Laboratory evaluation of essential oil constituents against the termites, *Acistrotermes latinotus*

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ABSTRACT

In continuous search for alternative insecticides to combat potential problem of termite infestation in household and tree plantations; the bioactivity of the essential oils extracted by hydro distillation of leaves of *Ocimum gratissimum*, *Gongronema latifolium* and *Piper guineensis* was assessed under laboratory conditions at 20 ± 3^0C, 70-75% R.H. and 12: 12h light: dark regimes for its biological activity against workers of the termites with Chlorpyrifos insecticide as a standard check. The botanicals were extracted using a Soxhlet extractor and serially diluted at different concentrations of 0.125%, 0.25% and 0.50% including 0% as the control. The essential oils at 0.25ml of the different concentrations of the botanicals and insecticide were applied using a syringe on twelve adult workers of termites in each Petri dishes. Mortality count or number of the termites knocked down after thirty, sixty, ninety and one hundred and twenty minutes respectively during the trial was recorded and expressed in percentages. The individual components of the essential oil were identified through GC, GC-MS and GC-Co injection with authentic standards. The identity of a total of 17 constituent compounds of the essential oil of each plant was confirmed and their relative proportion determined. The major compounds identified among the botanicals and the insecticide in their relative proportion included Alpha Pinene, Quinoline, 3- methyl and Benzylisoquinoline. Only Alpha Pinene was found in *O. gratissimum* and Chlorpyrifos insecticide, while Quinoline, 3 methyl and Benzylisoquinoline were recorded in the botanicals including the insecticide. The major components were found to be largely responsible for the toxic action of its essential oil against the termites. The highest dosage of the essential oils of the plant materials tested induced the highest mortality in the termites after 120 minutes. Within `120 minutes, *O. gratissimum* (55%) *P. guineensis* oil (52%) was significantly more effective than *G. latifolium* (47%) at the bioactive concentration of 0.25mg/L while Chlorpyrifos insecticide recorded the highest termite mortality. The termite mortality increased gradually with the increase in essential oil concentrations and with the time of exposure. There is need to diversify the use of botanicals especially in the agricultural pest management sector.

Keywords: Botanical; *Acistrotermes latinotus*, Chlorpyrifos insecticide, mortality, exposure

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INTRODUCTION

Termites erroneously referred to as "white ants" belong to the Order Isoptera. Approximately, 1900 living and fossil species of termites have been described, the vast majority being found in the tropics (Bignel and Eggleton, 2000). Termites live in nests of their own construction known as termitaria. The latter in addition to the social behavior of the termites themselves, tend to produce a condition of homeostasis by self-regulation of optimal conditions for development maintenance and reproduction in the terminarium or colony. The continual exchange of food substances through trophallaxis within the colony appears to guide the reciprocal behavior of individuals. This is because in addition to nutrient exchange, trophallaxis includes recognition of colony,

mates, inter-individual communications, distribution of pheromones involved in caste differentiation and caste elimination (by cannibalism). The individuals comprising a colony of termites are made up of several castes including the primary reproductives (king and queen), the reproductives and the sterile workers and soldiers. Termites of the genus Ancistrotermes, are a member of the fungus growing sub Family of Macrotermitinae and Family Termitidae. They are mostly mound building and are the largest termite species (Osipitan *et al*., 2012). These species of termite are economically important because of the damage they cause in agriculture and to agricultural products such as scarification of pods in groundnut. Groundnut damage is often aggravated by late harvest which is the most common termite damage at maturity. Erect plants covered by a sheet of soil are usually a sign of *Ancistrotermes* damage (Wright *et al*., 1994). Kannaiyan and Somporn (1989) reported that severe pod damage, particularly scarification due to termite, results in poor seed quality, increased contamination of mycotoxin-producing fungi and reduced germinability.

Termites have highly organized colonies and demonstrate group integration, division of labor among castes (the fertile (reproductives) and the sterile (workers, pre-soldiers, and soldiers) and their population could grow drastically with several overlapping generations (Osipitan *et al*., 2012, Watanbe *et al.,* 2014). They are eurytopic and distributed throughout the temperate, tropical, and subtropical regions of the world, with the highest diversity found in tropical forests (Eggleton, 2000). Termites are good scavengers and decomposers, feeding on a wide range of living, faeces, dead or decaying plant material and soil rich in organic matter. They help in recycling waste material (Freymann *et al*., 2008). They also alter soil composition and structure, improve drainage, and provide soil aeration (Donovan *et al*., 2001) due to burrowing activities. The objectives were to (i) evaluate the termicidal efficacy of some selected botanicals against *Acistrotermes latinotus* and (ii) determine the composition of the botanicals

MATERIALS AND METHODS

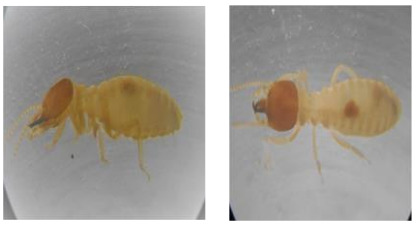
Location of Study

This experiment was carried out in the general laboratory of Crop and Soil Science, Faculty of Agriculture at the University of Port Harcourt. The laboratory is situated at a latitude of 6^{o}45'N to 7^{o}E under the ambient temperature of 20 ±3^{0}C and relative humidity of 70 ±5%.

Collection of Materials

Fresh and healthy leaves of *O. gratissimum, G. latifolium* and *P. guineense* were purchased at the common market in Port Harcourt. The plants were considered for this study based on the following criteria; relative absence of insect damage throughout the growing period, and taxonomic closeness to families known to possess biologically active compounds (Beaver, 1986). Also, the identification was done at the Department of Forestry and Wildlife, Faculty of Agriculture, Port Harcourt.

Insect identification



Ancistrotermes latinotus

Insect culture

The termites used for the study were cultured in their mounds by burying soft wood which enabled them to remain there and not migrate pending when they were used for the experiment. The workers of termites used for the study were collected from the mound with the aid of a spatula and soft camel hair brush into a tray and taken to the laboratory for the experiment.

Material and Reagents for Extraction and G.C/MS

G.C/ MS is the Agilent 6890N Gas Chromatograph with Agilent 5975 Mass Selective Detector Auto sampler vials, 150mL vial inserts, and crimp seals, vial crimper and decrimper 2.5 mL airtight syringe or 3mL disposable hypodermic syringe and 10mL autosampler syringe, 30-mm x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or

equivalent), 1-um film thickness Soxhlet extractor.

Reagents - Air-zero grade, Helium gas -- UHP grade, n-hexane, Anhydrous sodium sulfate and Standard plant chemicals (internal standard)

Extract Preparation

The plant extracts were prepared using a Soxhlet extractor. The collected leaves were thoroughly rinsed in distilled water to remove dirt and were allowed to air dry on a wire mesh for a period of 2 weeks. The dried leaves were then milled into powder using a grinding mill. Eighty grammes each of the dried and milled leaves of *O. gratissimum, G. latifolium* and *P. guineense* were weighed separately into vials and taken for extraction. Extraction was done with the use of n-hexane in the Soxhlet extractor to produce a stock solution of each of the botanicals that was used for the study. A 20g aliquot of sample was homogenized, and a 10g aliquot was spiked with the labeled compound. The sample was mixed with anhydrous sodium sulfate, allowed to dry for a minimum of 30 minutes and extracted for 18-24 hrs using methylene chloride in a Soxhlet extractor. The extract was evaporated to dryness and the liquid content was determined. For a 12mL volume of extract that was used for the experiment, the stock solutions were serially diluted (extract: water) into different concentrations 1:2v/v (50%), 1:4v/v (25%), 1:8v/v (12.5%) and 0% concentration which was water as control.

Procedure for Gas Chromatography

Extracts for phytochemicals analysis were subjected to a sequential methylene chloride - n-hexane (1:1) cleanup specifically for these analyses. 1uL of the sample was injected into a gas chromatograph equipped with either a narrow or wide-bore fused-silica capillary column and either an electron capture detector (GC/ECD) or an electrolytic conductivity detector (GC/ELCD).

Phytochemical Analysis

GC-MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5975 MS detector equipped 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-um film thickness (Agilent). The following temperature ramp was used: injector at 250 C, oven initially at 200C, held for 1 minute and heated to 230C (1.5C min-1, then held for 10 min). The characterization and identification of phytochemicals, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the helium as carrier gas was 1mL min-1; manual injection; the injection volume was 1mL. Interpretation of mass spectrum of GC-MS was done using data base of National Institution Standard and Technology (NIST). The mass spectrum of unknown component was compared with spectrum of the known component stored in the NIST library. Major components were identified by the authentic standards and by the recorded from computerized libraries film thickness.

Bio-assay

Comparative toxicity tests of essential oils and their constituents

These were conducted in Pyrex glass Petri dishes, 9 cm diameter (Weaver *et al*., 1991; Bekele and Hassanali, 2001). Twelve worker castes of the termite were picked at random and placed in each Petri dish from the collection tray with soft camel hair brush. There were four treatments per extract, namely 0.00%, 0.125%, 0.25% and 0.50%. 0.25 mL of each extract concentrations was applied to the filter paper lining the Petri dishes using an insulin syringe (1mL capacity) allowed to air dry for five minutes and the termite workers placed into each Petri dish. The mortality was recorded after 30, 60, 90 and 90 minutes of exposure. The control was water while the standard check was Chlorpyrifos 48EC and each was kept for 24 h in the laboratory maintained at 20 ± 3^0C and 70±5% relative humidity. The number of dead insects was counted after 24 hrs. Insects were considered dead if they were immobile and did not react to three probing with a blunt dissecting probe. A termite is considered dead when it was lying flat on its back and showing no sign of movement of its body after being touched with a soft camel hair brush. Each treatment including control was replicated three times in a Completely Randomized Design.

Data Collection and Analysis

The data obtained were converted to percentage (using Percentage Mortality = total number of dead termites after treatment/total number of termites before treatment x 100) and the percentage mortality per treatment was subsequently corrected (using Corrected Kill -Pt = Po - Pc/100 - Pc X 100; where Po is Percentage mortality observed and Pc is Percentage mortality in control). All data was subjected to Analysis of Variance (ANOVA) using SAS 2.0 version. Significant means were separated using Tukey's Honest Significant Difference test at 5% probability levels.

RESULTS

Botanicals and insecticide composition

The results of laboratory tests showed that termite mortality increased gradually as the concentration of each essential oil increased and the period of exposure. The highest dosage of the essential oil of the plant materials tested induced the highest mortality in the termites after 120 minutes. *Piper guineensis* and *G. latifolium* oil were not as effective as *O. gratissimum* and synthetic insecticide at the initial exposure at the lowest concentration. By the 120 minutes of exposure all the essential oils except *G. latifolium* oil had become toxic to the termite. Thereafter, all the essential oils became toxic and the percentage mortalities varied between 30 -75%, with *O. gratissimum* and *P. guineensis* oil producing the highest mortality within 60 minutes followed by *G. latifolium* in decreasing order. At 0.125% concentration, termite mortality was caused by essential oils irrespective of the plants and the relative toxicities of the various botanicals and insecticides were as shown in Table 1. The termite mortality increased gradually with essential oils concentration and with the time of exposure. Under laboratory conditions the proportion of the termites knocked down or dead at various concentrations of the essential oils increased as exposure time and dosages increased.

Thus, with 0.25% active ingredient, most of the essential oils except *G. latifolium* become more potent, knocked down and caused average mortality of the termite within ninety minutes of exposure; percentage termite mortalities also increased with exposure time. Within 120 minutes of exposure, *O. gratissimum* oil produced the highest mortality followed by *P. guineensis* oil and *G. latifolium* while the synthetic insecticide, the check recorded consistent highest termite mortality (100%). All the botanicals including the synthetic insecticide were most potent at 0.50% concentration causing over 40% mortality within 60 minutes of exposure. Only synthetic insecticide, the check killed the termite exposed to the solutions within 120 minutes. Within 120 minutes, average means of *O. gratissimum* and *P. guineensis* oil were significantly more effective than *G. latifolium* at the bioactive concentration of 0.25mg/l while Chlorpyrifos insecticide recorded the highest termite mortality. All the botanicals were most potent at 50% concentration causing over 40% mortality within ninety minutes of exposure of termites. The check insecticide killed all the termites exposed to the solutions within sixty minutes.

Table 1. Relative toxicities of botanical oil and synthetic insecticides to the termite in the laboratory

Concentration	**0.125%**				**0.25%**				**0.50%**				**Significance**
Exposure period (minutes)	**30**	**60**	**90**	**120**	**30**	**60**	**90**	**120**	**30**	**60**	**90**	**120**	
	Mean % Mortality				**Mean % Mortality**				**Mean % Mortality**				
O. gratissimum	30	40	45	60	45	55	65	70	50	60	65	75	55.00b
G. latifolium	35	35	40	50	35	40	45	50	55	60	60	60	47.08c
P. guineensis	35	40	45	60	45	50	55	60	55	60	60	65	52.50b
Standard insecticide	80	90	90	100	90	90	100	100	100	100	100	100	95.00a

Means are separated by Tukey's Honest Significant Difference Test at 5%. Means followed by the same letter in the same column are not significantly different from one another

The analysis of the botanicals revealed a complex mixture of constituents. A total of 31, 31 and 30 compounds were identified in *O. gratissimum*, *G. latifolium* and *P. guinensis*

respectively (Tables 2-4) while 18 compounds were identified in Chlorpyrifos (Table 5) by GC -MS using data based on NIST.

Table 2. Major constituents of *Ocimum gratissimum* and their relative proportion in the extract.

GC peak number	Component	Peak area (%)	Retention time
1	Ocimene	5.42	8.28
2	1,8-Cineole	6.68	8.69
3	Alpha-Silinene	18.74	9.17
4	Beta-Silinene	3.29	9.75
5	Alpha-pinene	2.44	10.42
6	Beta-pinene	2.62	10.63
7	Alpha-terpineol	1.76	10.92
8	Alpha-humulene	1.54	12.31
9	Copaene	5.93	12.75
10	Campesterol	3.27	13.26
11	Beta-Caryophyllene	2.48	13.85
12	Gamma-sitosterol	4.77	14.27
13	Lupenon	7.62	14.76
14	Bata-amyrene	3.44	16.24
15	Pyrrolidine,1-(1, 6-dioxooctadecyl)	1.76	16.79
16	Alpha-ergosterol	9.42	17.23
17	2-Pyrazoline,1-isopropyl-5-methyl	13.39	17.52
18	Resorcinol, bis(tert-utyldimethlsilyl)	17.71	18.31
20	Squalane	3.52	18.75
21	Euginol	1.87	19.32
22	Anabasine	6.29	19.87
23	Benzylisoquinoline	1.54	21.36
24	Carpaine	0.27	21.82
25	Phytol	9.26	22.24
26	Phytic acid	15.74	22.72
27	Myrcene	3.28	24.39
28	Lobeline	2.69	24.82
29	Beta-Caryophyllene	5.32	25.24
30	Sparteine	1.59	25.51
31	Pelletierine	1.44	25.87

Table 3. Major constituents of *Gongronema latifolium* extract and their GC peak number and retention time in the extract

GC peak number	Component	Peak area (%)	Retention time
1	Hexadecane	2.37	8.35
2	1-Hexadecane	5.42	8.76
3	Penta-triacotene	10.73	9.27
4	Ethyl-2,2-diethoxypropinate	3.69	9.84
5	Maltol	1.42	10.40
6	1,2,3- propanetriol, 1-acetate	5.71	10.77
7	Quinoline, 3- methyl	6.69	12.26
8	Coumaric	1.51	12.63
9	Copaene	3.77	12.92
10	Campesterol	1.83	14.29
11	Caryophyllene	5.91	14.74
12	Gamma- sitosterol	3.52	15.63
13	Lupenon	1.47	15.95
14	Bata-amyrene	4.62	16.26
15	Pyrrolidine,1-(1,6-dioxooctadecyl)-	0.31	16.68
16	Alpha-ergosterol	0.42	18.17
17	2-Pyrazoline,1- isopropyl-5-methyl	8.35	18.69
18	Resorcinol,bis(terbutyldimethlsilyl)	2.31	20.35
19	Sqalane	1.74	20.78
20	Euginol	3.29	21.25
21	Anabasine	1.24	21.87
22	Benzylisoquinoline	0.63	22.30

23	Carpaine	4.18	22.69
24	Phytol	3.95	23.25
25	Phytic acid	2.63	23.77
26	Myrcene	7.21	23.91
27	Lobeline	9.07	24.18
28	Beta-Caryophyllene	1.45	24.66
29	Sparteine	3.27	26.45
30	Pelletierine	4.31	26.76

Table 4. Major constituents of *Piper guineensis* extract and their peak area and retention time in the extract.

GC peak number	Component	Peak area (%)	Retention time
1	2,2-Dimethoxybutane	1.86	8.25
2	3-Furaldehydw	3.74	8.74
3	Neocurdione	6.69	9.28
4	Ethyl-2,2-diethoxypropinate	1.42	9.67
5	Maltol	2.58	10.32
6	1,2,3- propanetriol, 1-acetate	3.62	10.72
7	Quinoline, 3- methyl	1.72	10.98
8	Coumaric	0.58	12.32
9	Copaene	0.93	12.69
10	Campesterol	1.67	13.21
11	Caryophyllene	0.51	13.62
12	Gamma- sitosterol	3.62	14.34
13	Lupenon	3.28	14.78
14	Bata-amyrene	5.96	16.33
15	Pyrrolidine, 1- (1, 6-dioxooctadecyl)-	2.44	16.70
16	Alpha-ergosterol	7.67	17.21
17	2-Pyrazoline, 1- isopropyl-5-methyl	3.21	17.76
18	Resorcinol, bis (tert-butyldimethlsilyl)	1.40	18.35
19	Squalane	5.63	18.86
20	Euginol	2.84	19.20
21	Anabasine	3.72	19.74
22	Benzylisoquinoline	4.65	21.25
23	Carpaine	8.89	21.69
24	Phytol	1.17	22.37
25	Phytic acid	3.56	22.78
26	Myrcene	1.48	24.27
27	Lobeline	5.61	24.82
28	Beta-Caryophyllene	3.52	25.17
29	Sparteine	0.76	25.52
30	Pelletierine	2.86	25.88

Table 5. Major constituents of Chlorpyrifos insecticide and their relative proportion in the insecticide.

GC peak number	Component	Peak area (%)	Retention time
1	Dianizion	4.13	9.48
2	Emamectin	1.94	10.26
3	Parathion	2.52	10.74
4	Metoxychlorine	1.66	12.19
5	DDT, P, P-	0.45	12.43
6	Benzylisoquinoline	1.20	13.20
7	Endosulfan-sufate	1.34	15.51
8	Alpha Pinene	0.69	15.68
9	Primor	3.57	16.13
10	Thiamethoxan	1.25	16.42
11	Chlordon	0.73	17.38
12	Jasmolin	3.18	18.20
13	Cynarin	1.31	18.47
14	Veratridine	1.14	19.69
15	Alphamethrin	0.39	19.81
16	Quinoline, 3-methyl	0.58	20.34

17	Carbanil	1.69	20.17	**45**
18	Firponil	1.45	20.96	

Tables 2-5 showed the compounds that were found in the insecticide as well as the botanicals. Compounds such as Benzylisoquinoline which is an alkaloid was present in all the treatments. Alpha Pinene was also found in the insecticide as well as in *O. gratissimum* but the peak area was higher in *O. gratissimum* (5.42) compared to the insecticide (0.69). Quinoline,3- methyl was found in the insecticide as well as in *G. latifolium* and *P. guineensis*, with the peak area being higher in the botanicals than in the insecticide. These compounds are valuable bioactive compounds with therapeutic values (Adesina *et al*., 2003) for pest control.

Table 6. Major compounds common among the botanicals and the insecticide in their relative proportions.

Compound	Chlorpyrifos		*O. gratissimum*		*G. latifolium*		*P. guineensis*	
	RT (min)	PA (%)	RT (min)	P.A (%)	RT (min)	PA (%)	RT (min)	PA (%)
Alpha Pinene	15.68	0.693	10.42	5.42	_	_	_	_
Quinoline,3-methyl	20.34	0.582	_	_	12.64	6.69	10.98	1.72
Benzylisoquinoline	13.20	1.251	21.36	1.54	22.30	0.63	21.25	3.72

RT= Retention Time, PA= Peak Area

DISCUSSION

Farmers are encouraged to resort to botanicals that have the phyto-toxic effect which can maintain sustainable agriculture with low toxicity residual effect though eco-friendly.

Previous studies showed that the essential oils derived from the plants provided varying degrees of protection against termites (Azeez *et al*., 2022) This present study compared the toxic effects of major constituents of the essential oils of *Ocimum gratissimum, Gongonera latifolium and Piper guineensis*. From this study, soxhlet extracted botanicals of *Ocimum gratissimum, Gongonera latifolium and Piper guineensis* showed insecticidal activities through the topical and exposure test. The toxicity of the botanicals resulted in high mortality of the *Ancistrotermes latinotus*, in a dose dependent manner. These findings are in agreement with a similar work where powder and aqueous extract of tobacco and neem were used in the control of *Callosobruchus maculatus* (Enobakhare and Azeez, 2006). The optimum concentration at which control is achieved was 12.5% in all the botanicals as well as the Chlorpyrifos (Standard check). The extracts of the selected botanicals possess some insecticidal properties against *A. latinotus* but several variations occurred based on the extracting solvent (n-hexane) and the concentration of the extracts as these influenced the efficacy or biocidal activities of the plant materials. This was supported by Sakasegawa *et al*. (2003) who reported that some natural plants present toxic principles to insects including termites. Generally, the extract of *O. gratissimum* at 50% v/v concentration resulted in the highest mean mortality of *A. latinotus* and this was found to be significantly different from the other extracts at different concentrations used in this bio-assay. This suggests that apart from antimicrobial potency of the constituents of *O. gratissimum*, it also has some insecticidal properties. The sequential mortality rate in relation to the increase in concentration of plant extracts has been reported by many researchers which agrees with finding in this study. This may be due to the mixture of active compounds in the various essential oil. Phytochemical analysis of the botanicals showed that the botanicals constituted of valuable bio-active compounds which are alkaloids, amides, monoterpenes, sesquiterpenes, phenyl derivatives etc with therapeutic agents which can be attributed to the lethal effect of these plants. The plant component, terpenoids is implicated as responsible for the toxic effect exerted by the essential oils. The toxic effect of plant compounds has been reported by various authors (Jembere *et al*., 1995; Bekele and Hassanali, 2001; Bouda *et al*., 2001) who attributed their effect to different terpene and

alkaloid constituents of the botanicals. Similarly, toxicity of *O. gratissimum, P. guineensis* and *G. latifolium* against termites in the present study might be attributed to their constituents. The extract of these botanicals contained several monoterpenes, sesquiterpenes, alkaloids, amides, phenyl derivatives which supports the findings of Adesina *et al*. (2003), who said that extract of *P. guineensis* contains valuable compounds which could serve as therapeutic agents for the control of insects.

Also, according to Raupp *et al*. (2014) who reported the residual effect of synthetic insecticide on insect pests and natural enemies. In the same vein, Zacharia (2011), who reported on the inherent high mammalian toxicity and ecological hazard of the use of synthetic insecticides, the GC-MS analysis of Chlorpyrifos showed the presence of harmful compounds like DDT which could result in biomagnifications of these compounds.

Hence, synthetic insecticides should be discouraged in insect pest management and control and the extensive use of botanicals with insecticidal potentials such as those used in this study be encouraged. Furthermore, the analysis of the constituents in various insecticides should be done to determine the toxicity and effect on agricultural production and consumption so as to control and reduce the effects of this insecticide on the ecosystem.

This study demonstrates the potential of plant extracts against the test insect. Among the three botanicals determined *O. gratissimum* was the most bioactive botanicals against termites although the other botanicals had lethal effect on the test insect. The botanicals contained phytochemicals and valuable bio-active compounds that are effective in the control of the termite. The three botanicals are equally effective as the synthetic insecticide (Standard). Therefore, the higher the concentration of the botanicals the more effective control was achieved. We recommend the diversified use of botanicals especially in the agricultural pest management sector, as this could be of economically and eco-friendly benefit and to a great extent discourage the use of synthetic pesticides or use them only when necessary.

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