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Larvicidal Effect of *Zingiber officinale* and *Cyperusrotundus* Oils on The third Instar Larvae *Cephalopinatitillator* (L.)

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Cephalopina titillator (C. *titillator)* [Clark, Diptera: Oestridae] only attacks camels and damage tissues causing economic losses and reduction of fertility. The present work evaluated two common essential plant oils from *Cyperusrotundus* (C. *rotundus)* (Cyperaceae) and *Zingiber officinale* (Z .*officinale*) (Zingiberaceae), which are considered ecofriendly and have the ability to kill larvae. Scanning electron microscopic examinations were done to demonstrate alterations of the respiratory spiracles after exposure to the essential oils. Three different concentrations 30, 50, 100 µl/ml of oils in distilled water were prepared. Few drops of Tween 80 were added as an emulsifier to the used essential oils. Five 3rd instar larvae were used in each treatment then dipping procedure was applied five times. Each group of larvae was immersed for 60s in each dilution for each oil and the solution was continuously stirred. Dead larvae were counted after 24 hrs. Samples of treated and control larvae were fixed for Scanning Electron Microscope (SEM). The essential oils were very toxic to *C. titillator* larvae. Results of dipping bioassays showed that mortality of treated larvae was concentration-dependent. At the 100µl/ml concentration, there was 100% mortality of larvaefor both oils. The posterior spiracles were damaged and showed severe distortion of both lips. *C. rotundus* and *Z. officinale* oils have great potential to be developed as a novel larvicidea gainst *C. titillator* larvae and they could be used as nasal drench .They may be an effective, inexpensive control for this pest.

Keywords: *Cephalopinatitillator*, *Cyperusrotundus*, *Zingiber officinale,* Oils.

Introduction

Parasitic infections of camelsare the major causes of decreased performance, impaired milk productionor maylead to death. Some camel parasites represent a threat to human health [1]. One such disease of camels, nasopharyngeal myiasis, is caused by larvae of an obligate parasite of the family Oestridae (Diptera). The fly, *C. titillator* (Clark) (Diptera: Oestridae), attacksonly camels. During the fly life-cycle, the female enters the camel's nostrils and deposits its larvae into the nasal cavity.Few reports of the infection of camels with *C. titillator*in the world are found. A high incidence has been reported in Chad and other parts of West Africa as well as Sudan and Egypt [2-5] .Cephalopina infestations impair an animal's physiological functions, damage tissues and cause economic losses through abortion, loss of weight gain, reduction of camel fertility and hide quality[6,7] . Jalali et al. [5]demonstrated that infested camels show difficulty in breathing, restlessness and loss of appetite which may lead to a cessation of feeding. The severity of clinical signs depends on the amount of damage caused by migrating larvae [7].Camels exhibited congestion, swollen and edematous mucous membranes of the nasopharynx and frontal sinuses [2, 8]. Macrocyclic lactones are considered the systemic pesticide and have a larvicidal effect against nasal myiasis[3, 4] .Botanicals have been recognized for medicinal and insecticidal properties [9-13] since ancient Egypt [14] and can be useful for managing blow fly strike. Medicinal plants may be of some potential use in treatment because vaccinations

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do not work in most instances and the parasites have sometimes become resistant to the available synthetic therapeutics [15]. Natural essential oils have several properties that make them acceptable to the public and attractive to organic producers and environmentally conscious consumers [16]. EOs relatively safe, pleasant-smelling, and have anti-feedant and repellent. These properties make EOs a promising tool for management programs against a wide range of pests of medical and veterinary importance [17-20] including larval stages of *L. sericata* [21,22]. For example, *Z. officinale* (Roscoe), commonly called ginger, is used for a variety of purposes, including culinary and medicinal. In ancient times, ginger has been used in traditional medicine for respiratory troubles, stroke, inflammation, hyper-cholesterolaemia, helminthiasis, and larvicidal effect [23,5] .This plant still attracts extensive research attention. Researchers have reported that the deleterious effects of *Schistosoma mansoni* could be minimize after oral administration of ginger extract in experimentally infected mice [5]. *Cyperusrotundus* L. (Cyperaceae) is a weed that grows in fields and on the canal borders. Cyperus(known as Su'd) is consideredthe main weed species in cultivated soils in tropical areas[25,26]. *C. rotundus* is rich in alkaloids, nanthraquinone, coumarins, steroids, triterpenes, flavonoids, saponins, tannins, and resins. In addition, it has possible insecticidal properties and could be a repellent against arthropods [27].

The insecticidal and acaricidal efficacy of many essential oils has been well tested in a variety of pests. This efficacy is often attributed to the oil's major component(s); however, there is also evidence that the various oil components may work in synergy [28]. This may occur because some oil components aid cellular accumulation and absorption of other toxic components [29]. The mode of action of many essential oils or their components is largely unknown, although there is evidence of a toxic effect on the insect nervous system. For example, terpinen-4-ol, a monoterpenoid found at high concentrations in tea tree oil, inhibits arthropod acetylcholinesterase, an enzyme essential for transmission of action potentials [30, 31]. Alternatively, the hydrophobic nature of the oils may simultaneously exert mechanical effects on the parasite such as by disrupting the cuticular waxes and blocking the spiracles, which leads to death by water stress [32] or suffocation.

Spiraclesare the openings of the tracheal system on the integument of the insect.The main function of spiracles isdelivering sufficientoxygento all cells of the body and for removing carbon dioxidethat is produced as a waste product of cellular respiration. Exoskeletons of insects have spiraclesto allow air to enter thetrachea[33]. In addition, SEM has proved to be a useful tool for evaluating surface changes in the tegument and suckers of trematodes resulting from anthelmintic action[34].This paper evaluates the larvicidal effect of essential oils of *C. rotundus* and *Z. officinalis* against 3rdinstar C. *titilator.* SEM was used to demonstrate alterations in abdominal respiratory spiracles of larvae after treatment**.**

Materials and Methods

Collection of Larve

Larvae of *C. titillator* were collected from pharyngeal regionof camel (*Camelusdromedarius*) slaughtered in Nahya and El-Moneib abattoir Giza, Egypt. Larvae are used immediately after collection. They were collected in plastic petri dishes and washed with distilled water. The 3rd instar larvae was checkedand identified in the laboratory for dipping assay according to Khater et al. [3].

Essential oils

The essential oils of *C. rotundus and Z. officinale* were purchased from El Captain Company that specializes in extracting natural oils, herbs and cosmetics, Al-Obor City, Cairo, Egypt. Dilutions were made from each essential oil in distilled water. A few drops of Tween 80 were added as an emulsifier to the suspensions of essential oils. Three different concentrations (30, 50, 100 µl/ml) were prepared.

Larvalbioassay

To determine the efficacy of *C. rotundus* and *Z. officinale* oils against the third instar larvae larval immersion (dipping) tests were carried out according to Khater et al. [3]. The dipping procedure was applied to three groups of five larvae for each concentration, for a total of 15 larvae per concentration. Each group of larvae was immersed for 60s in each dilution for each oil and the solution was continuously stirred. The negative control (3 replicates, 5 larvae in each) was treated with distilled water and 1 to 2 drops of TWEEN 80. After treatment the larvae wereplaced in small glass containers having filter paper (Whatman No. 1). Containers were kept at 27±2°C and 80±5 % relative humidity (RH). The mortality of larvae was recorded after 3, 6, 9, 12, 24 and 48 hrs post treatment.

Scanning Electron Microscope

Third stage larvae were dipped for 60s in 100µl/ml of *Z. officinale* essential oil. Control larvae were immersed in distilled water for 60s. 24 hrs post treatment the last abdominal ring of treated and control larvae were sectioned and elucidated. After that, theywere immersed in 2.5% glutaraldehyde for 24 hrs, washed in phosphate buffer saline (PBS) then fixed in 1% osmium tetra oxide in 0.1M cacodylate buffer before being dehydrated in an ethanol series. Alternatively the last abdominal ring of larvae dehydrated in ethanol were dried in a CO2 critical point drier (Blazer Union F1-9496, Furstenum, Liechtenstein, Germany). Samples were then coated with 20nm of gold in a sputter coater (JXA 840, Electron Probe Micanalyzer, Jeol, Japan). Samples were then photographed by using SEM.

Results

Larvicidal effect of two tested essential oils on 3rd instar larvae of C. titillator

Mortality of treated *C. titilator* with each of the two oils was concentration dependent. Significant larval mortality (100%) was reached after 48hrs for the 50, 100, µl/ml concentrations of *C. rotundus* essential oil (Fig. 2). However, *Z. officinale* oil caused 100% mortality after 24hrs at all concentrations, 30, 50, 100 µl/ml(Fig .3). No mortality was observed within the control groups (Fig. 1). All concentrationsof *Z. officinale* oil produced greater and more rapid insecticidal effects than similar concentrations of *C. rotundus* oil (Fig.2 and 3).

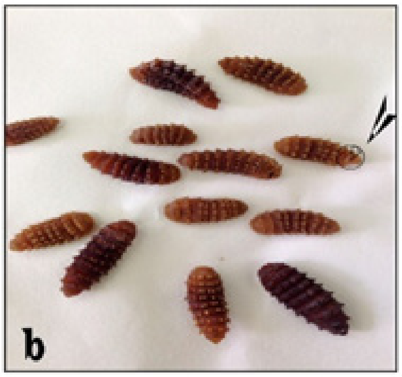

Fig.1. a) The appearance of normal *C.titillator* larvae b) the larvae after exposure to the essential oils, the larvae turn uniformly dark black after death (arrow pointing to the respiratory spiracle at last abdominal segment.

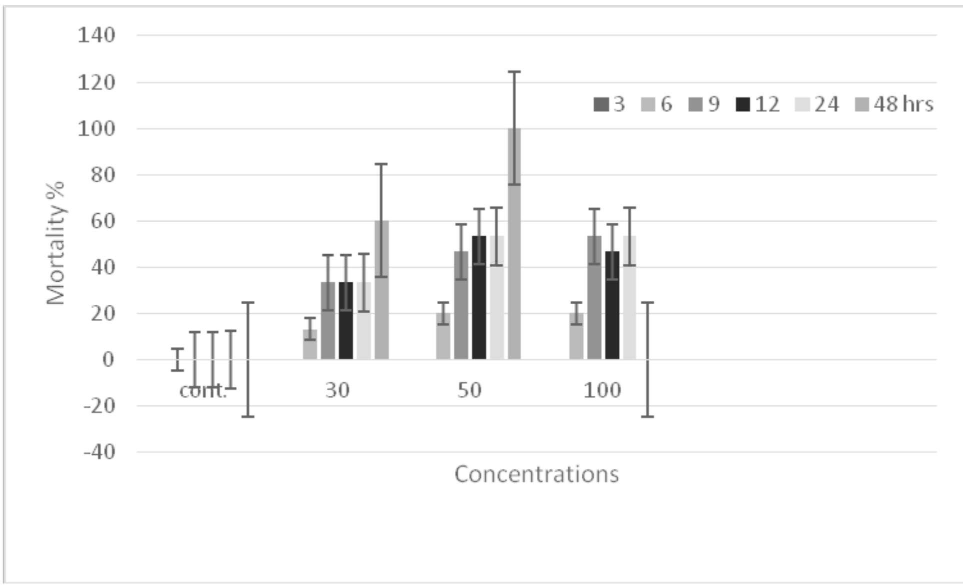

Fig. 2. Mean mortality percentages of *C.rotundus* oil on 3rd instar *C.titillator* larvae at different concentrations for 48 hrs. No mortality was recorded in the control group.

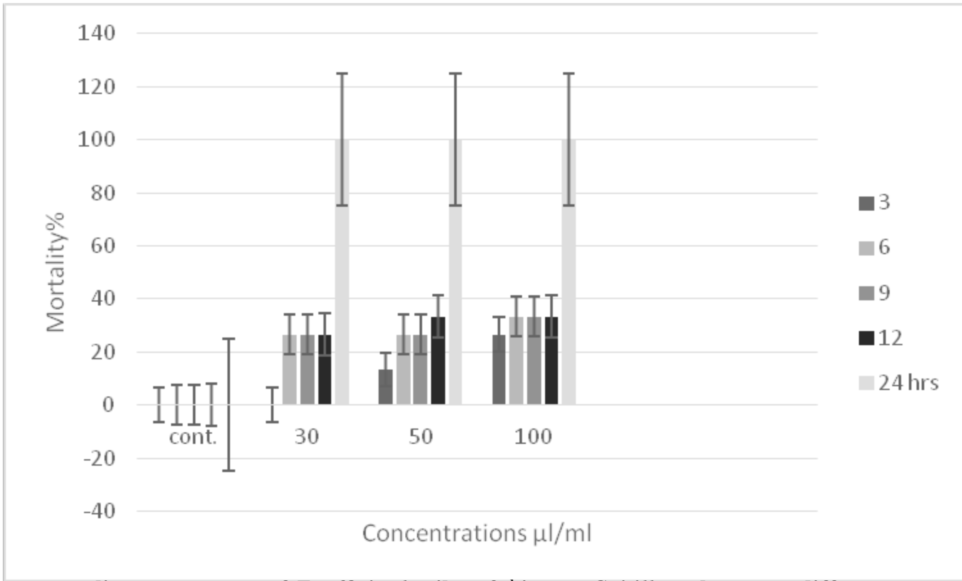

Fig. 3. Mean mortality percentages of *Z. officinale* oil on 3rd instar *C.titillator* larvae at different concentrations for 24 hrs.

Scanning Electron Microscope Finding

Normal posterior spiracles

SEM demonstrated that the posterior abdominal spiracles of 3rd instar *C. titillator* were composed of spinules and sensory papillae, surrounded by a layer of depressed cuticle as visualized by SEM (Fig. 4a). This cuticle consistedof a pair of dorsal and ventral lips. These lips were joined to each other medially to form a cuticle ring which enclosed the posterior spiracles (Figs.4a). The spiracular plate contained large numbers of respiratory units that were scattered irregularly on the spiracular plate. The respiratory unit were kidney shaped with a linear respiratory slit surrounded by rima (Fig. 4b).

Morphological deformities of posterior spiracles after treatment with tested essential oils

SEM was used to record morphological changes in 3rd instar larvae of *C. titillator* 24hrs after a 60sec exposure to a 100µl/ml concentration of *Z. officinale* essential oil(Fig. 5a-e). Third instar larva exhibited remarkably aberrant appearances. Extensive swelling, of the cuticle was evident in most specimens examined. As a result of the swelling the posterior spiracleswere damaged and showed severe distortion of both lips (Fig. 5 a-c). The respiratory units lost their normal aspect and appeared sunken (Fig. 5d, e).

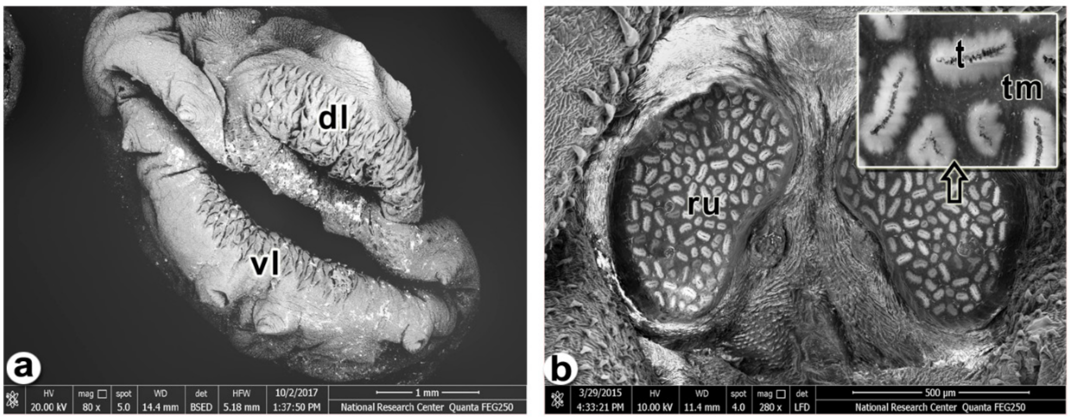

Fig. 4. Scanning electron micrographs of (a) normal control abdominal respiratory spiracles of 3rd instar *C.titillator*(L) showing normal kidney shaped posterior spiracles with spiracle units (b) , normal respiratory slite (arrow) and rima. Abbreviation: dl: dorsal lip, t: respiratory slit, vl: ventral Lip, rm: rima ,ru: respiratory unit.

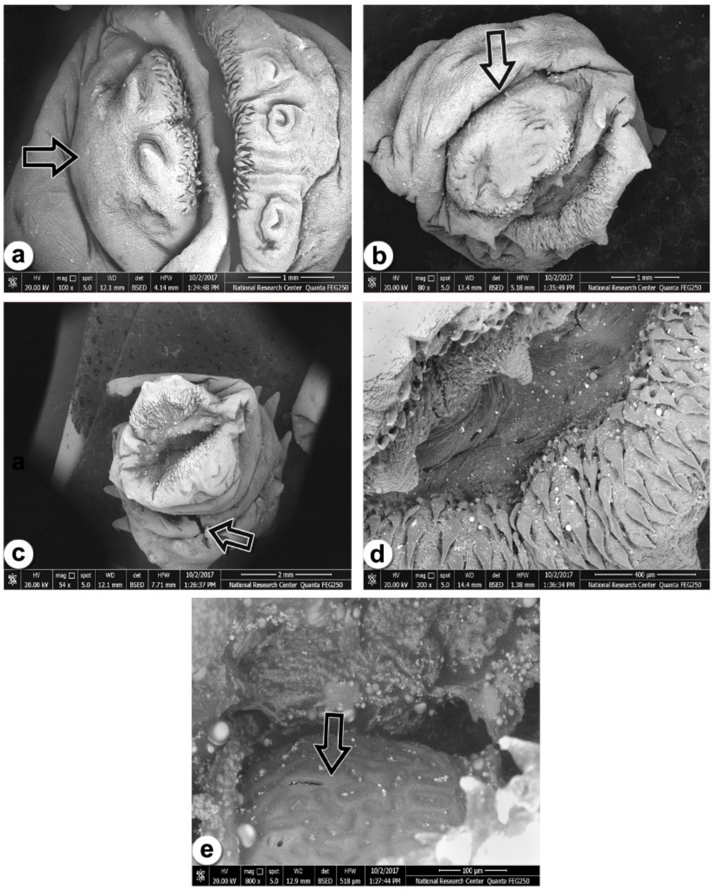

Fig.5. Scanning electron micrographs of abdominal respiratory spiracles of the 3rd instar C.titillator (L.) after 24 hrs treatment with 100 µl/ml ginger essential oil .(a, b, c) posterior end showing sever corrosion and swelling of the intersegmental spines and distortion of posterior spiracles (arrows), (d, e) respiratory units seemed to be sunken, respiratory slits lost their linear shape.

Discussion

This study investigated the larvicidal activity of *C. rotundus* and *Z. officinale* essential oils against *C. titillator* 3rd instar larvae .The alterations of the abdominal respiratory spiracles after treatment with *Z. officinale* essential oil were examined with SEM. Herbal medicine is the oldest form of medicine known to humanity [35]**.** Since ancient times Egypt has been renowned for herbal medication. Essential oils of Egyptian *Z. officinale* and *C. rotundus* are a well-known functional food and traditional herbal medicine[36,37] **.**Some researchers demonstrated that the whole essential oils are more efficient than individual constituents or even a combination of constituents [38], they effectively destroy several bacterial, fungal, and viral pathogens. The efficacy of veterinary products or medicines tested by researchers indicated that the overall efficacy of ectoparasiticides for the treatment of infestations by diptera species should be between 80 and 100 % [3,5,39]. The efficacy of *C. rotundus* and *Z. officinale* oils met these criteria in laboratory larval bioassays. Some essential oils, 2% pumpkin, 7.5% garlic, 30% lupine and (7.5%) peppermint oil, had the potential to produce mortality in 3rd*C. titillator* larvae 24hrs after exposure [4]. Also, Khater et al. [3] demonstrated that treatment with doramectin and lavender oil produced 100% mortality in 3rdinstar *C. titillator* larvae 24 hours after exposure.

Our study showed that the two tested essential oils had excellent insecticidal activity against 3rd instar *C. titillator* larvae at a range of concentrations. The essential oil of *Z. officinale* had significant, concentration dependant larvicidal and repellant effects against *Cx. quinquefasciatus and Cxpipiens* (Diptera: Culicidae) [40,41]. Shalaby et al. [42] investigated the effect of *Z. officinale* oil on the cuticle of the adult worm of *Toxocaravitulorum* (Nematoda; Toxocaridae). Application of the oil caused abnormal architecture of the surface of the worms. The greatest inhibitory effect on egg development of the *Z. officinale* extract was at the concentration of 100mg/ml.Lin et al. [43,44]characterized five constituents isolated from *Z. officinale* as larvicidal agents against *Angiostrongylus cantonensis* (Nematoda; Angiostrongylidae) and *Anisakis simplex* (Nematoda; Anisakidae)larvae. The individual components of the *Z. officinale* extract killed these nematode larvae or reduced their mobility in a time- and concentration-dependant manner. The anti-parasitic activity of *Z. officinale*might be linked to its constituents, 6-shogaol and 10-gingerol. These compounds killed the parasites by binding to parasite β-tubulin and inhibiting glucose uptake [24]. Topical treatment of *C. titillator* larvae with dilutions of the essential oil of *C. rotundus* produced 100% mortality at concentrations of 50µl/ml and above 48 hrs after treatment. Bajpay et al. [45] demonstrated that *C. rotundus* has a wide range of medicinal, insecticidal properties and pharmacological applications to treat diseases.

Exposure of 3rd instar larvae of *C. titillator* to 100µl/ml dilutions of *Z. officinale* oil produced significant changes in the posterior abdominal spiracles as visualized by SEM. The changes to the abdominal spiracles included swelling and alterations of the spiracular plate and respiratory units. Cuticular changes were observed in *Luciliasericata* (Diptera: Calliphoridae) larvae exposed to essential oils of lavender and camphor using light microscopy and SEM. There was severe distortion of the cuticle of larvae treated with lavender oil. The effects were less dramatic after treatment with camphor oil [39]. In addition , Abu El Ezz et al. (46) observed the morphological features of 3rd*C. titillator* that treated with camphor, ginger and cinnamon oils .the larvae showed a remarkable effect on cuticle in response to oil and were consisted of swelling which became pronounced and so severe with wrinkled and irregular curricular surface.

A literature survey of the mode of action of essential oils suggested that suffocation of the insect by blockage of the spiracles was the most likely cause of mortality [47].Stadler and Buteler[48] found evidence that petroleum distilled spray oils (PDSO) entered the trachea of *Anticarsiagemmatalis* (Lepidoptera: Noctuidae) larvae by observing the trachea-tracheole air-liquid interface. In larvae of *Blattellagermanica* (Blattodea: Blattellidae), oils produced mortality by asphyxia by blockage of trachea and tracheoles[49]. Najar-Rodriguez et al. [50] found that PDSO did not accumulate around the spiracular opening or in the tracheoles of adult cotton aphis, *Aphis gossypii* (Hemiptera: Aphididae) or the cotton leafworm, *Spodopteralitura* (Lepidoptera: Noctuidae).

Conclusion

This study suggests that essential oils of *Z. officinale* and *C. rotundus* may be effective and inexpensive larvicides against *C. titillator* infesting camels. Further study of the sub-lethal effects of these oils on longevity and fecundity of surviving adult flies will be important.

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التاثير القاتل للطور اليرقى لزيوت الزنجبيل والسعد على يرقات النغف الانفى *Cephalopina titillator* (L.)

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اهتمت هذه الدراسه باستخدام نوعين من الزيوت النباتية الشائعه ، والتي تعتبر صديقة للبيئة ولديها القدرة على قتل يرقات الحشرات ، وهي زيت الزنجبيل وزيت نبات السعد لمقاومه يرقات النعف الانفي التي تصيب الجمال وتسبب في خسائر اقتصادية وتلف لانسجه الانف. تم تحضير ثلاث تركيزات مختلفة ٣٠ ، ٥٠ ، ١٠٠ ميكرولتر / مل من الزيوت في الماء المقطرو تمت إضافة بضع قطرات من توين ٨٠ واستخدمت خمسة يرقات من الطورالثالث في كل تركيزلعمل المكررات. وأظهرت نتائج الاختبارات الحيوية أن وفيات اليرقات المعالجة تعتمد علي التركيز حيث اظهرت اعلي معدل للوفيات عند تركيز ١٠٠٪. كما تم تصوير الفتحات التنفسيهلليرقات بالميكروسكوب الالكتروني الماسح واظهرت تشوهات واضحه. توصي هذه الدراسه باستخام هذه الزيوت كمبيد جديد لليرقات ضد يرقات النغف الانفي للجمال التي يمكن تطويرها كجرعات انفيه.