ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 6(F), pp. 27633-27638, June, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EVALUATING TOXICITY OF MYRISTICA FRAGRANS vs SYZYGIUM AROMATICUM OILS AGAINST THE LARVAE OF ANTHRENUS VERBASCI

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2298

ARTICLE INFO

Article History:

Received 15th March, 2018
Received in revised form 25th April, 2018
Accepted 23rd May, 2018
Published online 28th June, 2018

Key Words:

Biodeterioration; Contact poison; LC90; Natural Biocides; Stomach poison; Stored products.

ABSTRACT

Stored products pest is refer to organism that infests and damages stored products found organic in nature included both plants and animals origin. Stored product pests are responsible for the severe loss of millions dollars every year through contaminated products, as well as for damage and destruction of important documents, household and heritage artifacts in offices, homes, museums, libraries and archives. Museum collections are severely vulnerable to pest infestation because much of the collection is composed of edible plant material cellulose or animal protein. The organic materials such as skin, leather, bone and parchment objects, form a substantial part of natural history collections of museums across the world, and are promptly infested by stored product pests like dermestid beetles, silver fishes, tenebrionides, clothes moths, psocids, cockroaches and other micro-organisms. In order to overcome the problems of biodeterioration, natural biocides should be taken in use. In this paper a comparative study has been shown between the toxicities of Myristica fragrans and Syzygium aromaticum oils with variation as contact and stomach poisons against the larvae of Anthrenus verbasci. Given an application rate in contact case of nutmeg oil LC 90 value is obtained at 19.11 whereas in stomach case LC 90 value is obtained at 16.62. other hand when used with clove oil in contact case LC 90 value is obtained at 8.97, whereas in stomach case LC 90 value is obtained at 4.59 which indicates use of clove oil in both cases of toxicity were much effective.

INTRODUCTION

Stored animal products include both food items like dried fishes and prawns, cheese etc and as well as non-food materials like silk, fur, feather, leather, wool, museum preserved collections, hides, honeycomb etc. Stored animals pest causes infestation to these animal products during storage and sometimes at the processing stages[1].Stored animal products possessing severe pest infestation problems, in spite this field has not received much attention unlike the infestation problem in stored products of plants origin. Most of the research work has been done in museum field to control and monitor pests of museum animal collections. Control with synthetic chemicals is an effective strategy used extensively in daily life [2]. Whereas the widespread use of synthetic insecticides led to many negative impacts[3], resulting in increasing attention to natural products [4]. However, generally pest controls were done through synthetic pesticides; their adverse effects have overweighed the benefits associated with their use. Due to overuse of pesticides and other non- degradable chemicals as well as products lead to high health risks factor and also causing several environmental problems, so government has taken severe initiatives on policy of banning it[5].

To overcome the above problems there is urgent need to develop safe, convenient, environmental friendly and low-cost alternative. However it is important to analyze the efficacy of various natural products for prevention & control of pest infestations, as a mean to reduce negative impacts on human health and environments, causes the disturbance in ecosystem [6, 7]. So, it is a prime concern to emphasize the research in this direction, to prevent and control the important museum collections from such harmful biological deterioration following the existing traditional conservation methods either indigenous on non indigenous method. Plants play very important roles in ecological systems [8]. It may impart potential alternatives to currently used insect-control agents, because they represent rich source of bioactive chemicals[9]. Nowadays considerable efforts have been focused on plant derived materials for potentially useful products as bioinsecticides[10] and also the indigenous method of control is highly emphasized globally, through which the use of extracts from medicinal plants and natural products have been considered, that doesn't show any harmful effects on human society, environment and further more commercially viable[11-17].Museum collections encompass valuable natural history collections belonging to various fields such as botany, zoology, archeology, ethnography, anthropology and archives. These valuable collections of museums are prized possessions of intellectual and cultural property. Natural history collections plays essential role in disseminating knowledge regarding, evolution, biodiversity, genetics, population and the environmental impacts of climate change, uses of pesticides and so on. These valuable collections are important not only for education and cultural reasons, but for wider environmental reasons as well. Hence, it is very important to maintain our biodiversity and understand changes in our environment that we need to sustain our natural history collections These valuable collections are very susceptible to pest damage. The pest does not infest every collection of museum objects in an equal ways. Mostly natural history collections such as dried insects, dry plants material in herbaria, stuffed animals, furs, feathers, wools and skeletons specimens are at a very high risk of infestation and damage[18].The cumulative effects of this damage can completely destroy museums precious collections. Museum pests are biological agents that can cause damage to organic objects found in

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museums. Pests are organisms that interfere with the management objective of the site. Pests come in a variety of forms: insects, rodents, bats, birds and mold[19]. Among all pests, insect are the most important agent of biodeterioration, they are notorious and destructive in nature and causes *serious damage to highly valuable and irreplaceable materials*[20, 21]. India have much favorable climatic condition for the development of biological agent, which causes biodeterioration [22, 23]. These insects are mostly scavengers in nature, they inhabited the nests of mammals and birds and feed on organic materials like wools, furs, feathers, bones, ivory, parchment membranes, valuable leather objects, books, journals, papers and dried animal materials etc. These objects ultimately form the significant parts of collections in museums, libraries and archives. Due to lack of proper care, handling and storage the important materials are regularly infested by insects like termites, dermestid beetles, silver fishes, tenebrionides, cockroaches and many other micro-organisms.[24-27].

Stored products of both origin animal and plants are infested by more than 600 species of beetle pests, 70 species of moths and nearly 355 species of mites causing quantitative and qualitative losses[28]. Many literatures have indicated most of the insect pests which feed on preserved skin and skin products are beetles and the research work which is carried on are mostly on bionomics of biological agents and control action of biodeterioration. Biodeterioration in stored animal collections are mainly caused by the insects belonging to Dermestidae family, there important species are Anthrenus, Attagenus, Dermestes, Trogoderma, Necrobia and Psocids[1, 29-31]. The extents of loss due to insect pests in museum collections are partially known [32]. Dermestid beetles have voracious appetite; they do not feed only on clothing and textiles but also occasionally feed on any sort of food eaten by pets, humans, or livestock. Many cases of carpet beetle infestation began with beetles brought into the home through bags of pets food [33]. Pests feeds on animal products can easily digest and utilize keratin, a chemically stable structural protein present in feather, wool, hair and horn [34]. Insects which feed on animal products rather than food grains are known to have higher proteinase and aminopeptidase enzyme activities [35]. Some of the insect pests of preserved animal products undergoes to diapauses during these period, they are more tolerant for extreme climatic conditions and pesticide treatment [36].

Syzygium aromaticum is commonly known as clove and it is belong to the Mirtaceae family. Whereas Myristica fragrans is commonly known as nutmeg which belongs to Myristiceae family.These both are cultivated in several countries throughout the world. Clove and nutmeg are important medicinal plants and both shows wide range of pharmacological effects consolidated from traditional uses for centuries and are reported in several literatures[37, 38]. They are some of the most valuable spices that have been used for centuries as food preservatives and for many medicinal purposes. Clove possesses antidiabetic, anti-inflammatory, antithrombotic, anesthetic, pain-relieving, and insect-repellent properties[39, 40]. Cloves possess comparatively higher antimicrobial and antioxidant activities than many fruits, vegetables and other spices and should deserve special attention [41]. Whereas Nutmeg have been reported to possess several advantages, such as anti-diarrheal activity, stimulant, anti-fungal, anti-diabetic, carminative and anti-inflammatory properties [37, 38].

In this study prevention and control is achieved with the toxicity of Myristica fragrans and Syzygium aromaticum oil individually against larval stage of A. verbasci. Essential oils are most effective substances tested against insects [42]. These compounds may act as fumigants[43], contact insecticides [44], repellents [45]and antifeedants [46]and also may affect some biological parameters, that can be and reproduction and life span [47]and growth rate [48]. In the following experiment a comparative observation is shown between the toxic effects of M. fragrans and S. aromaticum oils against the larvae of A.verbasci by the evaluation of LC 90 value of both oils through two different stomach and contact mechanisms, which shows much variation in the activities of larvae in both cases.

Bionomics

Larvae of varied carpet beetles are about the same length as adults when mature. They are generally showing fluffy appearance, body is covered with dense tufts of hair and having alternating light and dark brown transverse stripes. It boasts a complete or holometabolous development, which includes an egg, larva, pupa, and finally adult stages. Varied carpet beetles nearly hatched 35 to 40 eggs in its life cycle, it takes 10-20 days for hatching process and then it goes to their larval stages. These larval stages exceed nearly 220-630 days, after it turns to their pupation in next 10-13 days. There is a variation shows in development of adult female and male which takes nearly 2-6 and 2-4 weeks respectively. Larvae feed on a variety of dead animals and stored animal products, such as wool, silk, leather, fur, hair brushes with natural bristles and feathers; occasionally they feed on stored products such as certain spices and grains. They do not feed on synthetic fibers. They will also feed on linen, cotton, and rayon if these fabrics are soiled with juice, food, or animal excreta. They can be pests in cereals, stored grains, nuts and similar products. Carpet beetle larvae are frequently pests of insect collections and other museum specimens. The adult beetles do not feed on fabrics but seek out pollen and nectar. Different views of A. verbasci are clearly shown by Fig.1

Fig 1 Different views of adult *A. Verbasci*

Experimental approach
Collection of test oil

Myristica fragrans (nutmeg) oil and Syzygium aromaticum (clove) oil were purchased from herbal pharmacy in Aligarh-India. It was kept in proper air tighter glass container and placed in a cooled place for applying in the experiments.

Collection of test organism

The initial source of beetles culture was a infested “bull horn”, which we have collected from the natural history collections of MUSA Dakri museum of Aligarh Muslim University India, which is shown by Fig.2. These beetles were identified on the basis of their morphological characters in entomology section of zoology department, Aligarh Muslim University. 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.

Preparation of test concentration

we have done two different experiments in both cases with nutmeg as well as clove oil , firstly while doing with nutmeg oil different formulations has been tested which are given as 2.5%, 5%, 10.0% and 20.0% concentrated solutions 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. Another experimental setup with nutmeg oil is done for determining contact toxicity. We have taken different formulations of nutmeg oil which is given as 5.0%, 10.0%, 20.0% and 40.0% concentrated solutions. In this blotting papers of same size were impregnated with the above different concentrations of nutmeg oil A, B, C and D, and each box containing 10 specimens respectively. The representation of observing set up is shown by following Fig.3.1.

Fig 2 Bull horn infested by *A. verbasci* beetles

Whereas another experiment is done with S. Aromaticum oil in which we have adopted the same procedures as in the above experiment which is done with nutmeg oil in both cases of stomach and contact mechanism but with varied formulations.In stomach mechanism the formulation of S. aromaticum oil has been tested which are given as 0.625 %, 1.25 %, 2.50% and 5.0% concentrated solution. Another experimental setup is done for determining contact toxicity and procedure is same as above we have applied in nutmeg case. Its different formulations were tested and which are given as 1.25 %, 2.5 %, 5.0% and 10.0% concentrated solutions. The representation of observing set up is shown by following Fig.3.2.

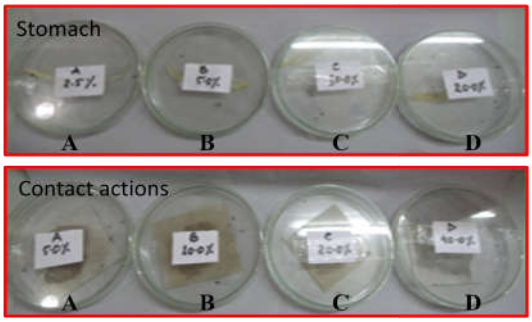

Fig 3.1 Experimental setup for mortality of *A. verbasci* larvae through toxicity of nutmeg oil by stomach and contact actions

RESULT AND DISCUSSIONS

The present study indicates the development of Anthrenus verbasci larvae is highly affected by the use of nutmeg oil and as well as clove oil individually. The effects of the nutmeg oil and clove oil for the mortality of the larvae were much higher in stomach case than contact case which are indicated by Table 1 & 2 respectively. In case of stomach mechanism 10.0-20.0% concentrated solution of used with nutmeg oil was found to record 90-100% mortality of the larvae within 24 hours of treatment in comparison to clove oil where 90-100% mortality of larvae was recorded at 2.5-5.0 % of concentrated solution treated for 24 hours. In case of contact mechanism 90-100% mortality of larvae was obtained at 20.0-40.0% concentrated solution of nutmeg oil kept for 24 hours. Whereas 90-100% mortality of larvae was achieved at 5.0-10.0% of concentrated solution of clove oil within 24 hours of treatment. 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, 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 case of nutmeg oil. The dead and alive ratio in case of clove oil with different formulation of concentration through the stomach mechanism is given A, B, C and D by 1:9, 4:6, 7:3 and 10:0, whereas dead and alive ratio for the contact mechanism on different formulation of concentration A, B, C and D by 1:9, 3:7, 7:3 and 10:0 respectively, which indicated that the given concentrations of clove oil in both means of toxicity was strongly affected the development of *A. verbasci* larvae. The result of experiments reveals that the insecticidal efficacies of clove oil and nutmeg oil individually at different concentrations were assessed by the mortality counts of the larvae during the given 24 hours of period. The mortality ratio of the larvae in both stomach as well as contact cases is comparatively higher in clove oil than nutmeg oil which shows by bar graph through Fig.4 and 5. Each experiment in this study was repeated nearly 3-4 times with each concentration of the intoxicant exposed for same duration of time. While counting the numbers of mortalities, the morbid larvae were also regarded as dead. The mortality percentage of the insects was calculated by the following method.

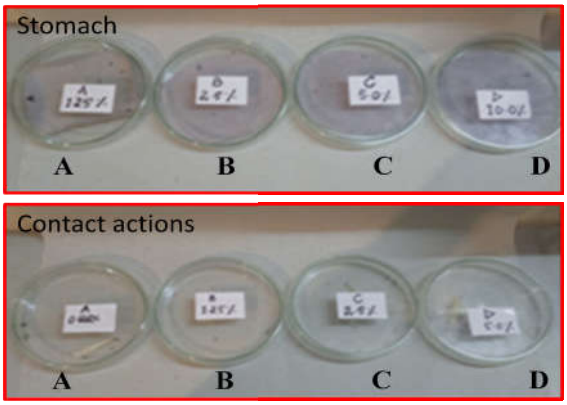

Fig 3.2 Experimental setup for mortality of *A. verbasci* larvae through toxicity of clove oil by stomach and contact actions

Mortality (X%) = N_t/N_o ×100

Where, Nt = Number of dead larvae,No =Total number of larvae

Table 1 Stomach toxicity through nutmeg oil and clove oil

Nutmeg Oil				
%	**A (2.5%)**	**B (5%)**	**C (10%)**	**D (20%)**
Dead	6	6	8	10
Live	4	4	2	0
Clove Oil				
%	**A (0.625%)**	**B (1.25%)**	**C (2.5%)**	**D (5%)**
Dead	1	4	7	10
Live	9	6	3	0

Table 2 Contact toxicity through nutmeg oil and clove oil

Nutmeg Oil				
%	**A (5%)**	**B (10%)**	**C (20%)**	**D (40%)**
Dead	2	4	6	10
Live	8	6	4	0
Clove Oil				
%	**A (1.25%)**	**B (2.5%)**	**C (5%)**	**D (10%)**
Dead	1	3	7	10
Live	9	7	3	0

The main constituent of nutmeg includes sabiene, alpha-pinene, beta-pinene, myrcene, limonene, terpenes, safrole, and myristicin compounds [49]. This bioactive agent could cause a depolarizing neuromuscular blocking action which may lead to the death of larvae[50]. The use of nutmeg oil has resulted to death of the A.verbasci larvae due the feeding as well as impairing respiration in larvae, which indicated the hallucinogenic characteristic of myristicin present in nutmeg oil [51]. FTIR analysis of nutmeg oil which is indicated by Fig.6, reveal 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. Clove consist of eugenol (50-87%), eugenyl acetate, tanene, thymol, and b-cariophyllene [52].Eugenol is the main bioactive compound found in clove. In one of important reports it is indicated that clove oil contained Eugenol (~75%), b-caryophyllene (~5%), eugenyl acetate (~16%), and other components (<1%) [53]. Clove represents one of the major vegetal sources of phenolic compounds as hidroxibenzoic acids, hidroxicinamic acids, flavonoids, and hidroxiphenylpropens [54].In clove there is higher antioxidant activity and polyphenol contents, (168.660±0.024) tetraethylammonium chloride (mmol of Trolox/100g dried weight) and (14.380±0.006) g of gallic acid (equivalents/100g of dried weight) respectively. The major types of phenolic compounds found were phenolic acids, flavonol glucosides,

eugenol, acetyl eugenol and tannins[55].Clove also serves as an anesthesia for a variety of fishes. However its lengthy exposures can cause mortality and sub-acute morbidity[56].FTIR analysis of clove oil which is indicated by Fig.7. The peak nearly 3400-2800 cm^{-1} represents OH group vibrations, the peak around 1600-1500 cm^{-1} shows C-H finger printing and C-C stretching vibrations respectively. While peaks nearly 1400-1200 cm^{-1} shows C=H bending vibrations of the present organic compounds

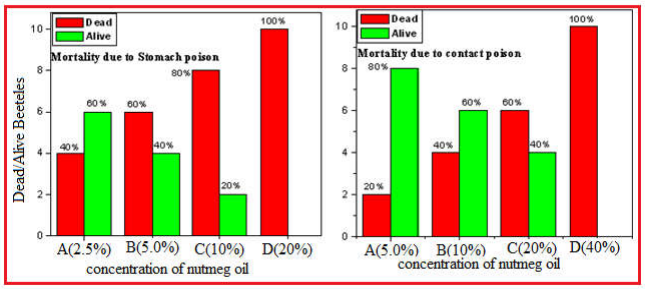

Fig 4 Percent of mortality in stomach and contact case through nutmeg oil against *A. Verbasci* (larvae) in 24 hours after treatment

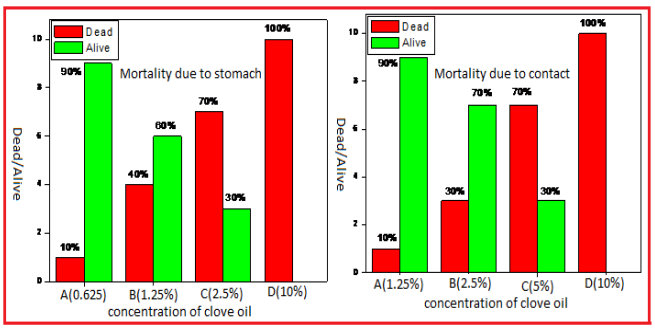

Fig 5 Percent of mortality in stomach and contact case through clove oil against *A. Verbasci* (larvae) in 24 hours after treatment

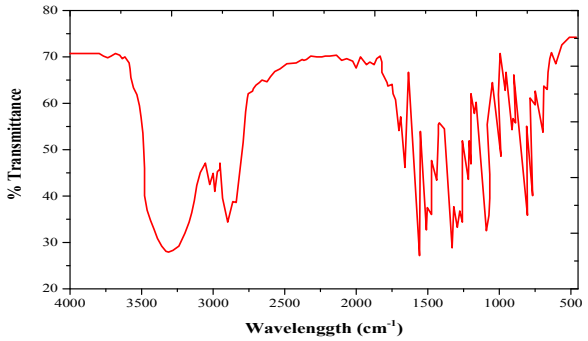

Fig 6 FTIR spectra of nutmeg oil

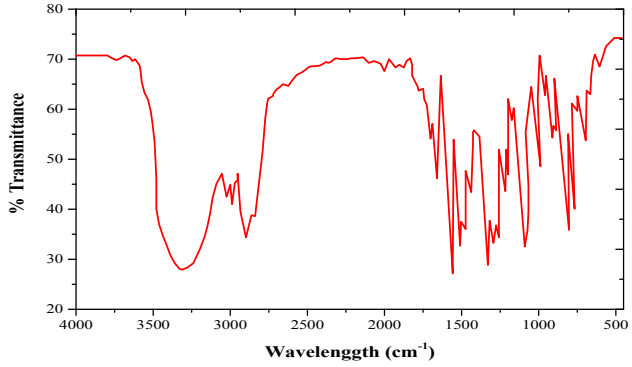

Fig 7 FTIR spectra of clove oil

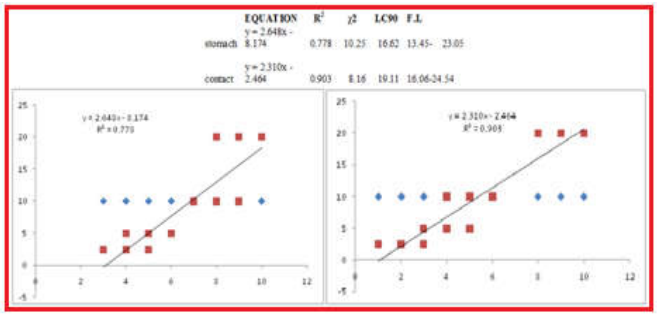

Fig 8 Regression line graph between log conc. and probit mortality in stomach and contact case of nutmeg oil

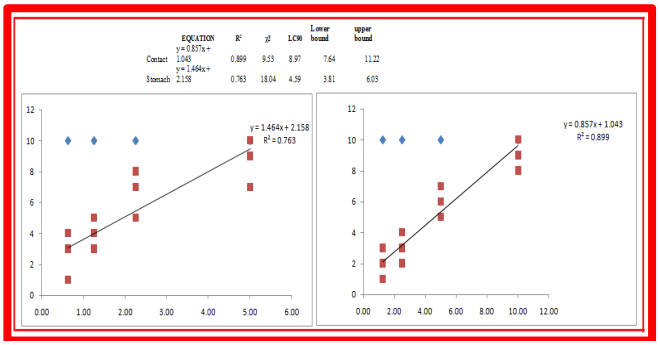

Fig 9 Regression line graph between log conc. and probit mortality in stomach and contact case of clove oil

Further the mortality data obtained in both cases were subjected to probit analysis [57] and used for drawing regression lines to determine LC90 values by using SPSS software(R). The regression line graphs and the most effective concentrations in both the cases are indicated by following Fig.8 and 9. A comparative study of the performance of the two different chemicals tested against the larvae of A. verbasci by means of two different toxicity mechanism indicates that in general, clove oil was the most effective at given all four different concentrations comparatively to nutmeg oil in both cases of toxicity mechanisms. Given an application rate in contact case of nutmeg oil LC 90 value is obtained at 19.11 whereas in stomach case LC 90 value is obtained at 16.62. On other hand when used with clove oil in contact case LC 90 value is obtained at 8.97, whereas in stomach case LC 90 value is obtained at 4.59, which indicates use of clove oil in both cases of toxicity mechanism were much effective than nutmeg oil. Hence clove oil at above the given concentrations in both stomach and contact case were sufficient to achieve 90% of mortality for over all larval stage of Anthrenus verbasci as compared to nutmeg oil.

CONCLUSION

As discussed earlier, there are several factors which may affect insecticidal performance and therefore it is more important to consider appropriate allowances such as increased quantities of the chemical agents or prolonged exposure of chemicals. Treatment of specimens or materials should be strictly followed by a suitable quarantine and inspection period before bringing it into the museum environment. Acute infestation problems have been experienced to items which are not properly stored in museums such as silk, leather and woolen materials. Insect damage to museums objects has been noted as a serious problem in several countries. In an ideal situation the use of synthetic chemicals in the museum environment would be eliminated completely. The synthetic chemicals which were mostly used are now justifiably discouraged by many museums experts on the basis of health and safety purposes, and also for being hazardous in nature. New techniques of control should have applied after thorough knowledge of bionomics of the insects, which are the main agent of deterioration in animal stored products. Therefore, it is important to study the complete life history of these insects to suggest the suitable remedies for control. Nowadays, there are well reported alternatives which focus on a healthy and non-toxic approach which is massively beneficial to both the valuable collections and the general public.With the safety of domestic application in mind, different herbal biocides can be used against these biological deteriorating agents.Natural plant products have been

traditionally used in insect control for stored animal products. There is a need to identify and separate the active molecules from promising plant sources by red spectroscopy so that high concentrated compounds can be easily extracted andfurther evaluated for the application at commercial level.

Acknowledgement

The authors are gratefully acknowledged Prof. Abdurrahim K, Department of Museology AMU-Aligarh for providing necessary research facilities like samples, reagents, analysis chamber instruments, glass wares etc. We are also thankful to UGC for providing financial assistance to complete my research work.

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How to cite this article:

Fatma Faheem and Abduraheem K.2018, Evaluating Toxicity of Myristica Fragrans vs syzygium aromaticum Oils against the larvae of anthrenus Verbasci. *Int J Recent Sci Res.* 9(6), pp. 27633-27638. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0906.2298

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