Development of Formulations to Improve the Controlled-Release of Linalool to Be Applied As an Insecticide

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ABSTRACT: In recent studies, insecticide activity of a monoterpene, linalool, has been demonstrated, finding, however, limitations in application because of its rapid volatilization. Potential effectiveness of microcapsules and effects of various types of matrices on its stability as controlled-release systems for the slow volatilization of linalool to be applied as insecticide were evaluated. To study controlled-release, linalool was entrapped into microcapsules, inclusion complexes, and beads, obtained by different methods, inverse gelation (IG1, IG2, IG3, IG4, and IG5), oil-emulsion-entrapment (OEE), interfacial coacervation (INCO), and chemical precipitation (Cyc5 and Cyc10). The encapsulation yield turned out to be different for each formulation, reaching the maximum retention for IG1 and OEE. In controlled-release, OEE followed by INCO presented a long time necessary for releasing as a result of the presence of glycerol or chitosan. These results pointed out remarkable differences in the release behavior of linalool depending on matrix composition and the method of encapsulation.

KEYWORDS: microcapsule, bead, inclusion complexes, monoterpene, release

1. INTRODUCTION
In the last decade, synthetic organic pesticides have been extensively used to prevent and control pests in agriculture, showing a high toxicity against insects, although presenting sometimes an important environmental impact, developing resistance, or even damaging human health. Therefore, other alternatives such as phytochemical, pheromones, biological control, or heat treatment are growing in the field of agriculture. Linalool, a monoterpene found in essential oils from some plants, has been proved to be an effective insecticide against some pests.1–4 However, application of this chemical turns out to be unsuccessful, due to its chemical and physical characteristics that involve low stability, high evaporation, and losses. As a result, it is necessary to develop formulations to improve the handling of chemicals such as linalool and be able to control the rate at which this compound leaves the microcapsules.

To study controlled-release, some aspects such as the nature of the compound to encapsulate and the selection of an adequate cross-linker as well as wall material or solvents are the main important points to take into account.5 According to this, molecular structures of monoterpens, including hydrocarbons, alcohols, aldehydes, ethers, ketones, and esters, which present variation in effects such as the degree of retention in the caps or the morphological distribution and characterization of the particles, achieve different responses in encapsulation and controlled release. For instance, cyclodextrins are a crown-like structures consisting of primary and secondary hydroxyls, atoms of hydrogen and carbon and glucosidic linkages, and have been widely employed due to their ability to form inclusion complexes with a wide range of nonpolar or unstable molecules5–9 being candidates for use with linalool since the inclusion of other monoterpens such as eugenol, cinamaldehyde, or thymol with cyclodextrins has been recently studied and demonstrated10–13 obtaining notable outcomes. In addition, wall material, as sodium alginate has been employed to make gel beads prepared through sol–gel transformation of alginate, which is brought by gelification of the alginate with divalent cations such as Ca2+14 for the delivery of biomolecules such as drugs, peptides, and proteins. Likewise, alginate–chitosan beads are prepared via ionic interaction between the carboxyl residues of alginate and the amino terminals of chitosan. Complexation of alginate with chitosan reduces the porosity of the alginate beads, and chitosan acquires a higher level of mechanical strength with the support of the alginate gel mass.15

Moreover, to play on the internal formulation it is interesting to add starch and starch-based ingredients that are widely used in the food industry to retain and protect volatile compounds such as linalool.16 They can act as carriers for aroma encapsulation, fat replacers, and also emulsion stabilizers.17 In fact, the behavior of volatile compounds as modulated release within starch matrices have been examined in recent works manifesting a great interest in this subject.18,19

The present work deals with developing new methods of microencapsulation of linalool using β-cyclodextrin, alginate, an alginate–chitosan blend, starch, and modified starch blends as coating materials obtaining nine formulations (IG1, IG2, IG3, IG4, IG5, INCO, OEE, Cyc5 and Cyc10) and observing the effectiveness of the release of linalool from these microcapsules, beads, and inclusion complexes in order to improve the application of this compound as an insecticide.

2. MATERIALS AND METHODS

2.1. Materials. Linalool (97%), β-cyclodextrin (98%), and chitosan low viscous were obtained from Sigma-Aldrich, whereas starch matrix

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(Meriten 100) and modified starch (Clear Gum CO 01) were, respectively, purchased from T&L (The Netherland) and Roquette (France). Algogel 3001 (sodium alginate powder, MW = 151,200 Da, M/G ratio = 0.64) was purchased from Panreac Quimica Sau (Panreac art n° 131232, Spain). Sunflower oil of commercial grade was obtained from Associated Oil Packers, France, and glycerol (99.5% pure) was obtained from Laborgros, France. Analytical grade solvents and surfactants were from Sigma-Aldrich.

2.2. Methods of Microencapsulation. 2.2.1. Preparation of β-Cyclodextrin/Linalool Complexes (Cyc). According to Reineccius, a chemical precipitation method was employed to prepare β-cyclodextrin—linalool complexes (Figure 1a). Forty milliliters of linalool was dissolved in 400 mL of ethanol. The solution was added slowly to a suspension of 5 or 10 g of β-cyclodextrin (Cyc5 and Cyc10 respectively) in 100 mL of ethanol/water (1:2, v/v). The blend was refrigerated overnight at 4 °C for 24 h. Each treatment was prepared in triplicate.

2.2.2. Microcapsules Prepared by Inverse Gelation (IG). Inverse gelation consisted of dropping a calcium suspension in an alginate solution (Figure 1b). By diffusion in the alginate solution, calcium was gelifying the alginate and forming a membrane around the droplets. The calcium suspension consisted of 80 mL of calcium chloride solution (40 g/L) dispersing in 200 mL (capsules IG1 and IG2) or 65 mL (capsules IG3, IG4 and IG5) of linalool/sunflower oil (50% v/v) with the help of a T10 basic ultraturax. The emulsion was dripped into a 10 or 6 g/L alginate solution. The internal diameter of tips was 0.38 mm. Modified starch was added to the calcium chloride solution (capsules IG4) or starch with alginate solution (capsules IG5) to increase the solid content in the membrane.

Table 1 summarizes the composition of the different phases. Alginate solution was continuously stirred at 350 rpm to avoid agglomerations, and the microcapsule curing time was 15 min. Capsules were filtered with wire mesh and washed with distilled water, and finally were allowed to air dry at room temperature (18 °C) overnight in order to reach their equilibrium moisture content.

2.2.3. Encapsulation of Linalool by Interfacial Coacervation (INCO). Microcapsules were produced by dripping an alginate suspension (polyanion) in a chitosan solution (polycation) (INCO) (Figure 1c). The alginate suspension consisted of an emulsion of 350 mL of alginate solution (10 g/L), a solution of linalool (100 mL), sunflower oil (100 mL), and surfactants (1.72 mL of SPAN85 and 2.28 mL of TWEEN85). This blend was dripped (0.38 mm internal diameter of tip) in a solution of chitosan (20 g/L, acetic acid 1% at pH 4) with continuous stirring. Migration of the polymers to the droplet interface led to the formation of the polymer complex and a membrane. The beads were filtered with wire mesh and finally were dried overnight at room temperature (18 °C).

2.2.4. Beads of Linalool by Oil-Emulsion-Entrapment (OEE). Beads were formed by dripping an alginate solution (containing a dispersion of linalool and glycerol) into a calcium solution (OEE) (Figure 1d). Diffusion of the calcium in alginate droplets led to their gelification. The preparation of the internal phase was carried out as follows: linalool (23 mL) was dispersed in glycerol (23.40 mL). The blend was dispersed in 350 mL of alginate (41 g/L) and starch (4.7 g/L) using an ultraturax. This dispersion was dripped into calcium chloride solution (19.36 g/L). The dispersion was dripped into calcium chloride solution (19.36 g/L). Beads were filtered with a wire mesh and finally were dried overnight at room temperature (18 °C).

2.3. Determination of Oil Volume Fraction in the Capsules and Membrane Thickness. For microcapsules, the diameter of the capsule (d1) and of the oil core (d2) was measured under an optic microscope, and the presence of oil was observed with bright-field optics. Selected photomicrographs (magnification 20×–100×) were taken (see Figure 2). The oil volume fraction (α) and membrane thickness (δ) were evaluated by the following equations:

\[
\alpha = \left(\frac{d_2}{d_1}\right)^3 \tag{1}
\]

\[
\delta = \frac{d_1 - d_2}{2} \tag{2}
\]

In the case of beads containing fine droplets, the distance between drops was evaluated assuming that each drop was centered in the matrix cube (side = L) and that all drops had the same diameter (d3) (Figure 3),

\[
V_{\text{cube}} = L^3 \tag{3}
\]

and

\[
V_{\text{drop}} = \pi/6d_3^3 \tag{4}
\]

with

\[
V_{\text{drop}} = \alpha V_{\text{cube}} \tag{5}
\]

Table 1. Composition of Linalool Encapsulated Microcapsules by Inverse Gelation

<table>
<thead>
<tr>
<th>method</th>
<th>IG1</th>
<th>IG2</th>
<th>IG3</th>
<th>IG4</th>
<th>IG5</th>
</tr>
</thead>
<tbody>
<tr>
<td>sunflower oil (mL)</td>
<td>100</td>
<td>100</td>
<td>34</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>linalool (mL)</td>
<td>100</td>
<td>100</td>
<td>32</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>calcium chloride (g/L)</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>modified starch (g)</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>sodium alginate (g/L)</td>
<td>1.72</td>
<td>1.72</td>
<td>1.72</td>
<td>1.72</td>
<td>1.72</td>
</tr>
<tr>
<td>starch (g)</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
</tr>
<tr>
<td>diameter of tips (mm)</td>
<td>0.38</td>
<td>0.25</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Figure 1. Schematic diagrams of the experimental setup corresponding to (a) chemical precipitation, (b) inverse gelation, (c) coacervation, and (d) oil-entraped-emulsion.
Combining these equations, $d_3$ and $\delta_2$ were calculated as follows:

\[
d_3 = \left( \frac{6\alpha L^3}{\pi} \right)^{1/3}
\]

\[
\delta_2 = (L - d_3) \cdot 2
\]

Through this work, porosity or macroporosity and microporosity were used to describe the different kinds of pores which were found. Macroporosity is referred to as the pores of alginate beads and capsules between 10 and 100 $\mu$m, whereas microporosity is referred to as smaller pores (<10 $\mu$m) found between alginate and starch capsules and beads or alginate and chitosan capsules and beads or alginate and other fillers.

2.4. Encapsulation Yield and Loading. The amount of linalool into the dry capsules was determined by weight loss in a chamber at 90 °C over 24 h for capsules and beads from IG, INCO, and OEE. However, the results were verified by GC/MS analysis in the case of $\beta$-cyclodextrin, as follows: 0.5 g of powder was dispersed in 8 mL of distilled water and 4 mL of hexane in 15 mL glass vials. Vials were heated and stirred in a hot plate at 75 °C for 20 min. The organic phase containing linalool was decanted, and the aqueous phase was exhaustively extracted with hexane 3 times ($4 \times 4$ mL). These 4 phases were combined. The hexane was removed using a nitrogen stream. The quantitative analysis of linalool was carried out using a model 5890 Series II equipped with a DB-Waxetr 30 m × 0.32 mm capillary column coated with a polyethylene glycol film (1 $\mu$m thickness) and an Agilent model 5972 inert mass spectrometry (MS) detector (Agilent, Palo Alto, CA). The initial oven temperature was held at 60 °C for 1 min. Afterward, it was increased by 3 °C/min to 225 °C, with injector at 250 °C, column head pressure at 8.00 psi, helium carrier gas, flow rate of 2.6 mL/min, and splitless with 2 $\mu$L of sample injected. The content of linalool was calculated according to the area of the chromatographic peak and using linear regression.

Encapsulation yield is defined as the ratio between the quantities of linalool in the capsules versus the initial amount of linalool. Loading is defined as the quantity of linalool per gram of dry microcapsules.

2.5. Controlled Release of Linalool through Different Matrix Blends. One gram of dry sample was placed into the vials without sealing. These vials were maintained in a humidity control chamber at 25 °C, and weight loss was monitored in an analytical balance as a function of time for 336 h (14 days). As a control, 1 g of linalool was set in a vial to study the weight loss for this time. The capsules were strictly maintained in dry conditions.

The release profile was fitted to the equation following a simple diffusion process out of the capsules:

\[
\% \text{release} = 1 - e^{-\ln(2)t/\eta/2}
\]
where \( T_{1/2} \) is the time to release 50% of linalool. Three replications were developed in this assay.

### 2.6. Statistical Analysis

Data were statistically analyzed by analysis of variance (ANOVA) using SPSS (PASW Statistic 18). Duncan’s multiple tests were applied for the calculation of the significant differences among the controlled release of the blends at the 5% level (\( P = 0.05 \)).

### 3. RESULTS AND DISCUSSION

#### 3.1. Encapsulation of Linalool in \( \beta \)-Cyclodextrin

On the basis of the work of other authors,\(^{21} \) first we assumed that \( \beta \)-cyclodextrin and linalool could interact when forming complexes. To carry out the entrapment of linalool in \( \beta \)-cyclodextrin, a chemical precipitation was followed according to literature recommendations,\(^{22} \) obtaining a drying fine powder (Figure 2d) which was finally analyzed. Encapsulation yield was very low reaching only 10% and 16% for the batch Cyc5 and Cyc10. Loadings were, respectively, 0.31 and 0.35 g linalool/g \( \beta \)-cyclodextrin. Assuming that one linalool molecule (154 g/mol) could be complexed by one \( \beta \)-cyclodextrin molecule (1135 g/mol), the maximum loading could be only 0.12 g linalool/g \( \beta \)-cyclodextrin. In this protocol, the linalool was largely in excess, and most of this volatile compound was trapped in the powder and not really complexed by the \( \beta \)-cyclodextrin.

In Figure 4, the release for cyclodextrins (Cyc5 and Cyc10) and free linalool as control for 336 h was compared. The time to release 50% of linalool (\( T_{1/2} \)) was relatively short, (24 and 22 h for Cyc5 and Cyc10, respectively, without statistical differences between Cyc5 and Cyc10 showing the low retention capacity of linalool by the powder, linked to the fact that linalool is mainly physically trapped in the cyclodextrin and not complexed.

Regarding the cost of the \( \beta \)-cyclodextrin, the low loading, and encapsulation yield, as well as the fast release, then, we decided to test other approaches for the encapsulation and controlled release of linalool.

#### 3.2. Encapsulation by Inverse Gelation

An inverse gelation technique (dripping calcium dispersion in alginate solution) was tested to form microcapsules. Data corresponding to the capsules prepared by inverse gelation are summarized in Table 2. Batch IG1 was defined as the control and batch IG2 had the same formulation but a smaller tip diameter. In batch IG3 to IG5, the quantity of organic phase (linalool and sunflower oil) was reduced (from 200 to 65 mL). In IG4 and IG5, starch was added, respectively, to the internal suspension or external solution, expecting that its incorporation in the membrane would increase the dry matter and reduce the permeability.

Capsules produced by inverse gelation were relatively spherical (Figure 2) with a diameter of 2.2 mm. While reducing the tip diameter (IG2 vs IG1), the diameter of capsules was slightly smaller (from 2.2 to 1.8 mm). The core of the capsule (oily phase) was 0.5 mm, leading to a loading of 0.70 for all the batches. The encapsulation yield varied from batch to batch reaching the maximum value for IG1 (89%) followed by IG2 (75%). The time to release 50% of linalool, \( T_{1/2} \), turned out to be 24 h for IG1 and IG2. For IG3, IG4, and IG5, the encapsulation yield was around 70%, and the \( T_{1/2} \) was shorter than that of IG1 and IG2 (15 h).

Several authors\(^{23} \) studied blends with starches to encapsulate cardamom oleoresin and proved it to be more efficient than these formulations. Furthermore, when the carrier was considered, it was shown that retention was influenced by its chemical functions, its molecular weight, and the state of the carrier. Also, an increase in the concentration of carbohydrates generally was proportional to the release of flavor compounds due to the salting out effect. Nevertheless, sometimes an increase in polysaccharide concentration had led to a decrease in the release of flavor compounds due to the complexation and viscosity effect of that polysaccharide itself.\(^{23} \)

Alginate microcapsules by inverse gelation using the same amount of linalool (IG3) showed a high release because of the high porosity of the alginate membrane (Figure 5), and at 24 h, basically all the content had been released. This level of porosity of the alginate membrane has been strongly proved by a lot of researchers.\(^{24,25} \) From Table 2, half time release (\( T_{1/2} \)) indicated how fast linalool was evaporated; thus, linalool was released more quickly by IG3, followed by the rest of capsules (IG1, IG2, IG4, and IG5). In addition by inverse gelation, two blends, IG4 and IG5, presented a quick liberation though release slower than that of IG3 since the presence of starch or

![Figure 4. Controlled-release (% of linalool by chemical precipitation (Cyc5 and Cyc10) for 336 h (14 days) using free linalool as a control.](image)

Table 2. Evaluation of the Inner Porosity in Capsules, Beads, and Inclusion Complexes

<table>
<thead>
<tr>
<th>method</th>
<th>dry capsules size (mm)</th>
<th>membrane thickness (mm)</th>
<th>linalool encapsulated (ppm)</th>
<th>loading (g linalool/g)</th>
<th>encapsulation yield (%)</th>
<th>( T_{1/2} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyc5</td>
<td>310</td>
<td>0.31</td>
<td>10</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyc10</td>
<td>350</td>
<td>0.35</td>
<td>16</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IG1</td>
<td>2.2 ± 0.20</td>
<td>0.230</td>
<td>700</td>
<td>0.70</td>
<td>89</td>
<td>21</td>
</tr>
<tr>
<td>IG2</td>
<td>1.8 ± 0.30</td>
<td>0.201</td>
<td>670</td>
<td>0.67</td>
<td>75</td>
<td>24</td>
</tr>
<tr>
<td>IG3</td>
<td>2.0 ± 0.20</td>
<td>0.220</td>
<td>700</td>
<td>0.70</td>
<td>71</td>
<td>14</td>
</tr>
<tr>
<td>IG4</td>
<td>1.9 ± 0.30</td>
<td>0.101</td>
<td>690</td>
<td>0.69</td>
<td>69</td>
<td>16</td>
</tr>
<tr>
<td>IG5</td>
<td>2.0 ± 0.20</td>
<td>0.152</td>
<td>680</td>
<td>0.68</td>
<td>69</td>
<td>14</td>
</tr>
<tr>
<td>INCO</td>
<td>1.8 ± 0.30</td>
<td>0.012</td>
<td>610</td>
<td>0.61</td>
<td>40</td>
<td>165</td>
</tr>
<tr>
<td>OEE</td>
<td>0.3 ± 0.05</td>
<td>0.010</td>
<td>870</td>
<td>0.87</td>
<td>86</td>
<td>1700</td>
</tr>
</tbody>
</table>

\(^{a}\)Distance between oil droplets in the case of beads. \(^{b}\)Linalool in the capsules. \(^{c}\)Percentage of initial linalool really encapsulated. \(^{d}\)Half time release.
modified starch made the membrane have less porosity because starch reduced the macroporosity. However, in spite of having IG1, IG2, and IG3 of the same membrane thickness with similar porosity, IG1 and IG2 showed a release slower than that of IG3 because its loading efficiency was greater. However, when comparing IG1 and IG2 to IG4 and IG5, differences between thickness and release are observed for the four treatments. These wall materials, alginate and starch, are economical, at least 10 times cheaper than β-cyclodextrin, and simple to employ. It would be notable to improve these formulations since when considering the cost of alginate and the remarkable loading and encapsulation yield, it would be interesting to improve the controlled release, increasing the time of release to achieve our objective.

### 3.3. Encapsulation of Linalool by Coacervation

The interfacial coacervation (INCO) technique was used to make beads using chitosan. The shape of beads from INCO was found to be partially disrupted, and most of them were not spherical (Figure 3). From Table 2, a low encapsulation yield (40%) was observed, mainly half of that in the case of inverse gelation. However, a slower controlled liberation was observed with time to release 50% of linalool at 165 h due to low porosity by using chitosan (Figure 6). This was between 7 to 10 times slower than that for inverse gelation. However, a drawback in this procedure was that chitosan was more expensive than the starch matrix or alginate; thus, it would be more interesting to improve formulations of starch or/and alginate, which involves the lowest possible cost. Comparing methods, inverse gelation showed a significant release at the beginning because the alginate membrane contained an elevated porosity easily allowing the release of the volatile. However, INCO presented a release of this chemical more slowly becoming more suitable in order to control this monoterpene for a long time, demonstrating that the presence of cross-linkers or coaters such as chitosan improved the time of release of linalool.

However, the cost of chitosan is very high, similar to β-cyclodextrin, thus turning out to be a great disadvantage for the use of this compound.

For Riyajan and Sakdapipanich neem capsules with sodium alginate matrix cross-linked by glutaraldehyde seemed also to show clear outcomes. In agreement with this, Lin and Ciou also have found that cross-linking with calcium and chitosan changed the properties and porous structures of the lyophilized alginate membrane. In general, the more cross-linked an alginate membrane was, the stronger and more stable it became, and the more suitable it was to be a controlled release carrier. However, Sao Pedro et al. found that there were few studies with the association of chitosan with essential oils or similar products, and more studies had to be performed for developing and characterizing new formulations, taking advantage of the potential of chitosan for essential oil entrapment.

### 3.4. Emulsion Entrapment in Alginate Beads

The last formulation carried out was oil-emulsion-entrapment (OEE), which was used to formulate beads having a spherical shape and a diameter of 0.26 mm. Starch and glycerol were added to the formulation to increase the solid content and solve one of the drawbacks of alginate beads, that is to say, porosity, since glycerol increased the viscosity and consequently improved the stability of the emulsion. Therefore, the volatile was better entrapped and the release was slower since glycerol reduced the porosity on alginate beads. This method showed a great encapsulation yield (86%), and the time to release 50% of linalool was very long (1700 h), but only 20% of linalool was, in fact, released at 336 h. After that, more experiments were needed to confirm it, but glycerol had already been reported to have a great affinity for flavor compounds and often had been used as a support for flavor preparations. Our results also indicated that glycerol improved the formulation, making linalool release slowly and optimized the bead, reducing the microporosity for applications requiring a long time. Nevertheless, the OEE blend (oil-emulsion-entrapment) showed liberation of this chemical extremely slowly, becoming a disadvantage to applying this monoterpene to control pests. These data showed, for the same volatile compound, that retention varied according to the nature and physical state of the carrier. Besides, depending on the type of blend used (alginate, starch, or modified starches), the properties of the capsules and beads presented different figures for the porosity since each type of carbohydrate presented different structures that influenced the interaction between flavor compounds and their structure and also the retention and release. According to this, our results of the release of linalool showed a great dependence on the formulation used.

To finish up, we can summarize that this preliminary screening of encapsulation methods gave us some interesting indications for further experiments. First of all, β-cyclodextrin encapsulation did not provide interesting performances (low loading, low yield, and low release control) associated with a high cost; therefore, this direction will not be maintained. Alginate is a cheap, environmentally friendly material, allowing the making of encapsulation in soft conditions. However, it did...
not provide good release control. The addition of starch and especially glycerol had a positive effect on the encapsulation yield, loading, and release rate of linalool. Nevertheless, in the oil-emulsion entrapment, only 20% of the linalool was released. Also, inverse gelation associated with glycerol and starch in the formulation may allow for the combining of advantages while avoiding drawbacks of the different methods tested in this study.

In conclusion, this work brings information, opening up the potential for succeeding in the encapsulation of molecules such as linalool, effective against pests using cheap, easy, and environmentally friendly methods.

Future works and new formulations involving methods of encapsulation, other carriers, or blends performing inverse gelation using glycerol should be tested to improve the encapsulation and controlled release for this chemical in order to apply it as an insecticide.

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