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# Chemical composition of Lavandula angustifolia Miller and Rosmarinus officinalis L. essential oils and fumigant toxicity against larvae of Ephestia kuehniella Zeller

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**Abstract:** The purpose of this study was to determine the chemical composition and fumigant toxicity of essential oils isolated from lavender (*Lavandula angustifolia* Miller) and Rosemary(*Rosmarinus officinalis* L.), harvested in 2014 in (Qudssaya Suburb)Damascus Governorate, Syria. The essential oils, isolated by steam distillation from sample plants at full flowering stage, were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The essential oil of *L. angustifolia* Miller analyzed contained as main components Linalool (35.12%), Borneol (17.69%), Champhor (14.31%)and Eucalyptol (1,8-Cineol) (7.60%),, while the essential oil of *R. officinalis* contains Borneol (16.25%) , Eucalyptol (1,8-Cineol) (12.69% ), Alpha-Pinene (8.5%), Champhor (7.13%) and Linalool (5.17%),. The fumigant toxicity effects of essential oils was evaluated on larvae (7 day old) of *Ephestia kuehniella*(Zeller)under laboratory conditions .Results demonstrated the fumigant toxicity of the two essential oils against larvae of *E. kuehniella*. The fumigant toxicity potential of *L. angustifolia* oil ($LC_{50}$ = 19 µl/$L^{-1}$ air) was greater than *R. officinalis* oil ($LC_{50}$ = 28µl/$L^{-1}$ air). The results suggest that essential oils from *L. angustifolia* and *R. officinalis* have a fumigant toxicity effects against *E. kuehniella* larvae under storage conditions.
**Keywords:** Lavandula angustifolia ; Rosmarinus officinalis, Essential oil; GC-MS analysis; fumigation, Ephestia kuehniella.

## Introduction

The use of synthetic insecticides in agricultural pest control practices to increase yields and protect stored products may involve serious health hazards for organisms in the class Mammalia. These insecticides are often associated with residuals that are dangerous for the consumer and the environment. In addition, the risk of developing insect resistance and the high cost–benefit ratio of synthetic pesticides pushes research towards investigating alternative insecticides[1].The concept of "Green Pesticides" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. They are safe and ecofriendly. They are more compatible with the environmental components than synthetic pesticides[2].Some plant extracts or phytochemicals are found to be highly effective against insecticide–resistant insect pests[3]. Many plant essential oils show a broad spectrum of activity against pest insects andplant pathogenic fungi ranging from insecticidal, antifeedant, repellent, ovi position deterrent, growth regulatory and antivector activities. These oils also have a long tradition of use in the protection of stored products[4]. Plant essential oils in general have been recognized as an important alternative source of pesticides. They represent a market estimated at US$ 7700,00 millions and total world production of 45,000 tons[5].The essential oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects[6]. Their action against stored product insects has been extensively studied[7]. Moreover, these natural derivatives are considered to be an alterna-tive means of controlling harmful larvae of lepidoptera. Recent research has demonstrated their larvicidal and antifeeding effects[8].

The *Lavenders* are a genus of about 25 - 30 species of flowering plants in the mint family, Lamiaceae, native to the Mediterranean region south to tropical Africa and to the many regions of Asia. The genus includes annuals, herbaceous plants, subshrubs, and small shrubs[9].*Lavandula angustifolia* Miller or true lavender is a perennial shrub of the family *Lamiaceae*[10]. *L. angustifolia* is an evergreen bushy shrub with straight, woody branches, the lower of which are leafless, putting out numerous herbaceous stems to a height of about 1 meter[11]. The main growing countries are Bulgaria and France and on smaller areas in Morocco, the former republics of Yugoslavia, Hungary, Italia, Russia, Spain, Romania, Ukraine, Turkey, and others[12]. This plant is cultivated primarily for its aromatic inflorescence from which the essential oil is isolated, although its fresh and dried flowers are also marketed[13]. Lavender oil is known for its excellent aroma and is extensively used in the perfumery, flavor and cosmetic industries. The oil is known to possess sedative, carminative, anti-depressive and anti-inflammatory properties[14].

Rosemary, *Rosmarinus officinalis* Linnaeus (Lamiaceae) is native of the Mediterranean countries, and now cultivated also in North America. Rosemary is one of the classic culinary herbs. Rosemary essential oils increase the circulation of blood and improve memory, concentration and mental alertness. Moreover rosemary oils have digestive, antiseptic, antispasmodic and anti-inflammatory properties[15,16,17].Earlier work on the chemistry of this species showed that it contains mainly abietane-type diterpenoids including some diterpenoid quinines[18,19], however some triterpenoids[20] were also isolated. Rosemary (*Rosmarinus officinalis* L.) with more than 240 active pharmacological and nutritional compounds is important from the botanical point of view[21].

The Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Lepidoptera, pyralidae) is a worldwide pest, particularly of stored grains, nuts, dried fruits, legumes, dates, cocoa beans and other stored food[22,23]. Larvae reduce product quality by their presence and they also cause direct damage by feeding[24]. The other author reported that apart from direct infestation of *E. kuehniella*, the faeces and webbings of larvae spoil the product[25]. Moreover, mandibular glands of the larvae secrete semiochemicals which are deposited on their food and silk[26].Methyl bromide and phosphine fumigants have been used for decades to control stored pests[27] and belong to the most effective treatments to protect stored food, feedstuffs, and other agricultural commodities. Growers are moving away from using methyl bromide as post-harvest fumigant because of its ozone-depleting nature[28] and phosphine, due to repeated use as it disrupts biological system leading to the development of pest resistance[29]**.** The toxicity of sulfuryl fluoride (Vikane®) to age groups of eggs of *E. kuehniella*[30]. Botanical insecticide composed of essential oils may be a sound alternative to the more persistent synthetic pesticides for managing the major pests of stored product insects[31]. The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used[32,33]. Essential oils produced in various external and internal glands of aromatic plants are a very complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds[34]. Many studies of the fumigant activity of such natural substances have been undertaken to establish new control practices with lower mammalian toxicity and low persistence in the environment[35,36].

### The objective of this study

The objective of this study was evaluate the comparing essential oil composition and essential oil yield of lavender (*Lavandula angustifolia* Miller) and Rosemary, (*Rosmarinus officinalis* L.)at full flowering stages, and evaluated the fumigant toxicity potential of the two essential oils against larvae of *Ephestia kuehniella* Zeller (Lepidoptera, pyralidae) at laboratory conditions.

## Materials and Methods

### Raw Materials

Aerial part and leaves of lavender (*L. angustifolia* Miller) and Rosemary, (*R.officinalis* L.) were harvested manually, at the maximum flowering stage during May and March, respectively , 2014, when the essential oils content and quality are considered the best[12,37].Growing in gardens of Qudssaya Suburb,Damascus

Governorate, Syria. The plants were identified and deposited in the Department of Renewable Natural Resources and Ecology- Faculty of Agricultural, Damascus University, Syria. The plant samples were air-dried at room temperature (20-25°C) and shad, for one week.

**Essential oil isolation:**

The dried samples of *L. angustifolia* and *R.officinalis* were ground in a coffee mixer, and subjected to hydrodistillation in Clevenger,s apparatus, (100 g of each sample in 500 ml of distilled water), for 3 hour for the extraction of the essential oil and to three replications. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and calculated average of essential oil yield, for three replication. The essential oils were stored at 4°C until bioassays tests and analysis by GC-MS.

**Gas chromatography–mass spectrometry analysis**

Analysis of oils were carried out by GC-MS chromatography (GC-agilent 7986, indictor: inert-MS) in Atomic Energy Commission(AECS)- Damascus, Syria. This instrument was fitted with HP-5MS capillary column (30cm×0.25mm i.d., film thickness 0.25µm). The temperature injector and indictor 250 °C .The oven temperature program was 60-270°C (2.5°C per min.). The identity of components was ascertained based on the spectra and compared with library and literature data. Also, the identification of each compound was confirmed by comparison of its retention index with those of authenticcompounds[38].

**Insect rearing**.

The Mediterranean flour moth was reared on wheat fine semolina (*Triticum aestivum*), in plastic boxes in 16 h. light/ day at 23 ± 1ºC and 70 ± 5% relative humidity. A stock culture was kept in the Laboratory of Biological Control Studies and Research Center, Faculty of Agricultural, Damascus University, Syria. For bioassays, seven day old larvae at the same weight and vigor were collected from the rearing colony and used.

**Fumigation bioassay:**

In order to test the toxicity of vapor of essential oils on the larvae of *E. kuehniella,* ten larvae (7 days old) were put into the 1000 ml glass jars. Essential oils were applied on a filter paper strip (Whatman No.1) measuring 2.5 x 2.5 cm which was attached to the lower side of the jar's lid. The larvae of *E. kuehniella* were exposed to vapor of essential oils at1, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µl/$L^{-1}$ air for 24 h. Control larvae were maintained under the same conditions without any essential oil, all treatments at three replicates. The number of dead and survivor larvae was counted after exposure and percentage mortality determined for each essential oils.

**Statistical procedures**:

Percentage larvae mortality was calculated using Abbot ́s formula[39] :

Corrected mortality (%) = [Mortality of treated larvae - Mortality of control larvae) / (100 - Mortality of control larvae)] × 100

The $LC_{50}$ values (as µl/$L^{-1}$ air) of the essential oils against larvae of *E. kuehniella* were calculated by Probit analysis[40].Means were separated at the 5% significance level by least significant difference (LSD) test, using SPSS.20 software.

## Results and Discussion

**Chemical composition of the essential oils in *Lavandula angustifolia* and *Rosemarinus officinalis* at full flowering stages.**

This study was undertaken to investigate a comparative chemical composition of essential oils obtained from two medicinal plant species, *L. angustifolia* and *R.officinalis* (Lamiacea), at the maximum flowering stage. We report first investigations on their fumigant toxicity against larvae of *E. kuehniella*In Syria. The essential oils isolated by water distillation were obtained in yield 5% v/w base on dry weight of sample for *L. angustifolia* and 2.8 % v/w for *R. officinalis.* Our results opposite of many studies were reported on the yield of oil, the essential oil content in the inflorescence of lavender (*L. angustifolia*) cultivated in the mid hills of Uttarakhand was found to be 2.8 % based on the fresh weight[41]. Also, in Romina[42] the yield of the essential oil (% v/w) was 2.75% for *Lavandula intermedia* and 1.13% for *L. angustifolia*. While, the volatile oil of rosemary reaches to 1.43%[43]. In case *R. officinalis,* our results are a good agreement to those obtained by[44] he's founded the yield of the oils obtained from *R. officinalis*was 2.6% w/w for Lalehzar and 2.1% w/w for Kerman (Iran).

The results obtained by GC-MS analysis of the essential oils in *L. angustifolia* and *R. officinalis*are presented in Table 1. Twenty eight and twenty five compounds were identified in the essential oils of *L. angustifolia* and *R. officinalis*, representing 98.28% and 91.63% of the total, respectively. The major constituents (> 1.0 %) of the *L. angustifolia* and *R. officinalis* oils in full flowering stage were Alpha-Pinene (4.06% & 8.5%), Camphene (0.51% & 3.41%), Bête-Pinene (1.26% & 4.15%), 3-Carene (0.38% & 3.02%), Limonene (1.52% & 5.25%), Eucalyptol (1,8-Cineol) (7.60% &12.69% ),Linalool (35.12% & 5.17%), Champhor (14.31% & 7.13%), Borneol (17.69% & 16.25%), Terpinen-4-ol (5.60% & 1.45%) and Caryophllene (1.33% & 3.45%), respectively. On the other hand, Our result detected that 4, Hexen-1-ol (1.34%), and Malonic acid (1.84%) are exited in *L. angustifolia* oil only. While, 3-Tyujen-2-ol (1.37%),and acetic acid (2.81%) are exited in *R. officinalis* oil only. Essential oils of *L. angustifolia* and *R. officinalis* have a similar chemical composition, the major components reported in the literature being *α*-terpineol, camphene, linalool, broneol and 1, 8-cineole . The essential oil of *L. angustifolia* was analysed by capillary GC and GC–MS, thirty seven constituents, representing 97.81 % of the oil were identified. The major components of the oil were linalyl acetate (47.56 %), linalool (28.06 %), lavandulyl acetate (4.34 %) and *α*-terpineol (3.75 %)[41]. Lavender oil, obtained from the flowers of *Lavandula angustifolia* composed mainly of linalyl acetate, linalool, lavandulol, 1, 8- cineole, lavandulyl acetate and camphor[10]. Twenty two components were identified in the essential oils obtained from *L. angustifolia* Miller, representing 99.9% of the total, the major components being caryophyllene 24.12%, beta-phellandrene 16% and eucalyptol (1,8-cineol) 15.69%[42]. A total of 47 compounds representing 98.4% - 99.7% of the oils were identified. 1,5-Dimethyl-1-vinyl-4-hexenylbutyrate was the main constituent of lavender essential oil (43.73%), followed by 1,3,7-Octatriene, 3,7-dimethyl- (25.10%), Eucalyptol (7.32%), and Camphor (3.79%).[45]. The essential oil of rosemary has been widely studied; Rosemary (*Rosmarinus officinalis* L.) The results showed that yield of Rosemary oil from Kerman (Iran) province was 3.2%. Forty-one compounds were identified in the essential oil concluded as 99.74% of the total oil. The major components were α-pinene (15.52%), camphor (11.66%), verbenone (11.10%) and 1, 8-cineole (10.63%)[21]. The other investigator demonstrated that chemical composition of essential oils obtained from *Rosemarinus officinalis* and *Lavandula angustifolia* were determined in two harvesting times. The results showed that harvesting time had significant effects on the oil content and compositions in both plants. The maximum essential oil percentage was obtained in full flowering stage in rosemary. Also, and in lavender maximum linalool percentage (19.2%) was obtained in full flowering, and minimum linalool percentage (0.2%) was shown in the other time. Also the concentration of β –pinene (2.1%), δ-3-carene (1.5%), β – phellandrene (6.6%), Camphor(10.6%), Cryptone (0.8%), α-terpineol (2.3%) and Linalool acetate (1.2%) were higher than before flowering stage[46]. Our result observed differences in the yield of *L. angustifolia* and *R. officinalis* oils may be due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants[45]. Also, these changes in the essential oil composition might arise from several environmental (climatic, seasonal, geographical) and genetic differences[47].

**Table 1. Main components of essential oils obtained from lavender (*L. angustifolia* Miller) and Rosemary, (*R. officinalis* L.) by GC/MAS chromatography.**

| Percentage % | | Retention time (min.) | | Component | No |
|---|---|---|---|---|---|
| *R. officinalis* | *L. angustifolia* | *R. officinalis* | *L. angustifolia* | | |
| 8.50 | 4.06 | 4.40 | 4.20 | Alpha-Pinene | 1 |
| 3.41 | 0.51 | 4.90 | 4.80 | Camphene | 2 |
| 4.15 | 1.26 | 5.76 | 5.86 | Bête-Pinene | 3 |
| 1.37 | - | 5.20 | - | 3-Tyujen-2-ol | 4 |
| 1.10 | - | 5.57 | - | Beta Phellandrene | 5 |
| 0.60 | 0.49 | 6.34 | 6.35 | 1-Octen-3-ol | 6 |
| 3.02 | 0.38 | 6.70 | 6.62 | 3-Carene | 7 |

| | | | | | |
|---|---|---|---|---|---|
| - | 0.41 | - | 6.88 | Butanoic acid | 8 |
| 5.25 | 1.52 | 7.82 | 7.64 | Limonene | 9 |
| 12.69 | 7.60 | 8.72 | 8.46 | Eucalyptol (1,8-Cineol) | 10 |
| 0.24 | 0.93 | 10.49 | 10.48 | Terpineol | 12 |
| 5.17 | 35.12 | 12.42 | 13.03 | Linalool | 13 |
| - | 0.33 | - | 14.41 | Prpanoic acid | 14 |
| 7.13 | 14.31 | | | Champhor | 15 |
| - | 1.34 | - | 17.45 | 4-Hexen-1-ol | 16 |
| - | 0.33 | | | Propanoic acid | 17 |
| 16.25 | 17.69 | 18.64 | 18.53 | Borneol | 18 |
| 1.45 | 5.60 | 18.81 | 18.91 | Terpinen-4-ol | 19 |
| 1.13 | - | 19.22 | - | Pinocamphone | 20 |
| 4.07 | - | 20.52 | - | Alpha-Terpineol | 21 |
| 0.33 | 0.27 | 21.88 | 21.94 | Thymol | |
| 0.06 | 0.14 | 23.32 | 23.44 | Carveol | |
| - | 0.21 | - | 24.52 | Cis-is-Verbenol | 22 |
| 7.94 | - | 25.02 | - | 2-Pinen-4-one | 23 |
| - | 1.84 | - | 25.99 | Malonic acid | 24 |
| 2.81 | - | 26.34 | - | Acetic acid | 25 |
| - | 0.48 | - | 26.90 | Propanal | 26 |
| 0.58 | - | 29.47 | - | Alpha-Cubebene | 27 |
| - | 0.69 | - | 32.80 | Tricyclo [2.2.1.0(2,6)] heptane | 28 |
| 3.45 | 1.33 | 34.25 | 34.08 | Caryophllene | 29 |
| - | 0.34 | - | 35.27 | Linalylisobutyrate | 30 |
| 0.47 | 0.18 | 37.46 | 37.47 | Alpha- Caryophllene | 31 |
| - | 0.15 | - | 41.42 | Alpha-Farnesene | 32 |
| 0.38 | 0.67 | 51.04 | 51.06 | Caryophyllene Oxide | 33 |
| 0.08 | 0.10 | 57.94 | 57.94 | Alpha- Bisabolol | 34 |
| **91.63** | **98.28** | **Total** | | | |

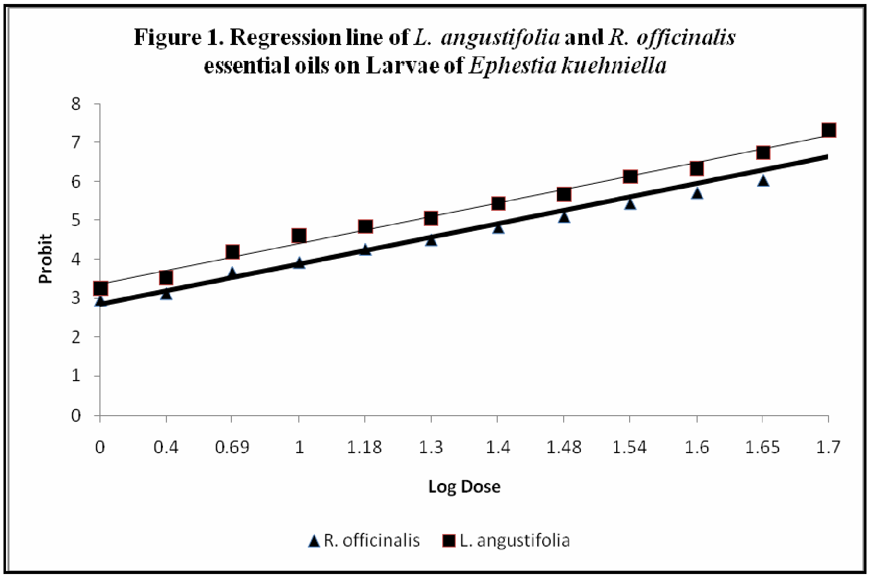
**Vapor toxicity of essential oils.**

The vapor toxicity of essential oils, obtained from two medicinal plant species, *L. angustifolia* and *R.officinalis* (Lamiacea), against larvae of *E. kuehniella* after 24 h exposure are presented in Table 2 and Figure 1. The oil obtained from two plants caused 100 % mortality on *E. kuehniella* larvae when exposed to 50 µl/L$^{-1}$air. However, the essential oil of *L. angustifolia* was significantly (L. S.D.$_{5\%}$ =4.23 ) more toxic than *R.officinalis* oil against larvae of *E. kuehniella* ,at all the lower doses. May be due to that the linalool and champhor are founded in *L. angustifolia* oil higher than *R.officinalis* oil .These result agreement with many studies, one of the important compounds of lavender oil is Linalool [46]. Additionally, insect toxic effects have been reported for linalool[48].Furthermore, linalool also acted as a deterrent to insects (mosqitoes and thrips)[49]. Moreover, our results showed that*L. Angustifolia* oil caused 87%, 91% and 96 % mortality on larvae of *E. kuehniella*at 35,40 and 45 µ l/L$^{-1}$air, respectively. On the contrary, *R.officinalis* oil caused 67%, 76% and 85% mortality on larvae at the same doses. In all cases, increasing mortality was observed on larvae of *E. kuehniella* with increasing dosage of two essential oils. The vapor activity of *L. angustifolia* and *R.officinalis* oils may be due chemical composition of the oil monoterpenoids such as limonene, linalool, terpineol, camphor and 1,8 - Cineole. Previous studies have shown the toxicity of essential oils from various aromatic plants against the *E. kuehniella.* Our results agreement with other studies, The insecticidal constituents of many plant extracts and essential oils against stored product insects are mainly monoterpenoids such as limonene, linalool, terpineol, carvacrol, and myrcene[3];. Also, the other investigators demonstrated that 1,8- cineole, camphor and linalool had effective insecticidal activity[50], Also,camphor, 1,8- cineole and carvacrol as compounds showing fumigant toxicity[51].The toxicity of essential oils to insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used[32,33]. Essential oils produced in various external and internal glands of aromatic plants are a very complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds[34].Theessential oil of *Elettaria cardamomum* L. toxic to the *Callosobruchus maculatus*, *Tribolium castaneum* Herbst (Coleoptera), andthe flour moth, *Ephestia kuehniella* Z eller (Lepidoptera). Adults of *E. kuehniella*weremore sensitive than the Coleoptera. Also, the highest mortality of these insects was seen after 12hours. The major constituents of cardamom were identified as 1,8-cineol, α-terpinyl acetate, terpinene and fenchylalcohol[52].

Probit analysis showed that $LC_{50}$ values for larvae of *E. kuehniella* were 19 µl/ $L^{-1}$air (*L. angustifolia* oil) and 28 µl/ $L^{-1}$air (*R.officinalis* oil) (Table 2).Similar results founded in Tunisia, the fumigant toxicity potential of Algerian *Laurus nobilis* oil ($LC_{50}$ = 20.77 µl/l air) was greater than Tunisian oil ($LC_{50}$ = 33.75, µl/l air)against adults of *E. kuehniella*. Also, the *Zataria multiflora* essential oil showed that the $LC_{50}$ values for *E. kuehniella* adults were 0.98 µl/l air and for larvae was 20.67 µl/l air[53].

**Table 2. The effect of different concentrations of volatile phases of essential oils of *L. angustifolia* and *R.officinalis* on Larvae of *Ephestia kuehniella.***

| Dose ($\mu l/L^{-1}$ air) | Mortality (%) | |
|---|---|---|
| | ***L. angustifolia*** | ***R. officinalis*** |
| 1 | 5 | 2 |
| 2.5 | 7 | 3 |
| 5 | 21 | 9 |
| 10 | 35 | 14 |
| 15 | 44 | 23 |
| 20 | 52 | 31 |
| 25 | 67 | 43 |
| 30 | 75 | 54 |
| 35 | 87 | 67 |
| 40 | 91 | 76 |
| 45 | 96 | 85 |
| 50 | 100 | 100 |
| **L.S.D 5%** | 4.23 | |
| **$LC_{50}$** | 19 | 28 |




## Conclusion:

The results showed that yield of *L. angustifolia* and *R. officinalis* essential oils at full flowering stage from Damascus (Syria) province were 5 % and 2.8 % respectively. Also, These results suggest that essential oils of *L. angustifolia* at full flowering stage a good choice for fumigation of *Ephestia kuehniella* larvae in stored products.

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