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Biopesticidal silo bag prepared by co-extrusion process

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ABSTRACT

Silo bag technology is used for storing grains in a hermetic plastic structure. The major limitation of this structure is resistance to improper handling and external aggression, thus promoting pest incidence due to alterations in its internal atmosphere. In this study, we developed a biopesticidal silo bag consisting of a co-extruded three-layer film made of polyethylene and essential oil of *Mentha piperita* (7% w/w) in the inner layer. A film with the same structure but without essential oil was produced to be used as a control. The presence of the biopesticide in the silo bag was confirmed by FTIR-spectroscopy. Thus, mechanical, optical and chemical properties were evaluated. Diffusion coefficient of biopesticide from silo bag at 15, 25 and 35 °C was estimated as $6.4 \pm 0.1 \times 10^{-11}$, $1.45 \pm 0.17 \times 10^{-10}$ and $1.30 \pm 0.2 \times 10^{-10}$ cm^2/s, respectively. Finally, the biopesticide silo bag was tested against *Rhyzopertha dominica,* primary pest of stored grain, showing 100 % of mortality during the time assayed (7 days). Hence, the incorporation of biopesticide by co-extrusion technology (low-cost and efficient machinery) into the inner layer of a silo bag could help to replace synthetic pesticides and avoid manipulation of these in the field, preventing biotic infestation.

1. Introduction

Grains, cereals and their industrial derivatives constitute one of the main food sources for humanity. The estimated world production grain and cereal for the period 2020/21 is of 2,765 millions of tons (FAO, 2020).

A silo bag is a hermetic plastic system used for storing grains. Today, this technology is considered as one of the best to store grains and forages in a safe, economical and profitable way (Stavisky, 2019). Therefore, the main advantages attributed to this hermetic system include reduction of storage cost and decrease of post harvest loss. For this reason, the silo bags are adopted in > 40 countries ranging from Brazil to Canada (Bartosik, 2012). In 2018 more than 40 million tons of grains were stored in silo bags in Argentina (INTA Informa, 2014; Abadía, Urcola, Ferrari, & Bartosik, 2019). Specifically, a silo bag is a structure comprising three-layers of polyethylene and anti UV additives of approximately 235 μm thick, 60 m long and 2.70 m wide (Castellón Petrovich, 2015; YPF, 2017) undergoing an extrusion process. In the field, the grains harvested can be stored in the silo bag from 6–12 months (Bartosik, Rodríguez, & Cardoso, 2008).

In the hermetic system, the respiration of biological agents such as grains, microorganisms, insects and mites reduces oxygen (O_2) and increases carbon dioxide (CO_2) (INTA Informa, 2014). Thus, an environment rich in CO_2 and poor in O_2 decreases its biological activity, promoting grain preservation. However, the internal atmosphere enriched with CO_2 could be affected by improper management and perforations in the plastic cover (Santa Juliana, Cardoso, De La Torre, Bartosik, & Abadía, 2019; Stavisky, 2019), promoting biotic activity. The attack of pests such as insects in the silo bags causes important loss, about 10 % in the production of grains in many countries worldwide (FAO, 2018). Phosphine and dichlorvos (DDVP) are the main pesticides used in silo bag as the fumigation method against the insect pests of

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stored products such as *Sitophilus sp*, *Tribolium castaneum* and *Rhyzopertha dominica* (US EPA, 2006; Cardoso, 2009; Abadía and Bartosik, 2013). Nevertheless, these synthetic pesticides have been listed as restricted or prohibited in some countries, affecting food-safety and causing environmental contamination and human diseases (Grandjean & Landrigan, 2006; Resolución-692-2017-Senasa - Servicio Nacional De Sanidad Y Calidad Agroalimentaria, 2020, 2018). An alternative for managing pest is the use of natural products like essential oils. Indeed, essential oils and their main components, such as monoterpenes, involve minimum risk as reported by the U.S. Environmental Protection Agency (EPA) (EPA, 2014) due to their low toxicity, reduced persistence in the environment and non-restricted use. Thus, the insecticidal effect of essential oils has been evaluated to control pests of stored grain using fumigation, contact, repellency and antifeedant tests *in vitro* (Kumar, Mishra, Malik, & Satya, 2011; Lashgari, Mashayekhi, Javadzadeh, & Marzban, 2015). Other studies have shown the potential of *M. piperita* essential oil in the control of crop pests having with an effect on the activity of the acetylcholinesterase (AChE) enzyme and oxidative stress (Lee et al., 2002; Bounoua-Fraoucene, Kellouche, & Debras, 2019; Rajkumara et al., 2019). Due to the volatility of essential oils, they have been incorporated into low-density polyethylene (LDPE) polymer through impregnation technology with supercritical CO_2. These films were developed as packaging materials of controlled release of the bioactive compounds (Goñi et al., 2017; Herrera, Gañán, Goñi, Zygadlo, & Martini, 2018). However, so far no reports have been published showing the incorporation of botanical pesticides into the inner layer of silo bag through the co-extrusion process in order to be released to the internal atmosphere to reduce grain loss caused by insects. Then, the objective of the present study was to manufacture a three-layer active film made of high-density polyethylene (HDPE) and LDPE (prototype silo bag) by co-extrusion process, adding essential oil of *M. piperita* as a biopesticide. Thus, the effect of the essential oil on the mechanical and optical properties of the active film was determined. In addition, the release of the biopesticide was monitored at different temperatures and the activation energy of the release was calculated. Finally, the insecticidal activity of biopesticide silo bag was tested against *R. dominica,* primary pest of stored grains (Faroni & García-Mari, 1992).

2. Materials and methods

2.1. Materials

HDPE and LDPE were provided by Braskem Idesa (Mexico) for the development of the silo bag. UV stabilizers and light blocking additives such as titanium dioxide (TiO_2) and carbon black, respectively, were supplied by Ampacet (Mexico). Biopesticide, the essential oil of *M. piperita*, was purchased from Young Living (therapeutic grade, USA), having menthone ($C_{10}H_{18}O$) (30 %), limonene ($C_{10}H_{16}$) (10 %) and menthol ($C_{10}H_{20}O$) (60 %). Pure internal standard, 1-octen-3-ol, was acquired from Sigma (USA). Methanol (HPLC grade) was obtained from JT Baker (Ecatepec, Mexico).

2.2. Insects

Adults of *Rhyzopertha dominica* Fab. were reared in laboratory conditions in a hermetic chamber under ambient temperature and humidity (30 °C and 60 %, respectively) with a cycle of light and dark of 12:12 h (FAO, 1974). Insects were fed with a diet of rice without pesticides.

2.3. Manufacture of biopesticide silo bag

A three-layer film was prepared by co-extrusion process, with a pilot size apparatus (Beutelspacher, Mexico CDMX, Mexico), at Centro de Investigación en Alimentación y Desarrollo, A.C. campus Hermosillo, Mexico. The biopesticide silo bag was manufactured from LDPE loaded with 7% w/w of essential oil from *M. piperita* in the inner layer, LDPE loaded with 4 % carbon black in the middle layer, and HDPE loaded with 20 % of TiO_2 in the external layer.

The operational conditions used for co-extrusion process were: Temperature: 100−150 °C for LDPE and 130−160 °C for HDPE; screw rate: 40 rpm. The essential oil was directly mixed with the pellet of polyethylene and the concentration of biopesticide was selected following Granda-Restrepo, Peralta, Troncoso-Rojas, and Soto-Valdez (2009). The control film was manufactured under the same conditions but without the biopesticide. Micrometer (E.J. CADY & CO, USA) was used to determine the thickness of the three-layer materials.

2.4. Characterization of biopesticide silo bag manufacture

2.4.1. Mechanical characterization of active silo bag

The mechanical properties of the biopesticide and control silo bags were measured using ASTM (D 882-02) norm. The penetration assay was performed by the compression mode with a TA.XT plus texture analyzer (Stable Micro Systems Ltd, United Kingdom) (TA Seetings program) relating the force applied (g) with specimen film (μm) until break (Castellón Petrovich, 2015). Tensile testing was assayed by the Elmendorf method (ASM International, 2004) with a United testing system (United Calibration Corporation, USA). Parameters were determined using the Instron program. Five specimens were tested from each material (biopesticide silo bag and silo bag control) and the means ± standard deviation values were calculated.

2.4.2. Spectrophotometric characterization of the silo bag

The UV–vis light transmission of the biopesticide and control silo bags was monitored in the interval of 400−500 nm with a Bio Spectrophotometer (Varian Cary 50, Mulgrave, Victoria, Australia). Five samples (1 × 6 cm) of each material were tested.

2.4.3. Chemical characterization

The presence of the biopesticide in the inner layer of the co-extruded film was confirmed with a FTIR spectrophotometer (Thermo Scientific, Nicolet IS50 FTIR, USA). Spectra were obtained following the methodology described by Herrera et al. (2018), using a wavenumber range of 600–4000 cm^{-1} with 32 scans. Furthermore, absorbance spectra were measured in different places of the biopesticidal silo bag, using a plate cell to evaluate homogeneity in the incorporation of biopesticide through the co-extrusion process.

2.4.4. Extraction and quantification of biopesticide in silo bag

The extraction of biopesticide from the silo bags was determined following the methodology described by Higuera-Barraza, Soto-Valdez, Acedo-Félix, and Peralta (2015), with some modifications. Previously, the extraction time was determined when the concentration of volatile compounds in the extract was constant. Thus, five samples (5 × 5 cm) were placed in amber vials (40 mL) with 10 mL of methanol. The vials were then placed in the sonicator (Branson Ultrasonic Corporation, USA) at 40 °C for 5 h. Finally, the identification and quantification of the principal compounds present in the essential oil of *M. piperita* were determined by GC-FID in a Varian Star 3400 CX (Australia) gas chromatography equipped with a 10 μL loop injector and a DB-1 capillary column (30 m x0.53 mm), following the conditions reported by Herrera, Goñi, Gañan, and Zygadlo (2017), with some modifications: the total run time was 32 min and nitrogen was used as the carrier gas with a flow rate of 0.8 mL/min.

The components were identified through retention indices with published data (Adams, 2007). The calibration curve for the biopesticide (R^2: 0.94) was built from 0.5 to 5 μL. Each treatment was performed three times. The 1-octen-3-ol was used as an internal standard with a concentration of 1 μL/mL, since the retention time (22.46 min) appeared before the retention times of the components of the essential oil (Moral Ruiz, 2016).

In addition, the efficiency of the co-extrusion process was calculated according to Eq. (1):

$$E(\%) = \frac{m_{quant}}{m_{load}} X\ 100 \tag{1}$$

where m_{quant} is the mass of biopesticide incorporated into the silo bag and m_{load} is the total amount of this compound loaded before the process.

2.5. Release assays

Samples of biopesticide silo bag and control (10 × 5 cm) were placed inside amber vials (40 mL) hermetically sealed. Then, the vials were incubated in chambers at 15, 25 and 35 °C, common temperatures in Argentine summer and winter, and relative humidity of 60 %. The release of the principal components (menthol and menthone) was monitored at 0, 24, 48, 72, 96 and 168 h (7 days). Samples of the head space were taken with a Hamilton syringe (10 μL) and injected into GC-FID in the conditions mentioned above. Calibration curve (R^2: 0.99) was built by placing 5, 10 and 20 μL of the biopesticide on a sample of silo bag control (2 × 2 cm). The experiments were carried out in triplicate.

2.6. Diffusion coefficient estimation

The simplified mathematical model (Eq. (2)) described by Hamdani, Feigenbaum, and Vergnaud (1997) and Soto-Cantu et al. (2008) was used to calculate the diffusion coefficient *(D)* of the biopesticide released from the inner layer of the silo bag film to the vial head space. The following assumptions were considered: the biopesticide diffuses only to the head space of vials; the concentration of the biopesticide in the film decreases with time; the volume of air in the vial (40 mL) is larger than the volume of the film (1.14 ± 0.16 mL); the release time is considered short.

$$\frac{\mathrm{mF,t}}{\mathrm{mF},\infty} = \frac{2}{\mathrm{L}}\sqrt{\frac{\mathrm{Dt}}{\pi}} \tag{2}$$

where mS_t is the mass of biopesticide diffused at time t, mS_∞ is the total released mass at equilibrium time, *L* is the inner layer thickness and *D* is the diffusion coefficient of the release of biopesticide components. Values of mS_t/mS_∞ were plotted vs $t^{½}$ and *D* was determined from the slope (SS_∞), according to Eq. (3) (Hamdani et al., 1997).

$$D = \frac{\pi}{4}\ (SS_\infty\ \ L)^2 \tag{3}$$

2.7. Insecticidal activity

Adults of *R. dominica* with no sex and age differentiation were used for insecticidal assays. Bioassays were carried out according to Herrera et al. (2018), with some modifications: thus, samples of silo bags (biopesticide and control) with a size similar to those used in the kinetic release experiment were placed in amber vials hermetically sealed (40 mL), with 1 g of rice and ten insects. After 7 days at 25 °C, the mortality percentage (%) was registered. The assays were performed in triplicate.

2.8. Statistical analysis

ANOVA tests were carried out and differences between the averages of the biopesticidal and control silo bag were determined by Tukey's test ($p < 0.05$), using INFOSTAT/Professional software (Di Rienzo et al., 2015). Normality was tested by the Shapiro-Wilk test.

3. Results and discussion

3.1. Characterization of biopesticidal silo bag

A prototype silo bag of 0.17 m wide and 6 m long was manufactured (Fig. 1). The total thickness of the co-extruded films was 235 μm. Individual layers were approximately 78 μm thick. A similar thickness is reported for commercial silo bags comprising three-layers of polyethylene and anti UV additives of approximately 235 μm thick, YPF, S.A. (YPF, 2017).

Table 1 shows the mechanical properties and transmission of light (400–500 nm) in the co-extruded materials. The structure of the silo bag was not affected by the incorporation of the essential oil (7 %) in the inner layer and did not modify the transmission of light and its mechanical properties. Similar values (light transmission (%): 0 and penetration (g-μm): 13) were reported for the commercially manufactured multilayer silo bags (PEBD) between 150 and 180 μm thick (Castellón Petrovich, 2015). The main function of the silo bag is resistance external aggression such as UV radiation (Stavisky, 2019). For this reason, the co-extruded material developed here proved to preserve their morphological and mechanical characteristics.

The presence of the components of the biopesticide in the inner layer of the silo bag was confirmed by FTIR. The spectrum shows characteristic bands associated to LDPE at 2952, 1471 and 729 cm^{-1}, corresponding to (CH_2—CH_2—CH_2) bands. The absorption bands for the biopesticide appear at 3500 cm^{-1}. There is a wide band attributed to the —OH functional group of menthol ($C_{10}H_{20}O$) (60 %). At 1705 cm^{-1} there is a sharp band characteristic of ketones, corresponding to the C═O functional group of menthone ($C_{10}H_{18}O$) (30 %) (Fuertes Ruitón & Munguía Chipana, 2001).

The main components of the biopesticide from silo bag were quantified as 11.57 ± 2.87, 5.85 ± 0.65 and 2.19 ± 0.56 μg/g for menthol, menthone and limonene, respectively (Young Living, 2018) (Fig. 2). Table 2 shows the retention times (RT) of the main compounds of biopesticide identified through retention indices with published data (Adams, 2007). No difference was found in the proportion of these three components after incorporation of the essential oil into the silo bag prepared by co-extrusion process.

On the other hand, the incorporation of biopesticide into the polymeric material by co-extrusion technology showed an efficiency of 27.79 ± 1.09 %. In agreement with our results, Galotto, Valenzuela, Rodríguez, Bruna, and Guarda (2012) reported for thymol a monoterpene phenol with a higher value of boiling point (232 °C), a 30 % incorporation after the extrusion process under the same operational conditions, in a unilayer polymeric matrix of LDPE with antimicrobial properties. In contrast, previous studies showed an efficiency between 1.72 and 14.73 % for biopesticides with physicochemical properties in

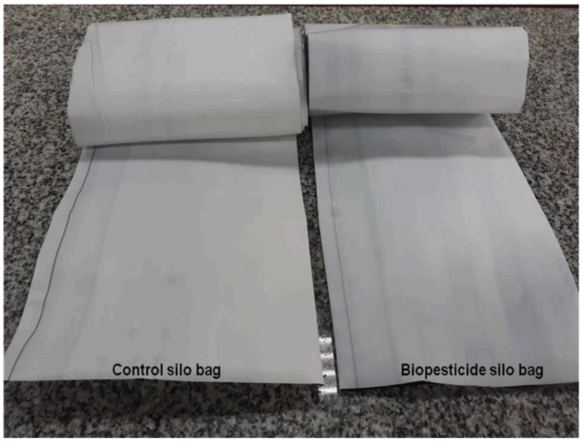

Fig. 1. Silo and control bags manufactured by co-extrusion process.

Table 1
Mechanical properties and transmission of light of manufactured silo bags.

Films	Thickness (µm)	*T (%)	Mechanical properties		
			Penetration (g-µm)	Deformation (%)	Elongation (mm)
Control	250 ± 19.70 ^{a}	0 ^{a}	10 ^{a}	389 ± 48 ^{a}	306 ± 13 ^{a}
Biopesticide silo bag	235 ± 20.70 ^{a}	0 ^{a}	10 ^{a}	402 ± 37 ^{a}	277 ± 1.5 ^{a}

Values show average ± standard deviation in five replicates, similar letters mean no significant differences ($p > 0.05$) between lines.

[*] Light transmission at 400–500 nm.

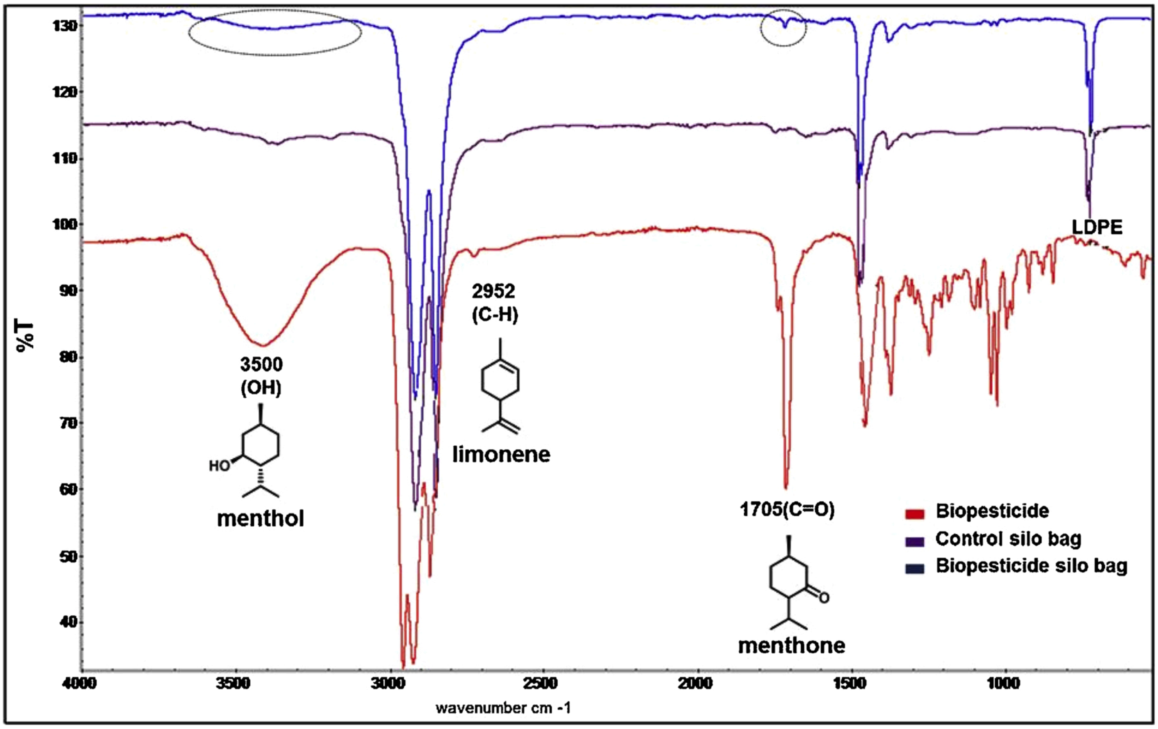

Fig. 2. FTIR spectra for biopesticide, control silo bag and biopesticide silo bag. The chemical structures and functional groups of each compound are included.

Table 2
Chemical constituents of biopesticide (*M. piperita*) silo bag.

Compound	RT*	Percentage (%)	Biopesticide quantified in the silo bag (µg g^{-1}) ^{a}
Limonene	24.4	10	2.19 ± 0.56
Menthone	28.29	30	5.85 ± 0.65
Menthol	29.2	60	11.57 ± 2.87

[*] Retention time on a DB-1 column.

[a] Values show average ± standard deviation of three replicates.

LDPE film by supercritical CO_2 -assisted impregnation for food preservation (Goñi et al., 2017; Herrera et al., 2018). Hence results suggested that the incorporation of biopesticides into the silo bag through the extrusion process improved efficiency as the essential oil is directly mixed with the pellets of polyethylene. The silo bag system could allow better retention of the active agent with a potential increase in release, due to the three-layer structure.

3.2. *Release of the components of the biopesticide from silo bag and diffusion coefficient*

Fig. 3 shows the effect of different temperatures on the release of menthol and menthone from the silo bag, during 7 days of storage. The response was temperature-dependent. At 15 °C the maximum release was lower (1.65 ± 0.37 µg/g), while at 25 and 35 °C it was higher (10.76 ± 0.12 and 9.03 ± 0.02 µg/g, respectively). However, no statistical difference was found between 25 and 35 °C after 7 days of release. Finally, at 168 h under the temperatures of 25 and 35 °C, the components were not completely released, showing a remaining concentration

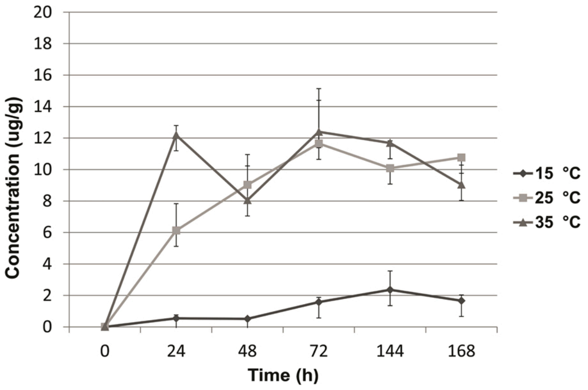

Fig. 3. Effect of temperature on the release of menthol and menthone from silo bag (HDPE + TiO_2/ LDPE + carbon black/LDPE + 7% *M. piperita*), at 15, 25, and 35 °C for 7days (168 h) storage. The values show average ± standard deviation of three replicates.

of 7 μg/g in the silo bag, quantified by GC-FID after extraction in methanol.

The diffusion rate of molecules in a polymeric matrix is regulated by the physicochemical properties of the migrants and the polymer used as the contact surface, in addition to environmental factors such as temperature. The diffusion of menthol and menthone components from LDPE inner layer was calculated according to Eq. (2) (Hamdani et al., 1997). Table 3 shows that *D* value at 15 °C was smaller than those at 25 and 35 °C, in one order of magnitude ($p < 0.05$). Thus, *D* values tend to increase with temperature (Herrera et al., 2018). However, at 25 and 35 °C no statistical differences were found. Granda-Restrepo, Soto-Valdez et al. (2009) showed, in a three-layer active packaging for whole milk powder, the diffusion of α-tocopherol, where *D* values at 30 and 40 °C were similar, although different at 20 °C. However, the molecule of α-tocopherol is bigger than menthol and menthone, hence, *D* values cannot be compared.

On the other hand, Higuera-Barraza et al. (2015) showed the migration of eugenol through co-extruded LDPE film at 5 and 25 °C, reporting *D* values with a similar order of magnitude, but with a higher *D* value at 25 °C. In addition, Kuorwel, Cran, Sonneveld, Miltz, and Bigger (2013) informed *D* values for monoterpenes increasing with the rise in temperature from 15 to 35 °C from the heat-pressed starch-based-films. Therefore, in a unilayer system as polyethylene films, the diffusion of active compounds depends directly on temperature (Graciano-Verdugo et al., 2010; Colín-Chávez, Soto-Valdez, Peralta, Mendoza, & Balandrán-Quintana, 2013).

In this study, a multilayer system developed by extrusion under high-temperature conditions with a mixture of different polymers (HDPE and LDPE) and additives, such as carbon black and TiO_2, could allow better retention of active agents. For this reason, the biopesticide incorporated into the inner layer of silo bag was not completely released, showing no statistical differences in the *D* values between 25 and 35 °C. The release of active agents from packaging depends mainly on the tortuosity of the matrix, which is related to the crystallinity and crosslinking degree and filler additives (Soto-Cantu et al., 2008). Further research should consider the thermal analysis of the biopesticidal silo bag samples.

The storage time of grains (between 6–12 months) in the silo bag depends on management of the system in the field and room temperature; hence, biological activity increases in summer (Bartosik et al., 2008). The diffusion of the biopesticide from the silo bag to the internal environment was higher at 25 and 35 °C, assuring release in summer. Accordingly, the effect of temperature on the release of biopesticide could be used as a tool to control pest populations in the field.

3.3. Insecticidal activity

The insecticidal effect of the silo bag with the addition of essential oil of *M. piperita* against *R. dominica* showed 100 % of mortality during the time assayed, having an estimated concentration of 300 μL/L air (data not shown), while the control silo bag (without biopesticide) showed 0 % of mortality. The essential oils and their major components act as fumigants towards weevils found in stored grain (Herrera et al., 2014). Previous studies showed the fumigant effect of *M. piperita* essential oil on *R. dominica, T. castaneum* and *S. oryzae* with lethal concentration 50 (LC_{50}) between 3.79 and 50.27 μL/L of air, whereas contact toxicity showed LC_{50} values between 3.6 and 8.4 $\mu g/cm^2$ (Lee et al., 2002; Bounoua-Fraoucene et al., 2019). The essential oil of *M. piperita* has two major components, terpene alcohol and ketone, such as menthol and menthone, respectively (Kumar et al., 2011; Moral Ruiz, 2016; Young Living, 2018; Pang et al., 2020). Previous studies showed the repellent effect of menthol against insects in products stored (Pang et al., 2020), while menthone is associated with strong insecticidal activity due to neurotoxic effect through inhibition of AChE enzyme (Herrera et al., 2015). Therefore, many researchers suggested that the presence of a ketone group increases toxicity by interaction with γ-aminobutyric acid receptors (GABA-R) (Abdelgaleil, Mohamed, Badawy, & El-Arami, 2009; Miguel, Sánchez-Borzone, & García, 2018). In addition, Herrera et al. (2015) reported that the fumigant effect of terpene ketones against *S. zeamais* is associated with morphological descriptors such as the branching of the carbon-atom skeleton, while the inhibition of AChE enzyme is linked to electronic parameters such as the orbital electronegativity of the carbonyl group.

Table 3
Diffusion coefficients (*D*) of biopesticide obtained at different temperatures through silo bag film.

Temperatures (°C)	15	25	35
D (cm^2/s) *	$6.4 \pm 0.10 \times 10^{-11a}$	$1.45 \pm 0.17 \times 10^{-10\ b}$	$1.30 \pm 0.20 \times 10^{-10\ \mathbf{b}}$

Values show average ± standard deviation of three replicates, different letters represent significant differences ($p < 0.05$).

* The coefficients were obtained according to Eq. (2), at 15 ($y = 0.001x+0.1348$; R^2:0.59), 25 ($y = 0.0017x-0.0133$; R^2:0.99) and 35 °C ($y = 0.0016x-0.0616$; R^2:0.73), respectively, and 60 % RH.

The silo bag with the added essential oil of *M. piperita* silo bag could therefore be used to control different pests in stored grain.

4. Conclusion

The addition of 7% w/w of *M. piperita* essential oil as a biopesticide to the inner layer of a three-layer silo bag prototype obtained by co-extrusion retained 27.8 % of the main components, improving the efficiency previously obtained with supercritical CO_2-assisted impregnation. The structure of the silo bag was not affected by the incorporation of the essential oil. The insecticidal effect of the biopesticide released from the silo bag showed 100 % mortality against *R. dominica* (primary pest of stored grain) during the time assayed. The diffusion of the biopesticide from the silo bag was higher at 25 and 35 °C, assuring release in summer. As a result, the effect of temperature on the release of biopesticide could be used as a tool for pest management in the field. The incorporation of biopesticide by co-extrusion technology (low-cost and efficient machinery) in the inner layer of a silo bag could help to replace synthetic pesticides, preventing biotic infestation and reducing grain loss.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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