Supplementary material:

Analysis of particle size regulating the insecticidal efficacy of phoxim polyurethane microcapsules on leaves

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Material

MDI (4,4-methylenediphenyl diisocyanate, AR) was purchased from Wanhua Chemical Group Co., Ltd. (Shandong, China). Xylene and ethylene glycol (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Polyethylene glycol (PEG 400) was purchased from Yunchuan Chemical Co., Ltd. (Zibo, China). Tween-80 was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Xanthan gum (food grade) was purchased from Zhongxuan Biochemical Co., Ltd. (Zibo, China). Sodium lignosulfonate (relative molecular weight, 1.0×104 to 1.2×104; sulfonation, 0.85 mmol/g) was purchased from MeadWestvaco Inc. (USA). The water used throughout the study was distilled water.

Insect source

The tested insects were *Agrotis ipsilon* (Rottemberg). The insects were successively reared in the laboratory and fed artificial feed at 25±1 °C, 70±5% relative humidity in 16:8 light/dark cycles. Fourth-instar larvae were selected for the bioassay and greenhouse experiments. Cabbage (*Brassica oleracea* L.) was cultivated artificially in the greenhouse without exposure to any chemicals.

Preparation of Microcapsules

Microcapsule samples of different sizes were prepared by interfacial polymerization. The specific operation flow is as follows: First, 0.5 g MDI, 36.5 g phoxim and 5.0 g xylene were mixed and dissolved as an oil phase. Then, an appropriate amount of Tween-80, 1.5 g PEG 400, 5.0 g sodium lignosulfonate and 45.0 g distilled water were mixed as the aqueous phase. After homogenizing the

mixture at 10,000 r/min for 2 minutes at room temperature, the oil-in-water emulsion was transferred to a three-neck flask and stirred at 300 r/min. Then, a mixture of 1.0 g of ethylene glycol and 0.2 g of xanthan gum was added. Finally, the reaction was maintained at 65 °C for 3 hours at a stirring speed of 300 r/min. The total capacity of the whole system was 100 g, and the remaining part was replenished with distilled water to ensure that the content of phoxim was 35%. It should be noted that the requirements of Tween-80 during the preparation of MC-S, MC-M and MC-L were 2.0 g, 1.0 g and 0.5 g, respectively. To ensure the consistency of the sample composition, Tween-80 was added to 2.0 g after the preparation process was finished. The dried microcapsules were obtained by centrifugation, washing and drying. Specifically, noteworthy is that in order to indicate the distribution of MC particles on the organism, MC-S, MC-M and MC+L were prepared in the same way by adding 0.1% solvent blue 35 in the oil phase.

Encapsulation Efficiency of Microcapsules

On the basis of methods MT 189, CIPAC, the encapsulation efficiency of the MC samples was tested.[1] Approximately 0.1 g of a microcapsule suspension sample (±0.0001 g; Sartorius; Göttingen, Germany) containing uniform phoxim (m_0) was weighed into a clean dry glass bottle (100 mL) and diluted with deionized water to a total volume of 10 mL. Then, 50 mL of hexane was added to the glass vial, which was quickly placed on the rolling device. The bottles were allowed to roll at 60 ± 10 rpm for 15 minutes. After 15 minutes (±10 seconds), the bottles were removed from the rolling device, and 1 mL of the upper hexane layer was transferred immediately. This

layer contained all unencapsulated phoxim (m_t) to be measured. The amount of phoxim was determined using HPLC system. The encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = (m_0 - m_t)/m0 \times 100$$

where m0 is the total amount of phoxim in the 0.1 g microcapsule suspension sample and mt is the amount of phoxim in the upper hexane layer.

Release properties of microcapsules

The assessment of release properties was referenced to the reported method. [2] A sample (0.2 g) was weighed into a clean, dry glass bottle (150 mL). The volume of formulation in the bottle was calculated, and sufficient water was added to give a total volume of 6 mL. A hexane/ethanol (120:10, v/v) mixture (100 mL) was added to the bottle via pipette. The bottle was capped and immediately placed on a roller set to roll the bottle horizontally at 70 ± 10 rpm, and a timer was started. Note that the speed quoted is that of the bottle and not the roller. Subsequently, 0.5 mL of liquid was removed at various time intervals and immediately added to the same volume of release media. The amount of phoxim (A_t) was measured, and the amount of phoxim in the 0.2 g phoxim formulation (A_0) was also determined by HPLC. The cumulative release ratio was calculated using the following equation:

$$\text{Cumulative release proportion (\%)} = (A_t / A_0) \times 100$$

Determination of A_0

The measurement of A_0 proceeded as follows: MC suspensions (0.2 g) were

accurately weighed and were transferred to glass bottles (100 mL). Methanol was added to a volume of 100 mL. Subsequently, the glass bottles were placed in a 25 ℃ water bath with ultrasonic treatment at 100 Hz for 20 min, and the phoxim that was encapsulated in the microcapsules could completely diffuse into the methanol solutions during this process. After that, the glass bottles were centrifuged at 3000 rpm for 5 min, and the upper methanol solutions were extracted for measurement. The response value of LC in methanol was measured using an HPLC system, and the amount of LC (A_0) in the 0.2 g MC formulation was determined.

UV irradiation experiment

The photostability of the MCs was tested according to a previously reported protocol. The samples were diluted with deionized water to a concentration of 3500 mg/L. Then, 1 mL of the dilution was evenly applied to a petri dish. After evaporation of the water, the dish was exposed to ultraviolet rays with an average irradiation intensity of 25 $\mu W/cm^2$. Then, the dish was removed at regular intervals. The residue in the dish was washed with methanol and transferred to a volumetric flask (100 mL). Then, the solution was filled to a constant volume of 100 mL using methanol. The amount of phoxim was detected using an HPLC system. The residual content was calculated using the following equation (1):

$$\text{Residual content (\%)} = (c_r / c_1) \times 100 \quad (1)$$

where c_1 is the initial concentration and c_r is the concentration after degradation.

HPLC analysis

The amount of phoxim was determined using an Agilent 1200 HPLC system

(Agilent 1200; Agilent Technologies; Santa Clara, CA), which was equipped with a UV detector. An Agilent Diamonsil C18 column (250 mm×4.6 mm i.d., 5 μm) was used to separate the analyte. An methanol/water mixture (85:15, v/v) was used as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 μL. The column temperature was maintained at 30 ℃, and the detection wavelength of the UV detector was set at 280 nm.

Field experiment of combined MC formulation

Field validation experiments were performed at the experimental field of Shandong Agricultural University, China (N 36°09', E117°09'), from May to June in 2018. One-meter-wide buffer zones were intended to separate each plot. The pesticides (EC and MCs of phoxim) were applied at 270 g a.i./ha with a spray volume of 450 liters per hectare. To promote foliage spreadability, 0.05% Silwet 408 (silicone additive, Momentive Performance Materials, USA) was added to diluted solutions. Cabbages in the untreated group were sprayed with an aqueous solution containing 0.05% Silwet 408. Each treatment had three replicates. Before treatment, the basic number of cabbageworm was counted. After treatment for 1, 3, 5, 7 and 9 days, the number of cabbageworm was investigated, and the control efficacy was evaluated.

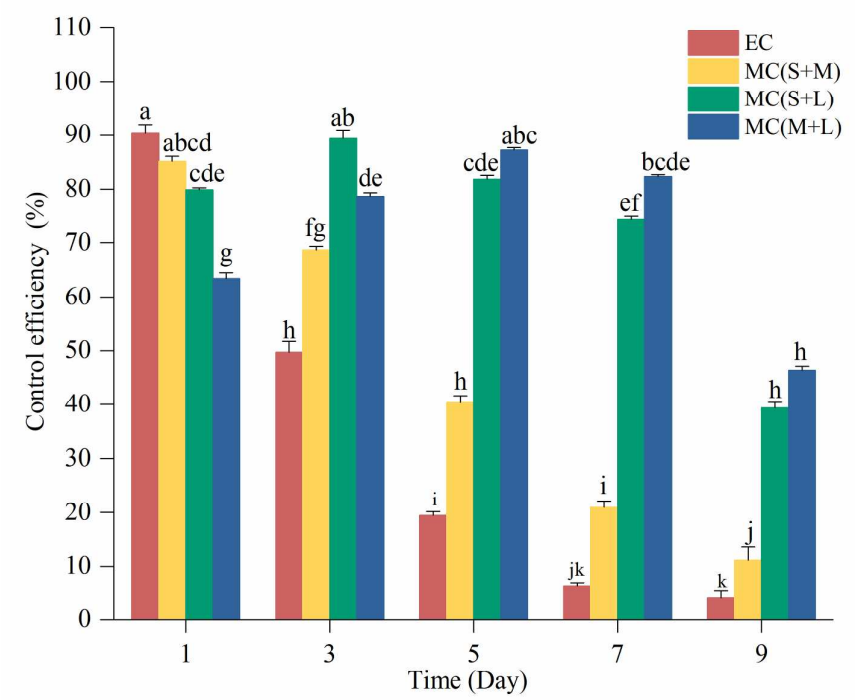

Figure S1. Control efficacy of EC, MC(S+M), MC(S+L) and MC(M+L) in the field. Data with different lowercase letters are significantly different at the $p < 0.05$ level by Tukey's multiple range test, and the data were arcsine square root transformed prior to statistical analyses. The error bars represent the standard errors of the means of three replicates.

Statistical analysis

The data from the experiments on the biological activity, wettability, release and photostability properties were statistically analyzed by using SPSS software (version 16.0) and were displayed as the means and SE (standard error) by Tukey's multiple range test ($p < 0.05$). The figures and data fitting analysis were obtained using OriginPro 2017 software.

(1) MT189. PAC-UK & Zeneca 539 Agrochemicals. CIPAC Handbook

Physio-chemical Methods for Technical Formulated Pesticides. 137-139 (2004).

(2) MT 190. CIPAC Handbook Physio-chemical Methods for Technical Formulated Pesticides; PAC-UK & Zeneca Agrochemicals, 140 (2004).