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RESEARCH ARTICLE

Increasing Efficacy of Eucalyptus Oil as Repellent Against *Rattus rattus* by Controlled Release Through Encapsulated Wax Blocks

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Abstract Plant based non-lethal repellents are most suitable for rodent control. Present studies were conducted to increase the efficacy of eucalyptus oil as repellent against black rat, *Rattus rattus* by its controlled release through encapsulated wax blocks. Mature and healthy *R. rattus* of both sexes were exposed to 5, 10 and 20 % eucalyptus oil encapsulated in wax blocks in laboratory pens in bi-choice tests. Each concentration was applied through three different modes of application i.e. daily, once a week, and every second day per week. Repellent effect of the oil was assessed based on food consumption from treated and untreated sides of a chamber for four days a week. Food consumption was found to be significantly ($P \leq 0.05$) low from treatment side as compared to that observed on untreated side at all the three concentrations tested indicating repellent effect of the eucalyptus oil against *R. rattus*. Percent repellency with eucalyptus oil encapsulated in wax blocks at all the three concentrations was found to be similar. Being the minimum effective concentration, present studies suggest the use of 5 % eucalyptus oil encapsulated in wax blocks as repellent against *R. rattus*. This method may be used as part of integrated pest management technology for managing rats in enclosed store houses.

Keywords Eucalyptus oil · Repellents · Food consumption · Laboratory pen · *Rattus rattus*

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Introduction

Rodents have gained the reputation as one of the most persistent and ubiquitous vertebrate pests. The black rat, *Rattus rattus* is the predominant commensal rodent pest species found worldwide [1, 2]. Conventional pesticides possess inherent toxicities that endanger the health of the farm operators, consumers and the environment [3]. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment [4]. They are more compatible with the environmental components than synthetic pesticides [5]. Essential oils derived from plants are easy to extract, biodegradable and do not persist in soil and water [6]. These can be useful for the prevention of rodent damage to food grains in storage, seeds and seedlings in crop fields and nurseries. Plant based non-lethal repellents are most suitable for rodent control [7–9]. Unpleasant taste and odour cues function as initial deterrents against ingestion of food leading to primary food aversion. Mills and Munich [10] defined rat repellents as substances placed in runways of rats that prevent them from going where they wish. Such substances may be used in protecting an area from rodent infestation or in protecting packed food, packing materials, electric cables and other important vulnerable materials as one component of integrated pest management (IPM) which involves various strategies that ideally have to be combined at different levels. IPM is defined as a decision support system for the selection and the use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and the impacts on producers, society and the environment.

Plants whose essential oils have been reported to have repellent activity include lemon grass, cedar, verbena, pennyroyal, eucalyptus, geranium, lavender, pine, cinnamon, rosemary, basil, thyme and peppermint. Plants with strong smells, such as French marigold and coriander, act as repellents and can protect the crops nearby [11, 12]. Eucalyptus oil is a colourless liquid, with a camphor-like odour and spicy, cooling taste. It is highly inflammable and contains compounds that are natural disinfectants and pest deterrents. Essential oils have been accepted as the main defensive trait in *Eucalyptus* spp. [13]. Among the various components of eucalyptus oil, 1, 8-cineole is the most important one and, in fact, a characteristic compound of the genus *Eucalyptus,* and is largely responsible for a variety of its pesticidal properties [14]. Eucalyptus oil has been placed under Generally Regarded as Safe (GRAS) category by Food and Drug Authority of USA and classified as non-toxic [15].

Relatively little work has been carried out on plant derived repellents as compared to other aspects of rodent control. Short term repellent effect of eucalyptus and citronella oils against *R. rattus* has been reported by Singla et al. [16] and Singla and Kaur [17]. The volatile nature of the oil is the limiting factor. To increase the stability of such compounds, it is necessary to formulate them in a way that they are protected from degradation by UV light and oxygen. Moreover, the formulation must ensure a controlled release of the compound. Several formulations and dispensers have been developed and commercialized with various slow-release capacities including the use of paraffin wax formulations [18]. The aim of present study was to increase the efficacy of eucalyptus oil as repellent against *R. rattus* for longer period of time by its controlled release through encapsulated paraffin wax blocks.

Material and Methods

The present work was carried out in the Department of Zoology, Punjab Agricultural University, Ludhiana, India located at an intersection of 30° 55′N parallel of latitude and 75°54′E line of longitude. Commercially available pure eucalyptus oil was used in present studies.

Collection and Maintenance of Animals

Rats of both sexes were collected from poultry farms using multi catch rat traps. In the laboratory, rats were acclimatized individually in cages of size 36 × 23 × 23 cm for 15–20 days before the commencement of experiment with food and water provided ad libitum. Food was prepared by mixing cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio 96:2:2. Animals were used and maintained as per the guidelines of Institutional Animal Ethics Committee. After acclimatization, healthy and mature rats of both sexes were weighed and selected for experimentation.

Experimental Setup and Treatment

A total of four laboratory pens (each of size 252 × 100 × 72 cm), were used for each concentration. Each pen consisted of three chambers of equal size. One rat was released in each chamber. Each chamber in a laboratory pen, on its opposite facing sides was connected with holes (each of diameter 6 cm) to two small nest boxes (each of size 20 × 15 × 15 cm). Rats had free access to these nest boxes. One oil encapsulated wax block was placed in the nest box of one side of each chamber. Repellent effect of the oil was assessed based on the consumption of WSO bait by the rat from the bowls kept in two nest boxes of a chamber in a laboratory pen.

Three different concentrations of eucalyptus oil i.e. 5, 10 and 20 % were tested. Different concentrations were prepared by diluting the oil in isopropyl alcohol. For each concentration, a total of 12 rats (6 of each sex) were taken. For preparing paraffin wax blocks encapsulating oil, molten wax along with 3–5 drops (1 ml) of each concentration of the oil were poured in a rectangular mould made by using L-pieces. The block was separated from the mould after the wax solidified. Rats were exposed to wax block containing each concentration of the oil for 3 weeks using three different modes of application i.e. applied daily, once a week and alternatively after every second day of the week. Blocks were always prepared afresh. Bait consumption was recorded daily after every 24 h from both treated and untreated sides for 4 days in a week to determine mean daily bait consumption (g/100 g bw). Based on mean daily bait consumption data, percent repellency was determined using the formula described in Singla et al. [16].

Statistical Analysis

Values were determined as mean ± SD. The data on food consumption for three concentrations of the oil, three modes of applications, 4 days of application, and from treatment and untreated sides was collected using factorial experiments in completely randomized design. Analysis was done using general linear model in SAS 9.3. All pair wise treatment comparisons were made using Tukeys' HSD test at 5 % level of significance.

Results and Discussion

Statistical analysis of the data revealed in overall, significantly ($P \leq 0.05$) low consumption of bait from treatment side as compared to untreated side at all three

Table 1 Food consumption in response to application of 5 % eucalyptus oil as encapsulated wax blocks by *Rattus rattus*

Mode of application	Days of treatment	Mean daily food consumption (g/100 g bw) [n = 12]	
		Treatment side	Untreated side
I	Day 1	2.76 ± 2.86^{a}	8.41 ± 3.46^{b}
	Day 2	3.18 ± 2.56^{a}	8.03 ± 4.09^{b}
	Day 3	2.74 ± 1.60^{a}	7.08 ± 3.05^{b}
	Day 4	3.25 ± 2.68^{a}	7.30 ± 3.46^{b}
	Average	$\mathbf{2.98 \pm 0.23^{A}}$	$\mathbf{7.70 \pm 0.53^{BC}}$
II	Day 1	3.72 ± 1.71^{a}	7.56 ± 1.92^{b}
	Day 2	5.01 ± 4.13^{a}	7.27 ± 2.75^{a}
	Day 3	6.79 ± 3.32^{a}	9.07 ± 3.85^{a}
	Day 4	4.07 ± 2.17^{a}	7.39 ± 2.59^{a}
	Average	$\mathbf{4.89 \pm 1.19^{B}}$	$\mathbf{7.82 \pm 0.72^{C}}$
III	Day 1	2.95 ± 1.97^{a}	7.45 ± 2.54^{b}
	Day 2	4.99 ± 2.48^{a}	8.94 ± 3.74^{b}
	Day 3	5.94 ± 2.56^{a}	6.43 ± 1.72^{a}
	Day 4	2.83 ± 2.21^{a}	8.77 ± 4.97^{b}
	Average	$\mathbf{4.17 \pm 1.33^{B}}$	$\mathbf{7.89 \pm 1.02^{C}}$

Values are Mean ± SD, *I* daily, *II* once a week, *III* alternatively every second day of the week

Values with different superscripts in a row for 4 days of treatment (a–b) and for average values (A–C) separately at each mode of application indicate significant difference at $P \leq 0.05$

Values with different superscripts (A–B) in a column for average values on treatment side for three modes of application indicate significant difference at $P \leq 0.05$

concentrations and modes of application (Tables 1, 2, 3) indicating repellency of eucalyptus oil applied as encapsulated wax blocks.

Effect of 5 % Eucalyptus Oil

When 5 % eucalyptus oil was applied as encapsulated wax blocks (Table 1), average mean daily food consumption of 4 days was found to be significantly low from treatment side as compared to untreated side at all the three modes of application. The average mean daily food consumption on treatment side at modes II (when applied once a week) and III (when applied alternatively after every second day) was found to be significantly high as compared to that observed at mode I (when applied daily) indicating reduced effect of the oil when applied once a week or alternatively. The mean daily consumption of food from treatment side was found to be significantly low from that of untreated side on all the 4 days at mode I of application, while the mean daily consumption of food from treatment side was found to be significantly low from that of untreated side only on day 1 at mode II of application. The mean daily consumption of food from treatment side was found to be significantly low from that of untreated side on days 1, 2 and 4 at mode III of application. This may be due to the reduced effect of the oil on days when the already kept wax block was not replaced by the freshly prepared block at modes II and III of application.

The average percent repellency caused by wax block encapsulated with 5 % eucalyptus oil (Fig. 1) was found to be significantly high at mode I (55.85 %) of application as compared to that observed at mode II (35.36 %) of application. At mode III of application, percent repellency was found to be significantly low on day 3 of treatment as compared to that observed on days 1, 2 and 4 of treatment (Table 2).

Effect of 10 % Eucalyptus Oil

When 10 % eucalyptus oil was applied as encapsulated wax blocks (Table 3), average mean daily food consumption of 4 days was found to be significantly low from treatment side as compared to untreated side at modes I and III of application. The average mean daily food consumption on treatment side at mode I (when applied daily) was found to be significantly low as compared to that observed at modes II (when applied once a week) and III (when applied alternatively after every second day) indicating reduced effect of the oil when applied once a week and alternatively. The mean daily consumption of food from treatment side was found to be significantly low from that of untreated side on all the 4 days at mode I of

Table 2 Percent repellency with eucalyptus oil when applied as encapsulated wax block using three different concentrations against *Rattus rattus*

Mode of application	Days of treatment	Percent repellency (n = 12 rats)		
		5 %	10 %	20 %
I	Day 1	64.30 ± 33.16[a]	67.34 ± 28.96[a]	50.72 ± 33.84[a]
	Day 2	44.47 ± 30.62[a]	47.26 ± 39.57[a]	27.76 ± 27.07[a]
	Day 3	54.70 ± 32.9[a]	60.72 ± 27.52[a]	43.73 ± 34.76[a]
	Day 4	59.94 ± 29.24[a]	59.01 ± 38.42[a]	53.09 ± 28.09[a]
	Average	**55.85 ± 7.39[A]**	**58.58 ± 7.23[A]**	**43.83 ± 9.89[A]**
II	Day 1	49.57 ± 28.75[a]	36.73 ± 22.71[a]	44.77 ± 27.08[a]
	Day 2	38.36 ± 30.53[ab]	18.70 ± 21.35[bc]	56.44 ± 32.17[a]
	Day 3	22.42 ± 32.68[ab]	12.33 ± 21.28[b]	29.31 ± 26.10[ab]
	Day 4	43.39 ± 26.94[a]	29.41 ± 21.67[ac]	34.63 ± 28.81[a]
	Average	**35.26 ± 15.27[B]**	**24.29 ± 9.42[B]**	**41.28 ± 10.36[AB]**
III	Day 1	56.62 ± 31.92[a]	52.31 ± 31.47[a]	59.03 ± 29.86[a]
	Day 2	38.14 ± 28.01[a]	47.68 ± 34.80[a]	54.37 ± 19.00[a]
	Day 3	17.06 ± 24.03[b]	17.43 ± 23.17[b]	17.77 ± 22.58[b]
	Day 4	56.20 ± 33.02[a]	40.89 ± 31.33[a]	42.56 ± 27.4[a]
	Average	**42.00 ± 16.21[AB]**	**39.57 ± 13.41[B]**	**43.43 ± 15.98[AB]**

The bold figures are for average values of four days

Values are Mean ± SD, *I* daily, *II* once a week, *III* alternatively every second day of the week

Values with different superscripts in a column for 4 days of treatment (a–c) separately at each mode of application indicate significant difference at $P \leq 0.05$

Values with different superscripts (A–B) in a column for average values at each concentration indicate significant difference at $P \leq 0.05$

Table 3 Food consumption in response to application of 10 % eucalyptus oil as encapsulated wax blocks by *Rattus rattus*

Mode of application	Days of treatment	Mean daily food consumption (g/100 g bw) [n = 12 rats]	
		Treatment side	Untreated side
I	Day 1	2.16 ± 1.58[a]	4.33 ± 3.73[b]
	Day 2	3.74 ± 2.30[a]	6.61 ± 2.47[b]
	Day 3	2.91 ± 1.47[a]	9.02 ± 2.89[b]
	Day 4	2.47 ± 2.08[a]	6.91 ± 3.08[b]
	Average	**1.01 ± 0.20[A]**	**6.71 ± 1.66[C]**
II	Day 1	2.83 ± 1.20[a]	4.34 ± 1.13[b]
	Day 2	5.22 ± 2.12[b]	5.73 ± 2.94[b]
	Day 3	6.18 ± 3.05[b]	5.45 ± 3.49[b]
	Day 4	4.67 ± 1.57[b]	6.53 ± 1.70[b]
	Average	**4.72 ± 1.22[B]**	**5.51 ± 0.78[BC]**
III	Day 1	2.67 ± 1.97[a]	5.28 ± 1.3[b]
	Day 2	2.66 ± 1.59[a]	5.58 ± 1.69[b]
	Day 3	4.71 ± 1.42[c]	5.66 ± 1.37[bc]
	Day 4	3.45 ± 2.02[ac]	5.51 ± 0.93[b]
	Average	**3.37 ± 0.83[B]**	**5.50 ± 0.14[C]**

The bold figures are for average values of four days

Values are Mean ± SD, *I* daily, *II* once a week, *III* alternatively every second day of the week

Values with different superscripts in a row for 4 days of treatment (a–c) and for average values (A–C) separately at each mode of application indicate significant difference at $P \leq 0.05$

Values with different superscripts (A–B) in a column for average values on treatment side at three modes of application indicate significant difference at $P \leq 0.05$

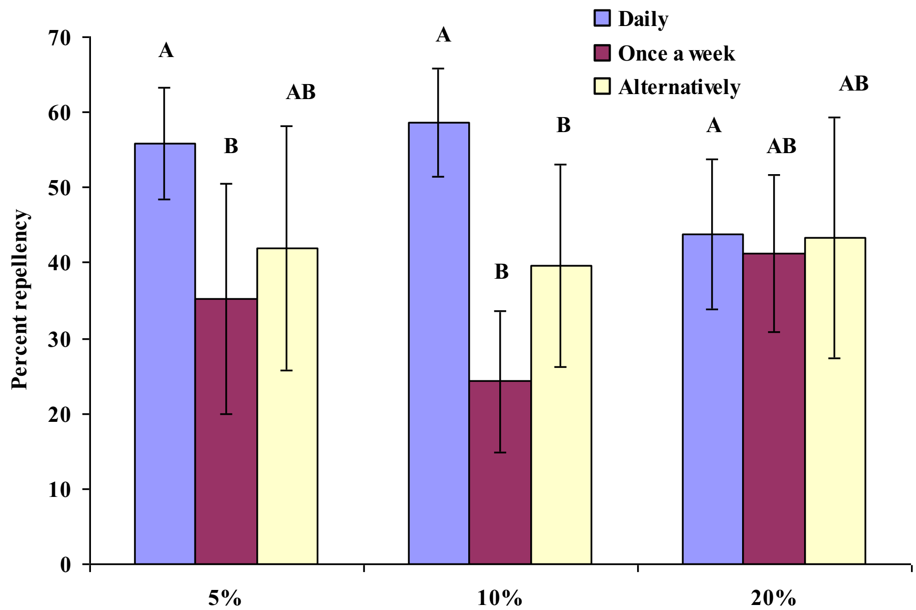

Fig. 1 Significant differences in average percent repellency of three concentrations of eucalyptus oil among three modes of application. *Bars* with *different superscripts* differ significantly at $P \leq 0.05$

application, while the mean daily consumption of food from treatment side was found to be significantly low from that of untreated side only on day 1 at mode II of application. The mean daily consumption of food from treatment side was found to be significantly low from that of untreated side on days 1, 2 and 4 at mode III of application. This may be due to the reduced effect of the oil on days when the already kept wax block was not replaced by the freshly prepared block at modes II and III of application.

The average percent repellency caused by wax block encapsulated with 10 % eucalyptus oil (Fig. 1) was found to be significantly high at mode I (58.58 %) of application as compared to that observed at modes II (24.29 %) and III (39.57 %) of application. At mode II of application, percent repellency was found to be significantly low on day 3 of treatment as compared to that observed on days 1 and 4 of treatment (Table 2). Percent repellency on day 2 at same mode of application was found to be significantly low from that observed on day 1. At mode III of application, percent repellency was found to be significantly low on day 3 of treatment as compared to that observed on days 1, 2 and 4 of treatment (Table 2).

Effect of 20 % Eucalyptus Oil

When 20 % eucalyptus oil was applied as encapsulated wax blocks (Table 4), average mean daily food consumption of 4 days was found to be significantly low from treatment side as compared to untreated side at all the three modes of application. The mean daily consumption of food from treatment side was found to be significantly low from that of untreated side on all the 4 days at mode I of application, while the mean daily consumption of food from treatment side was found to be significantly low from that of untreated side only on days 1, 2 and 4 at modes II and III of application. The mean daily consumption of food from treatment side was also found to be significantly high on day 3 as compared to that observed on days 1, 2 and 4 at mode III of application.

The average percent repellency caused at all three modes of application by wax blocks encapsulated with 20 % eucalyptus oil was found to be statistically similar (Fig. 1). At mode III of application, percent repellency was found to be significantly low on day 3 of treatment as compared to that observed on days 1, 2 and 4 of treatment (Table 2).

Essential Oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants. Many of these compounds deter insects from feeding, thereby showing an antifeedant effect. Relatively little work has been carried out on plant derived repellents as compared to other aspects of rodent control. Cinnamic aldehyde at 5 % concentration in bait was found effective as an antifeedant and secondary repellent against *R. rattus* [19].

Among the various components of eucalyptus oil, 1, 8-cineole is the most important one largely responsible for a variety of its pesticidal properties [14]. The presence of this essential oil also provides defense advantage to eucalyptus leaves against herbivory [20]. Repellency of eucalyptus oil has been recorded against the tick, *Ixodes ricinus* [21] and against acaricide-resistant mites [22]. Application of 1.0 % concentration of 1, 8-cineole reduced oviposition rate of *Thrips tabaci* by 30–50 % as compared to untreated

Table 4 Food consumption in response to application of 20 % eucalyptus oil as encapsulated wax blocks by *Rattus rattus*

Mode of application	Days of treatment	Mean daily food consumption (g/100 g bw) [n = 12 rats]	
		Treatment side	Untreated side
I	Day 1	2.52 ± 1.43^{a}	5.99 ± 1.83^{b}
	Day 2	4.82 ± 1.88^{a}	6.77 ± 2.05^{b}
	Day 3	4.03 ± 2.77^{a}	6.53 ± 2.42^{b}
	Day 4	2.84 ± 1.61^{a}	6.60 ± 2.15^{b}
	Average	$\mathbf{3.55 \pm 0.92^{A}}$	$\mathbf{6.47 \pm 0.29^{B}}$
II	Day 1	4.15 ± 2.04^{a}	7.76 ± 2.14^{b}
	Day 2	3.31 ± 1.91^{a}	8.43 ± 2.56^{b}
	Day 3	3.56 ± 2.10^{a}	4.87 ± 1.87^{ab}
	Day 4	3.90 ± 1.97^{a}	6.06 ± 1.90^{b}
	Average	$\mathbf{3.73 \pm 0.32^{A}}$	$\mathbf{6.78 \pm 1.40^{B}}$
III	Day 1	2.19 ± 1.54^{a}	5.51 ± 1.21^{b}
	Day 2	2.08 ± 0.99^{a}	5.12 ± 1.49^{b}
	Day 3	4.34 ± 1.49^{b}	4.52 ± 1.58^{b}
	Day 4	3.10 ± 1.65^{a}	5.32 ± 1.63^{b}
	Average	$\mathbf{2.92 \pm 0.90^{A}}$	$\mathbf{5.11 \pm 0.37^{B}}$

The bold figures are for average values of four days

Values are Mean ± SD, *I* daily, *II* once a week, *III* alternatively every second day of the week

Values with different superscripts in a row for 4 days of treatment (a–b) separately at each mode of application and for average values (A–B) at all the three modes of application indicate significant difference at $P \leq 0.05$

Values with different superscripts for 4 days of treatment in a column for treatment side (a–b) separately at each mode of application indicate significant difference at $P \leq 0.05$

controls [23]. Eucalyptus oil (1 %) added to sugar syrup, repelled honey bees [24].

Eucalyptus oil (2 %) on filter paper and wood floor repelled termites [25]. Eucalyptus oil can also protect plants against rice weevils, pine processionary moths and mushroom flies [6]. Essential oils of eucalyptus appear particularly potent as mosquito repellents [26]. Since eucalyptus oil possesses a wide spectrum of biological activity and is regarded as safer compound, there have been attempts to commercialize and market the insecticides/repellent products containing eucalyptus oil as such or based upon them. Quwenling is a eucalyptus-based product that has been successfully marketed as an insect-repellent in China [27]. It provides protection against *Anopheles* mosquitoes parallel to DEET (N, N-diethyl-meta-toluamide) and has, in fact, replaced the widely used synthetic repellent, dimethyl phthalate.

Prior to the present study, there was no report on efficacy of eucalyptus oil as repellent against rodent pests. The authors, for the first time evaluated the repellent potential of eucalyptus oil applied as paint and spray against *R. rattus* of both sexes and reported its highest repellent potential when applied daily [16, 28] and it was felt that there is a need to conduct further studies to enhance the persistence of repellent effect of eucalyptus oil for longer period of time. Other plant essential oils tested recently as repellents/antifeedants against rodents are citronella oil and cinnamic aldehyde [17, 29]. Some earlier studies have reported the potential of neem products (oil, seed powder, leaf powder) as antifeedants against rats [9, 30].

During present studies, the authors encapsulated eucalyptus oil in paraffin wax blocks so as to have its slow release with increased persistence of repellent effect. Moreover, the cost of eucalyptus oil used for preparing one wax block encapsulating 1 ml of 5 % eucalyptus oil comes out to be Indian Rs 2 (US $ 0.03), which can be considered cost effective if the extent of loss caused by *R. rattus* through damage and contamination of food is kept in view. In overall, the average percent repellency of eucalyptus oil at all the three concentrations tested was found to be similar at all the three modes of application though there were some differences on different days of treatment at different modes of application.

Conclusion

The present study is the first of its kind revealing the use of eucalyptus oil encapsulated in wax blocks for increasing the efficacy of its repellent effect against *R. rattus*. The study suggests the use of 5 % eucalyptus oil (being the minimum effective concentration) on encapsulation in wax blocks as repellent against *R. rattus*. This method may be used as part of IPM technology for managing *R. rattus* population in enclosed store houses.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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