(*E*)-Anethole Microspheres as an Alternative Insecticide in Funnel Traps

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ABSTRACT

Pyrethroids are the insecticides most commonly used inside traps, and the lack of alternatives poses the risk of pests developing resistance. In this paper we present two *(E)*-anethole formulations (spray drying (SD) and oil emulsion entrapment (OEE) processes) that provide a controlled release of their bioactive ingredient in the vapour phase with insecticidal potential in funnel traps.

An experiment with the two pyralid moths *Ephestia kuehniella* Zeller and *Plodia interpunctella* Hübner was set up at two pilot stores in Spain for a four-month period. The microspheres (4 g of SD powder/trap or 6 g of OEE beads/trap) remained effective for 100 days, killing the moths by volatile activity. The efficacy values were within the interval of 70–100% for the first half of the experiment, with a decrease afterwards. The OEE beads performed better than did SD powder in the long run: over 80% efficacy for the whole experiment. The OEE process gives more loading capacity (19.7 g of *(E)*-anethole per 100 g of beads) and entrapment efficiency (28.6 g of *(E)*-anethole encapsulated per 100 g of *(E)*-anethole added) and is slower in releasing the product.

In a laboratory study for *E. kuehniella*, the LC50 was 58.2 mg/L for SD after 24 h exposure to vapours and 111.6 mg/L for OEE after 48 h exposure to vapours. Therefore, the SD powder provides a quicker release of the bioactive ingredient.

The results indicate that encapsulated *(E)*-anethole could be a promising insecticide for mass trapping, mating disruption and attract and kill strategies.

KEY WORDS

Indianmeal moth, Flour moth, Botanical insecticidal volatile, Microencapsulation, Controlled release

1. INTRODUCTION

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, is a major pest in flour mills. Food contaminated by larvae and webbings of this insect are unacceptable for the consumer (Belda *et al*., 2011). The indianmeal moth, *Plodia interpunctella* Hübner, is a widespread pest of stored products, such as cereals, nuts and dry fruits. In a survey of pests in rice stores in Spain, the Lepidoptera *P. interpunctella* and the angoumois grain moth, *Sitotroga cerealella* Olivier were frequently caught in funnel traps (Pascual-Villalobos, 2006).

Today, sulfuryl fluoride is the main fumigant used in the food facilities, alongside with additional insecticide spot treatments using pyrethroids or natural pyrethrins. Applied heat treatments are also becoming more and more popular due to their lower cost. Essential oils are considered as non synthetic chemical control solutions for organic food production in developed countries and as home made products in developing countries (Stejskal et al., 2020). For instance leaf powder, wood ash or plant oils can be admixtured with grain for protection against pests due to insecticidal or repellent action. A review on the use of botanicals, with an emphasis on the classification, the mode of action and recent advances has been published by Trivedi *et al.* (2018).

Pheromone-based alternatives ((Z,E)-9,12-tetradecadienyl acetate (TDA)) for lepidopteran pests include mass trapping, mating disruption and attract and kill (Mueller, 2010). Trematerra and Gentile (2010) reported a five-year experiment of mass trapping *E. kuehniella* in a flour mill in Italy. The traps captured 85–95% of males and coupled with consistent hygienic practices in the store, the equipment and corners of the building, the pest population was able to be reduced over time. However, due to the many traps needed and the space required, mass trapping has limited practical

acceptance in commercial stores. According to Phillips *et al*. (2011), the attract and kill method was successful for the mass killing of *P. interpunctella* males. Sutherland *et al*. (2011) and Ryne *et al.* (2007) suggested that mating disruption could be effectively integrated in IPM programmes if the starting population of moths is not too large. Trials were conducted in Czech Republic, Greece and Italy with dispensers of TDA and subsequent monitoring of the adults. The pheromone-baited traps caught fewer males and there were fewer larvae in the area with dispensers.

In mass trapping, it is important that insects do not escape from the funnel traps once caught. An insecticide is used for this purpose. Previously, dichlorvos, acting in the vapour phase, was commonly used, but after it was forbidden, contact pyrethroids, such as deltamethrin or cypermethrin (painted on the lid or on the walls inside the trap), became the alternative in most countries. Dependence on just one type of insecticide poses some risks, such as pests developing resistance to the product, the pheromones and the attractants used, in addition to limited choice for the user of the traps. The reasons for the widespread use of pyrethroids in traps are the quick action and the efficacy of such compounds in simple surface contact assays. Campos and Phillips (2013) designed attract and kill formulations wherein the synthetic female pheromone was combined with either permethrin, cyfluthrin or natural pyrethrins as insecticides. Over a period of eight weeks, the most effective wax panels were found to be the ones containing 6% of either pyrethroid. Water has sometimes been used as an attractant for almond moths in mating disruption systems (Süss and Sovoldelli, 2011). Aside from attracting the moths, the water also served to drown the captured insects, resulting in a reduction of the number of mated females. However, when water is used in traps for pest monitoring, the observation of the catches becomes cumbersome and dirty if it takes several weeks. Therefore, the use of an insecticide becomes preferred. For

horticultural pests, the situation is quite similar, with just pyrethroids (deltamethrin, cypermethrin or permethrin) and natural pyrethrins used in newly released attract and kill traps, such as the one for the Southafrican mealybug, *Delottococcus aberiae* Delotto (Hemiptera: Pseudococcidae), of citrus trees.

In this work, we are testing the phenylpropanoid *(E)*-anethole, and since it is a volatile product, an encapsulation method is required. The formulations used are an improvement upon previous preparations (López *et al*., 2012; Pascual-Villalobos *et al*. 2015, 2020). In the work described by Pascual-Villalobos et al. (2020), we tested the insecticidal activity of *(E)*-anethole formulations against aphids, and the best was the oil emulsion entrapment (OEE) method. In this paper we provide new information on the potential of *(E)*-anethole as an insecticide in the vapour phase when formulated for a controlled release inside funnel traps. We conducted a practical experiment in pilot stores over four months with two stored product pest moths.

2. MATERIALS AND METHODS

2.1 Chemicals

A standard reagent of *(E)*-anethole (>99%) was purchased from Sigma-Aldrich (St. Louis, US). Other reagents used in the formulations were calcium chloride, maltodextrin, Arabic gum, glycerol, Tween 80 and sodium alginate. There were also obtained from Sigma-Aldrich (St. Louis, US).

2.2 Preparation of *(E)*-anethole formulations

2.2.1 Formulation of (E)-anethole by oil emulsion entrapment (OEE)

Beads were formed by dripping an alginate solution (containing a dispersion of *(E)*-anethole, glycerol and Tween 80) into a calcium solution (OEE); Table 1 depicts the

conditions. The diffusion of the calcium in the alginate droplets led to their gelation. The preparation of the internal phase was carried out as follows: *(E)*-anethole (50 mL) was dispersed in glycerol (50 mL) and Tween 80. The blend was dispersed in 50 mL of alginate (40 g/L) and 50 mL of maltodextrin (10%). This dispersion was dripped into calcium chloride solution (10 g/100 mL). Beads were filtered with a wire mesh and finally were dried overnight at room temperature (15 °C). The procedure is similar to the one described in Pascual-Villalobos *et al*. (2020) but further modified by adding maltodextrin.

2.2.2 Powder of (E)-anethole by spray drying (SD)

An optimization of the processes described by Pascual-Villalobos *et al*. (2020) was applied to obtain optimal conditions for drying. First, 60 g of *(E)*-anethole were mixed with 200 mL of maltodextrin, Arabic gum (30%, w/v and 1% w/v, respectively) and Tween 80 and stirred at 300 rpm for 2 h. The formulation of *(E)*-anethole was obtained by means of a spray dryer using a laboratory scale dryer (Mini Spray Dryer – B290, BÜCHI, Flawil, Switzerland). The emulsion was fed into the spray dryer at room temperature with a flow rate of 4 mL min-1. The inlet and outlet temperatures were maintained at 100 °C and 60 °C, respectively (Table 1). The dried powder was collected and stored in an opaque, airtight container at 4 °C for further analysis.

2.3 Encapsulation yield, entrapment efficiency and loading capacity of the formulations

The amount of *(E)*-anethole in the powder and beads was determined by GC-FID (6500GC System) by using a supelcowax column (30 m x 0.25 mm x 0.25 µm) as described in Pascual-Villalobos *et al*. (2020). The initial oven temperature was held at 60 °C for 1 min. Afterwards, it was increased by 3 °C/min to 225 °C, with injector at

250 °C, column head pressure at 8.00 psi, hydrogen carrier gas, flow rate of 0.8 mL/min, and 1/20 split with 2 μL of sample injected. The quantitative analysis of *(E)*-anethole was done using the linear regression of a standard. Prior to the quantification of monoterpene, the surface *(E)*-anethole in the formulation was washed.

Finally, the powder recovery (ratio between the quantities of microsphere or powder versus the initial mass solids), loading capacity (quantity of *(E)*-anethole per 100 grams of dry microspheres or powder) and entrapment efficiency (g *(E)*-Anethole encapsulated 100 g-1 *(E)*-anethole added) were calculated.

2.4 Controlled release of *(E)*-anethole through different formulations

A study of the controlled release through the microspheres (beads or powder) was carried out in the laboratory. In brief, 0.5 g dry samples were placed into the vials without sealing, and these vials were maintained in a humidity control chamber (60% RH) at 15 °C for 21 days. The release of *(E)*-anethole was monitored by means of GC-FID (6500GC System, Korea) containing a 30m x 0.25mm fused silica HP-5 column. The chromatographic conditions used were inlet 250 ºC and column 40 ºC for 2 min, followed by ramping at 5 ºC min–1 to 250 ºC. The quantification of *(E)*-anethole was carried out using a calibration curve of the standard compound (Sigma-Aldrich, St. Louis, Missouri, USA). A statistical comparison between the two formulations (three replications each) was performed.

2.5 Scanning electron microscopy (SEM) analysis

The evaluation of microspheres was done through a SEM Vega3 Easyprobe SBU (Tescan) (CMA, Universidad de Concepción). The samples were mounted (both entire structures and cross sections) on specimen stubs with double-sided adhesive tape. The specimens were coated with gold and examined at an accelerating voltage of 15 kV and

a working distance of 20 mm. Topographical images were captured at a magnification of 830x to 7370x for SD to visualize sizes of 5 µm to 50 µm and a magnification of 49x to 1350x for OEE to visualize sizes of 20 µm to 1000 µm.

2.6 Insects

E. kuehniella and *P. interpunctella* were maintained in laboratory cultures on a diet of whole wheat flour and yeast (15:1) for *E. kuehniella* and wheat bran, yeast, wheat germen and glycerine (9.7:2:1:1.5) for *P. interpunctella*. The cultures have been maintained in the laboratory for over 10 years. The chambers were kept at a constant temperature of 28 ºC and an RH of 75%, with a 16:8 h photoperiod of light:dark conditions. Newly unsexed emerged adults (<24 h) were used for the experiments.

2.7 Experiment in the stores with traps

2.7.1 Setup

Two food facilities located at Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) in Murcia and Institut de Recerca i Tecnologia Agroalimentàire (IRTA) in Barcelona were selected as pilot stores to test the formulations in *E. kuehniella* or *P. interpunctella,* respectively. They consisted of two houses with 5 rooms: 4 for the treatments (2 formulations x 2 replications) and 1 for the control. In each room, a funnel trap was hung at a height of 1.5 m from the floor. Data loggers registered the environmental conditions (T and RH) during the experiment.

2.7.2 Rounds and Periods

In each room, a funnel trap was hung at a height of 1.5 m from the floor. The microspheres were located inside the traps (except for the control traps), and then a group of adult moths (around 50 per trap, although the number varied according insect

availability). The number of alive and dead insects in the traps was counted after three days, and the insects were removed from the trap, and replaced by other newly emerged adults. In addition, the number of insects found outside the traps were also counted and sexed. The process was repeated. Firstly (round-1), twice a week, for 8 weeks, in total 16 observation periods. Secondly (round-2), once every two weeks, in total 4 observation periods for 10 weeks. Overall, 20 observations were done during a period of 129 days. Data loggers registered the environmental conditions (T and RH) during the experiment.

2.7.3 Microspheres

The amounts used per trap were 4 g of SD powder or 6 g of OEE beads, the two formulations of *(E)*-anethole. Samples were weighed at the beginning of the experiment and placed inside empty tea bags to avoid direct contact between the insects and the products so that only volatile insecticidal activity was studied. The same microspheres were used throughout the whole experiment (from 12 June to 19 October). The samples were weighed at the end of Round 1 and Round 2 to monitor the release of the bioactive volatile. The amount of *(E)*-anethole released from the formulations inside the traps was measured by the weight difference between the microspheres samples used at the beginning and at the end of the experiment.

2.7.4 Monitoring insecticidal activity

The efficacy of the *(E)*-anethole formulations was measured according to the formula of Henderson-Tilton (Henderson and Tilton, 1955):

$$Ef\ (\%)=[1-(Ata \times Acb)/(Aca \times Atb)]x100$$

Atb: Number of alive insects in the treatment before testing

209 Ata: Number of alive insects in the treatment after testing

210 Acb: Number of alive insects in the control before testing

211 Aca: Number of alive insects in the control after testing

212 The evolution of Henderson-Tilton efficacies over time were adjusted to a
213 binomial regression model, using as explanatory variables the time and the combination
214 of the formulations and insect species factors. It was established, a priori, that the
215 efficiency at infinite time is zero. The calculations were done by the drm function from
216 R statistical package drc v.3.0-1 (Ritz *et al*. 2015) in R software, v. 4.0.4 (R Core Team,
217 2021). The following models were tested: Log logistic (LL.3 function in drc), Logistic
218 (L.3), Log normal (LN.3), Weibull type 1 (W1.3), and Weibull type 2 (W2.3). Of the
219 tested models, the one that provided the lowest Akaike Information Criterion (AIC) was
220 used to calculate the efficacies over time, which were 90 (ET90) and 50 (ET50)—that
221 is, the time for which an efficacy greater than or equal to 50% or 90%, respectively, was
222 maintained.

223 *2.7.5 Lethal concentration (LC) and lethal time (LT)*

224 A laboratory assay was performed to calculate LC after 24 h exposure to *(E)*-
225 anethole volatiles in *E. kuehniella*. Three traps with 10 newly emerged adults (<24 h)
226 were prepared for each tested dose of each formulation. The doses tested were 4.44,
227 8.89, 22.22, 44.44, 88.89, 222.22 and 444.44 mg/L for SD and 6.67, 13.33, 33.33,
228 66.67, 133.33, 333.33 and 666.67 mg/L for OEE. The microspheres were placed in an
229 open Petri dish inside each trap. Every trap was enclosed in a plastic bag to prevent
230 insects from escaping. The mortality was observed after 24 h or 48 h (both dead and
231 dying insects were counted).

232 In addition, LTs were studied in a similar manner but with a fixed amount of 500
233 mg of SD powder or 750 mg of OEE beads (placed inside tea bags) per trap. Insect
234 mortality was counted after 6 h and daily for four days.

235 Probit analysis (Polo Plus, Probit and Logit Analysis, Version 1.0, LeOra
236 Software) was applied to the data to calculate the concentrations and time needed to kill
237 50% or 90% of the insects.

238

239 ## 3. RESULTS

240 ### 3.1 *(E)*-Anethole formulations

241 Both methods of encapsulation work well for *(E)*-anethole. The OEE method
242 gives more loading capacity and entrapment efficiency in comparison with the SD
243 method (Table 2). As for the release of the bioactive volatile in a laboratory study,
244 Figure 1 shows that at moderate temperatures (15 ºC), the SD formulation is faster in
245 releasing the product (90% after 21 days) than the OEE (only 25%) formula. This
246 means that OEE beads tend to have a more prolonged effect, but SD powder causes a
247 faster toxic effect against the pest for a given amount of formulated microspheres. There
248 were statistically significant differences ($P < 0.05$) in the amount of the volatile released
249 from the two matrix blends after one week.

250 The SEM micrograph of the particle samples is shown in Figure 2. These images
251 contributed to the determination of the morphology of the particles. For (A), the average
252 particle size is 2.5 mm. In addition, all micrographs of OEE formulations showed that
253 the bioactive was homogeneously dispersed in the alginate/maltodextrin matrix with a
254 few agglomerates. The addition of glycerol and Tween 80 to the polymers (alginate and
255 maltodextrin) increased the viscosity of the solution during the synthesis of beads and

decreased the penetration of water molecules. For (B), the micrographs showed an average particle size of 6.5 µm, although different sizes were observed. These particles appeared mainly spherical, as expected in spray-dried formulations. Nevertheless, some dents and slight agglomeration were observed. The addition of arabic gum improved the surface of the particles, as confirmed by the fact that rough surfaces were not detected. Beyond this, the arabic gum enhanced the homogenization and dispersion of the bioactive.

Inside the funnel traps, a controlled release of the volatile was produced during the four months. The average amount of *(E)*-anethole emitted inside each trap through the whole duration of the experiment in the stores was 0.5 g for the SD formulation and 0.5–0.9 g for the OEE beads (Table 3). At the end of the experiment, there was some compound left in the microspheres, since the release represented only 32% to 54.9% of the total amount of (*E)*-anethole encapsulated in the sample placed in the trap. During the first part of the study (periods 1 to 16), a quicker release of *(E)*-anethole (30.2% versus 21.7%) was obtained from the SD powders as opposed to the OEE beads. In summary, the total amount of the formulations tested—4 g of SD or 6 g of OEE samples per trap—proved to be enough to cover several months of insecticidal activity. Now it is important to derive the minimum amount needed or recommended for practical use.

3.2 Lethal concentration (LC) and lethal time (LT)

In Table 4, the results of the laboratory study with *E. kuehniella* are presented. The LC50 after exposure to the vapours inside the traps are 58.18 mg/L for SD (but 48 h of exposure was needed to obtain the value) and 111.56 mg/L for OEE microspheres. It must be noted that, in this experiment, the microspheres were placed in an open Petri dish (inside the trap), and it is possible that the insects were exposed not only to the vapours but to the product as well through direct contact. In addition, we counted both

dead and dying insects (those unable to escape from the traps). Campolo *et al*. (2018) in a recent review on the use of essential oils for stored product insect control have reported that oils of lamiaceae were the among the most active as fumigants, giving (unformulated oils) LC50 = 0.93 - 4.06 µL/L for larvae of *P. interpunctella* and LC50 = 3.27 - 7.52 µL/L for *E. kuehniella*.

It is common for the duration of exposure to be more important than the dose for fumigant products. In our case, we tried to obtain Lethal times (LT), testing 0.5 g of SD or 0.75 g of OEE samples per trap (in this case the microspheres were placed inside a tea bag and therefore there was no contact between the insects and the product) and monitoring mortality (dead insects only) after 6 h, 24 h, 48 h and 96 h. After 96 h of exposure, 39.2 (± 3.92)% mortality was obtained with the OEE beads, but 20 (± 13.33)% of insects were dead in the control (there was no mortality in both cases after 6 h). We conclude that the dose is more important than the duration of exposure in this case. However, it might be possible to further reduce the amount of formulations needed per trap, as dying insects (which cannot escape from the traps) are just as much a success as dead insects.

3.3 *(E)*-Anethole volatile insecticidal activity

During the experiment (12 June–19 October 2020), the temperature ranged from 19.4 ºC to 31.8 ºC and the relative humidity from 48.1% to 86.4% inside the facilities where the traps were located (Table 5). Values remained quite similar during the day and night. Temperatures reached their peak in July–August (27–32ºC) and were moderate (19–27ºC) during June and September–October. Overall, the conditions at Murcia (where *E. kuehniella* was tested) were warmer and dryer (rh ≤ 60.2 %) than in Barcelona (where *P. interpunctella* was tested).

The outcome of the experiment in the stores was very good. The *(E)*-anethole formulations were insecticides that killed the moths inside the traps. After three days of exposure of the moths to the microspheres, within the traps, the average mortality was higher than 70% in *E. kuehniella*, or 65% in *P. interpunctella* (less than 4% or 13% mortality respectively in the control, without exposure to the microspheres,Table 6). The values obtained of the Henderson-Tilton efficacy were promising, within the interval of 70–100% for the first half of the experiment in the stores and with a decrease afterwards. The OEE beads performed better than the SD powder in the long run for both insect species, and in addition, the activity was more consistent in replications. At Barcelona (*P. interpunctella*), the differences between SD and OEE at the end of the experiment (after 100 days) were more pronounced. For *E. kuehniella*, the insecticidal activity of both formulations followed the same pattern for 75 days. However, at both locations, the SD powder became a less efficient insecticide from day 77 onwards.

The results of the binomial modelling of the evolution of efficacy over time have been summarized in Table 7 and Figure 3. The model that provided the lowest AIC was Weibull type I (W1.3) (AIC = 668). Next were the Log-normal (AIC=669), the Logistic and the Log-Logistic (AIC=672 for both), and finally the Weibull type 2 (AIC= 680) for all curves. However, the OEE formulation with *P. interpunctella* had an efficacy of over 75% during the whole experiment, and therefore, only the Logistic model (L.3) allowed for an approximation of a curve.

Figure 3 and Table 7 show that, for *E. kuehniella*, both formulates had 100% efficacy at the beginning. The values of the "d" parameter of the model (see Table 7) were SD = 0.9904, with 95% CI of 0.9672–1.0136, and OEE = 0.9974, with 95% CI of 0.9923–1.0024. These values are maintained for 33 days for SD and for 61 days for OEE. We can also highlight that in *P. interpunctella*, SD had an average maximum

efficacy of 85%. The “d” parameter values (see Table 7) were 0.85, with 95% CI of 0.8367–0.8754. For 94 days, it maintained over 80% efficacy. On the other hand, the OEE with *P. interpunctella* gave an efficacy of 95% at the beginning, and for 126 days, the efficacy remained over 90% (Figure 3). This is possibly because the higher temperature values of Murcia (Table 5) allowed for a decrease in the amount of active volatiles inside the traps over time and therefore a decrease in the efficacy in *E. kuehniella* was also produced.

Few insects were able to escape the traps, but those that did were counted and sexed. In Table 8, these data are summarized in two groups: with *(E)*-anethole formulations (experimental) and without (control). *E. kuehniella* females tended to escape from control traps (75.22% of total insects outside) more often than from traps with *(E)*-anethole vapours inside (in this latter case, only 36.16% of the total insects outside were females). Perhaps this indicates a potential attractant effect of the compound. However, for *P. interpunctella*, the percentages of females outside the traps were quite similar for the treatment and the control (56.14% versus 60.24%, respectively), but in this location, the period of recording was shorter (Table 8).

Another observation worth mentioning is that male moths were more susceptible to *(E)*-anethole volatiles than females. For instance, the mortality with SD powder was 77.4% for males versus 63.6% for females for *E. kuehniella*. OEE beads gave higher mortality but there was less of a difference between males and females: 88.1% versus 87.9%, respectively. As for *P. interpunctella*, the differences in mortality between males and females existed but were small: 78.7% versus 76.9%, respectively, with SD and 94.7% versus 92.8%, respectively, with OEE.

4. DISCUSSION

The release of the volatiles of *(E)*-anethole from the microspheres inside the traps was enhanced by increased temperature and/or relative humidity values at both locations. With time, the amount of bioactive volatiles inside the traps possibly decreased, reducing the effectiveness. The traps required 0.4 g/L of SD powder or 0.56 g/L of OEE beads to kill 90% of *E. kuehniella* moths after 24 h or 48 h of exposure to the volatiles respectively. Currently, there are no insecticides acting in the vapour phase in use in traps. The development of a commercial product based on *(E)*-anethole would be advantageous for dealing with certain types of insects that are less mobile and might not come into contact with the commonly used contact insecticides. It is also convenient that there is a choice of more than one mode of action in the registered active substances to avoid insecticide resistance development.

Comparing both formulations, the OEE beads turned out to be more lethal than the SD powder to pyralid moths under the conditions studied. Comparing the two species of moths tested, *E. kuehniella* was harder to kill than *P. interpunctella* towards the end of the experiment, resulting in a greater number of alive insects inside the traps. The OEE beads present some advantages, such as homogeneous dispersion of the bioactive in the matrix blend and its prolonged insecticidal effect on account of the slower release. The efficacy of OEE beads on *P. interpunctella* remained over 80% for the whole experiment. Comparing the two locations, the conclusions about OEE and SD did not change. However, both formulations are suitable to encapsulate *(E)*-anethole and provide an improvement in relation to our previous works. The gradual slow release of a volatile substance from a matrix (plastic, plates, strips, pellets etc.) is an spontaneous process called by other authors as residual fumigation (Stejskal *et al*., 2020). In this way, DDVP was extentively used (now banned) for stored product pest protection.

Our group started to develop the OEE method to encapsulate linalool (López *et al.*, 2012) by adding glycerol and starch to solve the porosity problem of the alginate beads. A high encapsulation yield (86%) of the volatile and a better entrapment were obtained. However, the release of the bioactive took place very slowly, with only a 20% release after 336 h. In a previous published experiment in stores (Pascual-Villalobos *et al.*, 2015), beads of coriander and basil EOs were tested inside funnel traps as an insecticide. A similar number of dead insects was obtained with the encapsulated oils compared with dichlorvos insecticide, all of which act in the vapour phase. The main compounds of these oils were linalool and estragole. Solid beads were prepared with alginate and glycerol by an oil emulsion entrapment procedure described in López *et al*. (2012), but no starch was added. The encapsulation efficiency of the beads was 10.2% for coriander and 28.7% for basil, and 4 g of beads were used inside the traps.

Further experiments are required to fix the minimum amount of a formulation needed inside the trap (to see if less than 4–6 g works well) to give similar results to the ones we are reporting here. Not all the *(E)*-anethole was released inside the traps during the four-month period; in fact, at least 45% was left in the samples in the end, but this did not prevent the declining efficacy more clearly seen for the SD powder and for *E. kuehniella*. The time response curve, according the Weibull type 1 model, descends slowly from the upper limit (at time = 0, in our cases upper limit = 100% efficacy), but afterwards, the curve approaches the lower limit rapidly (Caffi and Rossi, 2018; Ritz *et al*. 2019).

The synthetic female sex pheromone has been used successfully in traps for monitoring, mass trapping, attract and kill or mating disruption strategies for stored product pest control (Phillips, 1997). Male pyralids respond to the single ZETA pheromone compound, but sometimes additional components are used (to improve the

activity), such as the corresponding alcohol to this acetate, ZETOH, which is also produced by females. However, there is still a need for a female attractant so that the traps target both sexes. Trematerra and Colacci (2020) point out the attractiveness of water traps to male and females of pyralid moth species, but this type of trap is not practical under some circumstances. In a paper published by Worthley and Nicholas (1937), female codling moths (*Cydia pomonella* L., Lepidoptera: Tortricidae) were more attracted than males to *(E)*-anethole added (1 cc) to a standard bait in the trap (33 out of 55 total catches—60%—versus 6 out of 13 —46.1%), indicating an attractant action. In our paper, we have also determined that *(E)*-anethole formulations inside funnel traps might be an attractant for female moths of *E. kuehniella* and *P. interpunctella,* in addition to their insecticidal effect. Some authors (Chang *et al*. 2009) have pointed out that if *(E)*-anethole and other bioactives are mixed with attractants, such as parapheromones, in an appropriate formula, they might be used as a natural insecticide. Therefore, if *(E)*-anethole acts as an attractant at described concentrations, this bioactive compound could be a promising insecticide, particularly if it can also be used in organic plant or stored product pest protection. Wang *et al.* (2021) have reported the toxic effects of the volatiles of star anise, *Illicium* verum Hook f. powder against the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and the main compounds were *(E)*-anethole and estragole. The possible attractant effect of *(E)*-anethole on female pyralid moths deserves further research in an experiment specially designed for this purpose.

According to Cox (2004) the attraction of only males means that a high proportion of the population has to be caught to have an impact on female fecundity, and this usually happens if the insect population is low. There are several factors to be taken into account, such as the most effective semiochemical, the most appropriate

delivery system, the dose and release rate and the trap design (e.g., adding vertical strips outside the funnel traps). A system that is being introduced is the combination of the autoconfusion Exosect SP Tab pheromone dispensers for males with water traps to eliminate the high number of *E. cautella* males and females. The pest was not eliminated, but a reduction in the percentage of mated females was achieved overtime in a dried fruit store (Trematerra and Colucci, 2020). Another approach that is being used is a coated polyester net that contains the insecticide alpha cypermethrin to cover tobacco during storage as a barrier against insects (the tobacco moth, *Ephestia elutella* Hubner, and the tobacco cigarette beetle, *Lasioderma serricorne* F.), similar to the mosquitocidal nets and insecticide treated nets used against agricultural pests in greenhouses (Paloukou *et al*., 2020).

The environmental conditions, the pest and the route of entrance of a compound determine the optimum formulation type. A fumigant enters by inhalation through the spiracles or the cuticle to the insect traqueal system. A slow and constant release of the bioactive compound is needed to obtain a prolonged action that is the objective of our research line.

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Worthley, N.H., Nicholas, J.E., 1937. Tests with bait and light to trap codling moth. Journal of Economic Entomology 30(3), 417-422.

550 **Table 1.** Summary of conditions of the preparation of (*E)*-anethole formulations

Conditions used	OEE	SD
Inlet air temp (°C)	--	100
Feed flow rate (mL min^{-1})	--	4
Wall material content (% wt vol^{-1})	34	40
Surfactant (g)	50	50

551 OEE: Oil emulsion entrapment (beads); SD: Spray drying (powder)

552 **Table 2.** Characteristics of beads and powders produced using two techniques of
553 encapsulation

Formulation Method[a]	Loading capacity[b]	Powder Recovery[c]	Entrapment Efficiency[d]
OEE	19.65a	62.05a	28.60a
SD	13.50b	57.51a	16.10b

554 [a]OEE: Oil emulsion entrapment (beads); SD: Spray drying (powder).
555 [b]Loading capacity was expressed as g *E*-anethole per 100 g^{-1} dry beads or powder.
556 [c]Powder Recovery was expressed as g beads or powder per 100 g^{-1} initial solids.
557 [d]Entrapment efficiency was expressed as g (*E*)-anethole encapsulated per 100 g^{-1} (*E*)-
558 anethole added. Different letters within each column are significantly different ($p < 0.05$)
559 according t.
560

561 **Table 3.** Release of (*E*)-anethole from microspheres inside the funnel traps

Formulation[a]	Periods[b]	weight loss of microspheres			
		IMIDA (Murcia)		*IRTA (Barcelona)*	
		g	% of total[c]	g	% of total[c]
SD	1 to 16	0.4210	30.2	0.5300	38.1
	1 to 20	0.5315	38.2	0.5055	36.3
OEE	1 to 16	0.3670	21.7	0.4770	28.2
	1 to 20	0.9275	54.9	0.5415	32.0

562 [a] OEE: Oil emulsion entrapment (beads); SD: Spray drying (powder)

563 [b] Periods 1 to 16 cover from 12 June till 3 Aug 2020 and Periods 1 to 20 cover from 12

564 June till the end of the experiment on 19 Oct 2020.

565 [c] The total amount of (*E*)-anethole in each trap at the beginning of the experiment was

566 1.392 g for SD or 1.69 g for OEE.

567

568

569 **Table 4.** Lethal concentrations of (*E*)-anethole microspheres (mg/L) for *E. kuehniella*

570 inside funnel traps.

Formulation[a]	LC50	95% CI	LC 90	95% CI	$\chi 2$
SD	58.18	50.04 - 67.65	398.08	296.45 - 510.66	18.046 ^{NS}
OEE	111.56	94.23 - 133.34	558.23	424.89 - 788.89	37.093 ^{NS}

571 Probit analysis fitting lethal concentration 50 (LC50) and 90 (LC90) after 24 h (SD) or

572 48 h (OEE) exposure to vapours in *E. kuehniella* and confidence intervals. $\chi 2$ non-

573 significant (NS). The number of insects per dose tested was 120.

574 [a] SD = Spray drying (powder) OEE = Oil emulsion entrapment (beads)

575

576

577 **Table 5.** Environmental conditions (average temperature and relative humidity) inside
578 the facilities

Period / Date	IMIDA (Murcia)				IRTA (Barcelona)			
	temp day (ºC)	temp night (ºC)	r.h. day (%)	r.h. night (%)	temp day (ºC)	temp night (ºC)	r.h. day (%)	r.h. night (%)
Round 1 (Observations every 3 days)								
1/12-15 June	24.8	24.9	54.3	56.9	23.4	23.5	60.3	61.9
2/16 -19 June	26.1	26.1	55.7	56.9	22.3	22.3	67.4	67.8
3/19-22 June	26.5	26.6	59.4	60.2	23.4	23.9	68.9	70.8
4/23-26 June	27.4	27.4	54.2	56.4	26.4	26.8	66.3	64.8
5/26-29 June	28.1	28.2	57.0	58.7	27.8	28.1	63.7	63.4
6/29 June-2 July	29.0	29.1	54.1	53.4	27.8	28.1	68.6	67.5
7/30 June-3 July	29.2	29.3	58.2	59.6	26.9	27.1	62.6	63.4
8/3-6 July	29.3	29.4	54.2	55.6	28.0	28.4	61.1	60.9
9/7-10 July	29.6	29.7	53.3	55.4	28.3	28.6	57.1	56.0
10/10-13 July	28.8	28.8	56.6	57.1	27.5	27.2	57.5	58.6
11/14-17 July	28.7	28.7	56.9	57.6	27.3	27.5	64.4	65.2
12/17-20 July	29.4	29.5	55.0	56.0	28.4	28.7	59.9	59.4
13/21-24 July	30.2	30.2	55.9	56.7	29.8	30.0	62.0	59.2
14/24-27 July	30.8	30.9	53	54.9	30.7	30.9	60.0	59.9
15/28-31 July	31.7	31.8	54.1	55.8	28.2	28.3	60.3	61.0
16/31 Jul-3 Aug	31.2	31.2	56.2	56.2	29.3	29.5	63.3	58.8
Round 2 (Observations every 15 days)								
17/25-28 Aug	30.6	30.7	52.1	51.5	29.4	29.6	67.5	68.4
18/11-14 Sept	27.2	27.3	54.2	53.7	26.6	26.7	66.3	67.3
19/29 Sept-2 Oct	25	25	51.3	51.5	17.3	18.7	54.0	50.5
20/16-19 Oct	21.1	21.1	48.1	49.2	19.4	19.6	85.7	86.4

579

580

581 **Table 6.** Average mortality (%) of *E. kuehniella* and *P. interpunctella* in funnel black
582 striped traps containing *(E)*-anethole microspheres of two formulations (SD or OEE,
583 mean of two traps for each formulation) or without any kind of insecticide (control, one
584 trap)**.**

	Ephestia kuehniella						*Plodia interpunctella*					
	Round -1		Round -2		Total		Round -1		Round -2		Total	
	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.
SD	81.91	951	26.62	263	70.85	1214	87.61	1146	65.15	396	83.12	1542
OEE	95.56	947	62.64	265	88.98	1212	94.57	1142	92.23	399	94.10	1541
Control	2.11	473	3.79	132	2.45	605	12.37	582	12.44	201	12.38	783

585 Round 1: twice a week, for 8 weeks, in total 16 observation periods.

586 Round 2: once every two weeks, in total 4 observation periods for 10 weeks.

587 no. = number of individuals tested

588

589 **Table 7.** Estimated efficacy over time of two *(E)*-anethole formulations (OEE and SD)
590 in two moth species inside funnel traps**.**

	Parameter estimates[a]			Estimated efficacy over time[b]			
	b	d	e	ET90	95% CI	ET50	95% CI
SD *E. kuehniella*	2.4142	0.9904	86.032	32.55	28.18-36.92	73.49	69.71-77.27
OEE *E. kuehniella*	3.1669	0.9974	124.51	60.69	56.02-65.35	110.8	103.7-117.8
SD *P. interpunctella*	10.521	0.8561	121.51	94.08[c]	87.22-100.9[c]	114.5	111.3-117.8
OEE *P. interpunctella*	0.02114	0.9464	267.16	126.9	--	--	--

591 [a] Binomial regression models (Weibull type I model was fitted for all curves, but for
592 OEE in *P. interpunctella*, the Logistic model was applied. According to the
593 parameterization of the DRC package, b = slope at "e" value, d = maximum value of
594 efficacy at start time, e = value of time, near inflection point in Weibull type I and in
595 inflection point in logistic model.
596 [b] Efficacy over time 90 (ET90) and 50 (ET50) are the times for which an efficacy
597 greater than or equal to 90% or 50%, respectively, is maintained. 95% CI = Confidence
598 intervals at 95%.
599 [c] ET80 was estimated because the maximum efficacy was <0.9.
600

601 **Table 8.** Percentage of female-sex moths in moths that escaped from the traps

Period	*Ephestia - IMIDA (Murcia)*		*Plodia - IRTA (Barcelona)*	
	Traps with anethole	Control traps	Traps with anethole	Control traps
1	50	-	-	-
2	0	100	-	-
3	0	100	-	-
4	100	0	-	-
5	-	100	-	-
6	0	100	-	-
7	-	0	-	-
8	100	100	-	-
9	0	-	-	-
10	-	-	-	-
11	100	100	-	-
12	-	100	-	-
13	0	75	-	-
14	16.7	100	-	-
15	28.6	100	-	-
16	50	33.3	66.7	61.5
17	0	25	66.7	46.4
18	0	45.5	36.4	42.9
19	100	100	60	100
20	33.3	100	50	66.7
Average	36.16%	75.22%	55.96%	63.5%

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603

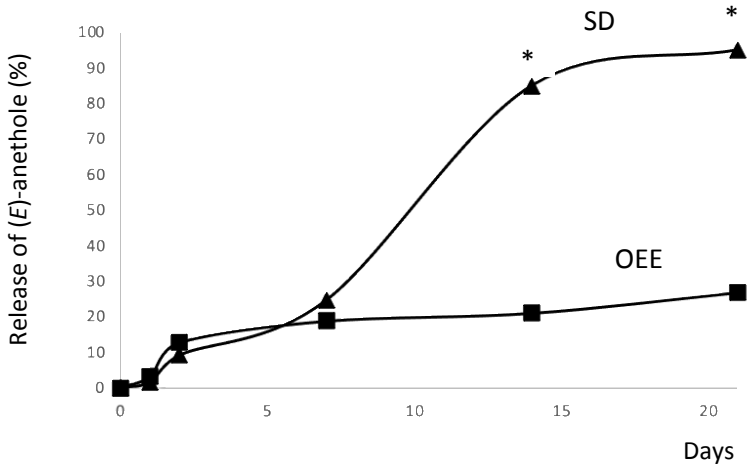
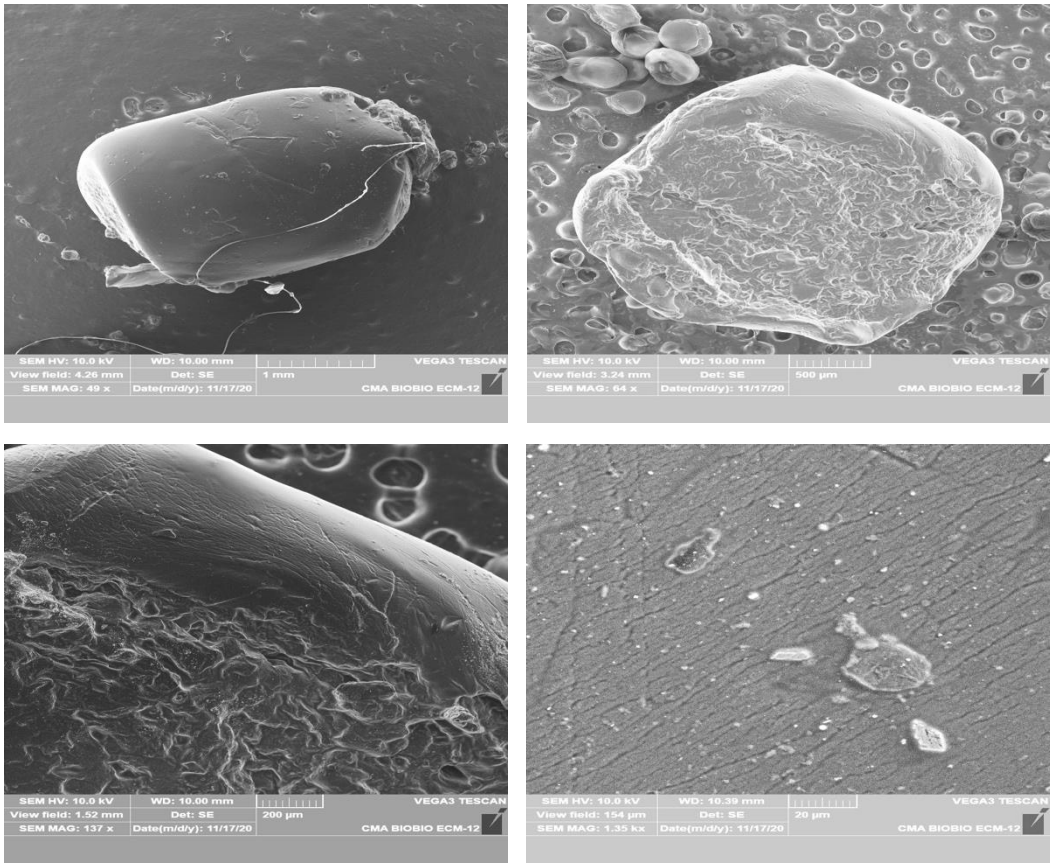


Fig. 1. Controlled release of (*E*)-anethole from different matrix blends during 21 days at 15 ºC (* = statistically significant differences between the SD and OEE formulations).

(A)


SEM HV: 10.0 kV
WD: 10.00 mm
VEGA3 TESCAN
View field: 4.26 mm
Det: SE
1 mm
SEM MAG: 49 x
Date(m/d/y): 11/17/20
CMA BIOBIO ECM-12
SEM HV: 10.0 kV
WD: 10.00 mm
VEGA3 TESCAN
View field: 3.24 mm
Det: SE
500 µm
SEM MAG: 64 x
Date(m/d/y): 11/17/20
CMA BIOBIO ECM-12
SEM HV: 10.0 kV
WD: 10.00 mm
VEGA3 TESCAN
View field: 1.52 mm
Det: SE
200 µm
SEM MAG: 137 x
Date(m/d/y): 11/17/20
CMA BIOBIO ECM-12
SEM HV: 10.0 kV
WD: 10.39 mm
VEGA3 TESCAN
View field: 154 µm
Det: SE
20 µm
SEM MAG: 1.35 kx
Date(m/d/y): 11/17/20
CMA BIOBIO ECM-12

(B)

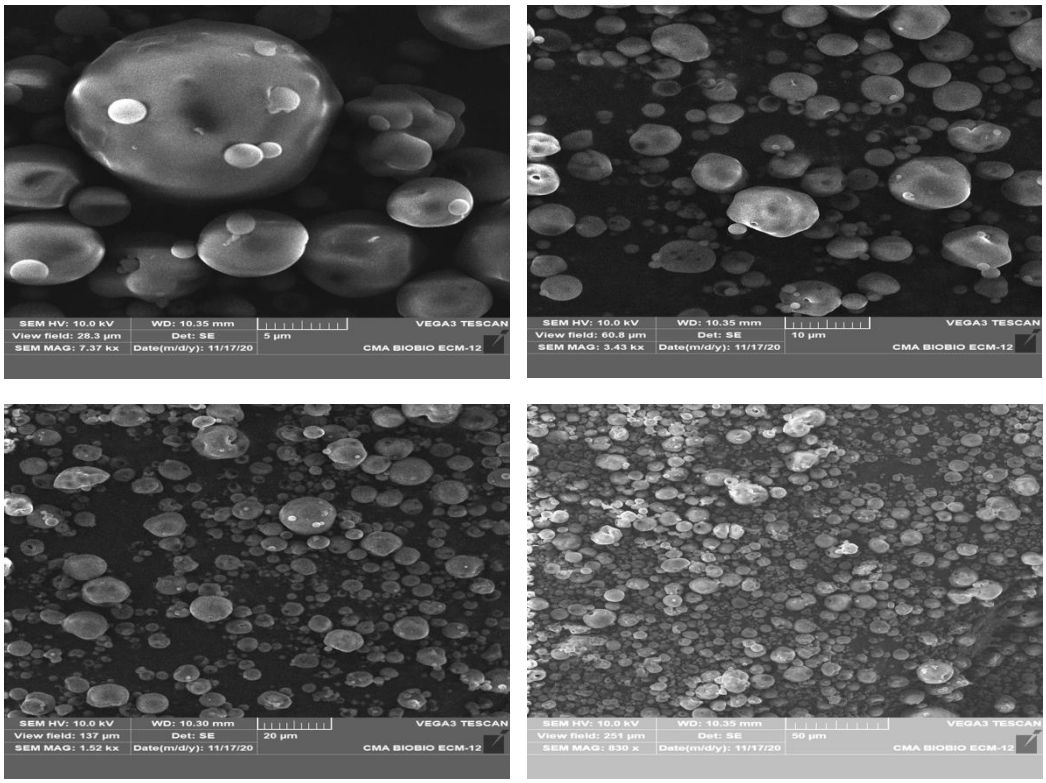
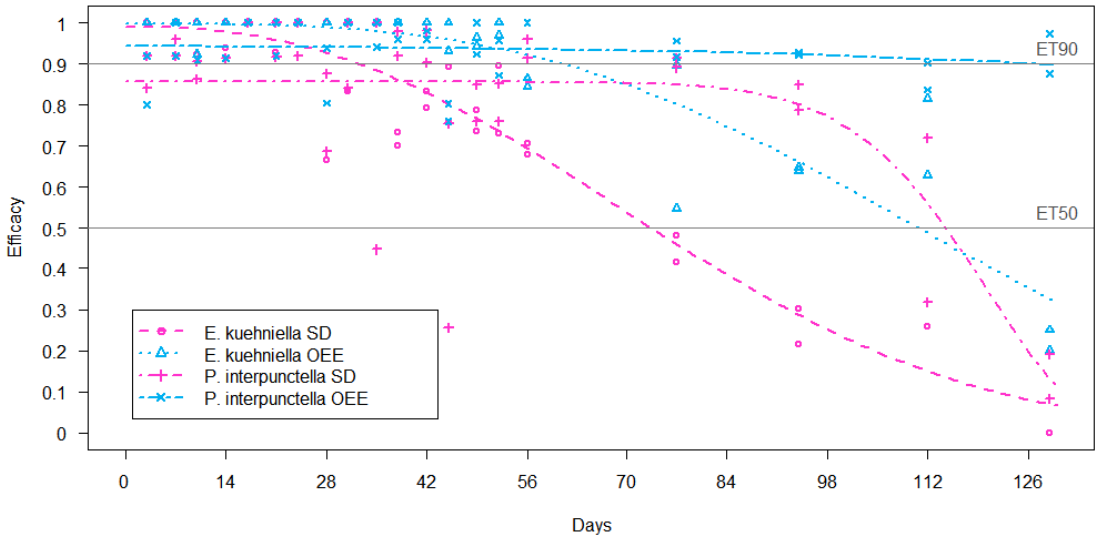


Fig. 2. SEM micrographs of microspheres: (A) OEE at 1000 µm, 500 µm, 200 and 20 µm and (B) SD at 5 µm, 10 µm, 20 µm and 50 µm.



639

640 **Fig. 3**. Evolution of efficacy over time of *(E)*-anethole formulations. The Weibull type I

641 model was used to fit both curves in *E. kuehniella* and *P. interpunctella* with SD, but *P.*

642 *interpunctella* with OEE was fitted with the Logistic model. ET90 = Efficacy over time

643 90 and ET50 = Efficacy over time 50.

644