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Biodegradable Films Containing Clove or Citronella Essential Oils against the Mediterranean Fruit Fly *Ceratitis capitata* (Diptera: Tephritidae)

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ABSTRACT

A concentration-response bioassay against the Mediterranean fruit fly, *Ceratitits capitata*, was performed to evaluate the insecticidal activity of biodegradable films containing clove (*Eugenia caryophyllata*, Myrtaceae) or citronella (*Cymbopogon nardus*, Poaceae) oils. We recorded the time of knock down flies after the exposure to soy protein-lignin films incorporated with 3% food grade clove or citronella essential oils, or control films without bioactive. The vapors of clove released from the film containing 3% essential oil were efficient to trigger the tumbling of the flies (40% after 4 h exposition) and after 20 h 90% of the flies died. However, films containing citronella essential oil had poor insecticidal activity. Citronella oil have a much higher insecticidal activity in a pure form, but probably one or both of their main bioactive compounds (citronellal and geraniol) were poorly released from the films. In this work, for the first time, a novel packaging system controlling the release of essential oil of clove with good insecticidal activity against *Ceratitits capitata* was achieved, that can be used to improve quality of fruit and for food products.

KEYWORDS: Biodegradable films; Diptera*; Cymbopogon nardus*; *Eugenia caryophyllata*; Insecticidal activity; Essential oil.

INTRODUCTION

The essential oils (EOs) comprised the steam-distilled fraction of the plant and are responsible for the distinctive smell of plants.[1] The main components of EOs are low-molecular-weight terpenes and phenolics, lipophilic molecules characterized by having a high vapor tension and hence a potential fumigant toxicity.[2]

In recent years, the use of EOs derived from aromatic plants as low-risk insecticides has increased considerably owing to their popularity with organic growers and environmentally conscious consumers.[3] The use of synthetic chemicals to control insects raises several concerns related to environment and human health and alternative natural products that possess good efficacy and are environmentally friendly.[4] Although from an economical point of view synthetic chemicals are still more frequently used as repellents than essential oils, these natural products extracted from Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae plants have the potential to provide efficient and safer repellents for humans and the environment.[4]

The EO have repellent, insecticidal and growth-reducing effects on a variety of insects and have been used effectively to control pre-harvest and post-harvest phytophagous insects and as insect repellents for biting flies and for home and garden insects.[5]

Clove oil from *Eugenia caryophyllata* Thunb (Myrtaceae) has insecticidal activity against some stored product insects, the American house dust mite, and the European house dust mite.[6] Park and Shine[7] reported that the main compounds of clove bud oils eugenol (86.1%) and α-caryophyllene (11.1%) has anti-termitic activity mainly attributed to the action of eugenol. Eugenol is a phenolic compound well known for its versatile pharmacological actions, including analgesic, local anesthetic, anti-inflammatory, antimicrobial, antitumor, and hair-growing effects.[8, 9]

Citronella oil, the essential oil from *Cympobogon* spp (Poaceae), is popular in mosquito-repellent formulations. Candles and incense containing citronella oil are sold as insect repellents in several countries. Despite the popular conception, it has been reported that citronella candles or incense were ineffective for reducing the biting pressure of mosquitoes.[10] The possible reasons could be uncontrolled release of the oil, i.e., either it is released into little amount and become ineffective, or it is released excessive with shorter duration of protection. Therefore, how to control the release of these essential oils is a key area worth investigating. [11] In addition, synthetic chemicals used for control of vectors are causing irreversible damage to the ecosystem, as some of them are non-degradable in nature.[10] These problems have highlighted the need for the development of effective alternatives.

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Biodegradable films are prepared from edible material that act as a barrier to external elements (factors such as moisture, oils, gases and vapors) and thus protect the product, extend its shelf life and improve its quality.[12] Different food ingredients, derived from meats, cereals, nuts, fruits and vegetables, are being used to produce edible films for strips and pouches. These films act as novel packaging systems and could control the release of active compounds such as antioxidants, flavors and antimicrobial agents in food protection and preservation since they offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly.[13] Its application as tunnel-film or to cover the fruit in plants is an interesting way to eliminate plastics from the field which led to an increase in environmental wastes and consumes a lot of energy, besides of conferring the function of repellent or insecticide if they contain the suitable active ingredient.[14]

Plant EOs may provide potential alternatives to currently used insect control agents because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages.[15] This paper describes a laboratory study to assess the potential of biodegradable films containing clove or citronella essential oils for use as protection of fruits or cultivars against insect attack. Insecticidal activities of essential oils were assessed using a fumigant bioassay against adults of *Ceratitis capitata*.

MATERIALS AND METHODS

Film preparation. The films were prepared by the casting technique using a two-step process for obtaining a bilayer film. The first layer was prepared as follows: soy protein isolate (SPI) (PRO FAM, ADM, Decatur, IL, USA) was dissolved in distilled water (4g /100mL) at room temperature until complete dissolution, then glycerol was added (1% w/v) and pH was adjusted at 8.0 with 2N NaOH, then formaldehyde (3.3 mmol/100mL) was added and stirred by 10 min and pH adjusted to 10.5 using 2N NaOH. Another solution was prepared dissolving commercial lignin powder (Protobind 1000, Granit R&D SA, Lausanne, Switzerland) in distilled water to a final concentration of 0.6% (w/v) in the film forming solution (FFS). This concentration was selected according to previous experiments [19]. The mixture was stirred at 40 ºC by 15 min and was alkalinized to pH = ~11.0 to obtain a blend with total solubility. Finally, the two solutions described above were mixed, and 25 mL of the FFS were spread over a plexiglass plate (Ø = 12x12 cm^2) and dried at 45 ºC in a forced-air oven (Binder FD 240, Tutlingen, Germany) for 6 h. The second layer follows the same method described above, except that the plasticizer (glycerol) was replaced in one assay by citronella (*Cymbopogon nardus*, Isabrubotanik S.A, Ambato-Ecuador) essential oil and in the other assay with clove (*Eugenia caryophyllata*, Isabrubotanik S.A) essential oil at 1.5 or 3% (w/w) and were homogenized with Ultra-Turrax (T25, IKA Werke GmbH & Co. KG, Staufen, Germany) by 3 min at 17500 rpm. The essential oils mostly contained 85% eugenol (clove) and 36% citronellal and 21% geraniol (citronella), (data provided by the company).

Thereafter, the lignin solution was added and mixed using magnetic stirring to prevent the bubbles formation. Each FFS containing the essential oils (25 mL) were slowly poured over the first layer and dried at 45 ºC in a forced-air oven (Binder FD 240, Tuttlingen, Germany) for 6 h. The resulting films were conditioned over a saturated solution of KBr (58% RH) for 5 d.

Insects. Wild-type *Ceratitis capitata* (strain "Mendoza") were reared in pumpkin-based medium[16] and kept in a Conviron chamber (CMP 3244) at 23°C, 50-60% RH, with a photoperiod of 16:8 (Light: Dark). These insects were used to lay eggs representative of the standard laboratory population. For all experiments adult flies less than 2 day old were collected under CO_2 and placed in flasks with free access to sucrose: dry yeast (3:1) and 1% agar as sources of food and water, respectively.

Bioassay. The effect of both EOs and films containing these oils was evaluated using a fumigation bioassay according to Tarelli et al. [17] with modifications.

Clove and citronella solutions (1, 3, and 6%) were prepared in 1% glycerol. The solution was distributed on a glass petri dish of 3.5 or 30 cm^2 covered with a piece of gauze (2 mm mesh) to avoid fly contact with the solution (Figure 1). The films of approximately 4 and 36 cm^2 containing 3% clove or citronella (5.2 mg EO/cm^2) were suspended from the top of the 1 L flask (18 cm height, 10.5 cm and 7.5 cm diameter at the bottom and top, respectively). To prevent the direct contact of the flies, each film was covered with a piece of gauze to avoid direct contact of the flies (Figure 1).

Groups of 25 virgin flies representing one laboratory population were cooled at 4ºC and quickly introduced in the experimental flask. Four replicas per treatment were performed. After a 20 min period of equilibration, the recording time of knock down flies began. Knocked down flies were counted at 30 min, 1, 2, 3, 4 hours. We considered that a fly is knocked down when is lying on the floor of the chamber in a supine position or when is unable to walk (the fly respond to mechanical stimuli).

The insecticidal effect of EOs and films was determined counting the dead flies after 20 hs exposition (none responds to mechanical stimuli).

Data analysis. The data were examined by analysis of variance (ANOVA) using the software Infostat (Universidad Nacional de Córdoba, Argentina).

RESULTS

The insecticidal activity of biodegradable films containing clove or citronella oil was evaluated against *C. capitata* using the experimental chamber described in Figure 1. Initially, the insecticidal activities of clove and citronella essentials oils incorporated to soy protein-lignin films were evaluated using 4 or 36 cm^2 of films by recording the mortality of *C. capitata* adult flies after 20 h of exposition. Table 1 shows that a high mortality was observed using 36 cm^2 films containing 3% clove EO (90% of the flies dead), while the incorporation of the same concentration of citronella EO presented only a 14% of dead flies ($p<0.0001$). Negligible percentage mortality of medfly was obtained by controls of 4 and 36 cm^2 films without EO incorporated.

Figure 2 shows the percentage of cumulative knockdown of flies exposed during 4 h to clove or citronella films (4 and 36 cm^2). We recorded the number of flies flipped during the first 4 h of exposition. Figure 2A shows that 3% clove EO knocked down 40% of the flies during the first 4 h of exposition using 36 cm^2 of the film, while no significant activity was observed using 4 cm^2 (differences between slopes are significant, $p= 0.001$). On the other hand, Figure 2B shows that the film containing 3% EO citronella did not present noticeable insecticidal effect at both 4 and 36 cm^2 of the film employed. However, the citronella oil altered the fly behaviour; flies were motionless distributed in different parts of the experimental chamber and cleaned the head and thorax with the anterior legs permanently. Previously, Muryati *et al*.[18], described that citronella showed disturbance effects on fruit fly.

In order to explore the possibility that this EO would be retained in the film, we analyse the effect of citronella and clove EO distributed on a glass petri dish (3.5 cm^2) or in paper. Figure 3A shows a 60% of cumulative knockdown flies achieved with 3% EO citronella on paper after 4 h of exposition. The clear insecticidal activity against the fly was registered even at a quarter of concentration of the bioactive that was included in the bioactive film. In addition, over 80% of the flies were knocked down assaying 6% EO of citronella (Figure 3A). The same results were obtained dispensing the citronella oil on the glass petri dishes (data not shown). Therefore, bioactive/s responsible for the fumigant activity present in the citronella oil seems to be hampered to release from the film.

In contrast, Figure 3B shows that 1 to 6% clove oil distributed on paper (3.5 cm^2) after 4 h showed a reduced cumulative percentage of knockdown *Ceratitis capitata*. Unexpectedly, only 10% of flies were registered with 6% EO clove. Likewise, 1 to 6% clove oil tested on glass Petri dishes exhibited similar percentages of flies as the obtained using paper. A final experiment was conducted to compare more accurately the insecticidal efficacy of 3% clove oil incorporated in the film (36 cm2) versus that of the oil distributed alone in a similar area. Table 2 showed the mortality of *Ceratitis capitata* flies after 20 h exposition using 3% clove oil distributed over 30 cm2 of glass. Results indicate that 3% clove EO alone tested on a petri dish showed noticeable mortality (55.94 %, $p<0.05$) confirming the insecticidal activity of the clove oil.

Our results indicated that a surface area of 36 cm^2 of soy protein-lignin films containing 3% clove oil may be useful as insecticide, while 3% citronella showed slighter insecticidal activity.

Table 1. Percentage mortality of *Ceratitis* flies tested with 3% of clove or citronella essentials oils incorporated to protein-lignin films. Mortality was registered after 20 h exposition. The assay was repeated four times with 25 adult per assay. No significant effects with 4cm^2 area ($p = 0.2755$); significant effect with 36cm^2 ($p<0.0001$).

Film (cm^2)	N	Treatments (% mortality ± SD)		
		Without EO	Clove EO	Citronella EO
4	4	5.04 ± 1.97	8.00 ± 9.24	13.0 ± 2.00
36	4	3.50 ± 2.52	90.00 ± 4.00	14.0 ± 6.93

Table 2. Percentage mortality of *Ceratitis* flies tested with 3% of clove essential oil. Mortality was registered after 20 h exposition. The assay was repeated four times with 25 adult per assay. Significant effect with 3.5 cm^2 area ($p>0.05$), and 30 cm^2 ($p<0.05$).

Glass Petri dish (cm^2)	N	Treatments (% Mortality ± SD)	
		Without EO	Clove EO
3,5	4	0	2.50 ± 2.89
30	4	4.02 ± 2.68	55.94 ± 17.09

Figure 1. Experimental chambers to evaluate fumigant activity of films with clove or citronella (A), and EO distributed on filter paper or glass (B). EOs on Watman Nº 3 paper was place inside a petri dish or on glass petri dish alone. The films and the petri dishes were covered with gauze to avoid direct contact with the flies (A and B). Arrow shows a knock down fly on the floor of the chamber.

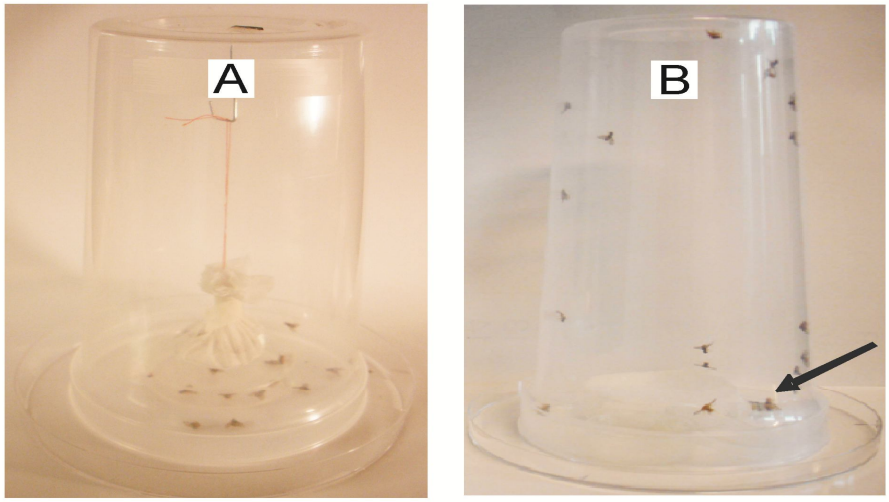
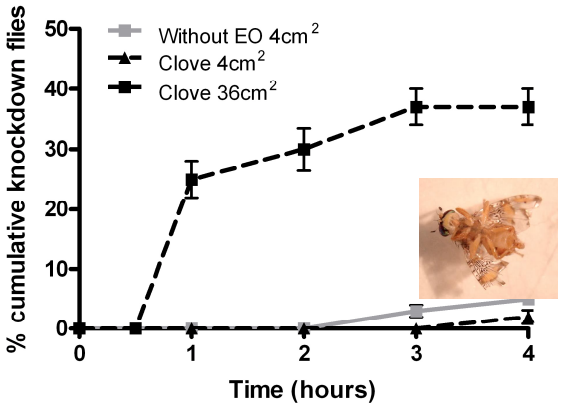


Figure 2. Cumulative percentage of knockdown *Ceratitis capitata* flies exposed or not to EO incorporated to protein-lignin films. (A) 3% clove film and (B) citronella 3% film. Controls without EO of 4 and 36 cm^2 films causes similar % of knockdown flies. The assay was repeated four times with 25 adult per assay.

A



B

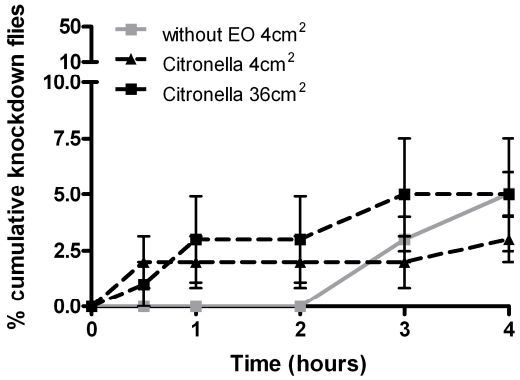
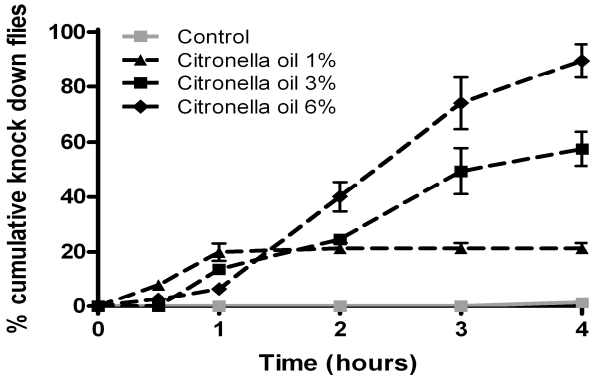
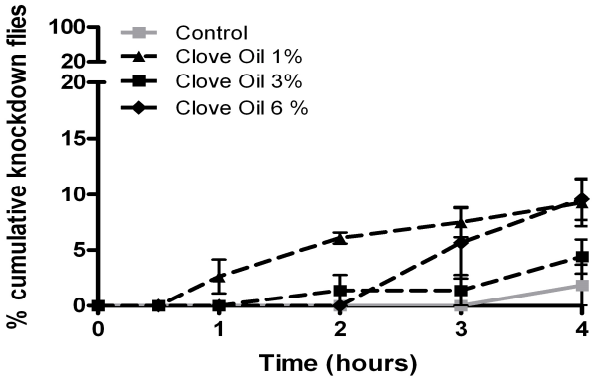


Figure 3. Cumulative percentage of knockdown *Ceratitis capitata* flies exposed to Whatman Nº 3 paper impregnated with citronella oil in 1% glycerol (A) or clove oil in 1 % glycerol (B). Controls were paper impregnated with 1% glycerol without EO. The assay was repeated four times with 25 adult per assay.

A



B



DISCUSSION

The results obtained showed for the first time a suitable method for entrapping clove essential oil to develop biodegradable composite films prepared with soy protein and lignin, which give them the insecticidal properties. Bilayer films were prepared with soy protein and lignin in a first layer containing glycerol (1% w/v) and the essential oil (3% w/v) was added in the second layer. These biodegradable films were active against the fruit fly *C. capitata* in the *in vivo* fumigation bioassay.

After incorporation of clove oil into the film the insecticidal activity of the film is clearly detected. Moreover, 36 cm^2 of soy protein-lignin films containing 3% clove oil showed superior knock down effect and mortality than that of the oil alone (Figure 3B, Table 2). Clove oil used here contains eugenol as the main compound in high concentration (85%). Zhang et al., [9] recently mentioned that the application of eugenol is greatly limited mainly because of its unwanted physicochemical properties, such as low solubility, liability to sublimation. So although we cannot rule that it is associated partially with the film; eugenol is in such concentration that still has plenty to act. In addition, it is reasonable to think that the increment in the insecticidal activity of the film containing 3% clove versus that of the oil alone (90% vs. 55%, Table 1 and Table 2, respectively) may be due to a possible change in the physicochemical properties of the clove oil bioactive/bioactives perhaps increasing the rate of evaporation of the clove essential oil via incorporation into the films. In this sense, previously, the antimicrobial activity of the clove essential oil was maintained when it was incorporated in bovine-hide gelatin and chitosan edible films, with no difference between the matrices employed.[19] Nevertheless, some differences were observed with regard to water solubility which could determine the release of bioactive compounds and affect the biological activity of the films, as the gelatin chitosan clove film is less soluble than the gelatin clove film. Therefore, films incorporating clove EO could be advantageous because the oil could then be more efficiently released assuring an extended bioactivity.

A different effect was observed using citronella oil, since a much higher insecticidal activity in a pure form was obtained, but after incorporation into the film it did not present any insecticidal effect. Therefore, citronella oil was well released from the Whatman paper and glass support, but probably one or both of their main bioactive compounds (citronellal and geraniol) were poorly released from the films. It was reported that citronella oil possess a great mosquito-repellent action and a rapid volatility, however the amount of citronella oil permeation was dependent on the type of ointment base used to microencapsulation.[20]

Currently, the use of synthetic chemicals to control insects raises several concerns related to environment and human health. An alternative is to use natural products that possess good efficacy and are environmentally friendly. Many plant EOs and phytochemicals are known to possess insecticidal or repellent activity. Among those chemicals, EOs from plants belonging to several species has been extensively tested to assess their repellent properties as a valuable natural resource.[4] These compounds can exert their activities on insects through neurotoxic effects involving several mechanisms, notably through gamma-aminobutyric acid, octopamine synapses, and the inhibition of acetylcholinesterase. With a few exceptions, their mammalian toxicity is low and environmental persistence is short. Registration has been the main bottleneck in putting new products on the market, but more EOs have been approved for use in the United States than elsewhere owing to reduced-risk processes for these materials.[3]

This paper presents an application of biodegradable films for a novel packaging system controlling the release of active compounds with fumigant activity and insecticide against the Mediterranean fruit fly *Ceratitis capitata*. For the practical use of clove oil and their constituents as novel fumigants, further study is necessary on development of formulations to improve the efficacy of biodegradable composite films and to reduce cost.

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REFERENCES

(1) Enan, E., 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol., 130: 325-337.

(2) Adams R. P., 2007. In Identification of essential oils components by gas chromatography 7 mass spectrometry; 4th Ed Allured Publishing, Carol Stream, IL.

(3) Regnault-Roger C.; Vincent, C.; J. T. Arnason, 2012. Essential oils in insect control: low-risk products in a high-stakes world. Annu. Rev. Entomol.**,** 57: 405-24.

(4) Nerio L. S.; Olivero-Verbel J.; E. Stashenko, 2010. Repellent activity of essential oils: a review. Bioresour Tech.., 101: 372-8.

(5) Tripathi, A. K.; Prajapati, V.; Verma, N.; Bahl, J. R.; Bansal, R. P.; S. P. S. Khanuja, 2002. Bioactivities of the leaf essential oil of *Curcuma longa* (Var. Ch-66) on three species of the stored product beetles (Coleoptera). J. Econ. Entomol., 95:183-189.

(6) Kim, E. H.; Kim H. K.; Y. J. Ahn, 2003. Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). J. Agric. Food Chem., 51: 885-889.

(7) Park, I. K.; S. C. Shin, 2005. Fumigant Activity of Plant Essential Oils and Components from Garlic (*Allium sativum*) and Clove Bud (*Eugenia caryophyllata*) Oils against the Japanese Termite (Reticulitermes speratus Kolbe). J. Agric. Food Chem., 53: 4388-4392.

(8) Pramod K.; Ansari, S.H.; J. Ali, 2010. Eugenol: a natural compound with versatile pharmacological actions. Nat. Prod. Comm.; 5: 1999-2006.

(9) Zhang, P.; Zhang, E.; Xiao, M.; Chen, C.; W. Xu, 2012. Enhanced chemical and biological activities of a newly biosynthesized eugenol glycoconjugate, eugenol α-D:-glucopyranoside. Appl. Microbiol. Biotech.**,** DOI 10.1007/s00253-012-4351-2.

(10) Fradin M. S.; J. F. Day, 2002. Comparative efficacy of insect repellents against mosquito Bites. New Engl. J. Med., 347: 13–18.

(11) Hsieh, W. C.; Chang, C. P.; Y. L. Gao, 2006. Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. Colloids Surf. B Biointerfaces, 53: 209–214.

(12) Suyatma, N. E.; Tighzert, L.; Copinet, 2005. A.Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films. J. Agric. Food Chem., 3: 3950-3957.

(13) Tharanathan, R., 2003. Biodegradable films and composite coatings: past, present and future. Trends Food Sci.Tech., 14: 71–78.

(14) Kyrikou I.; D. Briassoulis, 2007. Biodegradation of agricultural plastic films: A critical review. J. Polymers Environ., 15: 125-150.

(15) Isman, M. B., 2000. Plant essential oils for pest and disease management. Crop Prot., 19: 603-608.

(16) Pujol-Lereis, L. M.; Rabossi, A.; Filiberti, A.; Argaraña, C. E.; L. A. Quesada-Allué, 2006. *D. melanogaster and H. irritans* are more sensitive to Phloxine B phototoxicity than *C. capitata*. Dros. Inf. Serv., 89: 82-84.

(17) Tarelli G.; Zerba, E. N.; R. A. Alzogaray, 2009. Toxicity to vapor exposure and topical application of Essentials oils and monoterpenes on *Musca domestica* (Diptera: Muscidae). J. Econ Entomol., 102: 1383-1388.

(18) Muryati, Y. A.; Trisyono; Witjaksono; Wahyono, 2012. Effects of citronella grass extract on the oviposition behavior of Carambola fruit fly (*Bractocera carambolae*) in mango. J. Agric. Biol. Sci., 7: 672-679

(19) Gómez-Estaca, J.; López de Lacey, A.; López-Caballero, M.E.; Gómez-Guillén, M. C.; P. Montero**,** 2010. Biodegradable gelatin chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol., 27: 889-896.

(20) Solomon, B.; Sahle, F. F.; Gebre-Mariam, T.; Asres, K.; R. H. H. Neubert, 2012. Microencapsulation of citronella oil for mosquito-repellent application: Formulation and in vitro permeation studies. Eur. J. Pharm. Biopharm., 80: 61–66

Notes

The authors declare no competing financial interest.