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Article

Zein-Based Nanocarrier of the Insecticide Cyantraniliprole for the Control of *Bemisia tabaci*MEAM1 (Whitefly)

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ABSTRACT: *Bemisia tabaci*, also known as whitefly, is a sap-sucking polyphagous insect that severely affects important crops worldwide. The growing demand for environmentally friendly and effective pest control measures necessitates new approaches to reduce the volume and frequency of insecticide application to crops while maintaining the efficiency. This study aimed at developing and evaluating a biodegradable zein protein nanocarrier with the active ingredient (a.i.) cyantraniliprole (CNAP) for the control of *B. tabaci* in tomato (*Solanum lycopersicum*). Synthesis of the nanoformulation (ZeinCNAP) resulted in spherical structures with an average hydrodynamic size of 143.06 ± 1.03 nm and a surface charge of 40.36 ± 0.7 mV remaining stable over 56 days. When plants were treated with three doses of ZeinCNAP (1/10, 1/2, and full) and commercial CNAP, followed by the release of adult whiteflies, the insect mortality by ZeinCNAP full dose (30 ± 0.9%) did not differ significantly from the commercial CNAP (42 ± 0.8%) 48 h after spraying. However, 72 h after spraying, the ZeinCNAP dose of 1/10, insect mortality (32 ± 1.1%) remained statistically equivalent to the commercial CNAP at full dose (49 ± 0.6%). When the plants were treated with commercial CNAP, ZeinCNAP, or water, aspects of fresh mass, photosynthetic parameters, and oxidative stress were analyzed at 24, 168, or 360 h after application, and no significant negative effects were noted in the analyzed parameters. The developed nanoinsecticide based on the zein platform and the active ingredient CNAP has significant potential for controlling on *B. tabaci* MEAM1 at reduced doses and can be considered safe for tomato plants. This work adds to a growing body of evidence demonstrating the potential of nanoscale carriers to significantly reduce the environmental burden associated with agrochemical use while still maintaining equivalent efficacy to conventional strategies.

KEYWORDS: *zein, chemical control, nanoinsecticide, insect, protection, tomato*

1. INTRODUCTION

There is increasing concern about whether conventional agricultural practices can meet the rising global demand for food production, especially in a changing climate context. One of the undesirable effects described is related to the change in the dynamics of invasive species.[1,2] Biological invasions are diverse, and as climate change influences their behavior, new challenges in managing these species are expected to emerge.[3,4] It is estimated that, in the past 50 years, biological invasions have cost a minimum of USD 1.288 trillion in economic losses to human societies.[5] Among the most concerning invasive pests, the sap-sucking polyphagous insect *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), also known as whitefly, remains one of the most economically important pests in crops around the world.[6,7]

Adult whiteflies can indirectly cause significant reductions in photosynthesis rates due to the secretion of honeydew, which induces sooty mold, a fungal disease that grows on plants and other surfaces covered by sugary compounds.[8] However, the most deleterious effects of whiteflies on agriculture are their ability to transmit several plant pathogenic viruses belonging to the genera *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, and *Torradovirus*.[9−13] Some estimates suggest that plant diseases, including viruses, are responsible for annual losses of USD 30−50 billion in food production.[14] Plant viruses cause major reductions in the yield and quality of staple crops worldwide, imperiling global food security.[15]

Chemical insecticides are the broadly used methods to manage *B. tabaci* infestations in crops. Cyazypyr, a broad-spectrum anthranilic diamide insecticide with the active ingredient (a.i.) cyantraniliprole (CNAP), is one of the most widely used formulations in the control of these insects.[16,17] Although insecticides are essential in conventional agriculture, they can harm humans and other nontargeted organisms when overused.[18] For example, the excessive use of agrochemicals in crop fields has shown undesirable side effects, such as the development of pest resistance and toxicity to humans and animals alike.[19] Therefore, novel yet effective strategies to curtail the intensive use of insecticides in agricultural systems are much needed.

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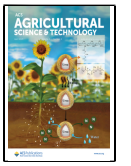

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Due to the difficulties in creating new agricultural chemicals (e.g., lack of novel molecules, increased cost of research and development, extensive regulation process, and public acceptance), research on developing novel formulations with increased efficacy at lower application rates is being widely pursued.[20] New methods to obtain more effective formulations are essential and should focus on environmentally safe and efficient pesticides to minimize the adverse effects on the environment.[21] In this context, nanotechnology offers great potential to transform agriculture and can be used as an innovative tool for delivering agrochemicals precisely and efficiently.[22−25] One novel strategy is based on the entrapment of the a.i. in tunable nanocarriers (NCs), which can then reduce the concentration of applied pesticides due to a more precise targeted delivery.[22,26−29]

Zein is a promising platform for developing NCs for the delivery of agrochemicals to plants. Zein is a class of prolamin protein derived from maize that can be processed into different forms of biopolymers and has a variety of uses, including in the pharmaceutical, and food industries, as well as coatings for drugs and in food products such as candy, nuts, and bakeries.[30] In the agricultural field, zein has been studied in combination with herbicides, fungicides, and insecticides.[31−33]

So far, few nanosystems incorporating CNAP as an active ingredient have been reported in the literature. Recently, Liu et al.[34] formulated a CNAP-based nanosystem through a hydrogen bond self-assembly technique with polylactic acid and a chemical deposition method with calcium carbonate for *Ostrinia nubilalis* control, being the first to demonstrate superior efficacy. Similarly, Li et al.[35] utilized star-shaped polycationic nanoparticles combined with CNAP through a self-assembly mechanism, targeting *Grapholita molesta* and *Cacopsylla chinensis*. Additionally, Gao et al.[36] encapsulated CNAP in a mesoporous silica nanosystem by physical entrapment for use against *Cnaphalocrocis medinalis*. However, there is limited investigation evaluating zein as a nanocarrier for insecticides for the control of pests, such as whitefly. Herein, we aimed to evaluate the effect of zein nanoparticles combined with the insecticide cyantraniliprole to control *B. tabaci* MEAM1. We investigated the effects of this nanoformulation on insect mortality in tomato plants and its possible effects on plant health indicators, including fresh mass production, photosynthetic responses, and oxidative stress. This work demonstrates the potential of nanoscale biopolymer carriers to deliver pesticides more precisely, leading to equivalent control at much lower application rates.

2. MATERIALS AND METHODS

2.1. Preparation of Zein Nanoparticles. All reagents used in this study were acquired from Sigma-Aldrich, USA, unless otherwise stated. The zein solution was prepared according to Hu et al.[37] using the nanoprecipitation method, with some modifications. First, the organic phase was prepared by dissolving zein in 80% ethanol with stirring overnight. The solution was centrifuged at 5,000*g* for 15 min at room temperature to remove the undissolved polymer. The supernatant was heated in a 70 °C water bath for 15 min, cooled to ambient temperature, and filtered with a 0.45 μm syringe filter. 10 mg of the analytical technical grade CNAP (95% purity, Sigma-Aldrich) active ingredient was added to the zein solution and magnetically stirred for 20 min. A pluronic solution (0.3 g in 30 mL of water) was prepared as the aqueous phase. Subsequently, the organic phase was quickly poured over the aqueous phase and kept under magnetic stirring for 30 min. A rotary evaporator was used to concentrate the solution to 10 mL, removing the ethanol. The final CNAP concentration was 1 mg mL^{-1}, and as a control nanoformulation, an equivalent batch was prepared without CNAP addition.

2.2. Physicochemical Evaluations. The particle size parameters of stability (*Z*-average), polydispersity index (PI), and zeta potential (ζ) were measured over time (days 0, 1, 7, 14, 21, and 56 after preparation) by dynamic light scattering (DLS), using a Malvern Zetasizer ZS (Malvern Panalytical, Westborough, MA), constantly comparing the ZeinCNAP and control. For DLS characterization, the formulation was dispersed at 1:1000 (v/v). Measurements were performed in triplicate.

Nanoparticle morphology was analyzed by transmission electron microscopy (TEM). The nanoparticle was diluted 1000 times in ultrapure water, and a 2 μL droplet was placed on a carbon film-supported copper grid and allowed to dry overnight at room temperature. The samples were then analyzed in a Hitachi HT7800 transmission electron microscope with a LaB_6 filament in high-resolution mode, with an acceleration voltage of 80,000 kV and a vacuum of 6.8×10^{-5} Pa.

The CNAP encapsulation efficiency was determined by an ultrafiltration−centrifugation method. The nanoparticles were subjected to centrifugation using a 10 kDa cellulose filter (Millipore). The filtrate is considered to contain the nonencapsulated CNAP. The efficiency of encapsulation was determined as

$$\text{Encapsulation efficiency (\%)} = (((\text{IA} - F) \times 100)/\text{IA})$$

where IA = initial amount of CNAP; F = filtrate.

The quantification of CNAP was performed by high-performance liquid chromatography (HPLC) with UV detection at 250 nm. The separation was performed using a HyperClone C18 column (250 × 4.6 mm, 5 μm) with a mobile phase consisting of a 95:5 (v/v) mixture of methanol and 1% (v/v) acetic acid. Quantification was obtained from an analytical curve ($y = 1.99x - 5.83$) with a correlation coefficient of 0.99903, limits of detection of 0.277 μg mL^{-1}, and a quantification of 0.926 μg mL^{-1}.

2.3. Evaluation of ZeinCNAP Effects on Tomato Plant Health Parameters: Photosynthetic Parameters and Oxidative Stress. The experimental design was completely randomized, with three treatments: water, ZeinCNAP, and commercial CNAP. There were five replicate tomato plants per treatment. Tomato seedlings of the Moneymaker cultivar (Eden Brothers Seeds) were germinated in 98-cell plastic trays containing ProMix BX substrate and fertilized once with Miracle-Gro soluble 24-8-16 fertilizer (NPK). At the first stage of true leaf development, the seedlings were transplanted into 2-L pots. When approximately 15 cm tall, seedlings were transplanted into 2-L pots filled with the same substrate, and plants were fertilized weekly with the NPK 04-14-08 formula and watered as needed. All plants were kept in a greenhouse: 28 ± 2 °C, 12 h/12 h (light/dark), and 60 ± 10% relative humidity until the end of the assays. Plants were treated with the formulations on the third and fourth fully expanded leaves at a dose of 2.5 mg a.i. of CNAP plant^{-1} by droplets on the leaves. The droplets were left to dry at room temperature for 2 h. The fresh weight of the aerial tissue was measured at 48,

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128, and 360 h after spraying. Photosynthetic parameters and oxidative stress were also measured.

At 2, 24, 48, 128, and 360 h after application, the following photosynthetic parameters were measured on the youngest leaf of each plant using a portable PhotosynQ (PHOTOSYNQ INC., USA): quantum yield of nonregulatory energy dissipation (PhiNO), nonphotochemical quenching (PhiNPQ), photosystem II (Phi2), relative chlorophyll, and linear electron flow (LEF).

The contents of malondialdehyde (MDA), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) were measured to determine the extent of oxidative stress upon exposure. Leaves and roots were sampled at 2, 24, 48, 128, and 360 h after application, frozen in liquid nitrogen, and stored at −80 °C until ready for use.

For the determination of APX, CAT, and SOD activity in the plants, 0.2 g of leaf or root tissue was used for extraction according to Tamez et al.[38] and Ma et al.[39] Briefly, 6 mL of 25 mM potassium phosphate buffer solution (KH_2PO_4, pH 7.4) was added and vortex mixed (12,000*g* for 2 min). The samples were centrifuged at 5,000*g* for 15 min at 4 °C, and 1.5 mL of supernatant was collected, frozen in liquid nitrogen, and stored at −80 °C for further analyses.

To determine MDA activity in tomato plants, 0.2 g of leaf or root tissue was used for extraction according to a protocol adapted from Ma et al.[40] A mixture of wet plant tissue and 0.1% (w/v) trichloroacetic acid (TCA) was homogenized through a vortex mixer (12,000*g* for 2 min). The samples were centrifuged at 5,000*g* for 15 min at 4 °C, and 1.5 mL of supernatant was collected, frozen in liquid nitrogen, and stored at −80 °C.

APX activity was determined by the decrease in H_2O_2 absorbance.[41] Briefly, 228 μL of 0.5 mM ascorbic acid and 532 μL of 0.4 mM H_2O_2 were added to 40 μL of the plant extract sample and vortex mixed (12,000*g* for 30 s). Then, 200 μL of each sample was added in triplicate in a 96-well plate and measured at 290 nm with a 2-min total run time using a UV−vis spectrophotometer (Molecular Devices, SpectraMax M2). A calibration curve of H_2O_2 (10−0.3 mM) was run for quantification.

For CAT activity, the method adopted was based on Medina-Velo et al.,[41] with some modifications. The analysis was performed by adding 40 μL of plant extract to 760 μL of 10 mM H_2O_2 and vortex mixed (12,000*g* for 30 s). Then, 200 μL of each sample was added in triplicate to a 96-well plate and measured at 240 nm with a 3-min total run time using a UV−vis spectrophotometer. A calibration curve of H_2O_2 (10−0.3 mM) was used for quantification.

For the determination of SOD activity, the method described by Medina-Velo et al.[41] and Tamez et al.[38] for the photochemical reduction of nitroblue tetrazolium (NBT) was adopted. Specifically, 707 μL of buffer (500 μM NBT, 78 mM L-methionine, 1.5 mM EDTA, and 100 mM potassium phosphate buffer—pH 7.8) and 80 μL of 0.02 mM riboflavin solution were added to 13 μL of plant extract and mixed by vortexing. Subsequently, 200 μL of each sample was added in triplicate to a 96-well plate and illuminated for 15 min in a box with compact fluorescent light. The plates were then analyzed with a UV−vis spectrophotometer (560 nm). The inhibition of NBT was determined by comparing samples with the control buffers only, under illuminated and nonilluminated conditions.

For MDA determination, the method of Ma et al.[40] for thiobarbituric acid reactive substances was adopted. A total of 160 μL of plant extract, 400 μL of trichloroacetic acid (2%), and 400 μL of thiobarbituric acid (0.5%) were mixed. An amount of 200 μL of each sample was added in triplicate in a 96-well plate, heated at 95 °C for 30 min, and immediately cooled on ice for 15 min. The sample's absorbance was measured using a UV−vis spectrophotometer (532−600 nm). The final MDA concentration was calculated based on Lambert−Beer's equation using an extinction coefficient of εM= 155 mM^{-1} cm^{-1}.

2.4. Evaluation of ZeinCNAP Absorption and Translocation on Tomato Plants. The experimental design was a random factorial 3 × 3 (formulations × plant portion). The three formulation treatments were water, ZeinCNAP, and commercial CNAP. The tomato plants were cultivated as described in section 2.3. The plant portion treatments consisted of the youngest fully expanded leaf ("upper leaf"), the third fully expanded leaf sprayed by the formulations ("sprayed leaf"), and the oldest fully expanded leaf ("lower leaf"). The CNAP-treated plants (ZeinCNAP or commercial CNAP) were sprayed with the formulations at a rate of 2.5 mg a.i. $plant^{-1}$, in the "sprayed leaf" treatment, with only water in the control. The plants were dried at ambient temperature for 2 h. At 2, 24, 72, 168, and 360 h after application, all leaves were cut and separated by the portions and kept at −20 °C. All "sprayed leaf" portions were washed with 4 mL of methanol: water (50:50) before sampling, and the rinsate was collected for determination of nonabsorbed CNAP.

For the estimation of CNAP absorption and translocation in tomato plants, 1 g of leaf tissue was used to extract CNAP according to Hazra et al.[42] using the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. Plant tissue was placed in 50 mL conical tubes amended with 15 mL of water and 10 mL of acetonitrile. The tubes were placed in a high-speed homogenizer for 30 s at 12,000*g*. 1 g of sodium acetate ($C_2H_3NaO_2$) and 4 g of magnesium sulfate ($MgSO_4$) were added to the tubes, which were homogenized by vortexing and centrifuged for 5 min at 12,000*g*. The supernatant was collected (10 mL) and transferred to a new 15 mL conical tube filled with 0.15 g of primary secondary amine (PSA) sorbent, 0.45 g of anhydrous $MgSO_4$ and 0.05 g of Cyclo[18]carbon (C_{18}). The samples were homogenized and centrifuged (5 min 12,000*g*). The supernatant (1 mL) was collected and kept at −20 °C. For the nonabsorbed CNAP analysis, 1 mL of the ethanol solution used to rinse the sprayed leaves was analyzed directly, without an extraction step.

CNAP quantification in plant tissues was analyzed by liquid chromatography−mass spectrometry using an Agilent 1200 series liquid chromatograph with a Thermo Hypersil Gold Aq 2.1 mm × 100 mm column packed with 1.9 μm particles interfaced to a Thermo Exactive high-resolution mass spectrometer. The initial mobile phase composition was 1% B, which was increased to 99% B for 6 min at a 0.25 mL/min flow rate. The calibration curves were obtained by using an external standard CNAP calibration curve. Continuing calibration verification (CCV) injections were performed at the beginning, throughout, and at the end of each analytical batch. Each extraction batch contained a method blank and a spike recovery sample. Extracts and standards were prepared gravimetrically. All solvents were HPLC-grade or better.

2.5. Testing ZeinCNAP for the Control of *Bemisia tabaci* MEAM1. *2.5.1. Tomato Test Plants and Bemisia tabaciMEAM1 Colony.* Tomato seedlings of the Moneymaker cultivar (Eden Brothers Seeds) were germinated in 98-cell

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Table 1. Physicochemical Parameters of the ZeinCNAP and Control Zein Formulations over 56 Days after Synthesis[a]

	Size (nm)		Zeta Potential (mV)		PI	
Evaluation Day	ZeinCNAP	Control Zein	ZeinCNAP	Control Zein	ZeinCNAP	Control Zein
Day 0	140.1 ± 1.39	143 ± 1.03	40.3 ± 0.70	38 ± 0.01	0.136 ± 0.02	0.172 ± 0.01
Day 1	144.3 ± 1.27	134.2 ± 0.5	35.8 ± 0.87	36.2 ± 0.01	0.188 ± 0.01	0.161 ± 0.01
Day 7	155.5 ± 2.31	167.4 ± 2.15	37 ± 0.52	40.4 ± 0.02	0.154 ± 0.007	0.138 ± 0.02
Day 14	162.4 ± 1.65	177 ± 1.87	27.9 ± 0.92	37.5 ± 0.02	0.136 ± 0.01	0.147 ± 0.02
Day 21	181.4 ± 1.30	173.9 ± 2.33	33.6 ± 0.69	40.2 ± 0.01	0.085 ± 0.005	0.119 ± 0.01
Day 56	161.9 ± 3.78	188.1 ± 2.13	28.3 ± 0.57	36.8 ± 0.01	0.075 ± 0.012	0.171 ± 0.01

[a](A) Average size (nm). (B) Zeta potential (mV). (C) Polydispersion index (PI). The parameters were obtained by using the dynamic light scattering (DLS) technique. The bars indicate the means ± standard error (n = 3)

plastic trays containing ProMix BX substrate and fertilized once with Miracle-Gro soluble 24-8-16 fertilizer (NPK). At the first stage of true leaf development, the seedlings were transplanted into 2-L pots. When approximately 15 cm tall, seedlings were transplanted into 2-L pots filled with the same substrate, and plants were fertilized weekly with the NPK 04-14-08 formula and watered as needed. All plants were kept in a greenhouse: 28 ± 2 °C, 12 h/12 h (light/dark), and 60 ± 10% relative humidity.

The whitefly *B. tabaci* Middle East-Asia Minor 1—MEAM1 was reared on collard plants (*Brassica oleracea*), Top Bunch 2.0 cultivar (Johnny's Selected Seeds), and maintained in an insect-proof cage in a different greenhouse under the above-mentioned conditions.

2.5.2. Evaluation of ZeinCNAP on Bemisia tabaci MEAM Mortality. The experimental design was randomized with three treatments: water, ZeinCNAP, and commercial CNAP. There were five replicate tomato plants per treatment. The third and fourth fully expanded leaves of tomato seedlings were treated with the formulations at a dose of 2.5 mg a.i. plant^{-1} of CNAP. The treatments were made as droplets on the leaves. Five droplets (50 μL each) of water, commercial CNAP (1 mg mL^{-1}), or ZeinCNAP (1 mg mL^{-1}) were deposited on the adaxial side of the leaves. The droplets were left to dry at ambient temperature for 2 h. Transparent plastic cages with top voile covers were used to confine the insects. Black card circles were placed on the base of the plant to facilitate visualization and counting of dead insects. Then, 20 adults of *B. tabaci* MEAM1, randomly mixed by age and sex, were transferred to each cage. The number of dead insects was counted at 2, 4, 24, 48, and 72 h.

2.5.3. Evaluation of Reduced ZeinCNAP Doses on Bemisia tabaci MEAM1 Mortality. The experimental design was randomized, with five treatments: water, ZeinCNAP 1/10 dose, ZeinCNAP 1/2 dose, ZeinCNAP full dose, and commercial CNAP. It is important to mention that the commercial CNAP was always maintained on the label rate when tested as a control, due to the agricultural practice of not using a subdose. There were five replicates per treatment with one plant per replicate. Each plant was sprayed with the treatments, covering the entire superficial foliar area, with the following doses: 2.5 mg a.i. plant^{-1} for the full dose of ZeinCNAP and commercial CNAP; 1.25 mg a.i. plant^{-1} for ZeinCNAP 1/2 dose; and 0.25 mg a.i. plant^{-1} for the dose of ZeinCNAP 1/10. The plants were allowed to dry for 2 h. Transparent plastic cages with top voile covers were used to confine the insects as described above, and black card circles were placed at the base of the plant to facilitate visualization and counting of dead insects. Twenty adults of *B. tabaci* MEAM1 were transferred to each cage as described above, and mortality was evaluated at 2, 24, 48, 72, and 96 h after application.

2.5.4. Evaluation of Bemisia tabaci MEAM1 Death Rates in Different Plant Portions. The experimental design was a random factorial 3 × 3 (formulations × plant portion) with water, ZeinCNAP, and commercial CNAP as the treatments. The treated tissue was the youngest fully expanded leaf ("upper leaf"), the third fully expanded leaf sprayed by the formulations ("sprayed leaf"), and the oldest fully expanded leaf ("lower leaf").

Tomato plants that were approximately 30 cm high were used. There were five replicates per treatment, consisting of one plant per replicate. The CNAP-treated (ZeinCNAP or commercial CNAP) plants were sprayed at a 2.5 mg a.i. plant^{-1} dose in "sprayed leaf". The droplets were allowed to dry for 2 h. Clip cages were made using plastic tubes (3 × 3 cm), with a top voile cover and hair clips, and were used to keep insects confined in each portion. Then, 20 adults of *B. tabaci* MEAM1, randomly mixed by age and sex, were transferred to each clip cage. By the end of 48 h, the clip cage was opened, and the whitefly mortality was recorded.

2.6. Data Analysis. All statistical analyses were conducted using R version 4.2.2.[43] The data were transformed by using the Box−Cox transformation and tested for homogeneity and homoscedasticity when necessary. The data for the cumulative number of dead were analyzed via a one-way ANOVA ($p < 0.05$ for the F test), and means were compared by Tukey's test ($p < 0.05$, <0.01, and <0.001). All figures were generated using the software Origin 2022b v 9.9.5.171 (OriginLab Corporation, Northampton, MA).

3. RESULTS

3.1. Characterization of ZeinCNAP Formulation. The ZeinCNAP stability parameters were analyzed 56 days after synthesis (Table 1). The average hydrodynamic diameter size of ZeinCNAP was 140.13 ± 1.4 nm, and the control (only zein) was 143.06 ± 1.03 nm. After 56 days, the dimensions remained comparable, with 161.9 ± 3.78 and 188.13 ± 2.13 nm for ZeinCNAP and the control, respectively. The ζ-potential was 40.36 ± 0.7 and 38 ± 0.1 mV for ZeinCNAP and control, respectively, decreasing to 28.33 ± 0.57 mV and 36.83 ± 0.31 mV at day 56. The polydispersity index (PI) remained close to 0.1 over time. The encapsulation efficiency of CNAP into the nanocapsules was 95 ± 1.2%.

Figure 1 shows TEM images that exhibit the spherical shape and nonaggregation characteristics of the ZeinCNAP NPs.

3.2. Effects of ZeinCNAP on Tomato Plant Health. *3.2.1. Effects on Plant Fresh Weight.* Figure 2 shows the fresh weight of plants treated with commercial CNAP, ZeinCNAP, or water at 24, 168, or 360 h after application. Importantly,

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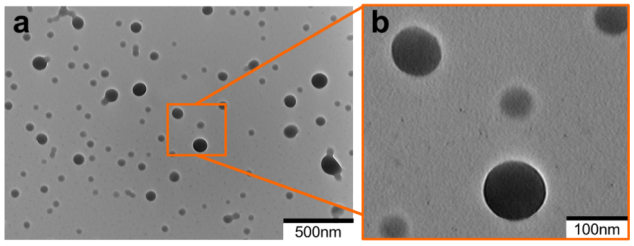

Figure 1. TEM micrographs of ZeinCNAP NPs solutions that were drop-cast on copper grids (a) and individual particle images highlighting the spherical shape of the ZeinCNAP NPs (b).

there was no difference in root or shoot mass as a function of treatment, demonstrating no phytotoxicity of the nanoscale CNAP.

3.2.2. Photosynthetic Parameters and Oxidative Stress. The photosynthetic parameters PhiNO, PhiNPQ, Phi2, LEF, and relative chlorophyll of tomato plants at 2, 24, 48, 168, and 360 h after spraying with ZeinCNAP, commercial CNAP, and water are shown in Figure 1. Similar to biomass, none of the parameters showed significant differences as a function of treatment at each time point.

The oxidative stress parameters SOD, APX, CAT, and MDA content of tomato plants at 2, 24, 48, 168, and 360 h after spraying with ZeinCNAP, commercial CNAP, and water are shown in Figure 2. Nearly all enzymes were unaffected by treatment, except for the CAT activity in the roots. At 2 h, the plants treated with commercial CNAP showed 50% higher CAT activity than plants treated with ZeinCNAP and water. However, at 6 h, all the treatments exhibited similar activity for all the enzymes. Notably, this pattern changed at 24 h when ZeinCNAP exhibited a greater effect on CAT levels than water and commercial CNAP. Specifically, at 168 and 360 h, CAT activity was higher in plants treated with ZeinCNAP (51.56 ± 1.87% and 52.32 ± 1.62%) than commercial CNAP (30.64 ± 2.23% and 30.02 ± 2.46%), with water-treated activity the lowest at 6%.

3.3. ZeinCNAP Absorption and Translocation in Tomato Plants. CNAP absorption by tomato plants is shown in Figure 3. Generally, the commercial formulation showed 1.68 times higher absorption values, with 37.66 ± 7.74 μg/g and 22.29 ± 3.97 μg/g for the ZeinCNAP (Figure 3a). The opposite pattern was observed when analyzing the

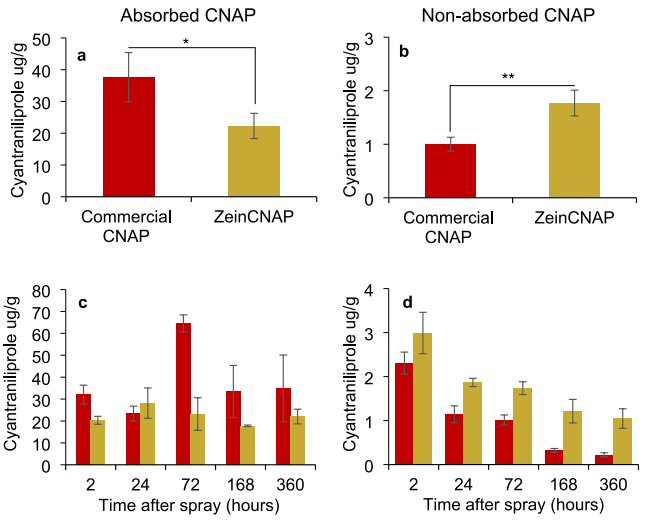

Figure 3. Mean of the total amount of CNAP in ZeinCNAP or commercial formulation absorbed (a) and nonabsorbed (b) in tomato plants. Mean of the total amount of CNAP in ZeinCNAP or commercial formulation absorbed (c) and nonabsorbed (d) at 2, 24, 72, 128, and 360 h after application. The asterisks indicate the difference in the amount of CNAP absorbed depending on formulations, regardless of the time (a,b) $*p < 0.01$ and $**p < 0.001$ are significant using a one-way ANOVA with a Sidak pairwise test. The bars indicate the means ± standard errors. The absence of asterisks (c,d) indicates no significant differences.

nonabsorbed fraction, with ZeinCNAP being 1.77 times higher (1.77 ± 0.24 μg/g) than the commercial formulation (1 ± 0.12 μg/g) (Figure 3b). The plants showed the highest CNAP content at 72 h after application, with the commercial CNAP (64.51 ± 4.32 μg/g; Figure 3c). Regarding nonabsorbed CNAP, a greater amount was detected 2 h after application (ZeinCNAP 2.99 ± 0.47 μg/g; Figure 3d). CNAP was not detected in other parts of the plant, such as the upper and lower leaves (untreated).

3.4. Mortality of *Bemisia tabaci* Using ZeinCNAP. The number of dead *B. tabaci* MEAM1 adults on tomato plants treated with water, ZeinCNAP, and commercial CNAP at 2, 4, 24, 48, and 72 h after spraying is shown in Figure 4. At 2 h, no differences in the mortality rates of the whiteflies were detected across all treatments. However, 4 h after application, the

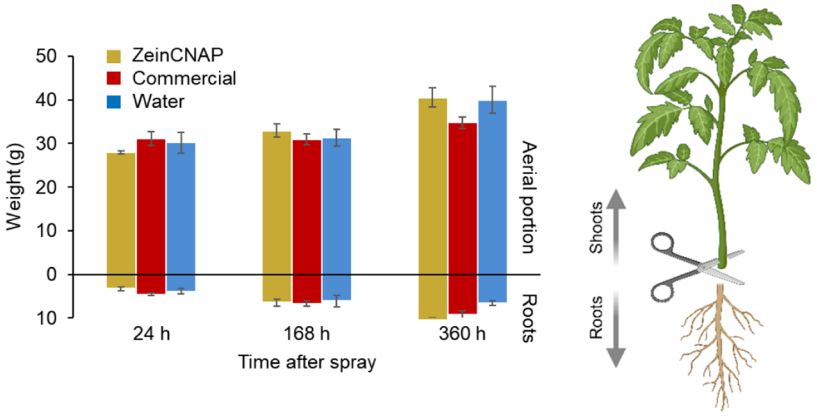

Figure 2. Fresh weight (g) of tomato plants at 48, 168, and 360 h after spraying with ZeinCNAP, commercial CNAP, and water ($n = 5$). No significant difference between treatments was determined by a one-way ANOVA and Tukey's test ($p < 0.05$) at each time point. Data above x-axis are for shoots, and below the x-axis are for roots. The bars indicate the means ± standard errors.

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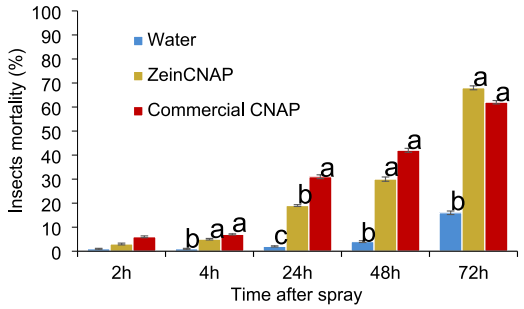

Figure 4. Number of dead *Bemisia tabaci* MEAM1 adults on tomato plants treated with water, ZeinCNAP, and commercial CNAP at 2, 4, 24, 48, and 72 h after application (n = 5). Lowercase letters indicate the significant differences between treatments according to a one-way ANOVA with Tukey's test ($p < 0.05$). The bars indicate the means ± standard errors.

commercial CNAP and ZeinCNAP caused statistically higher insect mortality rates (5 ± 0.31% and 7 ± 0.24%, respectively) than water-treated plants (1 ± 0.2%). After 24 h, the commercial CNAP, ZeinCNAP, and water caused 31 ± 0.8%, 19 ± 0.37%, and 2 ± 0.24% whitefly mortality, respectively. Interestingly, after 48 h, the mortality rate of ZeinCNAP was statistically equivalent to that of the commercial CNAP; 30 ± 0.89% and 42 ± 0.81%, at 48 h, and 68 ± 0.81% and 62 ± 0.7% at 72 h, respectively.

3.4.1. Bemisia tabaciMEAM1 Plant Portion-Dependent Mortality. The number of dead whiteflies in tomato plants 48 h after spraying with ZeinCNAP, commercial CNAP, and water that were confined inside clip cages placed on the upper leaf, sprayed leaf, and lower leaf is shown in Figure 5. On the

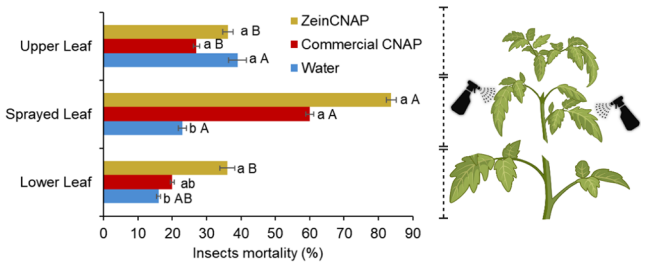

Figure 5. Number of dead *Bemisia tabaci*MEAM1 adults on tomato plants at 48 h after spraying with ZeinCNAP, commercial CNAP, and water. Insects were confined inside clip cages distributed between plant portions: upper, sprayed, and lower leaf (n = 5). Capital letters indicate significant differences between portions inside each treatment. The absence of letters indicates no significant differences between treatments in the same portion of the plants. Both were submitted to Tukey's test ($p < 0.05$). The bars indicate the means ± standard errors.

upper leaf, no differences were recorded across treatments, and the majority of the dead insects were on the sprayed leaf. No significant differences were detected between commercial CNAP and ZeinCNAP, although both were more effective than water. On the lower leaf, insect death rates with ZeinCNAP were statistically equivalent to those of the commercial CNAP (20 ± 0.63% and 36 ± 2.19%, respectively) and higher than water (16 ± 0.58%). Notably, on average, the insecticides killed more whiteflies on the treated leaves (71.8%) than on the leaves above (28%) or below (31.6%).

3.4.2. Dose-Dependent Mortality of Bemisia tabaci-MEAM1 with ZeinCNAP. The number of dead whiteflies on tomato plants at 2, 24, 48, 72, and 96 h after spraying with three doses of ZeinCNAP (1/10, 1/2, and full), commercial CNAP, and water is shown in Figure 6. No treatment

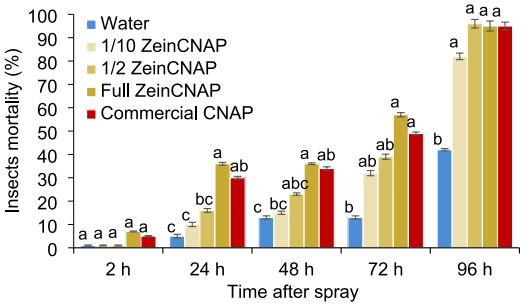

Figure 6. Number of dead *Bemisia tabaci*MEAM1 adults on tomato plants at 2, 24, 48, 72, and 96 h after spraying with three doses of ZeinCNAP (1/10, 1/2, and full), commercial CNAP, and water. Insects were confined inside cages (n = 5). At each time point, lowercase letters indicate the significant differences between treatments according to a one-way ANOVA with Tukey's test ($p < 0.05$). The bars indicate the means ± standard errors.

difference in mortality was observed at 2; however, at 24 h, commercial CNAP and full-dose ZeinCNAP killed 36 ± 0.6% and 30 ± 0.6% of insects, compared to 5% ± 0.8% in water-only treated plants. This pattern was sustained for 48 h. At 72 h, mortality rates at the 1/10 and 1/2 doses showed an increasing trend in mortality, although not statistically different from that for the water treatment. At 96 h, no mortality rate differences between the insecticide source nor dose (92% average on insect death rates) were evident, although all were significantly greater than water (42 ± 0.5%). Importantly, the 1/10 dose of ZeinCNAP resulted in mortality at the same rate (82 ± 1.4%) as the nonencapsulated full-dose commercial insecticide (96 ± 1.8%) at this time point.

4. DISCUSSION

Cyantraniliprole (3-bromo-1-(3-chloro-2-pyridyl)-4′-cyano-2′-methyl-6′- (methylcarbamoyl)pyrazole-5-carboxanilide (IUPAC)) is a second-generation diamide insecticide, aimed mainly at controlling some Lepidoptera and sucking pests by activating their ryanodine receptors.[44] Given its widespread use and efficacy, this model insecticide was chosen to evaluate increased activity upon incorporation into a nanoformulation. Importantly, several prior studies have demonstrated the potential enhanced control of zein nanoformulations on insects. Oliveira et al.[21] described enhanced repellent activity of geraniol and R-citronellal against *Tetranychus urticae* using zein as a nanocarrier, compared with water. Furthermore, Bonser et al.[33] found similar effects of the commercial insecticide methoxyfenozide and the same a.i. carried by zein on the mortality rate of *Chrysodeixis includens*.

In this study, we prepared a formulation of ZeinCNAP and conducted in vivo bioassays by spraying the synthesized ZeinCNAP on tomato plants, exposing insects at different intervals to the product, with a direct comparison to the commercial cyantraniliprole (CNAP) formulation and water (control). To the best of our knowledge, this is the first study to evaluate the effects of zein nanoencapsulated CNAP on the mortality of *B. tabaci* MEAM1. Nanoparticle morphology

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parameters are fundamental indicators of the synthesis success and eventual performance. The mean size of the measured ZeinCNAP nanoparticles was as expected, generally ranging between 10 and 1,000 nm.[45,46] Similar size results can be found in the literature with respect to zein-based nanoparticles.[33,47−49] Importantly, minimal changes in these parameter values were noted over time, indicating excellent potential for ZeinCNAP storage due to its stability.

We showed that ZeinCNAP has the same effects as commercial CNAP when the droplet is applied on the leaves (Figure 4). When sprayed over the total area, not only does the same dose have a similar effect, but also lower doses of ZeinCNAP have the same efficacy as commercial CNAP; specifically, 10-fold less a.i. ZeinCNAP compared to the label dose caused the same whitefly mortality as commercial CNAP at the label rate after 72 h of spray (Figure 6). After 72 h, the dose−effect on insect mortality decreases, likely due to the time required for lower insecticide doses to reach the whiteflies' feeding sites. By this point, the minimum lethal threshold has been met, suggesting that doses above 1/10 may not be necessary for effective control. A similar lower dose effect can be found in previous studies, wherein the LC_{50} was reduced 6.25 times compared to the commercial formulation when the nanoformulation of pyridalyl + alginate was used against *Helicoverpa armigera*.[20] Meyer et al.[50] also found that an imidacloprid + poly(lactic-*co*-glycolic acid) nanoformulation could kill *Diaphorina citri* as effectively as the a.i. commercial formulation, even at 200 times lower doses.

CNAP exhibits a high systemic distribution in plants compared to other similar insecticide classes.[51] However, according to Barry et al.,[17] while translaminar and xylem translocation of CNAP was evident, phloem content seemed limited. Here, CNAP was not detected in leaves above and below the treated leaf, which can also be indirectly confirmed by the absence of significant insect death rates on these portions in the in vivo test (Figure 5). The effects on insect mortality seem to be restricted to the location of application (Figure 5). As also mentioned, CNAP was only detected on the sprayed leaves (by commercial or ZeinCNAP), which in turn was the only site protected against the insects. Interestingly, the amount of CNAP absorbed by the sprayed leaves was lower when nanoformulated, although equivalent amounts remained available on the leaf surface and there was no difference in efficacy (Figure 3).

Although there was lower CNAP absorption when nanoformulated with zein, the insecticidal effect was equivalent to that of the commercial product. This effect can likely be related to an improvement in the delivery of insecticide molecules via the nanocarrier. Importantly, phloem cells of plant leaves constitute the feeding sites for whiteflies.[52,53] Chen et al.[54] noted that a system designed to deliver effective molecules specifically to the phloem could greatly enhance overall efficacy, thereby reducing the rate of pesticide use in agriculture. The same authors showed a promising approach based on designing stimuli-responsive polymeric nanoparticles carrying phytohormones. When analyzing the release of the a.i. under different pH conditions (7−8.5), the authors reported rapid release at an increased pH, similar to conditions in the phloem of plants. This improvement in the spatial targeting of insecticides and the fact that a large amount of ZeinCNAP remained on the leaf surface could explain the equivalent rate of insect mortality observed in the current study.

Importantly, the general lack of effects on tomato plant health parameters demonstrates the nanoformulation's safety. Shalaby et al.[55] and Dimkpa and Bindraban[56] noted that the impact of nanoparticles on plants depends on the composition, concentration, size, exposure duration, exposure route, physical and chemical properties of the engineered nanoparticles, and plant species. A number of studies have, in fact, reported toxic effects from polymeric nanoparticles on plant health in the literature. Salinas et al.[47] evaluated the health effects of zein- and lignin-based nanoparticles on the overall health of soybeans and found that nanoparticles had a minimal impact at low and medium doses (0.02 and 0.2 mg/mL). Notably, there were some impacts on select endpoints with treatment, such as CAT activity in the aerial tissues. CAT influences antioxidant defense by catalyzing the conversion of H_2O_2 accumulated by plants to O_2 and H_2O, thereby reducing oxidative damage to plant tissues.[57] A similar effect on CAT activity in the shoots of soybean plants was described by Salinas et al.,[47] which occurred 14 days after treatment with zein-based nanoparticles. Notably, other health parameters analyzed in the tomato plants, such as fresh weight, photosynthesis, and additional oxidative enzymes or biomarkers (APX, SOD, and MDA), were not significantly affected in the presence of ZeinCNAP.

Taken together, these findings demonstrate that this zein-based nanoinsecticide has minimal impact on tomato plants at doses used in commercial formulations. The insecticidal effect of ZeinCNAP on whitefly mortality was equivalent to commercial CNAP even at a ten times lower dose. Notably, the absorption of the CNAP was reduced when nanoformulated, although the efficacy of CNAP increased, most likely because greater amounts of CNAP remained available at the leaf surface. Hence, the systemic movement of CNAP was more limited in the plant, limiting the potential for pesticide translocation and biomagnification in edible tissues. Future work on ZeinCNAP should focus on achieving a greater understanding of insecticide fate in plants, including distribution and metabolism. Also, further evaluation of the efficacy of the ZeinCNAP in controlling *B. tabaci* on different crops and under field conditions should be undertaken. Importantly, this work further advances the developing field of nanotechnology-enabled agriculture, continuing the demonstration of increased efficacy with lower overall inputs and highlighting this strategy as an important tool in the effort to combat rising global food insecurity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.4c00751.

Effects on photosynthesis parameters and oxidative stress (PDF)

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Notes

The authors declare no competing financial interest.

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