-value		0.000*		0.000*		0.000*		0.000*	
				p-value = 0.000*				p-value = 0.001*	

Values are the means of 3 replicates

Knockdown and contact toxicity effects of the oils

Table 3 shows the knockdown and contact toxicity effects of the oils on *M. bellicosus* at the 60th minute of exposure. Results obtained revealed that for both test oils the effect was concentration dependent. At the least volume of 0.05 ml of both oils, 3.33 % mortality was recorded for *C. odorata* while *U. chamae* recorded 13.33 % mortality of the termites. The highest oil volume of 0.15ml however resulted in percent mortality of 86.66 and 100 % for *C. odorata* and *U. chamae* respectively. There was no mortality in the

control experiments. For the knockdown test, 16.65 % of the insects were knocked down on exposure to 0.05 ml of *C. odorata* oil while 40 % of the termites were knocked down on exposure to the same volume of *U. chamae* oil. At the highest volume of 0.15 ml of both oils, 56.67 % and 86.67 % knockdown were recorded for *C. odorata* and *U. chamae* respectively. There was however no knockdown recorded in the control experiments. Results obtained for knockdown ($p = 0.000$) and toxicity ($p = 0.001$) effects revealed significant difference ($p < 0.05$) in the activity of the two oils

DISCUSSION

The significant roles played by some plant essential/volatile oils in the management of insect vectors and pests, including some termite species have been well documented (Okweche *et al.*, 2015; Nta *et al.*, 2017). The escape attempt and loss of agility/weakness observed in this study is corroborated by the report of Mehmood and Shahzadi (2016), who made similar observation when they tested the essential oil of *Boenninghausenia albiflora* against black garden ants. The mandible extension of termites on exposure to test oils was described by Seid *et al* (2007) as “mandible strike”, a form of defense mechanism. The brownish secretion observed in this study was described earlier by Prestwich (1979) as spray from the frontal gland of the termites that acts as defense mechanism when either soldier or worker termites feel threatened.

Termites damage to buildings in tropical countries is a serious concern. Ugbomeh and Diboyesuku (2019) carried out a study on termite infestation of buildings in Aso, a rural community in the Niger Delta of Nigeria. They reported that of the 106 houses inspected, 35.85% were infested with termites. A study on the incidence and severity of termites' infestations on *Azadirachta indica* used as avenue trees in University of Port Harcourt, Nigeria was conducted by Adedeji *et al.* (2015). Two termite species incriminated in the infestations of these trees were *Amitermes evuncifer* and *M. bellicosus*. They further added that because of this infestation *A. indica* could not sustain the expected environmental service functions of avenue trees over time. The activity of mound-building *M. bellicosus* around Kwara State University Campus, Nigeria, was investigated by Ajao *et al.*, (2018). Results obtained revealed the abundance of *M. bellicosus* mounds and called for precautionary measures to be taken to ensure the protection of the buildings in the campus.

Earlier, Aisagbonhi (1989), carried out a survey of the destructive effect of *M. bellicosus* on coconut seedlings in the Nursery of Nigerian Institute for Oil palm Research (NIFOR), Benin, Nigeria. He found out that 10.8 % of 1300 coconut seedlings were attacked by termites and *M. bellicosus* was implicated as the main termite species. Also, nest-mounds of termites including those of *M. bellicosus* often obstruct agricultural machinery and have to be removed with explosives or bulldozers, thereby increasing

the cost of mechanized farming, road construction and building site clearance (Oyedokun *et al.*, 2011). Thus, *Macrotermes* is an important agricultural, forestry and household pest (Ojianwuna *et al.*, 2016). Serious damage to crops such as maize, sugar cane, millet, rice, yam, groundnut, etc by *M. bellicosus* has been reported (Ito and Ighere, 2017). Also termites species of *Macrotermes subhyalinus* and *Macrotermes herts* have been reported to cause 50 % pre and post-harvest damage on maize, teff, eucalyptus, grasses, wheat, barley, pepper, tomato and other vegetable crops in several parts of Wallage and Asossc zones of Ethiopia (Abdurahman *et al.*, 2010).

Essential oils of the leaves of *C. odorata* and the roots of *U. chamae* tested in this research demonstrated appreciable potency against *M. bellicosus* in terms of repellency, knockdown and insecticidal effects. The knockdown effect of the test oils observed in this study agrees with the findings of Okweche and Nnah (2018) who assessed the efficacy of *Jatropha curcus* on *M. bellicosus* and Manimaran *et al.* (2012), who also observed similar effect of essential oils of *Pinus radiata*, *Citrus sinensis*, *Eucalyptus globulus* on their test insects. The potential of oils and leaf extract of *C. odorata* in the control of insect pests of the species *Callosobrunchus maculatus*, *Callosobrunchus subinnotatus* and *Periplanata americana* have been documented (Udebuani *et al.*, 2015; Nyamador *et al.*, 2017). Similarly, the efficacy of *U. chamae* in insect pest management have been documented by Negbenebor *et al.*, (2018). They reported the insecticidal effect of the powdered stem bark and ethanol extract of *U. chamae* against *Callosobrunchus maculatus*, *Rhizopertha dominica* and *Sitophilus zeamais*.

Chemical analysis of these oils revealed the presence of some chemical constituents that possess insecticidal properties. For instance, monoterpenes that were detected in both oils have been reported by Erol *et al.* (2013) to possess insecticidal property. Jija and John (2011) also detected sesquiterpenes in the essential oils of four species of *Salvia* (*Salvia splendens*, *Salvia schult scarlet*, *Salvia elegans* and *Salvia dorisiana*) that they studied. They attributed the insecticidal activities observed to the presence of sesquiterpenes in the oils. Benzyl benzoate that was detected in the oil from the roots of *U. chamae* has notable potency against the human itch mite, *Sarcoptes scabiei*. Its insecticidal efficacy is also well known. Walton *et al.*, (2000) in their *in vitro*

study reported the efficacy of benzyl benzoate in the control of *Sarcoptes scabiei*. Thus, the repellent, knockdown and contact toxicity of the test oils against *M. bellicosus* as reported in this research are attributable to these chemical constituents. Their effects which may have been additive or synergistic or otherwise require further evaluation. In terms of knockdown and toxicity efficacy, *U. chamae* was more potent than *C. odorata*. Similar differential potency was documented by Saeidi and Pezham (2018), when they tested essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis* against the maize weevil, *Callosobrunchus maculatus*. They observed that *E. globulus* was more potent than *E. camaldulensis* against their test insect.

CONCLUSION

The need for the control of termites has become imperative owing to their devastating damage in agriculture and destruction of properties. Synthetic insecticides have remained the commonest method of control of these termites over the years. However, the continuous use of synthetic insecticides has given rise to problems such as resistance, persistence in the environment and high mammalian toxicity. Current research efforts on product development should focus more on ecologically tolerable control measures such as the use of plants products. Results obtained from this preliminary study reveal that essential oils from *C. odorata* and *U. chamae* could be used by resource poor farmers in the management of *M. bellicosus*. Adequate formulations of these oils could thus be prepared as eco-friendly repellents and also as contact insecticides for the control of this pest species.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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