International Journal of Food Science and Agriculture, 2022, 6(4), 433-442
https://www.hillpublisher.com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Advanced Integrated Pest Management: Chemical Control *Via* Microencapsulation

Borjana Lubura*, Miguel Gimeno

Jesmond Bioscience GmbH Weimarerstrasse 104, Vienna 1190, Austria.

How to cite this paper: Borjana Lubura, Miguel Gimeno. (2022) Advanced Integrated Pest Management: Chemical Control *Via* Microencapsulation. *International Journal of Food Science and Agriculture*, 6(4), 433-442.
DOI: 10.26855/ijfsa.2022.12.012

Received: October 30, 2022
Accepted: November 28, 2022
Published: December 30, 2022

***Corresponding author:** Borjana Lubura, Jesmond Bioscience GmbH Weimarerstrasse 104, Vienna 1190, Austria.
Email: bl@jesmond.com

Abstract

The goal of the development of the current formulation based on 1R-trans Phenothrin and Prallethrin was to show how the microencapsulation can be applied to control the release rate in form of Capsule Suspension (CS) versus "free" non-encapsulated form, therefore to obtain a safer, more sustainable and less toxic biocidal product for its use in household or certain agricultural areas. In this paper, we primarily present the analytical data for the determination of the release rate by applying our microencapsulation technology versus the release of the free material including the biological data for the residual efficacy. In general, this approach represents an effective tool to describe the characteristics of the microcapsule wall in our CS formulation. The release rate was quantified by adopting the Franz cell diffusion method (OECD Test Guideline 428) in order to provide information on release of the active in CS form by using a membrane with a pore size of 0.20 µm.

Keywords

Type of formulation, capsule suspension, 1R-trans Phenothrin, Prallethrin, insecticide

1. Introduction

The further development of pest management strategies coupled with specific active ingredients (A.I.s), increases the success rate of insect pest's eradication, using the controlled release concept of a formulated biocide. Certain biocidal products with a controlled release of A.I. and safer form of formulation based on Capsule Suspension (CS) belong to different types of microencapsulation techniques. Several of these products that deliver the release of A.I. by triggering of external forces like electric relay, heating, destruction of capsules' membranes, evaporation in function to humidity, coacervation release etc. are not based on the same principle as the microcapsulation technique mentioned in this paper. The application of free materials in conventional formulations in a form of EC (Emulsifiable Concentrate), D (Dust), EW (emulsion in water) or SC (Suspension Concentrate) provides a 100% release of the A.I.s at time zero - representing a first order release - therefore creating an over dosis well superior to the lethal dose.

In case of a zero order release - in which the insecticide is released at a constant rate - the capsules will release steadily only the required amount of active ingredient after application in a controlled release manner (Figure 1), and will biodegrade after some weeks. It offers several advantages such as the reduction in the frequency of insecticide re-application.

In this regard, when insecticides are applied in a larger quantity for an extended period of time, insect pests can become more resistant [1].

Controlled release Capsule Suspension formulations are designed to improve the delivery of the active ingredient while release of a "free" active ingredient based on first order kinetics can lead also to insecticide dissipation in the environment [2].

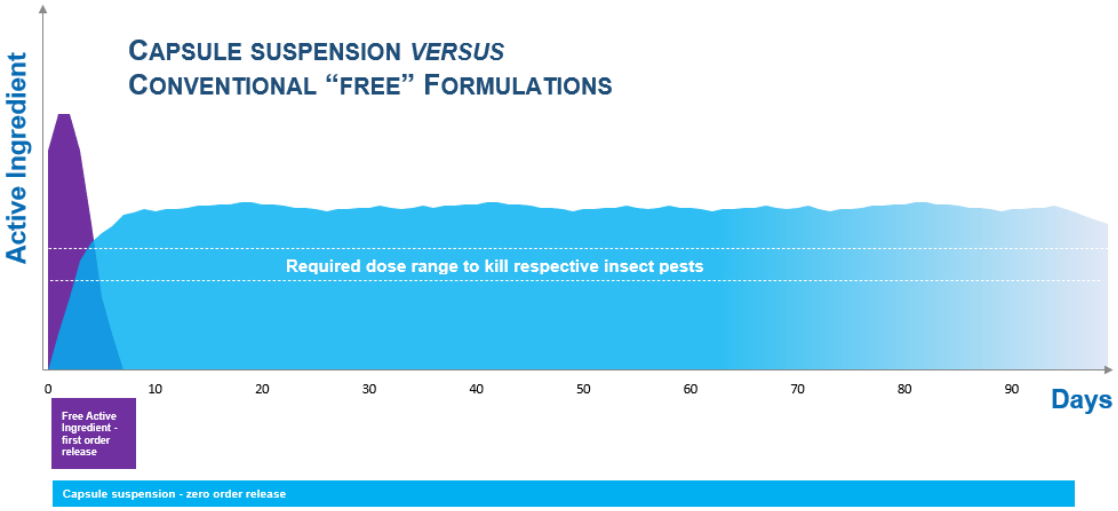

Figure 1. Comparison between formulations of different release kinetics.

Development of innovative microencapsulated sprayable formulations enables their safer application by reducing toxicity or environmental pollutions [3] since no high loading of the microencapsulated A.I. in the formulated product is required.

The combination of alternate classes of A.I.s with different mode of action and the use of microencapsulation technique enhance the killing effect of insect population, and ensure a residual effect avoiding the overdose of the lethal level of the applied A.I.

The use of formulations with a controlled release provides a steady application of A.I. at a constant level avoiding the peak application of the A.I. that will produce the non-desired effect of creation of resistance.

In practice, this technology with a controlled release allows fewer applications avoiding unnecessary overuse of insecticides.

The formulated product described in this paper, contains two different A.I.s, 1R-trans Phenothrin (PHE) at a concentration of 0.1% in a Capsule Suspension form and Prallethrin (PRA) as emulsion in water at the concentration of 0.01%.

The process of making the capsule suspension was based on interfacial emulsion polymerization of oil in water, followed by wall formation of a cross-linked polymer which was a combination of Polyurea and Glycoluril, by crosslinking of their reactive groups at the interphase of water and oil [4].

This formulated product shows some of the key benefits of the technology, including a fast and residual efficacy, and a controlled release rate with less frequent re-application intervals.

In addition, there were no toxicity concerns toward human or warm-blooded organisms present for the formulated product which findings were based on acute oral toxicity study [5] and acute inhalative study [6].

The studies main focus presented in this paper was to show differences in release rate between the free active ingredient and the formulated product as Capsule Suspension and to give an overview of results for the sustainable efficacy profile.

Therefore, an *in vitro* release testing method - the Franz diffusion cell system - was applied [7].

In general, this kind of *in vitro* release testing reflects the combined effect of several physical and chemical properties of the tested product, including solubility in order to assess the quantity of the released active ingredient over a certain period of time.

The efficacy testing was done in two sets: in the laboratory in a test chamber with materials simulating the real conditions of use against 12 different insect pests in total [9] and in the field against 14 different insect pests in total [10].

2. Materials and methods

2.1 *IN VITRO RELEASE TESTING*

2.1.1 Preparation of calibration solution

The analytical standards of 1R-trans Phenothrin and Prallethrin were weighed, dissolved and diluted according to the expected concentrations as given in table 1 and 2. The analytical standard stock solutions were homogenized in an ultrasonic bath for 15 minutes. The analytical standards were prepared in triplicate using water and isopropyl alcohol (IPA).

Table 1. Preparation of the calibration solutions – 1R-trans Phenothrin

ASTD Stock solution		Concentration [mg/mL]	Purity [wt-%]
ASTD stock solution	52.97 mg 1R-trans PHE in 50 mL 80% H_2O/20% IPA	1.00	94.4
	Calibration solutions	**Concentration [mg/mL]**	
Calibration solution 1	0.20 mL ASTD stock solution to the total of 1.00 mL with 80% H_20 / 20% IPA	1R-trans Phenothrin: 0.2	
Calibration solution 2	0.20 mL calibration solution 1 to the total of 1.00 mL with 80% H_20 / 20% IPA	1R-trans Phenothrin: 0.02	
Calibration solution 3	0.02 mL calibration solution 2 to the total of 1.00 mL with 80% H_20 / 20% IPA	1R-trans Phenothrin: 0.002	

Table 2. Preparation of the calibration solutions – Prallethrin

ASTD Stock solution		Concentration [mg/mL]	Purity [wt-%]
ASTD stock solution	6.75 mg PRA in 50 mL 80% H_2O/20% IPA	0.13	96.3
	Calibration solutions	**Concentration [mg/mL]**	
Calibration solution 1	0.20 mL ASTD stock solution to the total of 1.00 mL with 80% H_20 / 20% IPA	Prallethrin: 0.026	
Calibration solution 2	0.026 mL calibration solution 1 to the total of 1.00 mL with 80% H_20 / 20% IPA	Prallethrin: 0.003	
Calibration solution 3	0.003 mL calibration solution 2 to the total of 1.00 mL with 80% H_20 / 20% IPA	Prallethrin: 0.000	

2.1.2 Franz Cell diffusion method

The release rate method represents an effective tool to evaluate the characteristics of CS formulations in order to design the controlled release pattern of A.I.s. to differ the wall composition of the microencapsulated A.I.

The most common *in vitro* release testing method employs a Franz diffusion cell system, as depicted in Figure 2 and can be used with a synthetic membrane or even with a recuperated human or animal skin.

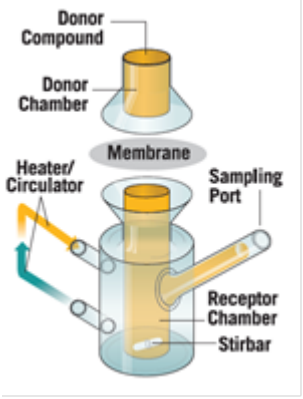

Figure 2. Diffusion cell test with PermeGear Franz cell.

In the case of 1R-trans Phenothrin CS and Prallethrin EW, this method can be used to study the release rate from a matrix of an aqueous capsule suspension. This release is defined as a function of time and the level of active ingredient before starting the release which can be attributed to free material.

The study has been primarily focused to determine the release rate of both technical grade A.I.s, 1R-trans Phenothrin and Prallethrin for a specific time while using a mixture of an organic solvent (IPA) with water (50% H_20 / 50% IPA). For the formulated product, a direct dispersion in water was applied.

In this regard, four sample preparations of the formulated product and two samples of the technical grade of both A.I.s have been tested in order to obtain reproducible analytical results.

The quantity of each sample of the formulated product containing 0.1% of PHE and 0.01% of PRA (SPT01 RTU PH08 141015, PH09 141015, PH10 141015 and PH11 141015) was 5000 mg, dissolved in 5 mL water (table 3) in the donor chamber and by stirring the receptor fluid on the bottom, the free active ingredient has diffused through the

membrane filter into the receptor chamber. This chamber was tempered at 30°C by a heating circulator. The membrane of a pore size of 0.20 μm was selected in order to avoid the transfer of capsules through the pores and to have more homogeneous transfer of the free material.

The quantity of the technical grade of 1R-trans Phenothrin applied was 50.04 mg, which was dissolved in a solution of 50% water and 50% IPA (table 3). It gave a transparent solution in the donor chamber at the temperature of 30°C. By stirring the receptor fluid on the bottom, the technical grade of 1R-trans Phenothrin has diffused through the membrane filter into the receptor chamber.

The quantity of 5000 mg of each sample of the formulated product was used in order to achieve enough amount of 1R-trans Phenothrin - which is present as Capsule Suspension - and thus obtain a proper and significant response to the UV-HPLC detector.

Table 3. Preparation of the sample for the determination of technical grade of 1R-trans Phenothrin diffused as free material or in the formulated product in a CS form

Sample preparation	Formulation [mg]	Dissolved [mL]	Dissolved [solvent]	Formulation [mg/5mL]	A.I. content [wt-%]	A.I. [mg/5mL]	Receptor fluid
1R-trans PHENOTHRIN technical	50.04	50	50% H_20 / 50% IPA	5	94.4	4.72	50% H_20 / 50% IPA
SPT01 RTU PH08 141015	5000	5	applied directly	5000	0.10	5.00	50% H_20 / 50% IPA
SPT01 RTU PH09 141015	5000	5	applied directly	5000	0.10	5.00	50% H_20 / 50% IPA
SPT01 RTU PH10 141015	5000	5	applied directly	5000	0.10	5.00	50% H_20 / 50% IPA
SPT01 RTU PH11 141015	5000	5	applied directly	5000	0.10	5.00	50% H_20 / 50% IPA

Table 4. Preparation of the sample for the determination of Prallethrin technical and the formulated product in emulsion in water form

Sample preparation	Formulation [mg]	Dissolved [mL]	Dissolved [solvent]	Formulation [mg/5mL]	A.I. content [wt-%]	A.I. [mg/5mL]	Receptor fluid
PRALLETHRIN technical	5.72	50	50%H_20 /50% IPA	0.57	96.3	0.55	50% H_20 / 50% IPA
SPT01 RTU PH08 141015	5000	5	applied directly	5000	0.01	0.50	50% H_20 / 50% IPA
SPT01 RTU PH09 141015	5000	5	applied directly	5000	0.01	0.50	50% H_20 / 50% IPA
SPT01 RTU PH10 141015	5000	5	applied directly	5000	0.01	0.50	50% H_20 / 50% IPA
SPT01 RTU PH11 141015	5000	5	applied directly	5000	0.01	0.50	50% H_20 / 50% IPA

For the determination of the transfer of technical grade of Prallethrin through the membrane, 5.72 mg was dissolved in a solution of 50% water and 50% IPA (Table 4). It gave a transparent solution in the donor chamber at the temperature of 30°C. By stirring the receptor fluid on the bottom, the technical grade of Prallethrin has diffused through the membrane filter into the receptor chamber.

The fours samples of the formulated product containing 1R-trans Phenothrin in the CS form were emulsified in a homogeneous way in water guaranteeing the homogeneity and the continuous transfer after it is extracted from the capsule. The release was observed over a period of 7 hours in order to show the profile of release rate curve.

In addition, the release rate of the technical grade of Prallethrin has been also determined in order to compare the quantities of Prallethrin alone and when used in the formulated product present as EW [8].

2.2 EFFICACY TESTING: SIMULATED USE TRIAL

2.2.1 Preparation of trial chamber

The trial was conducted in four 15 m^3 (6 m^2 floor) test chambers. The test chambers were maintained at a temperature of 26°C+1°C and a relative humidity of 70%+5% during the period of testing. The test chamber materials were washable and unporous material on the walls/ceiling and on the floor (respectively epoxy-painted steel and ceramic tiles).

To simulate what happens in premises, some polystyrene blocks and cardboards were set into the test chamber to be harbourages including a water and food source. The insects were able to reach water and food sources without being in contact with the formulated product (Figure 3).

Only the half of the area was treated, therefore the insects had the choice not to be in contact with the formulated product.

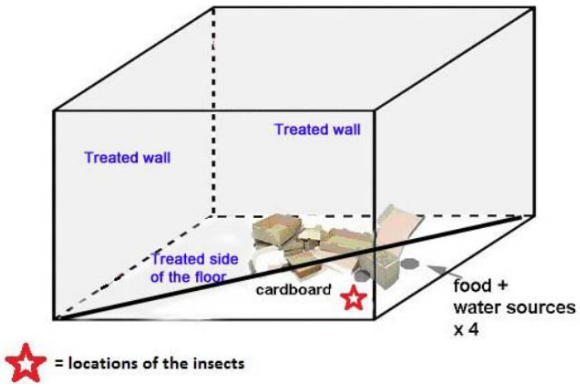

Figure 3. Treated surface to measure the residual efficacy.

For each mode of treatment and repetition, batches of 25 of each species of insects were used, except for house spiders, which were very difficult to find and for whom only 5 were used per replicate.

Assessments were carried out 56 days after treatment. The experimenter was entering the test chamber to count dead/alive organisms. The application rate was at 50 mL/m^2 [9].

2.3 EFFICACY TESTING: FIELD TRIAL

2.3.1 Preparation of the experimental sites

For field trials, only the data for German and Oriental cockroaches have been included in the paper to serve as a representative insect pest.

The trial was conducted in multi-family public accommodation buildings with high level of cockroaches' infestation.

The apartments were not isolated, so a re-invasion could be possible. All apartments were occupied (area from 40 m^2 to 390 m^2).

The sticky traps were placed overnight in a test site, collected the next morning and returned to the laboratory for processing. Five trap placement sites were used:

- Under the kitchen sink,
- Beneath or behind the stove,
- On/in cabinets.

So in total 5 sticky traps were settled per site.

The application of the formulated product was done using a professional sprayer (GLORIA 141T, anti-leak sprayer, 1.5 bar pressure). The application rate was at 50 mL/m^2.

Assessments were carried out 1, 7, 14, 28 and 56 days after treatment. At each date, new sticky traps were placed 24 hours for a count [10].

3. Results

3.1 Franz Cell diffusion method

As shown in Table 5, the technical grade of 1R-trans Phenothrin has diffused very fast through the membrane being available in 7 hours at the level of 98.26% in the receptor compartment.

1R-trans Phenothrin in CS form has shown an average exponential and slow release rate of 1.9% in the receptor compartment in 7 hours. These results clearly indicate that a higher residual activity by applying less quantity can be obtained in a controlled and timely tailored release manner in order to achieve an effective and high killing level in target insect pests.

Table 5. Release rate of the technical grade of 1R-trans Phenothrin and in the formulated product (4 samples)

	1R-trans PHENOTHRIN tech	Sample: SPT01 RTU PH08 141015	Sample: SPT01 RTU PH09 141015	Sample: SPT01 RTU PH10 141015	Sample: SPT01 RTU PH11 141015
A.I. applied [mg]	4.72	5.00	5.00	5.00	5.00
fluid on top	50% H_2O / 50% IPA	Applied directly dispersed in H_2O	applied directly dispersed in H_2O	applied directly dispersed in H_2O	applied directly dispersed in H_2O
receptor fluid	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA
time [h]	recovery [%]	recovery [%]	recovery [%]	recovery [%]	recovery [%]
0.5	8.77	0.00	0.00	0.00	0.00
1	17.16	0.00	0.00	0.00	0.00
2	37.03	0.07	0.00	0.00	0.00
3	54.90	0.24	0.06	0.02	0.02
5	80.98	1.52	1.01	0.49	0.96
7	98.26	1.66	2.64	1.45	1.85

As shown in Figure 4, the release rate of technical grade of 1R-trans Phenothrin and in four tested samples of the formulated product was compared.

The results have revealed a clear difference between the release behavior between the two forms.

The technical grade of 1R-trans Phenothrin in “free” form has shown a complete release in 7 hours.

Clearly, the release rate of technical grade of 1R-trans Phenothrin did not correlate with the release rate of the active ingredient available in CS form in the formulated product.

Based on the outcomes from Table 6 for technical grade of Prallethrin, 99.70% of the total recovered dose of Prallethrin has been released into the receptor chamber in 7 hours, when solubilizing the samples using a mixture solution of IPA in water as shown in Figure 5.

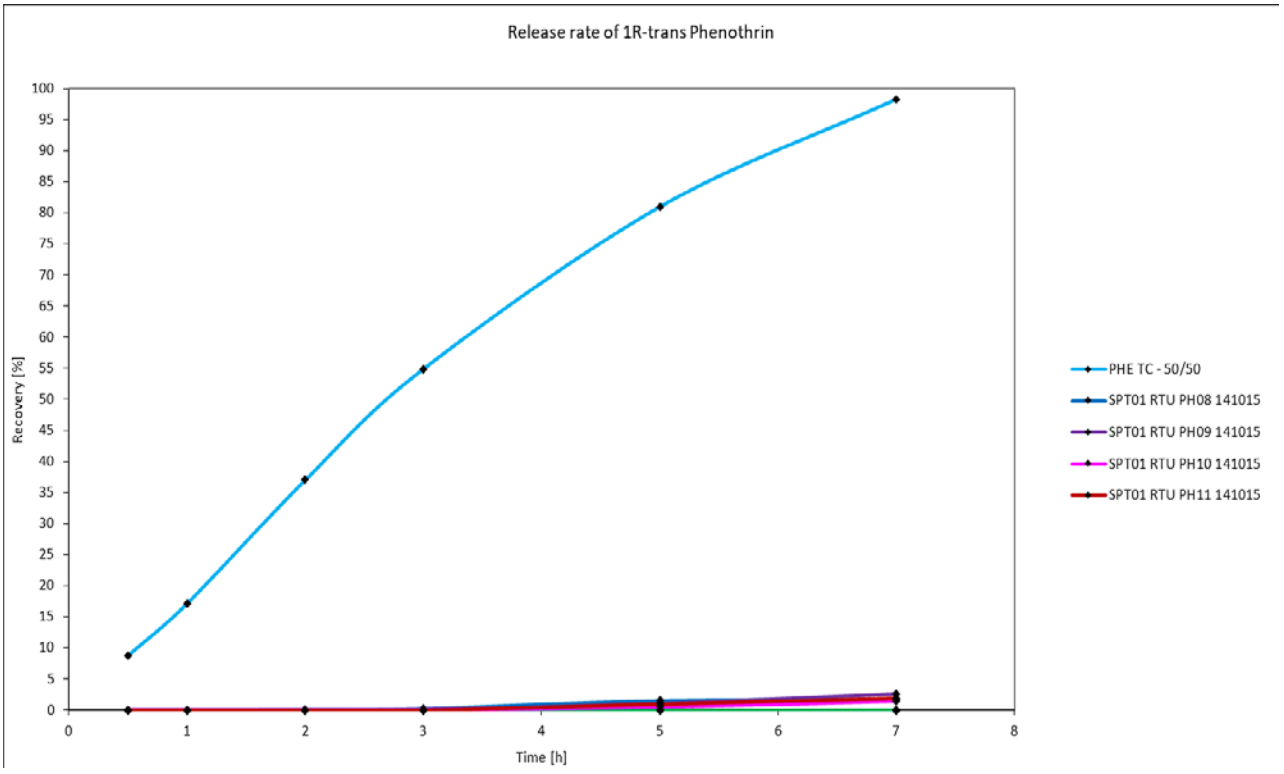

Figure 4. Release rate curves of technical grade of 1R-trans Phenothrin and in the formulated product.

Table 6. Release rate of the technical grade of Prallethrin and in the formulated product (4 samples)

	PRALLETHRIN tech	**Sample:** SPT01 RTU PH08 141015	**Sample:** SPT01 RTU PH09 141015	**Sample:** SPT01 RTU PH10 141015	**Sample:** SPT01 RTU PH11 141015
AI applied [mg]	0.55	0.50	0.50	0.50	0.50
fluid on top	50% H_2O / 50% IPA	applied directly dispersed in H_2O	applied directly dispersed in H_2O	applied directly dispersed in H_2O	applied directly dispersed in H_2O
receptor fluid	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA	50% H_2O / 50% IPA
time [h]	**recovery [%]**	**recovery [%]**	**recovery [%]**	**recovery [%]**	**recovery [%]**
0.5	9.33	0.00	0.00	0.00	0.00
1	18.25	0.00	0.00	0.00	0.00
2	38.13	0.00	0.00	0.00	0.00
3	55.97	7.45	6.92	5.51	8.12
5	82.23	17.70	21.29	17.47	26.19
7	99.70	22.89	34.03	30.14	28.98

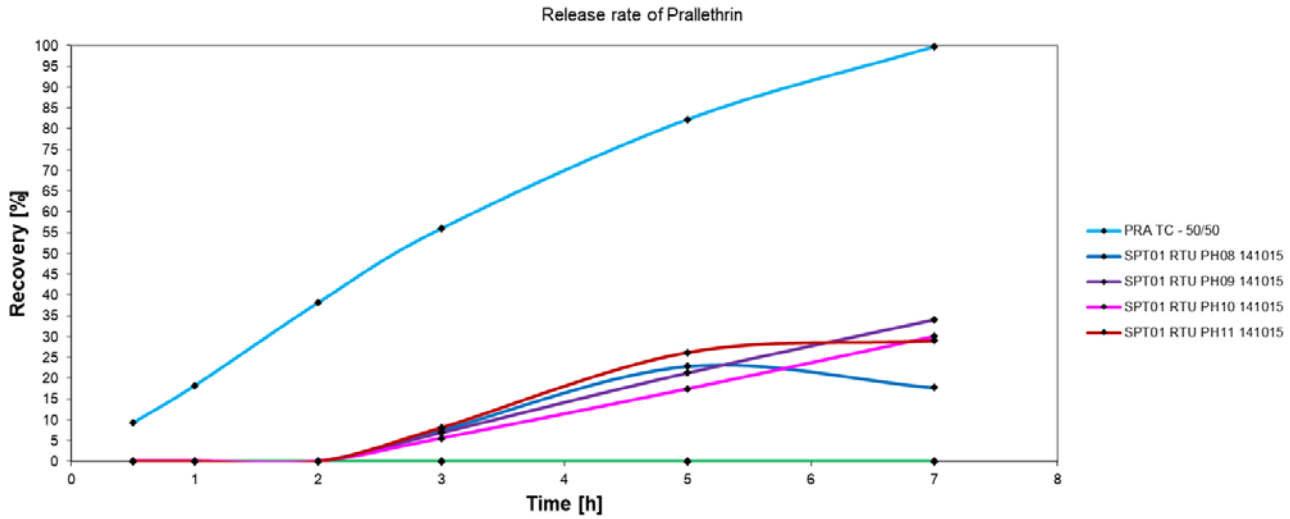

Figure 5. Release rate curves of technical grade of Prallethrin and in the formulated product.

Prallethrin formulated as EW in the formulated product contains some protective colloids that are part of the water phase of the capsule which produce the effect of micellisation and retarding the diffusion through the membrane and after its application in the field have the effect of controlling the evaporation.

However, once Prallethrin is solubilized using a mixture solution of IPA in water, the active becomes available in the receptor chamber immediately.

In fact, Prallethrin is 100% bioavailable upon application and evaporates quickly so that no residues should remain.

3.2 Simulated use trial

Under the conditions of this simulated-use trial, with samples provided and methodology used, the formulated product, applied at a rate of 50 mL/m^2 has proven to have a residual efficacy which remained sufficient also 56 days after treatment (Figure 8).

3.3 Field trial

The formulated product has proved a very good control of both species (German and Oriental) of cockroaches with more than 95% populations reduction 56 days after treatment.

Similar results were observed also for other tested insect pests [10].

CALIBRATION CURVES

1	2	3
Sample name	**A.I. concentration total (in solution)** [mg/mL]	**Area** [mAU]
1R TRANS PH ASTD_solution 1	0.002	49.31
1R TRANS PH ASTD_solution 2	0.020	596.89
1R TRANS PH ASTD_solution 3	0.200	6157.74

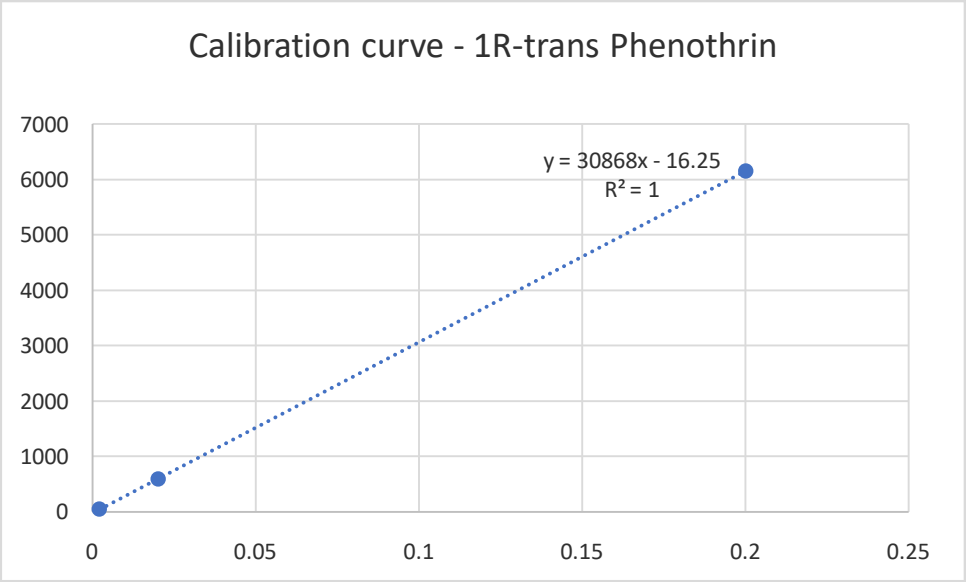

Figure 6. Calibration curve for 1R-trans Phenothrin (PHE) analytical standard (ASTD).

1	2	3
Sample name	**A.I. concentration total (in solution)** [mg/mL]	**Area** [mAU]
PRA ASTD_solution 1	0.000	0.00
PRA ASTD_solution 2	0.003	130.11
PRA ASTD_solution 3	0.025	1363.02

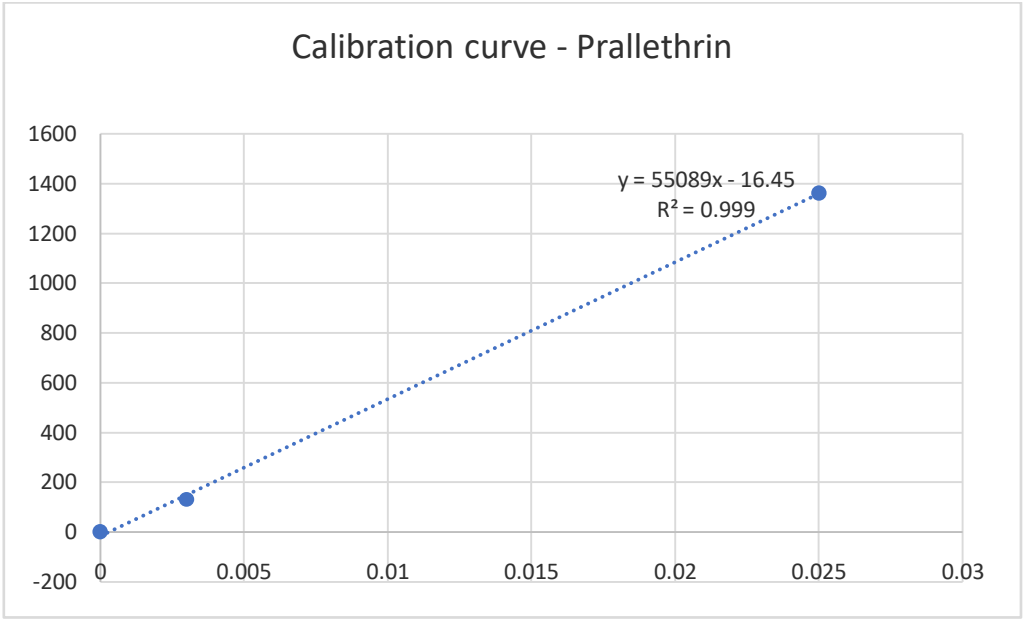

Figure 7. Calibration curve for Prallethrin (PRA) analytical standard (ASTD).

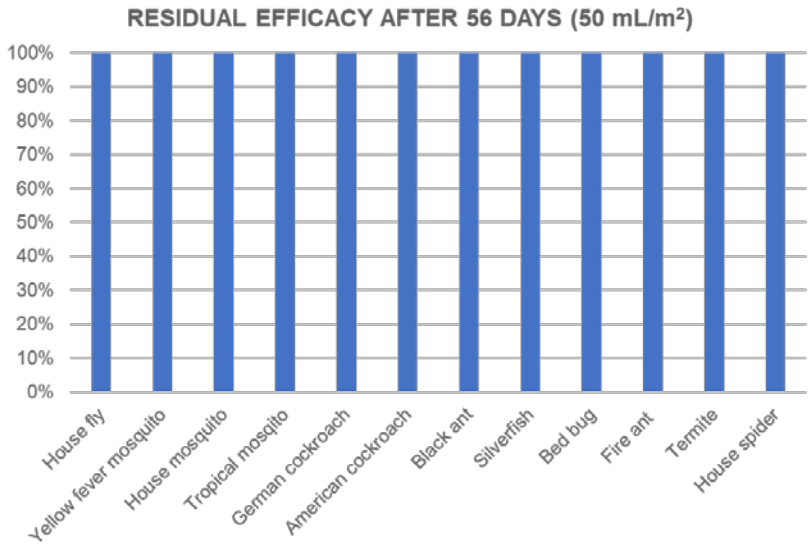

Figure 8. Overview of the results for the efficacy after 56 days.

4. Discussion

The intention to conduct the presented *in vitro* release studies according Franz Cell method was to determine the release rate of each active ingredient at a given time. Analytical results shown the release profile, confirms its influence on the performance of the formulated product which at the end correlates to the results on the efficacy revealing a long residuality.

The results obtained in 7 hours for technical grade of 1R-trans Phenothrin and in the formulated product as Capsule Suspension, have shown that the release rate of A.I. in CS form was ~ 52 times less in comparison to technical grade, as shown in Table 5 and Figure 4.

The kinetic explanation of these results is based on the physical properties of 1R-trans Phenothrin, which is very lipophilic and highly insoluble in water. When 1R-trans Phenothrin starts to release through the pores of the capsules, the tiny droplets of the active ingredient cover the pores of the microcapsule wall and since 1R-tans Phenothrin is not soluble in the water media, the tiny droplets of the active ingredient remain covering the pores of the microcapsule wall avoiding the release of further droplets. Even if tiny quantities are migrating outside the capsules - due to the motion of the water media provoked by the stirring system - the membrane between the donor and the receptor in the Franz cell will not be crossed since a coalescence of the tiny droplets will block the membrane until the donor solution which is in contact with the membrane will dissolve and allow the transfer and the determination of the release 1R-trans Phenothrin is possible.

However, the results are magnified due to the enhancement of the solubility in the media and by displacing the onset of the release and the quantity. The mixture of 50% H_2O / 50% IPA in the receptor compartment triggers the dissolution of the active ingredient which is in the membrane, making a pulling effect to transfer the active ingredient into the receptor, provoking that more product will be released. This is a consequence of the dissolution of the active ingredient which is lipophilic and not prone to be dissolved in water.

At the end, the applied system should give a correlation picture of what will happen in the environment, but since the environment is not dissolving in the same proportion as in the Franz cell mixture system but with a longer period of time than in this artificial setup, which can be measured as a correlation between the activity as reported for more than 56 days for the residual effect.

The results obtained at 3 hours can be correlated to the free material, which is outside the capsule, but until then there was no amount of free material available (0.00%).

In addition, also the physical properties of the formulated product after 3 years storage stability [12,13] does not show any differences in the particle size distribution and in the sieving which indicates that no oil of 1R-trans Phenothrin released out of the microcapsules. If some oil goes out of the microcapsules, it will be free oil that will influence in the particle size or sieving results indicating release with the time. This explain that as far as the formulated product remains in the ready to use form and it is not applied in the field, the capsules do not release the active ingredient and therefore the chemical stability is improved which was also confirmed by the results of the storage stability studies. The same was observed in the case of the accelerated storage stability after 2 weeks at 54°C [11].

References

[1] Huang BB, Zhang SF, Wu G. Release and Degradation of Microcapsulated Spinosad and Emamectin Benzoate. Sci Rep. 2017; 1-10.

[2] Wilkins R. Controlled Release Formulations of Pesticides. Encyclopedia of Agrochemicals, 2003; 1-10.

[3] Hazra DK, Purkait A. Role of Pesticide formulations for sustainable crop protection and environment management. Journal of Pharmacognosy and Phytochemistry. 2019; 1-8.

[4] Lubura B, Gimeno M. Microcapsules of Phenothrin in a mixture with Prallethrin and their mode of application and advantages. EPO. EP2020/082164. 2020; 1-89.

[5] Lowe C. BIO KILL micro-fast Ready To Use; Acute Oral Toxicity. PSF. 2014; 1-14.

[6] Lowe C. BIO KILL micro-fast Ready To Use; Acute Inhalation Toxicity. PSF. 2014; 1-23.

[7] OECD Guideline No. 428 for the testing of chemicals. Skin absorption in vitro Method. 2004; 1-8.

[8] Gimeno M. Determination of the free material and release rate of 1R-trans Phenothrin and Prallethrin from the formulated product Bio Kill micro fast Ready To Use by Liquid Chromatography - Mass spectrometry (LC-MS). GAT Microencapsulation. 2015; 1-22.

[9] Serrano B. Simulated use trial of the efficacy of a residual insecticide against various pests. TEC. 2015; 1-17.

[10] Serrano B. Fiel trial of the efficacy of an insecticidal residual treatment to control German and Oriental cockroaches. TEC. 2018; 1-17.

[11] Gimeno M. Accelerated storage stability of Bio Kill micro fast Ready To Use, a mixture of aqueous capsule suspension and an oil in water emulsion. GAT Microencapsulation. 2014; 1-115.

[12] Gimeno M. Long-term storage stability 1R-trans Phenothrin 1 g/L and Prallethrin 0.1 g/L ZW, a mixture of aqueous capsule suspension and an oil in water emulsion. GAT Microencapsulation. 2016; 1-154.

[13] Belussi C. Shelf-life stability study at 25°C/60% RH for 12 months on the test item “Bio Kill micro fast Ready To Use”. Eurofins Biolab S.R.L. 2018; 1-50.