Article

JOURNAL OF ELASTOMERS & PLASTICS

Journal of Elastomers & Plastics
1–12

DOI: 10.1177/0095244317729554
journals.sagepub.com/home/jep

SAGE

Recyclable amitraz-ethylene vinyl acetate strips used for beehives treatment against *Varroa destructor*

Federico Karp, Julio A Luna and Luciano N Mengatto

Abstract

In this work, a new recyclable ethylene-vinyl acetate (EVA)-based strip impregnated with amitraz (AMZ) was prepared, characterized, and evaluated for the treatment of *Apis mellifera* against *Varroa destructor*. Bees are important for natural pollination, honey, and related goods production. *Varroa destructor* is currently considered one of the major pests and important efforts around the world are focused on methods for varroasis treatment. The procedure of strips preparation presented in this work consisted of two steps: impregnation and molding of impregnated pellets. Differential scanning calorimetry and gas chromatography–mass spectrometry analyses confirmed that AMZ molecule resisted the impregnation and molding conditions. The strips were sufficiently strong to resist destruction by the bees. The final infestation was lower in the hives treated with AMZ/EVA strips than in those treated with commercial strips. In order to check the possibility of recycling, strips were cut into little pieces and were subjected to total AMZ extraction. Finally, the fragments were exposed to re-impregnation and molding. The strips prepared after the recycling process presented the same shape and AMZ load than fresh strips.

Keywords

Ethylene-vinyl acetate, *Varroa destructor*, strips, amitraz, recyclable

Laboratorio de Química Fina, Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional del Litoral (CONICET-UNL), Centro Científico Tecnológico, Santa Fe, Argentina

Corresponding author:
Luciano N Mengatto, Laboratorio de Química Fina, Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional del Litoral (CONICET-UNL), Centro Científico Tecnológico, Colectora Ruta Nac. No. 168, Santa Fe, Argentina.
Email: lmengatto@santafe-conicet.gov.ar

Introduction

Honey bees, *Apis mellifera*, are essential pollinators for the maintenance of natural biodiversity and agriculture. The hives can be easily maintained and transported between different productions zones allowing pollinator-dependent crops reproduction.[1,2]

Like all living systems, honey bees die and this can be due to many causes: wintering mortalities, starvation, queen-related issues, parasites, viruses, and bacteria. There is a growing consensus that colony mortality is the product of multiple factors, both known and unknown, acting singly or in combination.[3] Three species of parasitic bee mites are of economic importance due to their destruction capacity on honey bee colonies around the world. Between them, the mite *Varroa destructor* is currently considered the major pest of honey bees. The pathology it causes is commonly called varroasis. Some signals of its presence in a hive are as follows: mites are seen on white pupae, colonies are weak with a spotty brood pattern, and adults present morphological deformities. Additionally, the mites can affect the bee's immune system leading to other kinds of pathologies.[4] Because *V. destructor* populations increase in proportion to the available bee larvae, the mites can quickly overrun a colony. Without treatment, infested colonies usually die within 6 months to 2 years.

Insects, particularly bees, are the primary pollinators of most agricultural crops and wild plants. It is noteworthy the negative impact of their loss on local, regional, and even world economy. The magnitude of the matter is such that a group of scientific professionals are working in a world network for the prevention of honey bee colony losses. In this way, one of its task forces is *V. destructor* control which works on developing and encouraging sustainable solutions for management of this ectoparasitic mite.[5]

Chemical and biological/cultural controls are some of the methods proposed to treat varroasis. On one hand, a wide range of substances including oils (thymol, eucalyptol), pyrethroids (fluvalinate, flumethrin), organophosphates (coumaphos), amidines (amitraz), and organic acids (formic, oxalic, lactic) are used in chemical methods. On the other hand, tobacco smoke or smoke from other plant materials that cause mite knockdown and heat are used as alternatives treatment over chemical methods. In addition, bees remove mites from each other (grooming) and eliminate dead or dying brood which reduce the mite levels.[6–8] Some comparisons were done among mites control options to identify the best ones. However, more comparative studies are required to be performed under laboratory and field conditions taking into account ecological situations and all possible factors impacting treatment efficacy. Chemicals can be applied in the form of gels, vermiculite tablets, plastic polymer strips, combustible strips, spray, or mixed with syrup as a suspension. Between the main differences, other than the composition of the formulation and cost, it is notable the method, amount and interval of application, and the need or not of removal. For example, strips based on plastic polymer and containing amitraz (AMZ) were placed into the hive and removed after 8 weeks.[9] Applied in this manner, chemicals are released slowly and dispersed by adult bees. The bees come in contact with the strips as they move, and then pass the chemical on to other bees as they rub against each other in the hive. Examples of the polymer materials include polyvinyl chloride, polyvinylpolypyrrolidone, polyurethane,

and ethylene-vinyl acetate (EVA).[10–12] These polymers are widely used in medicine and pharmaceutical applications due to their biocompatible properties. Researchers study the preparation and characteristics of drug delivery systems based on these materials for human and animal health treatment.[13,14] In addition, other types of materials as wood and cardboard are used. However, these kinds of materials are susceptible to different natural factors (humidity, heat, insect attacks).

The continuous worldwide problem of bee mites makes necessary the research on detecting, monitoring, and controlling them. In this context, the aim of this work was to prepare polymer-based recyclable strips impregnated with AMZ intended for the treatment of *A. mellifera* against *V. destructor*.

Materials and methods

Materials

AMZ (purity > 98%) was supplied by MSD Salud Animal (Argentina) and ethylene-vinyl acetate (ELVAX®460, EVA) was obtained from DuPont (Argentina). The organic solvents (Ciccarelli, Argentina) were analytical grade. Methanol and acetonitrile were HPLC grade (JTBaker, USA). The water was ultrapure and was obtained using a Milli-Q lab water system.

Quantification of AMZ by HPLC

The quantification of AMZ was carried out with an HPLC system (Shimadzu model Prominence Serie 20A) with UV detection by diode array. The chromatographic system and conditions of analysis were as follows: a C18 column, the mobile phase was a mixture of methanol and water (90:10), oven temperature 30°C and the absorbance was measured at wavelength of 314 nm.

AMZ was dissolved in acetonitrile (500 ppm). Then, this solution was diluted to prepare the standard solutions which were filtered through a membrane filter of 0.45 μm before HPLC analysis. In order to verify the linearity of the analytical procedure within a concentration range of 5–60 ppm of AMZ, five concentration levels were prepared and analyzed three times each. The calibration curve (peak area vs AMZ concentration) was fitted to a straight line, using linear regression analysis.

Manufacture of the strips and quantification of AMZ load

Manufacture of the strips. AMZ was incorporated in the EVA pellets by impregnation.[11,14] Pellets were immersed into a solution of AMZ in an organic solvent. After mixing, the solvent was evaporated with a rotary evaporator, causing the entrance of the acaricide into the pellets. Then, the impregnated pellets were placed in an oven until dryness.

During the molding step, impregnated pellets were placed into a mold and were heated in an oven at 130°C until the material was in the molten state. Then, the mold was placed in a pneumatic hot plate press machine (130°C, 30 min). Finally, the mold was retired from the oven, cooled to room temperature, and the strips were easily removed.

Quantification of AMZ load. In order to find out the conditions to extract all the AMZ impregnated in the strips, a screening of variables that affect extraction was carried out. The variables were as follows: extraction time (1–5 h), stirring (yes: 100 rpm – no: without stirring) and temperature (25–37°C). Strips from different batches of manufacture were cut into little pieces and were immersed into a bottle containing the same organic solvent used for impregnation. Eight different experiences of extraction were carried out based on the combination of the three variables. After extraction, the solution was heated under reduced pressure until the solvent evaporation left a solid residue. The solid was dissolved in acetonitrile and assayed by HPLC. The response variable *extracted AMZ* (%) was calculated as

$$extracted\ AMZ\ (\%) = \frac{extracted\ AMZ(\mathrm{g})}{theoretical\ AMZ(\mathrm{g})} \times 100 \tag{1}$$

where *extracted AMZ* (g) was the amount obtained from HPLC assays and *theoretical AMZ* (g) corresponds to

$$theoretical\ AMZ\ (\mathrm{g}) = \frac{AMZ\ (\mathrm{g})}{AMZ + EVA_{imp}(\mathrm{g})} \times AMZ + EVA_{strip}(\mathrm{g}), \tag{2}$$

where AMZ (g) was the weighted amount of acaricide for impregnation, $AMZ{+}EVA_{imp}$ (g) was the weighted amount of acaricide and EVA pellets after impregnation, and $AMZ + EVA_{strip}$ (g) was the weighted amount of pieces of cut strip subjected to extraction.

Strips recycling. In order to check the possibility of recycling, fragmented strips were immersed into a bottle containing methylene chloride until total AMZ extraction. Then, the fragments were cut into more little pieces and were subjected to re-impregnation and molding to prepare new strips. Finally, the strips were tested for AMZ load.

Control of AMZ stability during impregnation/extraction procedures

Differential scanning calorimetry (DSC): DSC thermograms were obtained on samples of about 10 mg with a Mettler TA 3000 instrument equipped with a DSC 30 measuring cell. The samples were AMZ solid reactive, EVA pellets, and a portion of a strip. Each sample was subjected to the following heating cycles: from –30 to 110°C at a rate of 10°C/min, from 100 to –30°C at a rate of 3°C/min, and finally from –30 to 150°C at a rate of 10°C/min.

Gas chromatography–mass spectrometry: The experiments were performed using a GC Clarus 600-MS Clarus 600T (Perkin Elmer, Shelton, USA) system. The samples were dissolved in methylene chloride and were introduced across an Elite-5 column (5% diphenyl/95% dimethyl polysiloxane, 0.25 mm i.d. × 30.0 m, film thickness 0.25 μm) under a temperature program that began at 60°C, held for 1 min, then increased at 15°C min^{-1} to 280°C with a 5-min hold. The injection port and detector temperature was 280°C. Helium was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. The samples were AMZ solid reactive and AMZ extracted from a strip. Identification was performed with the NIST 15 Mass Spectral Library.

Apiary trial

The trial was carried out in an apiary of thirty hives of *A. mellifera* bees in the rural area of San Wendelino (San Jerónimo Norte, Santa Fe, Argentina). Sixteen hives were organized as follow: 10 hives which were treated with AMZ/EVA strips (one strip per hive) and 6 hives which were treated with commercial strips made of cardboard (Varrotraz, extended-release strips containing 1 g of AMZ per strip, one strip per hive was used for treatment). In every case, each strip was hanged in the heart of the beehive, between two comb frames using a toothpick. The alcohol shaking method was used for counting mites.[15,16] Infestation level (%) determined as the ratio of the amount of counted mites to the amount of counted bees was calculated for each hive before and after treatment (28 days). Then, the strips were removed and were subjected to the evaluation of residual AMZ.

Results

The evaluation of performance of the quantification method showed that the model can explain approximately 98.06% (R^2) of the variation in the response variable. The corresponding correlation coefficient (R) was 0.9903 and indicated a large relationship between the variables. The p-value of the model was smaller than 0.05 ($p = 0.0000$) and indicated a statistically significant relationship between peak area and AMZ concentration for a significance level of 95.0%. The p-value of the lack-of-fit was higher than 0.05 ($p = 0.3691$) and indicated that the proposed lineal model fitted well for a significance level of 95.0%. Therefore, the analytical procedure can be used to quantify AMZ.

In general, polymeric systems can be manufactured in two ways: the strips are painted with a viscous mixture containing the acaricide or they are impregnated with a solution of the chemical and an organic solvent. In combination with the acaricide, some systems include other substances which act as stabilizers or possess the property to attract bees. The procedure of AMZ/EVA strips preparation consisted of two steps: impregnation and molding of EVA impregnated pellets. The time needed to complete it depends on the amount of pellets to be impregnated and molded. As an example, to prepare 10 AMZ/EVA strips, the procedure took 2 days. The amount of EVA pellets impregnated depended on the number of the strips to be prepared. The mold was specifically designed to obtain strips of 15.0 × 2.5 × 0.3 cm (L × W × T) due to commercial strips are of similar dimensions (Figure 1). The weight of each strip was approximately 10.0 g; 1.0 g corresponds to AMZ and the rest to polymer. Due to the solvent could be completely eliminated before molding, there was no unusual smell in the strips. In addition, strips were flexible and easily manipulated during AMZ extraction and apiary assays.

Table 1 shows the independent factor combinations and response values of the screening phase when optimization of AMZ extraction was carried out. The analysis of variance showed that the effect of the temperature on the *extracted AMZ* (%) ($p < 0.05$) was significant. The effects of extraction time ($p = 0.1374$) and stirring ($p = 0.3274$) were not significant in the evaluated levels; 1–5 h for extraction time and yes: 100 rpm – no: without stirring for stirring.

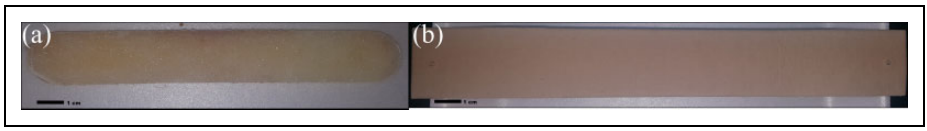

Figure 1. (a) AMZ/EVA strips and (b) commercial strips made of cardboard. AMZ: amitraz; EVA: ethylene-vinyl acetate.

Table 1. Screening design to study variables affecting extracted AMZ (%).

Experiment	Temperature (°C)	Stirring (rpm)	Extraction time (h)	Extracted amitraz (%)
1	25.00	100	1.00	99.22
2	25.00	0	5.00	109.76
3	37.00	0	1.00	78.91
4	37.00	0	5.00	71.38
5	37.00	100	1.00	72.48
6	25.00	100	5.00	113.01
7	25.00	0	1.00	103.53
8	37.00	100	5.00	68.03

AMZ: amitraz.

The strips prepared after the recycling process presented the same form, dimension, and AMZ load than fresh strips.

DSC thermograms (Figure 2) show that there were no important interactions between AMZ and EVA that could result in retention of the acaricide molecules in the strips decreasing the release. In the thermograms of EVA pellets and of the portion of strip, an endothermic peak corresponding to the melting temperature of EVA was observed around 88°C.

As was mentioned, the mold was designed to obtain strips with specified dimensions. The AMZ load (1 g per strip) was accomplished controlling the amount of pellets that can fill the mold. Nevertheless, the amount of extracted AMZ was of 1 g per strip; gas chromatography–mass spectrometry experiments were performed in order to evaluate the stability of AMZ during impregnation and molding. Figure 3 shows GC chromatograms corresponding to AMZ solid reactive (Figure 3(a)) and AMZ extracted from a strip (Figure 3(b)). Both chromatograms presented the peak at approximately 17 min related to AMZ. Figure 4 shows mass spectra corresponding to AMZ solid reactive (Figure 4(a)) and AMZ extracted from a strip (Figure 4(b)). In the mass spectra, the molecular ion peak of AMZ was located at 293 m/z and was in agreement with the NIST 15 Mass Spectral Library. AMZ molecule resisted the impregnation and molding conditions.

For the purpose of evaluating the efficacy of the new AMZ/EVA strips in the treatment of varroasis and the reception by the bees in their hive environment, an apiary trial was conducted. The initial infestation of the 16 hives was in the range of 4–6%. The final infestation was 0% and 4.16% in the hives treated with AMZ/EVA and with the commercial strips, respectively. Figure 5 shows pictures of AMZ/EVA (Figure 5(a)) and commercial strips (Figure 5(b)) after their use in hives during 28 days. AMZ/EVA strips

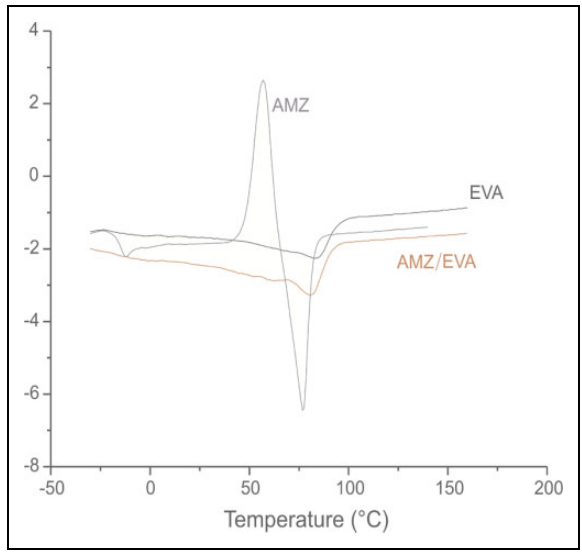

Figure 2. DSC thermograms of AMZ solid reactive (AMZ), EVA pellets (EVA), and a portion of a strip (AMZ/EVA). DSC: differential scanning calorimetry; AMZ: amitraz; EVA: ethylene-vinyl acetate.

were subjected to the extraction process to the evaluation of residual AMZ. The average percentage of AMZ was 85%.

Discussion

Nevertheless, resistance to AMZ was reported, it is still currently used to treat varroasis and other parasitic problems. EVA is one of the mostly used polymers in the preparation of drug delivery systems for human and animal health treatment. It is inexpensive, biocompatible, and biologically inert than other nondegradable polymers, such as silicone and polyurethane, used in the fabrication of veterinary drug delivery systems. EVA is a thermoplastic polymer; therefore, it can be processed by conventional thermoplastic processing techniques, such as injection molding, sheet and shape extrusion, and casting. In addition, it does not require a curing process. Then, based on these properties, AMZ and EVA were selected for the manufacturing of a new strip for varroasis treatment. A composition containing AMZ as acaricide and EVA was reported for varroasis treatment.[12] In this case, AMZ was incorporated to melted EVA and then the mixture was spread over a cardboard surface. The strips were flexible, presented good adherence of the acaricide to the surface, and were ready to use in a hive. Cappadoro and Luna[14] reported a method for the development of an injection molded EVA copolymer intravaginal insert for the delivery of progesterone to cattle. In this work, pellets were impregnated with the hormone and then molding by injection. In the current work, to avoid the use of another component as support, EVA pellets were first impregnated and the strips were obtained by using a pneumatic hot plate press machine. As another

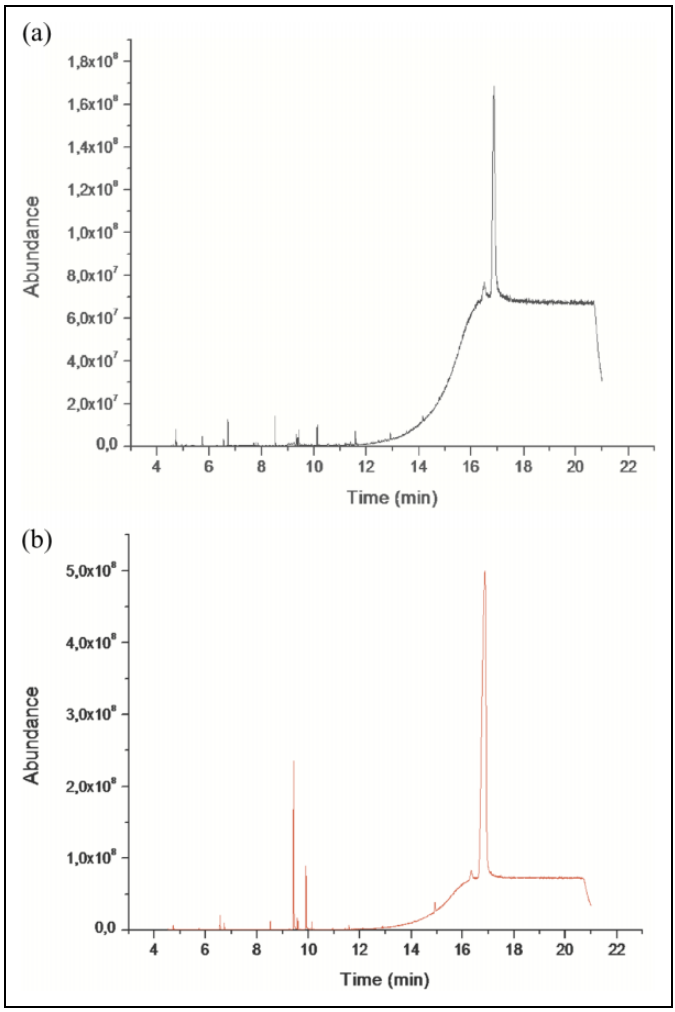

Figure 3. GC chromatograms corresponding to (a) AMZ solid reactive and (b) AMZ extracted from a strip. AMZ: amitraz.

alternative of manufacture, pellets without AMZ were used to obtain strips. Subsequently, the strips were immersed into a solution of AMZ in an organic solvent. After the solvent was completely evaporated, the strips presented an irregular form having a wavelike surface and edge. In addition, the AMZ load per strip was heterogeneous. Therefore, this alternative was discarded and the method consisting of molding of impregnated pellets was used. It was mentioned that as an example to prepare 10 AMZ/EVA strips, the procedure took 2 days. This time can be reduced by the use of extrusion method instead of oven/hot press machine. A reduction in manufacturing cycle time is important during scaling up.

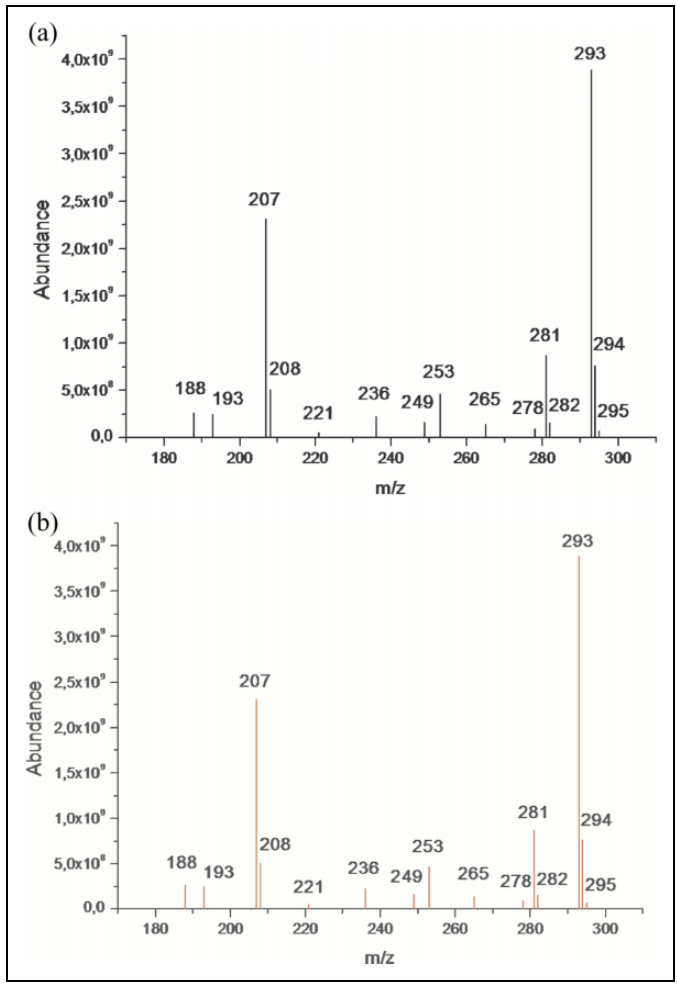

Figure 4. Mass spectra corresponding to (a) AMZ solid reactive and (b) AMZ extracted from a strip. AMZ: amitraz.

According to the results of the screening experiments, the effect of the temperature on the extracted AMZ was significant and the type of influence was negative. Then, an increase in temperature from 25 to 37°C decreased the percentage of AMZ extracted. In the impregnation step during the manufacture of the strips, the organic solvent used must cause swelling but not dissolution of the pellets. In addition, it must have a low boiling point to evaporate easily, forcing the entrance of AMZ into the pellets. On the other hand, for the quantification of AMZ load, methylene chloride was used as extraction solvent and its boiling point (approximately 40°C) is near to the superior level of the variable evaluated ($T = 37$°C). Therefore, an effect caused by evaporation of the solvent was proposed as explanation. During extraction, a phase transition from liquid to vapor could take place

Figure 5. (a) AMZ/EVA strip and (b) commercial strips; after their use in hives during 28 days of varroasis treatment. AMZ: amitraz; EVA: ethylene-vinyl acetate.

affecting the gradient of AMZ concentration. Consequently, the driving force necessary for a complete extraction could not be strong enough. This is a possible explanation for the negative impact of the increase in temperature on the AMZ extracted. The effects of extraction time and stirring were not significant in the evaluated levels, but the percentage of AMZ extracted was 100% with or without stirring during 1 or 5 h of extraction. Therefore, the conditions of extraction selected were as follows: 1 h, without stirring at 25°C. Taking into consideration that a fabrication method and quantification of AMZ load were performed, the possibility for strips recycling was tested. Strips ($n = 3$) were subjected to total AMZ extraction; the fragments were cut into more little pieces and were exposed to re-impregnation and molding as if they were pellets. The strips were flexible, with the form and dimensions as expected and an AMZ load of approximately 1 g per strip. This finding suggests the possibility of recycling, decreasing the cost of manufacture, and avoiding residues to be treated by beekeepers. In addition, this result points out the idea of study the possibility of recycling other similar commercial systems based on thermoplastic polymers such as preventive tick collars for dogs which also contain AMZ.

A serious problem of the chemical options is that repeated exposure to the same pesticides selects for resistant mites.[16] Therefore, one of the tools is acaricide rotation, and in this course of action, it is possible that the method of strips preparation proposed in the current work could be used for other chemical such as coumaphos.

At the beginning of the apiary trial, the average infestation was 5%. Similar results were reported when evaluating the efficacy of other parasitic control method such as pulverized sugar dusting on knocking-down *V. destructor* mites in honey bee colonies.[17] At this point, it is worth to mention that it is difficult to compare results of different studies since researchers use various alternatives to assess treatment efficacy. The percent of dead mite or final infestation after treatment application can be used as standard indicators for treatment efficacy when a comparison between different treatments is carried out.[8] The final infestation was 0% and 4.16% in the hives treated with AMZ/EVA

and with the commercial strips, respectively. Nevertheless, commercial strips, which are made of cardboard, were severely damaged at the end of the treatment (Figure 5(b)). This finding suggests the following interpretation: the AMZ/EVA strips were sufficiently strong to resist destruction by the bees in comparison to commercial strips and they were able to control mite infestation, but it is not possible to confirm that they were better than commercial strips. The strips work by direct contact while they are suspended. The bees can walk on both sides of the strips covering their bodies with the active principle. AMZ migrates from the inside of the strip to the surface upon contact with bees, which pick up AMZ molecules causing a concentration gradient. Bees distribute the AMZ through contact with each other. Finally, mites on the bees are exposed to the acaricide. It is important to note the fact that bees mortality did not increase during the application of treatment, meaning that AMZ/EVA strips were harmless for bees. In addition, beekeepers showed conformity with AMZ/EVA strips regarding design, easy applicability, and results.

The recommended dosage is 1 g of AMZ per beehive due to effectiveness is not guaranteed with a lower dose and a higher dose may increase the risk of leaving residues. The possibility of using the AMZ strips in the colonies containing brood is clearly an advantage. In addition, releasing AMZ for a long time allows the acaricide to act on the successive parasite generations.[9] Regarding toxicity, studies were carried out to evaluate AMZ residue in honey. No or permitted low residue values were detected, even when AMZ is directly administered in the hive. This is probably due to its fast degradation.[18,19]

Conclusion

New AMZ/EVA strips for varroasis treatment were prepared, characterized, and evaluated at laboratory scale. The procedure of strips preparation consisted of two steps: impregnation and molding of EVA impregnated pellets. With this procedure, the strips can be recycled. The treatment with AMZ/EVA strips showed good efficacy in the control of infestation by *V. destructor*, strips were harmless for bees and resisted their natural attack. Additional experiments should be carried out with more hives and commercial strips made of a material resistant to the destructive behavior of bees.

Acknowledgements

The authors thank the Group of Polymers and Polymerization Reactors (INTEC) for technical assistance.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional del Litoral (UNL) for the financial support.

References

1. Potts S, Biesmeijer J, Kremen C, et al. Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 2010; 25: 345–353.
2. Aebi A and Neumann P. Endosymbionts and honey bee colony losses? *Trends Ecol Evol* 2011; 26: 494.
3. Neumann P, Yañez O, Fries I, et al. Varroa invasion and virus adaptation. *Trends Parasitol* 2012; 28: 353–354.
4. Le Conte Y, Ellis M, and Ritter W. Varroa mites and honey bee health: Can Varroa explain part of the colony losses? *Apidologie* 2010; 41: 353–363.
5. Neumann P and Carreck N. Honey bee colony losses. *J Apicult Res* 2010; 49: 1–6.
6. Sammataro D, Gerson U, and Needham G. Parasitic mites of honey bees: life, history, implications, and impact. *Annu Rev Entomol* 2000; 45: 519–548.
7. Mahmood R, Waghchoure E, Raja S, et al. Control of *Varroa destructor* using oxalic acid, formic acid and Bayvarol® strip in *Apis mellifera* (Hymenoptera: Apidae) colonies. *Pak J Zool* 2012; 44: 1473–1477.
8. Abou-Shaara H. Continuous management of varroa mite in honey bee, *Apis mellifera,* colonies. *Acarina* 2014; 22: 149–156.
9. Semkiw P, Skubida P, and Pohorecka K. The amitraz strips efficacy in control of *Varroa destructor* after many years application of amitraz in apiaries. *J Apic Sci* 2013; 57: 107–121.
10. Robert JE, Champseix H, Maby J, et al. Acaricidal composition suitable for use against varroatosis in bees and device containing same. United States Patent. US005230894A, USA, 1993.
11. Arther R, Grissom J, Pennington R, et al. An improved process for combating parasitosis in bee colonies. World Intellectual Property Organization. WO 00/72683A2, 2000.
12. Higes Pascual M and Prat Aran J. Dispositivo para combatir la varroosis de las abejas. Oficina Española de patentes y marcas. ES2251304A1, USA, 2006.
13. Yasin MN, Svirskis D, Seyfoddin A, et al. Implants for drug delivery to the posterior segment of the eye: a focus on stimuli-responsive and tunable release systems. *J Control Release* 2014; 196: 208–221.
14. Cappadoro A and Luna J. Development of an injection molded ethylene-vinyl acetate copolymer (EVA) intravaginal insert for the delivery of progesterone to cattle. *Anim Reprod Sci* 2015; 158: 104–108.
15. Hood M and McCreadie J. Field tests of the varroa treatment device using formic acid to control *Varroa destructor* and *Acarapis woodi*. *J Agr Urban Entomol* 2001; 18: 87–96.
16. Dietemann V, Nazzi F Martin S, et al.Standard methods for varroa research. In: Dietemann V, Ellis JD, and Neumann P (eds) *The COLOSS BEEBOOK, Volume II: standard methods for Apis mellifera pest and pathogen research*, 2013. http://dx.doi.org/10.3896/IBRA.1.52.1.09
17. Stanimirovi Z, Nevenka A, Jevrosima S, et al. The influence of pulverised sugar dusting on the degree of infestation of honey bee colonies with *Varroa destructor*. *Acta Vet-Beograd* 2011; 61: 309–325.
18. Floris I, Satta A, Garau V, et al. Effectiveness, persistence, and residue of amitraz plastic strips in the apiary control of *Varroa destructor*. *Apidologie* 2001; 32: 577–585.
19. Marcangeli J, García MC, Vega C, et al. Estudio sobre la eficacia a campo del Amivar® contra *Varroa destructor* (Mesostigmata: Varroidae) en colmenas de *Apis mellifera* (Hymenoptera: Apidae). *Rev Soc Entomol Argent* 2005; 64: 29–33.