TOXIC EFFECT OF *MYRISTICA FRAGRANS* ESSENTIAL OIL AGAINST THE MUSEUM PEST *ANTHRENUS VERBASCI (COLEOPTERA: DERMESTIDAE)* TO CONTROL BIODETERIORATION OF ANIMAL COLLECTIONS

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ABSTRACT

Museums are custodians of natural and cultural heritage. Objects like tribal dresses, headgears, weapons, musical instruments and other ethno-cultural materials housed in museums are prized possession of intellectual and cultural property of people. Tropical countries like India have a favorable climatic condition for the biological agents of biodeterioration. Organic materials such as leather and parchment objects form substantial part of collections in museums across the world which are promptly infested by insects like dermestid beetles, tenebrionides, silver fishes, cockroaches and other micro-organisms. From the last two to three decades the environmental problems are increases due to the over use of pesticides or other non-decomposing chemicals as well as products. Synthetic products and pesticides are very expensive and also highly toxic for humans and its environment. In order to overcome the above problems, there is urgent need to develop safe, convenient, environmentally friendly and low-cost alternatives. The aim of present study is to evaluate toxicity of nutmeg oil through contact and stomach mechanism against the larvae of *Anthrenus verbasci* under laboratory conditions (25±29 °C and 60±68% RH). The mortality data thus obtained in both cases of mechanisms were first subjected to profit analysis using SPSS software and then later the transformed data were used for drawing regression lines graphs between probit mortality and concentration to determine LC 90 values in each cases of test. In contact case LC 90 value is 19.11% whereas in stomach case LC 90 value is obtained at 16.63%.

Keywords: Biodeterioration; Contact Toxicity; Cultural Heritage; Dermestid beetles; Natural Heritage; Stomach Toxicity.

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1.INTRODUCTION

The environmental problems caused due to the overuse of pesticides and other non-decomposing chemicals as well as products have been the matter of serious concern for both the scientists and public in recent years. Although, pesticides were used initially to benefit the human life for increasing the agricultural productivity by controlling infectious disease, their adverse effects have overweighed the benefits associated with their use. So this is the time that necessitates the proper use of pesticides to protect human beings and their environment [1]. To overcome the above problems natural products are an excellent alternative to synthetic pesticides, as a mean to reduce negative impacts to human health and environment [2, 3].

Museum objects encompass valuable natural history collections belonging to various fields such as botany, zoology, entomology, paleontology, mineralogy, archeology, ethnography, anthropology and archives. These valuable items of museums are prized possessions of intellectual and cultural property. There are various environmental factors such as light, humidity, atmospheric pollutants, dust particles, temperature, hazardous gaseous and biological agents causing damage and decay of organic objects present in museums, libraries, archives and similar institutions. The agents which responsible for biodeterioration are insects, fungus, bacteria, lichens, algae, birds, rodents and even human being also. Among all, insect is the most notorious and destructive in nature [4, 5]. Tropical countries like India, Pakistan and Bangladesh have much favorable climatic condition for the biological agent, which causes biodeterioration [6, 7].

It has been observed that the rich wealth of manuscripts in many countries faces great threat due to the various causes of deterioration, particularly by the biodeterioration. Biodeterioration is defined as the damage caused by living organisms such as insects, fungi or algae which endangers the cultural property of all around the world. The damage results show "enormous economic loss" and irreversible cultural and artistic loss of information of the objects which are affected. [8]

Biodeterioration of cultural property is a major problem in almost all the parts of world, likewise art works and artifacts are constantly being destroyed by biological agents. Since organic materials, which are most vulnerable to biological attack, are used for many traditional objects, and since the high heat and humidity in tropical countries are weaken the organic materials and foster the growth and reproduction of biodeteriogens. Museum of India faces critical problems in protecting its natural history collections from biodeterioration. This chapter will discuss the major biodeteriogens, the negative effects of environmental conditions on human health, and the threats of biodeterioration to the skin and skin products most commonly found in the museum collections. The information is summarized briefly with an emphasis on

biodeterioration of skins and hides artifacts. Many research projects have been carried out on biodeterioration of organic objects in museums, archives and libraries [9-16]. During the course of those studies, it was revealed that entomological collections in different museums were found to have undergone various degrees of decay and damage by Dermestes [5]. The extent of losses in museum collections due to insect pests and their control are inadequately researched [18]. So it is a prime concern to emphasize the research in this direction, to prevent the important products from such harmful biological deterioration either by indigenous or non-indigenous methods.

Most literature survey has indicated that, the research which carried on bionomics and control action of biodeterioration. It is mainly caused by the insects belonging to *Dermestidae* family, there important species are *Anthrenus, Attagenus, Dermestes, Trogoderma, Necrobia and Psocids* [17-19]. Species like *Anthrenus and Attagenus* have been noted to breed in bird's nests and animal burrows in the vicinity of museums, and then which moves from their breeding sites to museum collections of animal objects, resulting causes severe infestation [20]. In addition to these above, beetle pests of *Tenebrionidae, Anobiidae and Ptinidae* are also occasionally observed which deteriorated many important animal products [21]. Mostly these insects are scavengers, they inhabited the nests of birds and mammals and feed on organic materials like feathers, wools, furs, parchment membranes, valuable leather objects, books, journals, papers, dried animal materials etc. These objects entirely form the significant parts of collection in museum, libraries and archives. Such types of important materials are regularly infested by the insects like termites, dermestid beetles, silver fishes, tenebrionides, cockroaches and many other micro-organisms also [22-25]. Insect pests which feed animal products are having unique ability to digest and utilize keratin, a chemically stable structural protein present in wool, feather, hair and horn [26]. Unlike insects feed on food grains, the pests infested on animal products are known to have higher proteinase and aminopeptidase enzyme activities [27]. Some of the insect pests of preserved animal products diapauses these periods, they are more tolerant for extreme climatic conditions and pesticide treatment [28].

In order to overcome the problems of biodeterioration, natural biocides should be used. The use of pesticides and synthetic products in museums and libraries are not only expensive but also highly toxic, which results to show very harmful effects on human beings and entire environment that causes the disturbance in ecosystem. Due to the high health risk factor government has taken some severe initiatives on policy of banning it. On the basis of above drawbacks; nowadays the indigenous method of control is highly emphasized [29-35].

One of the potential sources for producing natural biocides is the substances produced by plants. The

essential oils extracted from plants are usually decomposed very easily in the environment, so they have lower toxicity for humans and other mammals which show lower harmful effects on the environment [36]. Even Xie et al, 1995 [37] reported, that plants are a rich source of insecticidal compounds and the effectiveness of these compounds has easily been demonstrated against many stored product insects. Scientists in different parts of the world are working for the development and establishment of plant-based pesticide, usually called as Phyto-pesticide, Botanical pesticide, Bio-pesticide, Green or Natural pesticides etc [38-47].

Historically botanicals were used before other kinds of pesticides. They are mentioned in Hieroglyph, Chinese, Greek, and Roman antiquity and also in India the use of neem tree (*Azadirachta indica Juss.*; *Meliaceae*) was reported in the Veda, a body of manuscripts written in archaic Sanskrit dated at least 4,000 years ago [48] The numbers of indigenous flora in different ecosystems are still remains unknown. Over 6000 species of medicinal plants have been identified in India alone [49]. Apart from wild plants cultivated spices and condiments are routinely used in traditional medicines [50].

Essential oils are produced by the secondary metabolism in plants. Their constituents are used in human consumption as functional food, food additives, medicines, nutritional supplements and also for the manufacture of cosmetics [51]. Essential oils are most effective substances which are tested against many insects [52]. And it is defined as a concentrated hydrophobic liquid from plants having a volatile aroma known as essential oil. These are ethereal oils also known as "oil of" the plant from which it is isolated like oil of rosemary. The oil is called as essential because it possesses a distinctive smell of the plant [53]. These are usually known as "life force" of plants. It is reported that he compounds present in essential oil may act as fumigants, [54] contact insecticides, [55] repellents, [56] and anti-feedants [57] and also may affect some biological parameters like reproduction, life span [58] and even growth rate etc [59].

Myristica fragrans is commonly known as nutmeg which belongs to the *Myristiceae* family. It is cultivated in several countries throughout the world. It is some of the most valuable spice that has been used from centuries as food preservatives and for many medicinal purposes. It shows wide ranges of pharmacological effects consolidated from traditional uses for centuries which are reported in several literatures. It has been reported that nutmeg shows several advantages such as anti-diarrheal activity, stimulant, anti-fungal, anti-diabetic, carminative, anti-inflammatory properties etc [60, 61]. The main constituents of nutmeg are alkyl benzene derivatives like myristicin, elemicin, safrole, terpenes, alpha-pine, beta-pinene, myristic acid trimyristin etc [62-65]. Generally, it is believed that myristicin is the major component responsible for intoxications [66]. In a study, control with essential oil of nutmeg has

shown much insecticidal activity against larvae of *Lycoriella ingenua* [67] and *Callosobruchus chinensis* [68]. Whereas, in another important study it was observed that excessive doses may lead to narcotic effect; symptoms of delirium and epileptic convulsions appear after 1-6 hours [69,70].

In present study, a series of experiments were conducted to examine the toxicological effects of nutmeg essential oil against the larvae of *Anthrenus verbasci* with different modes of action. The mode of actions of pesticides is described on their modes of entry [71-73]. In the insecticide mode of action, contact and ingested or stomach mechanism have been discussed in this study. It is revealed that the prevention and control can be achieved with the toxicity of *Myristica fragrans* oil against larval stage of *A.verbasci*. In the following experiment toxic effects of *M. fragrans* essential oils is determined against the larvae of *A.verbasci* by analyzing LC 90 value after 24 hours of exposure via stomach and contact mechanisms respectively. The bioactivity shows much variation comparatively in both cases.

2.Bionomics

Dermestid beetles have a complete metamorphosis, with the larvae, the only stage which causes serious damage to proteinecious material whereas the adults consume less food comparatively to larvae and therefore causes less damage. Adults may be found indoors or outdoors and are frequently found on flowers where they feed on pollen. Sometimes they are also found feeding on fibers such as rayon, linen, cotton and jute. The insect feeds on animal products can easily digest and utilize keratin, a chemically stable structural protein present in feather, wool, hair and horn [74]

Anthrenus verbasci species commonly known as varied carpet beetle infests the usual range of household articles and museum collections such as carpets, woolen items, silks, skins, furs, feathers, hair, horn, cereals, red pepper, fishmeal, or any processed animal or plant food. In common with other carpet beetles, this species will feed on cottons, linens, and synthetic fibers if they are contaminated, but it will not feed on rayon acetate. The adult carpet beetle feeds only on pollen and nectar of garden flowers but lays its eggs accumulated animal stuff in buildings.

This species has a number of varieties. They differ in shape, size, color, and pattern of the scales, but in general the adults are 2 to 3.5 mm long, and have a varied pattern of white, brownish, and yellowish scales on the back and fine, long, greyish-yellow scales below. Where the elytra terminate, they do not form a cleft as do those of the furniture carpet beetle, *A. flavipes*. The mature larva is 4 to 5 mm long, and has a series of light- and dark-brown transverse stripes. If it is suddenly alarmed, the larva erects the 3 dense tufts of bristles and hair located on each side of the rear end of the body, spreading them out to form beautiful, round plumes. Different views of *A.verbasci* are clearly shown by following Fig.1. The larvae of *Anthrenus verbasci* are commonly known as woolly bears. They are broadest near

the rear, and become narrower toward the front end, unlike other carpet beetles.

Fig.1. Ventral and dorsal view of adult *Anthrenus verbasci*

Dorsal view of larvae of *Anthrenus verbasci* is shown below in the given Fig.2.

Fig.2. Dorsal view of larvae *Anthrenus verbasci*

The female lays about 40 eggs. There are usually 7 to 8 larval instars, but the number may vary from 5 to 16. The numbers of days for the various stages are as follows: egg, 17 to 18; larva, 222 to 323; and pupa, 10 to 30. The period from egg to adult is 249 to 354 days, and the adult may live another 14 to 44 days [75]. The cuticles of larvae have more long-chain hydrocarbons and a higher degree of unsaturation of hydrocarbons as well as esters than in adults comparatively. These differences in composition have been considered responsible for reduced penetration of the larval cuticle by insecticides and thereby the higher tolerance of that particular developmental stage.

3. Experimental approach

3.1. Collection of test oil

Nutmeg (*Myristica fragrans*) essential oil was purchased from herbal pharmacy in Aligarh. It was kept in proper air tighter glass container and placed in a cooled place for applying in experiments.

3.2. Collection of test organism

The initial source of beetles culture was a infested "bull horn", which was collected from the natural history collection of MUSA Dakri museum of the Aligarh Muslim University, India, indicated by Fig.3. These beetles were identified on the basis of their morphological characters in entomology section of zoology department, Aligarh Muslim University.

Fig.3. Bull horn affected by Dermestid Beetles

3.3. Identification of test organism

The rearing beetles were species of *Anthrenus verbasci.* It was identified based on the morphological characters in the entomology section of zoology department, Aligarh Muslim University**.**

Insect Profile

Binomial name: *Anthrenus verbasci*	
Kingdom	*Animalia*
Phylum	*Arthropoda*
Class	*Insecta*
Order	*Coleoptera*
Family	*Dermestidae*
Genus	*Anthrenus*
Genus	*verbasci*

3.4. Rearing of Insect Culture

During the month of April 2014 these identified beetle along with the infested bull horn was kept in rearing box covered with muslin clothes in dark storage area. The culture of insects was carried out in the rearing box **(48cmx27cmx34cm)** in order to obtain a homogeneous and sufficient population of *Anthrenus verbasci* larvae for various biological tests, shown in the given Fig 4.

Fig.4. Rearing box covered with muslin cloth

3.5. Bioassay for stomach and contact toxicity

In the first experimental setup, with different formulation of nutmeg oil has been tested which given as 2.5%, 5%, 10.0% and 20.0% concentrated solution prepared in distilled water, shows the effectiveness of toxicity by means of ingestion (or stomach) against the larvae of A. *verbasci*. The four feathers were impregnated with the above different concentrations of nutmeg oil in four different boxes A, B, C and D, while each box contain 10 specimens of larvae. Another experimental setup with different formulation of nutmeg oil are given as 5.0%, 10.0%, 20.0% and 40.0% concentration which also been tested for the showing the effectiveness of contact toxicity against larvae. In this blotting papers of same size were impregnated with the above concentration of nutmeg oil A, B, C and D respectively, with each box containing 10 specimens respectively. The entire experimental set up and the controls were arranged in the laboratory with the temperature fluctuating between 25±29 °C and humidity 60±68% RH in a Completely Randomized Design (CRD). The representation of observing set up is shown by following Fig.5.

Fig.5. Experimental setup for mortality of *Anthrenus verbasci* larvae through toxicity of *M. fragrans* oil by stomach and contact actions

4. Result and Discussions

The present study indicates the development of Anthrenus verbasci larvae is highly affected by the use of nutmeg oil. The effect of the nutmeg oil on the mortality of the larvae was much higher in stomach case than contact case. In case of stomach mechanism 10.0-20.0% concentrated solutions with nutmeg oil was found to record 90-100% mortality of the larvae within 24 hours of treatment in comparison of contact mechanism where 90-100% mortality of larvae was recorded at of 20.0-40.0% concentrated solution treated for 24 hours. By the analysis it is realized that the used larvae have been dead or alive according to the concentration percentage, feeding and contacting point of views. It shows dead and alive ratio through the stomach mechanism of A, B, C and D by 4:6, 6:4, 8:2 and 10:0 respectively in Table1, where through contact means it is given as A, B, C and D by 2:8, 4:6, 6:4 and 10:0 respectively in Table2.

	A (2.50%)	B (5.0%)	C (10.0%)	D (20.0%)
Dead	**4**	**6**	**8**	**10**
Live	**6**	**4**	**2**	**0**

Table.1. Toxic effect of stomach action by *Myristica fragrans* essential oil against the larvae of *A.verbasci* after 24 hours days of exposure

	A (5.0%)	B (10.0%)	C (20.0%)	D (40.0%)
Dead	**2**	**4**	**6**	**10**
Live	**8**	**6**	**4**	**0**

Table.2. Toxic effect of contact action by *Myristica fragrans* essential oil against the larvae of *A.verbasci* after 24 hours of exposure

The present study indicates the development of Anthrenus verbasci larvae is strongly affected by the use of nutmeg oil through both means of contact and stomach toxicity mechanism. The result of experiments reveals that the insecticidal efficacies of nutmeg oil at different concentrations were assessed by the mortality counts of the larvae during the given 24 hours of period. The mortality of the larvae in stomach case is comparatively higher than contact case. The ratio of dead and alive larvae in both cases is clearly demonstrated through bar graph in Fig.6.

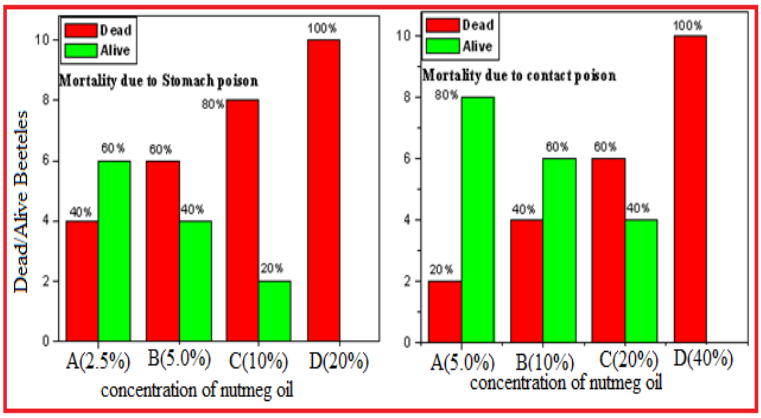

Fig.6. Percent of mortality in stomach and contact case through *M. fragrans* oil against *A. verbasci* (larvae) in 24 hours after treatment

Each experiment done in this study was repeated for 3 times with each concentration of the intoxicant exposed for same duration of time. While counting the numbers of mortalities the morbid larvae was also regarded as dead. The mortality percentage of the insects was calculated by the following method given below.

Mortality (X %) = (Nt/No) ×100

Nt = Number of dead insects pests

No = Total number of insects pests

The main constituents of nutmeg include sabiene, alpha-pinene, beta-pinene, myrcene, limonene, terpenes, safrole, and myristicin compounds [76]. These bioactive agents could cause a depolarizing neuromuscular blocking action which could lead to the death of insects [77]. The use of nutmeg oil has resulted to death of the A.verbasci larvae due the feeding as well as impairing respiration, which indicated the hallucinogenic characteristic of myristicin present in nutmeg oil [78]. By FTIR analysis indicated by Fig.7, shows that there are many peaks present which assigned different functional groups of nutmeg oil. The peak at 3355cm^{-1} represents OH group vibrations, 1701.7 cm^{-1} shows carbonyl group stretched (C=O) vibration. The peak at 1209.2 cm^{-1} are attributed ether group (C-O-C), while 2927-2874 cm^{-1} indicated (C-H, stretching bond), 1452, 1376 cm^{-1} shows (CH bends of CH_2 and CH_3 group) and lastly the peaks of 3064 cm^{-1},1608 cm^{-1} and 1496.5 cm^{-1} are indicated the aromatic CH bond vibration.

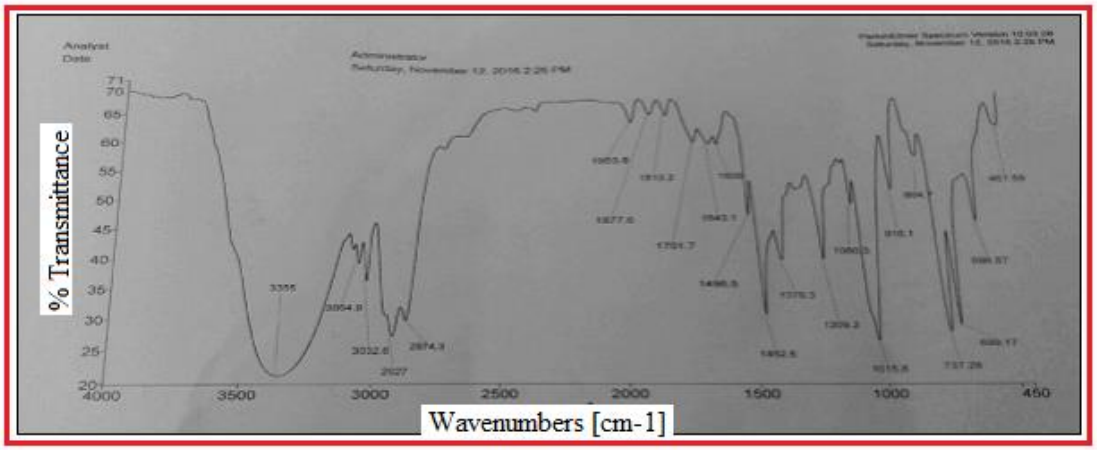

Fig.7. FTIR spectra of *M. fragrans* essential oil

The mortality data thus obtained in both cases of toxicity were subjected to profit analysis and used for drawing regression lines to determine LC90 values by using SPSS software, which is given below in Table.3.

Insecticide mode of action	Name of Botanical	(x^2)	R^2	LC 90 (%)	Fiducial Limits		Regression Equation(y)
					Upper	Lower	
Stomach toxicity	*Nutmeg oil*	10.25	0.778	16.62	23.05	13.45	2.648x-8.174
Contact toxicity	*Nutmeg oil*	8.16	0.903	19.11	24.54	16.06	2.310x-2.464

Table. 3. Values of LC 90 given for stomach and contact action with nutmeg oil for killing 90% larvae of *Anthrenus verbasci after 24 hours of exposure*

The regression line graphs and the most effective concentration in both cases are shown by following Fig.8. In contact case LC 90 value is 19.11% whereas in stomach case LC 90 value is obtained at 16.625%. Therefore, stomach toxicity is more effective than toxicity by contact, they are sufficient to kill 90% of larvae at less concentration comparatively to contact case.

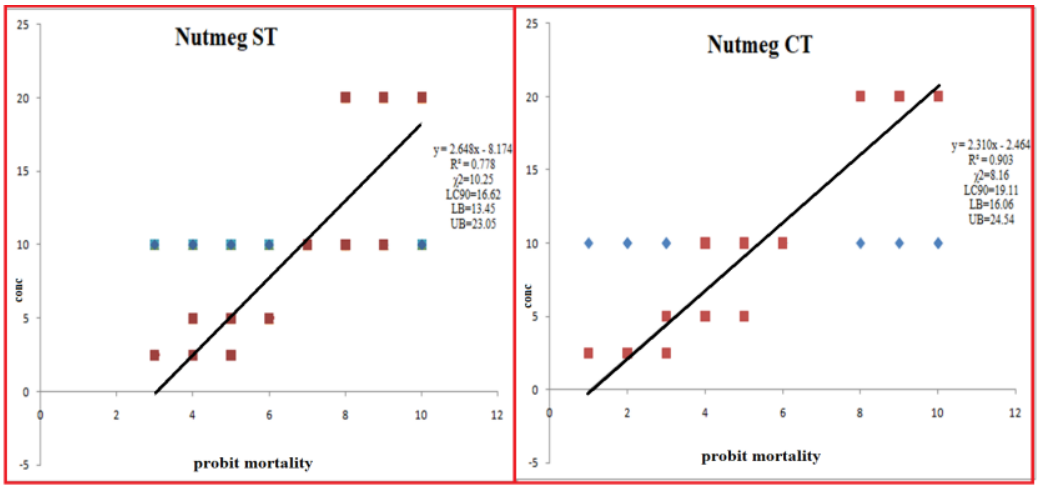

Fig.8. Regression line graph of *Myristica fragrans* essential oil toxicity action in stomach and contact case shown between log conc. and probit mortality

Conclusion

With the safety of domestic application in mind, different botanicals can be used against these biodeteriogens. Application of *M. fragrans* essential oil as stomach and contact insecticides to control infestation against the larvae of *A.verbasci* was found effective for conservation of animal collections in museums. But in case of stomach, efficacy of *M. fragrans* essential oil lead to higher mortality rate than in contact toxicity. By the above analysis and experiment it is very clear that the mortality rate of insects are directly proportional to the concentration of used *M.fragrans* essential oil in both cases of toxicity. New techniques of control have applied after thorough knowledge of bionomics of the dermestid beetles, which is main agent of deterioration in skin and leather products in museums. Therefore, it is important to study the complete life history of these beetles to suggest the suitable remedies for control. The effective constituents of the botanicals should be also study in detail. The botanicals active constituents would be separated by infra-red spectroscopy so that the high concentrated compounds can be easily extracted and which will be more effective instead of using entire products.

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