

Insecticidal Activity of Essential Oils of Four Medicinal Plants Against Different Stored Grain Insect Pests

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Abstract.- Studies were carried out to investigate the insecticidal activity of essential oils of four locally grown plants such as *Datura stramonium*, *Eucalyptus camaldulensis*, *Moringa oleifera* and *Nigella sativa* against three major insect pests viz., *Tribolium castaneum*, *Trogoderma granarium* and *Cryptolestes ferrugineus* responsible for economic loss to stored commodities. Test insects were fumigated with concentrations of 5, 10, 15 and 20 µl/L under laboratory conditions at 30±2°C and 65±5% relative humidity. Essential oils fumigation significantly affected the mortality at all levels of concentration and exposure periods. Among essential oils *D. stramonium* was found to be the most toxic against *T. granarium* (14.46%) and *C. ferrugineus* (28.49%) while *N. sativa* showed the highest fumigant mortality (20.06%) against *T. castaneum*. Overall results show that *C. ferrugineus* was found to be the most susceptible test insect with 23.79 % mean mortality followed by *T. castaneum* (17.11%) and *T. granarium* (12.27%). Higher mortality was found to be concentration and exposure time dependent. The results demonstrate that the essential oils of investigated plants can be used as fumigant to control insect pests of stored products.

Keywords: Plant essential oil, fumigant toxicity, *Tribolium castaneum*, *Trogoderma granarium*, *Cryptolestes ferrugineus*

INTRODUCTION

After harvesting, approximately 1660 insect species attack the agricultural produce during different phases like transportation, processing, marketing and storage (Hagstrum and Subramanyam, 2009). Losses caused by these insect pests may reach up to 30% during storage (Haubruge *et al.*, 1997). Among these insect pests *Tribolium castaneum* (Mondal, 1994; Danahaye *et al.*, 2007), *Trogoderma granarium* (Burgess, 2008; Mark *et al.*, 2010) and *Cryptolestes ferrugineus* (Suresh *et al.*, 2001; Mason, 2003) are documented to be the most damaging and destructive pests of stored products throughout the world. About 2-6% food grain production of Pakistan is lost every year during storage by stored grain insect pests (Avesi, 1983)

Synthetic insecticides (pyrethroids and organophosphates) and fumigants (methyl bromide and phosphine) are commonly used to control these insect pests throughout the world. Out of these control strategies, fumigants (because of their broad

spectrum action and rapid penetration without residues) are known to be convenient and economical control measure (Mueller, 1990; Varma and Dubey, 2001; Ogendo *et al.*, 2008). Methyl bromide is completely phased out as it was found one of main causes of ozone depletion (Butler and Rodriguez, 1996; Shaaya and Kostyukovsky, 2006; Tayoub *et al.*, 2012). The control of stored product insect pests mainly depends on application of phosphine (Varma and Dubey, 2001). There are also many problems associated with its application, such as adverse effects on non target organisms and environment, human health safety concerns and pest resistance and resurgence (Ogendo *et al.*, 2008). Almost all major pests of stored products have developed resistance against phosphine (Pimentel *et al.*, 2007; Lorini *et al.*, 2007; Nayak *et al.*, 2012).

This situation demands a serious effort to find out some safe, viable, biodegradable, environment friendly and effective substitute to these conventional fumigants. Botanicals extracted from higher plants have been found suitable after investigating their fumigant insecticidal properties by many scientists (Isman, 2006, 2008; Rajendran and Sriranjini, 2008; Sagheer *et al.*, 2013). A wide number of plant essential oils and their constituents have been proved for their fumigant insecticidal action against stored product pests (Kim and Ahn,

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2001, Singh *et al.*, 2005; Opolot *et al.*, 2006; Tripathi *et al.*, 2009; Lopez and Pascual-Villalbos, 2010).

In many studies *Eucalyptus camaldulensis* have shown its fumigant potential against *T. castaneum* (Tunc *et al.*, 2000; Channoo *et al.*, 2002; Negahban and Moharramipour, 2007). Similarly, Ogendo *et al.* (2008) performed space fumigation tests to evaluate bioactivities of *O. gratissimum* against five major pests of stored grain. *T. castaneum* and *S. oryzae* were found to be the most tolerant among the insects as they showed 23 and 94% mortality at 10 µl/air for 168 hr of exposure, respectively, while *R. dominica*, *O. surinamensis* and *C. chinensis* exhibited 98, 99 and 100% mortality, respectively, even at 1µl/air concentration with 24 h of exposure time.

Though effectiveness of volatile oils as fumigant has been well investigated but their activity at different concentration levels and exposure time against local strains of stored grain insect pests in Punjab, Pakistan, is yet to be evaluated.

MATERIALS AND METHODS

Plant materials

Leaves from the locally grown medicinal plants, *Datura stramonium* (Dhatora), *Eucalyptus camaldulensis* (Sofaida), *Moringa oleifera* (Sohanjana) and seeds from *Nigella sativa* (Kalwanji) were collected from University of Agriculture, Faisalabad, Punjab, Pakistan (Longitude 73°74 East; Latitude 30°31.5 North; Altitude: 184 m). These fresh plant parts were brought to laboratory (Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad and dried in shade. Dried plant parts were grinded to powder using stone electric grinder (Machine No. 20069, Pascall Engineering Co. Ltd.). This grinded material was then sieved through 40 mesh sieve to obtain fine powder.

Extraction of essential oils

Soxhelt extraction apparatus (Model WHM12295, Daihan Scientific Co., Ltd.) was used to prepare essential oils. Soxhelt thimble was filled

with 50 g of fine botanical powder and placed in flask. Acetone was used as solvent in bottom flask. This process of extraction oil from all plant powders was repeated many times to achieve enough quantity of essential oil. Extracted essential oil was then purified by evaporating solvent by using electric rotary evaporator. These pure extracted essential oils were preserved in glass vials at 4°C and then used to prepare appropriate concentrations of 5, 10, 15 and 20 µl/L by mixing acetone as solvent. These prepared concentrations were used for subsequent experiments.

Test insects

To collect three strains of *Cryptolestes ferrugineus*, *Tribolium castaneum* and *Trogoderma granarium*, Infested grain samples from three districts, Faisalabad (FSD strain) (Longitude 73°74 East; Latitude 30°31.5 North; Altitude: 184 m), Sahiwal (SWL strain) (Longitude, 73-06; Latitude, 30-40 north; Altitude 150 m) and Toba Tek Singh (TTS strain) (Longitude, 72°08' to 72°48'; Latitude, 30°33' to 31°2 north; Altitude, 162 m) of central Punjab, Pakistan were collected and brought to laboratory. Populations of each test insect were sorted out as a strain from the collected samples of each district.

Collected insects were kept in the jars of 9.5cm diameter having commodity (wheat grains for *T. granarium* and wheat flour for *C. ferrugineus* and *T. castaneum*) sterilized for 30 min at 70°C using oven (Lab Line Instruments Inc. Model no 3512-1) and covered with the muslin cloths. After three days adults were sieved out from the commodity. The rearing commodity along with eggs of the insects was put into jars which were kept at 30±2°C and 65±5% relative humidity in incubator (MIR-254 Sanyo) to obtain F₁ population which was considered as a homogenous culture (Mujeeb and Shakoori, 2012). This homogenous culture was used for different bioassays using plant essential oils.

Bioassays

Space fumigation method (Shaaya *et al.*, 1991) was used for the evaluation of fumigant toxicities of essential oils. 0.5-L flat-bottom air tight plastic space fumigation chambers with a small amount of food were used for experiment. Twenty

adults of each test insect species were introduced in each test chamber. Essential oils were applied separately on small pieces of Whatman No. 1 filter paper to provide dosages of 5, 10, 15 and 20 µl/L air. Control was treated with acetone alone. Treated filter papers were kept in air and allowed to evaporate the solvent (acetone) for 10 min. Fumigation chambers were sealed to make them air tight after suspending the treated filter papers in the middle of chamber below the lid to avoid physical contact and to assure uniform distribution of fumes. Data for adult mortality was recorded after 72 (3 days), 120 (5 days) and 168h (7 days) of treatment.

Statistical analysis

The data were arranged in tabulated format. The mortality (%) was corrected by Abbot's (1925) formula:

$$\text{Corrected Mortality} = \frac{M_o - M_c}{100 - M_c} \times 100$$

where, M_o = Observed mortality; M_c = Control mortality

The collected data were subjected to Analysis of Variance using Statistica software (8.0, Stat Soft, Inc. 1984-2008). Means of significant treatments were separated using Tuckey-HSD test at $\alpha = 5\%$.

RESULTS

Three major insect pests of stored grains viz., *T. granarium*, *C. ferrugineus* and *T. castaneum* were fumigated with essential oils of *D. stramonium*, *E. camaldulensis*, *M. oleifera* and *N. sativa* to evaluate fumigation potential of essential oils. Insecticidal efficacy of essential oils was found significant against all test insects (*T. castaneum*, $F_{(3,436)} = 96.761$, $p = 0.000$; *T. granarium*, $F_{(3,436)} = 69.247$, $p = 0.000$; *C. ferrugineus*, $F_{(3,436)} = 67.291$, $p = 0.000$). Results revealed that maximum fumigation toxicity was depicted by *D. stramonium* against *T. granarium* (14.43 %) and *C. ferrugineus* (28.49 %) whereas against *T. castaneum*, *N. sativa* was observed the most toxic (20.06 %) essential oil. There was not considerable difference found in fumigant toxicities of *D. stramonium* (14.34%) and *E. camaldulensis* (14.25%) against *T. granarium*.

While *M. oleifera* was found least toxic with mean mortality of 12.03, 9.38 and 20.44% against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively. According to susceptibility, following order was observed in the test insects, *C. ferrugineus* (23.79% mortality) > *T. castaneum* (17.11% mortality) > *T. granarium* (12.25% mortality).

Effect of concentrations of each essential oil on mortality of test insects was found significant (*T. castaneum*, $F_{(3,436)} = 346.40$, $p = 0.000$; *T. granarium*, $F_{(3,436)} = 226.03$, $p = 0.000$; *C. ferrugineus*, $F_{(3,436)} = 481.51$, $p = 0.000$). Higher mortality was achieved with higher concentration that is maximum mortality against *T. castaneum*, *T. granarium* and *C. ferrugineus* recorded at 20µl/L concentration was 25.69, 17.80 and 35.48 %, respectively. Overall mean mortality of essential oils against all test insects at 5 µl/L concentration was recorded 10.5% which was significantly increased to 26.72% with 20 µl/L concentration. It is obvious from Table III regarding plant and concentration interaction that the highest fumigant toxicity (29.55 %) was depicted by T_8 against *T. castaneum* and T_4 (45.14%) against *C. ferrugineus*.

Fumigation with essential oils was performed for three different exposure times which significantly affected the mortality against *T. castaneum* ($F_{(2,436)} = 814.41$, $p = 0.000$), *T. granarium* ($F_{(2,436)} = 572.21$, $p = 0.000$) and *C. ferrugineus* ($F_{(2,436)} = 728.55$, $p = 0.000$). Mean mortality after 72hr (lowest exposure time) of treatment was 9.12, 6.19 and 14.19% which significantly increased to 26.56, 18.53 and 33.21% after 168hr (highest exposure time) against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively. Essential oils and exposure time interaction significantly increased mortality of all test insects (Table I).

When the effects of exposure time for all the oils was checked, the maximum mortality was observed in *D. stramonium* after 168hrs of treatment against *C. ferrugineus* (41.51±2.81), *T. castaneum* (33.83±1.72) and *T. granarium* (24.98±1.17) (T_3 , Table I).

All the results showed that the mean fumigant toxicity was concentration and exposure time dependent (Table II). Fumigation with higher

concentration of essential oil for prolonged period of time (T_{12}) resulted in 40.84, 27.26 and 51.90 % mortality against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively.

Table I.- Percent mortality of *T. castaneum*, *T. granarium* and *C. ferrugineus* at different exposure periods against plant essential oils.

Treatments Exposed for (h)	Mortality (%)		
	<i>T. castaneum</i>	<i>T. granarium</i>	<i>C. ferrugineus</i>
<i>D. stramonium</i>			
72	07.7±0.5 g	06.0±0.5 ge	14.6±0.8 f
120	14.8±0.8 e	12.0±0.6 ef	29.0±1.6 c
168	33.8±1.7 a	25.0±1.2 a	41.5±2.8 a
<i>N. sativa</i>			
72	10.3±0.7 fg	06.1±0.4 ge	10.0±0.5 g
120	19.1±1.0 d	10.5±0.6 ef	23.6±1.5 de
168	30.4±1.7 b	16.1±1.0 c	34.9±2.2 b
<i>E. camaldulensis</i>			
72	10.6±0.6 fg	07.5±0.5 g	16.1±0.7 f
120	16.7±1.0 de	14.8±0.8 cd	21.8±1.0 de
168	24.9±1.8 c	20.2±1.1 b	31.8±1.6 bc
<i>M. oleifera</i>			
72	07.8±0.5 g	05.0±0.5 e	16.0±0.8 f
120	11.0±0.7 f	10.2±0.6 f	20.6±0.9 e
168	17.0±1.1 de	12.8±0.9 de	24.6±1.2 d

Means within the same column followed by the same letter are not significantly different. ANOVA, HSD ($P > 0.05$).

Fumigant toxicity against *T. castaneum*

The essential oils significantly exhibited fumigant toxicity against *T. castaneum*. *N. sativa* was found the most lethal fumigant against *T. castaneum* with mean mortality of 20.06% followed by *D. stramonium* (18.90%), *E. camaldulensis* (17.43%) and *M. oleifera* (12.03%). Fumigation for longer time with higher concentration (T_{12} , Table II) exhibited maximum mortality (40.84%). Three strains of *T. castaneum*, investigated in this experiment significantly affected mortality ($F_{(2,436)} = 16.233$, $p = 0.000$). Susceptibility of TTS strain was found high (18.43% mortality), whereas moderate in SWL (16.93% mortality) and low susceptibility to essential oils was observed in FSD strain (15.96% mortality). Table IV, regarding exposure time and strain interaction also indicated the same trend with maximum mortality in T_9 .

Table II.- Percent mortality of *T. castaneum*, *T. granarium* and *C. ferrugineus* against different concentrations of all plant essential oils at three exposure times.

Exposed for dose (μ L)	Mortality (%)		
	<i>T. castaneum</i>	<i>T. granarium</i>	<i>C. ferrugineus</i>
72 h			
5	5.1 ± 0.37 i	3.1 ± 0.4 h	8.8 ± 0.5 h
10	7.5 ± 0.4 hi	4.9 ± 0.4 gh	12.6 ± 0.5 g
15	10.2 ± 0.5 gh	7.3 ± 0.4 f	16.1 ± 0.5 ef
20	13.5 ± 0.5 ef	9.4 ± 0.4 ef	19.3 ± 0.7 de
120 h			
5	9.3 ± 0.5 h	7.2 ± 0.4 fg	14.0 ± 0.5 fg
10	12.6 ± 0.5 fg	10.5 ± 0.5 e	19.8 ± 0.5 d
15	17.2 ± 0.7 d	13.2 ± 0.5 d	26.1 ± 0.7 c
20	22.7 ± 0.8 c	16.7 ± 0.6 c	35.3 ± 1.1 b
168 h			
5	15.6 ± 0.9 de	11.0 ± 0.8 de	20.3 ± 0.5 d
10	22.2 ± 1.0 c	16.5 ± 0.8 c	27.3 ± 0.7 c
15	27.5 ± 1.6 b	19.3 ± 1.1 b	33.3 ± 1.8 b
20	40.8 ± 1.5 a	27.3 ± 0.9 a	51.9 ± 2.1 a

Means within the same column followed by the same letter are not significantly different. ANOVA, HSD ($P > 0.05$).

Table III.- Percentage mortality of *T. castaneum* and *C. ferrugineus* against four concentration of plant essential oils.

Fumigant	Dose (μ L)	Mortality (%)	
		<i>T. castaneum</i>	<i>C. ferrugineus</i>
<i>D. stramonium</i>			
	5	12.7 ± 1.4 fgh	16.11 ± 1.2 jk
	10	15.7 ± 1.7 def	22.41 ± 1.3 gh
	15	19.7 ± 2.1 bc	30.23 ± 2.1 cd
	20	27.4 ± 2.9 a	45.13 ± 3.6 a
<i>D. stramonium</i>			
	5	11.8 ± 1.0 ghi	12.52 ± 1.0 k
	10	16.5 ± 1.3 cde	18.58 ± 1.5 hij
	15	22.3 ± 1.9 b	25.01 ± 2.1 ef
	20	29.5 ± 2.2 a	35.62 ± 3.0 b
<i>E. camaldulensis</i>			
	5	9.0 ± 0.6 ij	15.32 ± 0.9 jk
	10	14.3 ± 1.0 efg	20.39 ± 1.2 hi
	15	18.8 ± 1.4 cd	24.85 ± 1.4 ef
	20	27.6 ± 1.9 a	32.63 ± 1.9 bc
<i>M. oleifera</i>			
	5	6.6 ± 0.5 j	13.49 ± 0.7 k
	10	9.9 ± 0.7 hij	18.12 ± 0.7 ij
	15	13.4 ± 1.0 efg	21.56 ± 1.0 ghi
	20	18.2 ± 1.2 cd	28.52 ± 1.1 de

Means within the same column followed by the same letter are not significantly different. ANOVA, HSD ($P > 0.05$).

Fumigant toxicity against *T. granarium*

Though fumigant effect of essential oils was significant but *T. granarium* was found less

susceptible with overall 12.27% mean mortality as compared to other test insects in this study. The higher mortality was exhibited by *D. stramonium* (14.46%) and *E. camaldulensis* (14.27%) followed by *N. sativa* (10.94%) and *M. oleifera* (9.41%). Effect of different strains on mortality was found significant ($F_{(2,436)} = 17.269$, $p = 0.000$). According to fumigant mortality in different strains following order was observed FSD (11.01%) < SWL (12.62%) < (13.12%) TTS. Essential oil and exposure time interaction was found significant which indicated maximum mortality (24.98%) in T_3 against *T. granarium* (Table I).

Table IV.- Percentage mortality of three strains of *T. castaneum* and *C. ferrugineus* against essential oils at different exposure times.

Treatments time (h)	Mortality (%)	
	<i>T. castaneum</i>	<i>C. ferrugineus</i>
72 h		
FSD	8.4 ± 0.5 d	13.5 ± 0.7 d
SWL	9.3 ± 0.5 d	14.9 ± 0.7 d
TTS	9.7 ± 0.5 d	14.1 ± 0.7 d
120 h		
FSD	14.4 ± 0.8 c	22.4 ± 1.2 c
SWL	15.5 ± 0.8 c	24.2 ± 1.1 c
TTS	16.4 ± 0.9 c	24.8 ± 1.2 c
168 h		
FSD	25.1 ± 1.5 b	31.6 ± 1.7 b
SWL	25.5 ± 1.6 b	32.2 ± 2.1 b
TTS	29.2 ± 1.7 a	35.8 ± 1.8 a

Means within the same column followed by the same letter are not significantly different. ANOVA, HSD ($P > 0.05$).

Fumigant toxicity against *C. ferrugineus*

Strong fumigant insecticidal bioactivities of essential oils were recorded against *C. ferrugineus* as compared to *T. granarium* and *T. castaneum*. Essential oils depicted 23.79% overall mortality against *C. ferrugineus* while *D. stramonium* was found the most active fumigant (28.49%) followed by *E. camaldulensis* (23.31%), *N. sativa* (22.95%) and *M. oleifera* (20.44%). Effect of the strains on mortality was also found significant ($F_{(2,436)} = 11.224$, $p = 0.00002$) that is, mean mortality of 22.52, 23.97 and 24.89% was recorded in FSD, SWL and TTS strain, respectively. Significant interaction of strain and exposure time is given in Table IV which indicated maximum mortality

(35.78%) in T_9 against *C. ferrugineus*. Highest individual mean mortality of essential oils against *C. ferrugineus* was observed at 20% concentration after 168hr of treatment which was 70.46, 55.93, 45.76 and 35.44% against *D. stramonium*, *N. sativa*, *E. camaldulensis* and *M. oleifera*, respectively.

DISCUSSION

This experiment was designed to evaluate fumigant potential of some indigenous medicinal plants. Effect of essential oils from *D. stramonium*, *E. camaldulensis*, *M. oleifera* and *N. sativa* was found significant against *T. granarium*, *C. ferrugineus* and *T. castaneum*. Insecticidal activities of *D. stramonium* (Mahfuz and Khanam, 2007), *E. camaldulensis* (Negahban and Moharrampour, 2007), *M. oleifera* (Anita *et al.*, 2012) and *N. sativa* (Chaubey, 2007) are extensively investigated against stored grain insect pests.

In present studies *D. stramonium* (20.62%) was found the most effective fumigant followed by *E. camaldulensis* (18.34%), *N. sativa* (17.98%) and *M. oleifera* (13.96%). Whereas the highest mortality in *T. castaneum* (20.057 %) was recorded against *N. sativa*. These results are also in agreement with that of Chaubey (2007) who evaluated fumigant toxicity of *Anethum graveolens*, *Nigella sativa* and *Trachyspermum ammi* against *T. castaneum* adults found *N. sativa* the most toxic among these essential oils. Among essential oils *M. oleifera* exhibited least mortality against *T. castaneum* (12.03%), *T. granarium* (9.38 %) and *C. ferrugineus* (20.44%).

T. granarium (12.25%) was found tolerant insect followed by *T. castaneum* (17.11 %) and *C. ferrugineus* (23.79%). These findings are similar to those of Ali *et al.* (2012) who evaluated insecticidal efficiencies of *Datura alba* and found *T. granarium* more tolerant than *S. oryzae* with 33.5 and 45% mortality, respectively. In another study Abdel-Sattar *et al.* (2010) investigated insecticidal activities of *Schinus molle* against *T. granarium* and *T. castaneum* and found LC_{50} s 915.1 and 779.1 μ l/L which indicated higher tolerance in *T. granarium* than *T. castaneum*.

Fumigation with higher concentration of essential oils enhanced the mortality in insects. It is evident from Table III that treatment with higher

concentrations, T₈ (29.55%) and T₄ (45.13%), resulted in maximum mortality against *T. castaneum* and *C. ferrugineus*, respectively. These findings are in accordance with earlier reports documented by Rozman *et al.* (2006), Negahban *et al.* (2006), Liska *et al.* (2010) and Theou *et al.* (2013).

Insects were fumigated with essential oils for 72, 120 and 168 h and exposure time was found directly related to mortality. All investigated essential oils exhibited strongest fumigation action at 168 h of exposure to all tested insects. It is easily understandable from Table I that maximum mortality was observed in T₃ against *T. granarium*, *C. ferrugineus* and *T. castaneum*. These results are confirmatory to those of Ebadollahi *et al.* (2010) who evaluated fumigant toxicity of essential oil extracted from *Lavandula stoechas* against *T. castaneum* and observed decrease in LC₅₀ by increasing exposure time i.e., LC₅₀ was recorded 39.68, 29.41 and 26.77 µl/L after 24, 48 and 72 h, respectively. Similar trend was observed by Liska *et al.* (2010) who recorded 5.0 % fumigant toxicity of camphor at 2 h and 13.2% after 4 h of treatment against *T. castaneum*. These findings were also in accordance with previous studies carried out by Shukla *et al.* (2002), Lee *et al.* (2004), Rozman *et al.* (2006) and Xie *et al.* (2010).

Three strains of *T. granarium*, *C. ferrugineus* and *T. castaneum* investigated in this experiment were found significantly different in susceptibility to essential oils. FSD strain was found tolerant as it showed less mortality (16.5%) against all essential oils followed by SWL (17.84%) and TTS (18.81%) strain. Results are quite in line to those of Khalequzzaman and Sultana (2006) who also reported the significant difference in sensitivity of different strains of *T. castaneum* against essential oils. Similar conclusions have also been drawn by Sagheer *et al.* (2013) who investigated Vehari and Faisalabad strains of *T. granarium* against some essential oils which resulted higher mortality against Vehari strain and proved Faisalabad (FSD) strain tolerant.

In summary, the botanicals have a potential to control the stored grain pests at higher concentrations and for long periods of exposures. This study along with some other earlier studies opens a way for grain-storage protectionists to use

natural oils in the huge storage structures instead of the synthetic fumigants to overcome the problem of insect resistance.

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