UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA AGRÍCOLA

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AVALIAÇÃO *IN VITRO* E SEMI-CAMPO DE LUFENUROM NANOENCAPSULADO PARA CONTROLE DE *RACHIPLUSIA NU*: UMA ABORDAGEM INTERESSANTE VISANDO UMA AGRICULTURA SUSTENTÁVEL

Santa Maria, RS
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Dissertação apresentada ao Programa de Pós-Graduação em Engenharia Agrícola, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Engenharia Agrícola**.

Orientador: Prof. Dr. Adriano Arrué Melo

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Lenz, Marcos
AVALIAÇÃO IN VITRO E SEMI-CAMPO DE LUFENUROM NANOENCAPSULADO PARA CONTROLE DE RACHIPLUSIA NU: UMA ABORDAGEM INTERESSANTE VISANDO UMA AGRICULTURA SUSTENTÁVEL / Marcos Lenz.- 2024.
39 p.; 30 cm

Orientador: Adriano Arrué Melo
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós Graduação em Engenharia Agrícola, RS, 2024

1. Controle químico 2. Inseticida 3. Nanotecnologia 4. Controle de pragas I. Arrué Melo, Adriano II. Título.

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Marcos Lenz

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Aprovada em 09 de setembro de 2024

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Santa Maria, RS

2024

AGRADECIMENTOS

A toda minha família, em especial aos meus pais, Lucia Strieder e Elio Erico Lenz e irmão Giuvan Lenz. Por todo amor e carinho, apoio e incentivo, paciência e compreensão. Sem vocês eu não teria conseguido.

Ao meu orientador, professor Dr. Adriano Arrué Melo, pelas contribuições realizadas na elaboração do trabalho e por me orientar durante o mestrado, além de um grande orientador um grande amigo.

Aos professores Leonardo Fernandes Fraceto e Walter Boller pelas contribuições na elaboração e correção do trabalho e pelo aceite na participação da minha banca examinadora, além destes, um agradecimento ao Jhones Luiz de Oliveira por todas as contribuições e auxílio na escrita do artigo.

Agradeço a Universidade Federal da Santa Maria (UFSM) e ao Programa de Pós-Graduação em Engenharia Agrícola (PPGEA) pela oportunidade e acolhimento.

A Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) pela concessão da bolsa de mestrado durante a realização do mestrado.

Agradeço aos colegas do grupo Manejo Inteligente em Tecnologia de Aplicação (MITA) Matheus Mota Lanzarin, Leonardo Marques de Almeida Mariano, Renata Bencke Gross, Manoel Peres Zinelli, Matheus Henrique Lobao de Sousa e Bianca Goularte Dias, por todos os auxílios durante a condução do experimento, agradeço também ao Felipe Melo, Caue Oliveira, Leonardo Schwarz, Luana Lopes, Thaís Pires Roso, Eduardo Weber, Renata Simon e Eduarda Severo pelo convívio e amizade adquiridos através do grupo de pesquisa.

A todos os amigos feitos durante a pós-graduação, pelos momentos de estudo, ajuda, risadas e conversas jogadas fora. Sem vocês seria muito difícil.

Agradeço a todos que direta ou indiretamente fizeram parte de minha jornada.

RESUMO

AVALIAÇÃO *IN VITRO* E SEMI-CAMPO DE LUFENUROM NANOENCAPSULADO PARA CONTROLE DE *RACHIPLUSIA NU*: UMA ABORDAGEM INTERESSANTE VISANDO UMA AGRICULTURA SUSTENTÁVEL

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A nanotecnologia está revolucionando as práticas de controle de pragas, e este estudo explora uma nova abordagem utilizando formulações do inseticida lufenurom baseadas em nanotecnologia para combater a lagarta Falsa-medideira (*Rachiplusia nu)*, uma praga que causa sérios danos às plantações de soja. As lagartas desse inseto atacam as folhas das plantas, inicialmente criando pequenas lesões superficiais, mas à medida que crescem, tornam-se extremamente destrutivas, consumindo as folhas e até danificando caules mais finos e para seu controle pode ser utilizado o inseticida lufenurom. O objetivo desse trabalho foi desenvolver uma nanoformulação de lufenurom, avaliar suas características de estabilidade e propriedades físico-químicas com o intuito de controlar com eficiência a lagarta falsa-medideira em dieta artificial e semi-campo. Para isso, o lufenurom foi nanoencapsulado em nanopartículas de policaprolactona (PCL), utilizando um método de nanoprecipitação. As formulações desenvolvidas foram submetidas a uma análise detalhada das propriedades físico-químicas, incluindo o tamanho das partículas, potencial zeta, eficiência de encapsulação e morfologia. Além disso, o desempenho das formulações foi testado tanto em dieta artificial quanto em condições de semi-campo para avaliar sua eficácia no controle da *R. nu*. Os resultados mostraram que a nanoencapsulação do lufenurom em nanopartículas de policaprolactona (PCL), na concentração de 1,0 mg/mL, resultou em um índice de polidispersidade inferior a 0,2, com um potencial zeta médio de -40 mV e boas características físico-químicas, apresentando variações mínimas ao longo do tempo de armazenamento. Os bioensaios confirmaram a eficiência do lufenurom nanoencapsulado, demonstrando controle eficaz em doses equivalentes e uma eficácia notável em doses reduzidas, alcançando 98,5% de controle em dieta artificial. Esses resultados indicam que a nanoencapsulação do lufenurom não apenas garante a estabilidade do inseticida, mas também melhora significativamente seu desempenho, contribuindo para práticas agrícolas mais sustentáveis e eficazes no combate às pragas.

Palavras-chave: Controle químico. Inseticida. Nanotecnologia. Controle de pragas.

ABSTRACT

IN VITRO AND SEMI FIELD EVALUATION OF NANOENCAPSULATED LUFENURON FOR *RACHIPLUSIA NU* CONTROL: AN INTERESTING APPROACH AIMING SUSTAINABLE AGRICULTURE

AUTHOR: Marcos Lenz

ADVISOR: Adriano Arrué Melo

Nanotechnology is revolutionizing pest control practices, and this study explores a new approach using nanotechnology-based lufenuron insecticide formulations to combat the soybean looper (*Rachiplusia nu*), a pest that causes significant damage to soybean crops. The larvae of this insect attack plant leaves, initially creating small superficial lesions, but as they grow, they become extremely destructive, consuming leaves and even damaging thinner stems. Lufenuron is commonly used as an insecticide to control this pest. The objective of this work was to develop a nanoformulation of lufenuron, evaluate its stability characteristics and physicochemical properties, with the aim of efficiently controlling the soybean looper in an artificial diet and semi-field conditions. To achieve this, lufenuron was nanoencapsulated in polycaprolactone (PCL) nanoparticles using a nanoprecipitation method. The developed formulations underwent a detailed analysis of physicochemical properties, including particle size, zeta potential, encapsulation efficiency, and morphology. Additionally, the performance of the formulations was tested in both artificial diets and semi-field conditions to evaluate their efficacy in controlling *R. nu*. The results showed that the nanoencapsulation of lufenuron in PCL nanoparticles at a concentration of 1 mg/mL resulted in a polydispersity index below 0.2, with an average zeta potential of -40 mV and good physicochemical characteristics, exhibiting minimal variations during storage. Bioassays confirmed the efficiency of nanoencapsulated lufenuron, demonstrating effective control at equivalent doses and remarkable efficacy at reduced doses, achieving 98.5% in artificial diet. These findings indicate that the nanoencapsulation of lufenuron not only ensures the stability of the insecticide but also significantly improves its performance, contributing to more sustainable and effective pest control practices in agriculture.

Keywords: Chemical control. Insecticide. Nanotechnology. Pest control.

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1 INTRODUÇÃO

A soja, é uma leguminosa dicotiledônea, pertencente à família Fabaceae, subfamília Faboideae, gênero *Glycine* e, espécie *Glycine max* (L) Merrill (SEDIYAMA; TEIXEIRA; BARROS, 2009). Possui grande importância econômica e social, sendo cultivada em mais de 100 países do mundo e utilizada para diversas finalidades, como fabricação de óleo, biocombustíveis, ração animal e para alimentação humana (GAZZONI, 2018).

A cultura da soja é atacada por diversos insetos-praga durante o seu ciclo de desenvolvimento. A Lagarta Falsa-medideira (Guenée, 1852) de nome científico *Rachiplusia nu* (Lepidoptera: Noctuidae: Plusiinae) tem tomado destaque nas últimas safras dessa cultura. Além disso, é considerada uma das maiores desfolhadoras em alguns países da América do Sul, tais como, Paraguai, Bolívia, Argentina, Chile, Uruguai e Sul do Brasil (SPECHT *et al*., 2019).

Essa espécie é comumente confundida com a *Chrysodeixis includens* (Lepidoptera: Noctuidae) também chamada de Lagarta Falsa-medideira, no entanto, sua identificação se dá pela observação do lado interno da mandíbula, que não apresenta dentes, além disso, apresenta micro espinhos na região superior à inserção das três pernas torácicas (SOSA-GÓMEZ *et al*., 2014). Além disso, possuem um corpo com coloração verde intensa, com um comprimento médio de 27 mm quando adulta, tendo um escudo cervical pouco evidente e uma área ocular em tonalidade castanho clara (MOSCARDI *et al*., 2012). Os seus danos em soja são caracterizados pelo rendilhamento dos folíolos, esse efeito é resultado da alimentação das lagartas, que mostram preferência pelo parênquima foliar, esse padrão de alimentação preserva as nervuras, causando danos mais acentuados, especialmente nos estágios finais do desenvolvimento (RUSSO *et al*., 2024).

Desde o desenvolvimento da tecnologia de cultivares de soja Bt (*Bacillus thuringiensis*), esta tem sido a principal forma de controle deste lepidóptero. No entanto, na safra 2020/21 em um estudo da Empresa Brasileira de Pesquisa Agropecuária (Embrapa), verificou-se a ocorrência de espécies resistentes em cultivares de soja com esta tecnologia, que antes não existia, devido a inserção da proteína Cry1Ac nestas plantas (BUENO; SOSA-GÓMEZ, 2022). A resistência é resultado de processos evolutivos, alguns insetos de uma população alvo podem sobreviver à exposição inicial a uma planta Bt projetada para eliminá-los, devido a características genéticas, e transmitir essa capacidade aos seus descendentes (BERNARDI *et al*., 2016).

Tendo em vista os pontos citados, o controle químico vem se tornando peça chave no manejo integrado desse inseto-praga. Atualmente existem cerca de 31 produtos comerciais registrados no Ministério da Agricultura Pecuária e Abastecimento (MAPA) para controle deste inseto na cultura da soja, entretanto, apenas 14 ingredientes ativos (MAPA, 2023).

A nanotecnologia tem diversas aplicabilidades, dentre elas, sua utilização na formulação de agrotóxicos. Estes são compostos formulados, que após esse processo, a molécula ativa é potencializada, em decorrência da estrutura utilizada para a construção da nanoformulação (LIMA, 2020). Nos últimos cinco anos, a nanotecnologia tem se destacado na formulação de agrotóxicos, com várias pesquisas focadas em aumentar a eficiência e reduzir os impactos ambientais desses produtos.

Um exemplo é a utilização de nanoformulações para melhorar a solubilidade, estabilidade e biodisponibilidade dos ingredientes ativos em pesticidas, resultando em maior eficácia no controle de pragas e menor toxicidade ambiental (BAMISAYE *et al*., 2023; LI *et al*., 2023). Outro avanço significativo é a aplicação de nanopartículas em pesticidas para obter um controle mais direcionado e uma liberação controlada dos ingredientes ativos.

Esses avanços mostram o potencial das nanopartículas em transformar o setor de agrotóxicos, tornando-o mais eficiente e menos impactante ao meio ambiente, ao mesmo tempo em que se enfrenta o desafio de garantir a segurança dessas novas tecnologias para os ecossistemas e a saúde humana. Dessa forma, é fundamental validar os efeitos desses nanopesticidas em diferentes aplicações e modelos. Portanto, o objetivo desse estudo foi desenvolver uma formulação de lufenurom baseada em nanotecnologia e avaliar sua caracterização físico-química, eficiência de encapsulamento, morfologia e cinética de liberação, com o intuito de controlar a *Rachiplusia nu* em condições de dieta artificial e semi-campo.

2 ARTIGO

In vitro and Semi Field Evaluation of Nanoencapsulated Lufenuron for *Rachiplusia nu* Control: an interesting approach aiming sustainable agriculture[1]

Abstract

Nanotechnology is transforming pest control strategies. This study focuses on developing nanotechnology-based lufenuron (an insecticide) formulations to control *Rachiplusia nu*, a significant pest in soybean crops, the caterpillars attack the leaves, initially rasping them and causing small light spots, as they grow, they become voracious and ultimately destroy the leaves, potentially damaging even the thinner stems. Lufenuron was nanoencapsulated using polycaprolactone (PCL) nanoparticles prepared by a nanoprecipitation method. The formulations were characterized by their physicochemical properties, including particle size, zeta potential, encapsulation efficiency, and morphology. Additionally, evaluate its performance in controlling this arthropod in artificial diet and semi-field conditions, offering improved control against *R. nu*. The experiments demonstrated that nanoencapsulation of lufenuron using PCL nanoparticles at a concentration of 1.0 mg/mL exhibited polydispersity index below 0.2, an average zeta potential of -40 mV and good physicochemical properties with minimal parameters variations during storage time. Bioassays showed that lufenuron nanoencapsulation is efficient in control at equivalent doses and significant control at reduced doses, especially *in vivo* with 98,5%. In conclusion, it is possible to obtain nanoencapsulated lufenuron with stability and effective control and in this way, contributing to a more sustainable agriculture.

Keywords: Nanotechnology**;** Pest Control; Chemical control, Insecticide; Sustainable Agriculture

[1] Artigo com pretensão de submissão e publicação na revista *Journal of Agricultural and Food Chemistry*.

INTRODUCTION

Recent agricultural advancements play a significant role in the economies of numerous countries. The growing human population, along with shifting environmental conditions, intensifies the need to boost agricultural food production to meet the rising demand.[1] The use of pesticides is an important tool to increase crop yield and quality, reduce pest management workload and improve prospects for long-term sustainable food production.[2] However, the excessive use of these products has resulted in detrimental effects on the ecosystem, leading to the contamination of soil, surface water, and groundwater. There is now widespread consensus that new technologies are urgently needed to protect crops from insect damage.[3-5]

The Looper Caterpillar (Lepidoptera: Noctuidae), scientifically named *Rachiplusia nu* (Guenée, 1852), primarily occurs in the southern region of the American continent, especially in Uruguay, Argentina, and southern Brazil.[6,7] This caterpillar causes the most damage in its later developmental stages on soybean, characterized by feeding on the leaf parenchyma, leaving the leaf with a lace-like appearance.[7] A concerning aspect of this species is its high tolerance to chemical insecticides, as well as its history of developing resistance to these products in the United States.[8] And lufenuron is one of the most important insecticide for *R. nu* management.

Lufenuron, with a molecular weight of 511.2 g/mol, exhibits a pKa value of 10.18 ± 0.05, indicating its acidic nature, a key physicochemical property of lufenuron is its low solubility in water, which varies slightly with changes in pH (54 μg/L at pH 5.0 and 64 μg/L at pH 9.0, all measured at 25 °C).[9,10] These low solubility values highlight lufenuron's hydrophobicity, which is a critical factor in its behavior in biological and environmental systems.[11] The hydrophobic nature of lufenuron contributes to its persistence and distribution characteristics, influencing its efficacy and environmental impact as an insecticidal agent.[12]

Therefore, the integration of techniques, like nanotechnology and pesticides, to combat pests and maintain an economic and harmonious relationship with the environment has gained attention from numerous researchers and farmers.[13] The distinctive properties of materials at the nanoscale make them ideal candidates for designing and developing innovative tools to support sustainable agriculture.[14] Nanotechnology is an emerging field with numerous applications across various domains of modern science, including physics, pharmacology, chemistry, agriculture and engineering.[15]

Nanopesticides are pesticides that are formulated using nanomaterials, which can be applied in agriculture through various methods such as immobilization on hybrid substrates, encapsulation within matrices, or incorporation into functionalized nanocarriers that respond to external stimuli or enzymatic triggers.[16] The nanoscale size, shape, and distinctive properties of these particles are utilized to enhance pesticide effectiveness; these innovative formulations often use materials such as silica, lipids, polymers, metals, carbon, and others.[17]

Developing chemical formulations associated with nanotechnology is an important tool for pest control, achieved by developing new technologies.[18] These techniques allow us to develop stable particles that improve interesting characteristics such as better control.[19] For instance, numerous studies have illustrated that nano-Imidacloprid outperforms conventional insecticides in combatting pests like *Sitophilus granarius*.[20] Also, when controlling adults of *Bemisia tabaci*, it was observed that nanoetofenprox was more efficient than the commercial formulation, enabling a dose reduction.[21]

However, it is crucial to validate the effects of these nanopesticides in different applications and models. Therefore, the objective of this study was to produce a formulation based on nanotechnology to evaluate your physicochemical characterization, encapsulation efficiency, morphology, and release kinetics aiming to control *Rachiplusia nu*. This system may also prove beneficial for enhancing pest control efficiency and ultimately contribute to increased agricultural productivity.

MATERIALS AND METHODS

Nanoparticles preparation

Polycaprolactone nanoparticles with lufenuron (NP_PCL_LFN) were prepared by the nanoprecipitation method. The organic phase was prepared by combining polycaprolactone (PCL) (100 mg), Mirytol 810 (a mixture of capric and caprylic acid triglycerides, 200 mg), SPAN 60 (sorbitan monostearate surfactant, 40 mg), and lufenuron (LFN) (10 mg). Subsequently, an aqueous phase was prepared using Tween 80 (polysorbate 80) at a concentration of 2.0 mg mL^{-1} in 30 mL of water. To form the nanocapsules (NP), the organic phase was slowly added to the aqueous phase, which was maintained under constant agitation. The resulting suspension was stirred for 10 minutes. Water was then evaporated under reduced pressure, yielding a final volume of 10 mL. The final concentration of LFN was 1.0 mg mL^{-1}. Furthermore, a formulation was developed for comparison with only polycaprolactone nanoparticles (NP_PCL).

Physicochemical characterization of the PCL nanoparticles

The size distribution and polydispersity index (PDI) were measured using Dynamic Light Scattering (DLS). Zeta potential was determined by microelectrophoresis with a ZetaSizer Nano ZS90 system (Malvern Instruments, UK), operating at a fixed angle of 90° and 25 °C with samples diluted 200-fold. Additionally, nanoparticle concentration, size distribution, and polydispersity were analyzed using nanoparticle tracking analysis with a NanoSight instrument (Malvern Instruments, UK). This instrument, equipped with a green laser (532 nm) and an sCMOS camera, was controlled by NanoSight v.3 software, with samples diluted 20,000-fold. Results were obtained as the average of triplicate analyses. The formulations were stored at room temperature (25 °C) and their stability was assessed over time with measurements taken at 0, 15, 30, 60, and 90 days.

Encapsulation efficiency

The encapsulation efficiency was determined using the ultrafiltration/centrifugation method with Microcon 10 kDa regenerated cellulose ultrafilters (Millipore), which permit only the passage of non-encapsulated substances. Encapsulation efficiency was calculated by subtracting the quantity of non-encapsulated substances from the initially added quantity. For the quantification of LFN in the ultra-filtered, a Phenomenex Gemini (Phenomenex, Torrance-USA) C18 reverse phase column (250 × 4.6 mm, 5.0 μm) was used. The mobile phase was methanol:

water (1:1 v/v) pumped at flow rate of 1.0 mL/min. Chromatographic analysis were performed using an Ultimate 3000 instrument (Thermo Fisher Scientific, Waltham, USA) with UV detection (225 nm). The calculations were based on the LFN calibration curves with the equations $y = 1.28395x + 2.60255$ ($R^2 = 0.99894$), respectively.

Morphology of nanoparticles by atomic force microscopy

Morphological analysis of nanoparticles containing lufenuron (NP_PCL_LFN) was conducted with an Easyscan 2 atomic force microscope (AFM) (Nanosurf) operating in TapAl-G (BudgetSensors) mode at a scan rate of 90 Hz. For sample preparation, the formulation was diluted 10,000-fold in ultrapure water and then dried on a silica grid at room temperature. Surface images and three-dimensional information were captured using Easyscan 2 v3.10.0 control software and subsequently processed with Gwyddion v2.0.

Release kinetics

The *in vitro* release kinetics was studied using the dialysis method. Briefly, the NP_PCL_LFN and non-encapsulated LFN (0,5 mL) were placed in dialysis membrane bags (1.0 kDa exclusion pore size) and immersed in Pluronic solution (2.0 % w/v) (250 mL), under mechanical stirring (shaker). Aliquots were periodically collected for 210 minutes, and the LFN was quantified as described above. The release assays were performed at room temperature and 25 ºC.

Evaluation of biological activity in artificial diet

The system initially prepared was submitted to biological activity assays against *R. nu*. Diet-overlay bioassays were conducted in 100 mL plastic pots (Coposan, Orleans, SC, Brazil). Each received 1.0 mL of an artificial diet based on white beans, wheat germ and yeast (adapted from Greene et al., 1976).[22]

Different system concentrations were prepared with distilled water and the surfactant Triton™ X-100 (Sigma-Aldrich, São Paulo, SP, Brazil) at 0.1% to spread the solution over the diet surface. Six treatments were tested (Control; NP_PCL; 7,5 g of LFN; 7,5 g of NP_PCL_LFN; 1,5 g of NP_PCL_LFN; 0,75 g of NP_PCL_LFN). Values are based on the dose of lufenuron active ingredient. The control treatment was distilled water and surfactant.

A 30 microliters volume of the system concentrations was applied to the diet surface in each plastic pot and allowed to dry. Two hundred (200) caterpillars were used in instar L3 for each treatment, divided into 20 replications, totaling 1200 caterpillars. The experiment was conducted in a Completely Randomized Design. Insects were maintained at 25 ± 2°C for five days. Mortalities were submitted to Bartlett test and analysis of variance (ANOVA), and means were compared by the Scott–Knott test ($P < 0.05$) using the R software (R Development Core Team 2017).[23]

Evaluation of biological activity in semi-field

The experiment was conducted at the Federal University of Santa Maria, Santa Maria – RS, Brazil, in the 2023/24 harvest, the soybean cultivar used was BMX Valente® sown on December 1st, 2023. The same six treatments from the artificial diet assay were applied under field conditions in 25 m² plots in soybean at phenological stage R5.5, using a CO_2-pressurized side boom, a spray volume of 100 L ha^{-1}, and TT110.01 nozzles at a pressure of 2.1 bar. The weather conditions at the time of application were 25°C with 80% relative humidity, wind speed of 3.0 meters per second and partly cloudy skies. One hour after the treatments were applied, approximately 160 leaflets from the upper third of the plants were collected from each treatment.

In the laboratory, 360 pots were prepared with about 1.0 mL of carrageenan to maintain moisture levels during the experiment. Each pot received two leaflets, subsequently infested with L3 instar *R. nu* larvae. Each treatment had 200 caterpillars divided into 20 replications. After setup, the assay was maintained at 25 ± 2°C in a completely randomized design. Seven days after the experiment setup, the evaluation was conducted, using the number of dead larvae per replicate as the criterion. The data were tested for homogeneity of variances using the Bartlett test and then subjected to analysis of variance (ANOVA). Their means were compared using the Scott-Knott test ($P < 0.05$) with the R software (R Development Core Team 2017).[23]

RESULTS AND DISCUSSION

Nanoparticle preparation and characterization

Initially, several tests were conducted using different carrier systems to determine the optimal system for encapsulating LFN. Zein nanoparticles, lignin nanoparticles, zein/lignin nanoparticles, and nanostructured lipid carriers were tested. It is worth noting that none of the carrier systems investigated yielded promising formulations. The systems exhibited precipitation during solvent evaporation processes and showed low interaction with the active ingredient (data not shown).

In this context, PCL nanoparticles were tested, which showed promise for encapsulating LFN. It should be emphasized that different concentrations of LFN ranging from 1.0 to 5.0 mg/mL were tested in the PCL nanoparticle formulations. However, formulations with concentrations higher than 1.0 mg/mL did not exhibit stability due to precipitation of the active ingredient (data not shown). Therefore, further work proceeded with the formulation of PCL nanoparticles containing 1.0 mg/mL of LFN (NP_PCL_LFN), and the initial characterization data are presented in Table 1.

Table 1: Physicochemical characterization of PCL nanoparticles. The parameters analyzed were size by dynamic light scattering (DLS), size by nanoparticle tracking analysis (NTA), polydispersity index (PDI), zeta potential (mV), and pH. The values represent the means of three determinations.

Sample	DLS (nm)	NTA (nm)	PDI	ZP (mV)	pH	Concentration (10^{13} Particles/mL)
NP_PCL	246 ± 4	199 ± 2	0.120 ± 0.022	- 33 ± 1.5	5.2 ± 0.6	1.06 ± 0.12
NP_PCL_LFN	264 ± 5	205 ± 5	0.081 ± 0.013	- 44 ± 1.2	5.7 ± 0.7	1.59 ± 0.76

According to Table 1, both the control formulation (NP_PCL) and the formulation containing lufenuron (NP_PCL_LFN) exhibited good physicochemical properties. Regarding the particle size, a slight increase in the average diameter of the particles was observed when the active ingredient was added for both techniques, most likely due to the encapsulation process of the active ingredient. Furthermore, the formulations showed a polydispersity index below 0.2, an average zeta potential of -40 mV, and a pH of 5. These data are consistent with findings in the literature for

PCL-based formulations, reinforcing the favorable physicochemical properties of the systems.[24,25] These formulations were then stored at room temperature for stability evaluation over time.

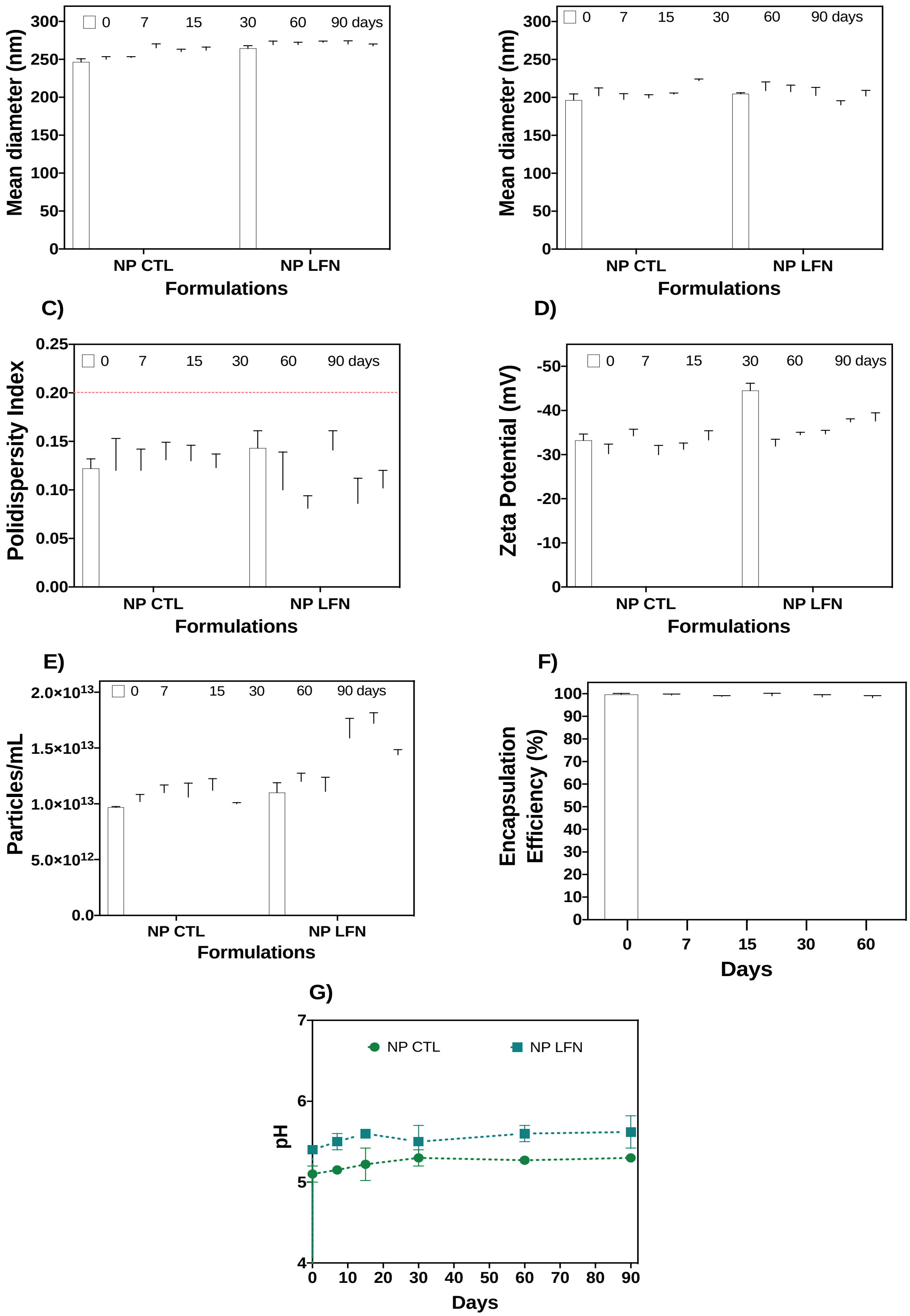
Figure 1 presents the results of physicochemical stability, analyzing the parameters described in Table 1, as observed, there were no significant variations in the average diameter of the formulations during the storage time, assessed by both DLS techniques (Figure 1-A) and NTA (Figure 1-B). The formulations maintained polydispersity index values (Figure 1-C) below 0.2 throughout the storage period, indicating good system stability. The zeta potential (Figure 1-D) was another parameter that demonstrated stability over time. The formulation containing lufenuron (NP_PCL_LFN) had a value of -44 ± 1.2 mV at time 0, showing a decrease to -32 ± 1.2 mV after 7 days and remained stable until the final analyzed time of 90 days. Regarding the particle concentration (Figure 1-E), the control formulations showed greater stability than those containing LFN.

The LFN-containing formulations showed a significant increase in particle concentration after 30 days, remaining stable until the final time. It is worth noting that even with the concentration increase, no changes were observed in the other analyzed parameters. PCL nanoparticles proved promising for lufenuron encapsulation, with values exceeding 99%, and these values remained stable throughout the analyzed stability period (Figure 1-F). The pH of the formulations was also evaluated over time (Figure 1-G) and showed stability. The formulations containing LFN had higher values than the control formulation due to the addition of the active ingredient, but like the control, the values remained stable over time.

PCL nanocapsules have shown good properties for encapsulating chemical actives, and these results are consistent with findings in the literature. Diyanat et al. (2019)[26] encapsulated the herbicide pretilachlor in PCL nanocapsules. The authors obtained nanocapsules with a size range of 70 to 200 nm. Similar to the observations in this study, the encapsulation efficiency of pretilachlor was higher than 99%. Additionally, in line with our results, the studies on physicochemical stability over 60 days showed that the nanocapsules remained stable in suspension without aggregation.

Figure 1: Physicochemical characterization of NP_PCL and NP_PCL_LFN in function of storage time (0, 7, 15, 30, 60 and 90 days). **A)** Size by DLS (nm); **B)** Size by NTA (nm); **C)** polydispersity index (PDI); **D)** Zeta potential (mV) **E)** Nanoparticle concentration (particles/mL) **F)** Encapsulation efficiency (%) and **G)** pH. The values represent the means of three determinations.

3.

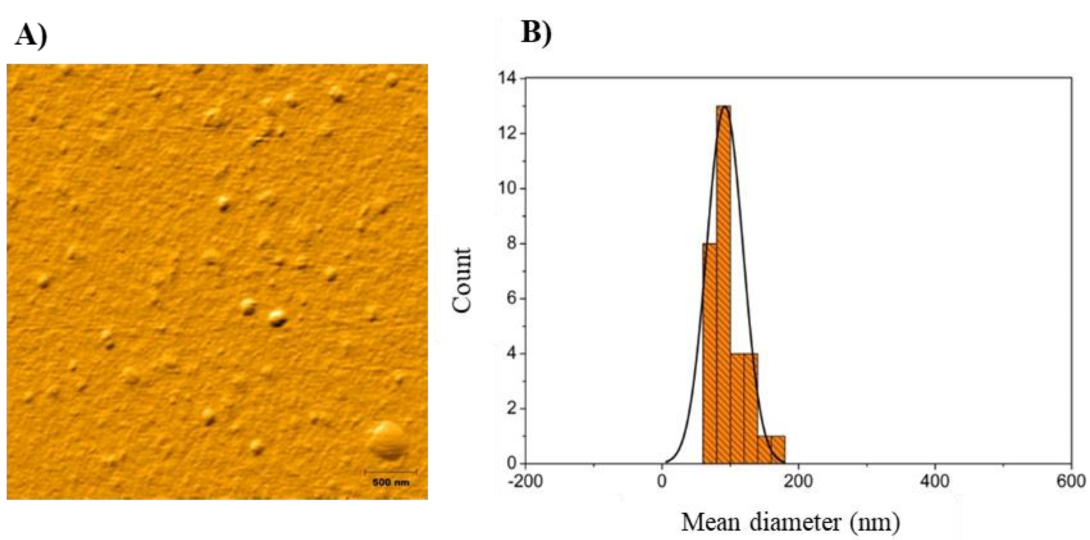


Morphology of nanoparticles by atomic force microscopy

The atomic force microscopy (AFM) technique was also used to characterize the LFN nanoparticle formulation. As shown in Figure 2, the nanoparticles exhibited a spherical morphology with an average diameter of 94 ± 20 nm. The average diameter of the formulations was smaller than the analyses performed by DLS and NTA. However, it is important to note that for AFM analysis, the formulations are dried, unlike the other studies where the hydrodynamic diameter of the nanoparticles is measured. These data further support the results of polydispersity, as we can observe highly uniform particles in the size distribution graph (Figure 2-B). Furthermore, no pores were observed in the analyzed particles, which is significant when aiming to protect the actives, and this finding is consistent with the encapsulation efficiency (EE) data, where values above 99% were observed.

In the literature, several studies demonstrate that Atomic Force Microscopy (AFM) is a rapid technique with excellent resolution, useful for determining both the size and morphology of lipid nanoparticles.[27] In the study by Abrantes et al. (2021), the authors employed this technique to characterize nanostructured lipid carriers containing the active ingredient icaridin. The authors were able to determine the size of the nanocarriers, corroborating the data obtained from other laser-based techniques. Moreover, they observed the spherical morphology of the nanoformulations, which were identifiable even within a hydrogel matrix.[28]

Figure 2: Morphology and size distribution of PCL nanoparticles containing LFN. **(A)** AFM micrograph of PCL nanoparticles containing LFN; **(B)** Size distribution by AFM techniques.

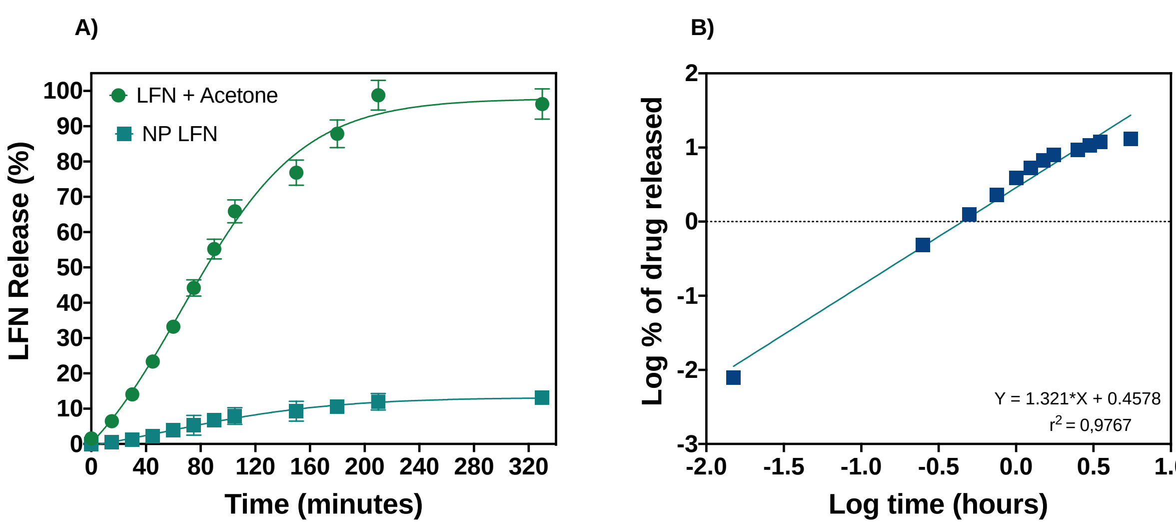


Release kinetics

The release of LFN was studied using dialysis bags under sink conditions at room temperature (25 °C). The release profiles of encapsulated and non-encapsulated LFN over time are shown in Figure 3-A. Due to the low aqueous solubility of LFN, the control group (non-encapsulated LFN) was prepared in acetone at the same concentration as the nanoparticle formulation.

As observed in the results presented in Figure 3-A, the PCL nanoparticles demonstrated the ability to modulate LFN release, resulting in a lower release rate compared to the non-encapsulated compound. After 1 hour and 30 minutes of the assay, the non-encapsulated compound reached a release of 33.21 ± 1.85%. At the same time, the formulation containing encapsulated LFN exhibited a release of only 6.71 ± 1.02%, which is nearly eight times less.

Figure 3: A) Kinetics of release of LFN from the PCL nanoparticles and LFN in acetone; **B)** Application of the Korsmeyer-Peppas mathematical model. The release assay was performed using a dialysis bag system at (room temperature and 25 °C), in Pluronic solution (2% w/v). The analyses were performed in triplicate and quantified by HPLC.



These results are consistent with findings in the literature for certain hydrophobic active ingredients. Campos et al. (2015)[29] also observed low release levels for the fungicide carbendazim when using the same model to evaluate the release of PCL nanoparticles. The authors reported release values below 10% after more than 6 hours of the assay, reaching 47% release after 6 days

(144 hours) of testing. Similar to LFN, carbendazim has low water solubility, with a value below 1.0 mg/L.

It is important to note that modifications in the co-solvent concentration (4% Pluronic F-68) were made; however, no significant changes were observed in the release profile of the active ingredients (data not shown). This indicates a strong interaction between the active compound and the PCL nanocapsules, reinforcing the high encapsulation efficiency.

Based on the release results presented in Figure 3-A, different mathematical models were applied to elucidate the mechanism of active ingredient release from the studied formulation. The applied mathematical models included zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Through the application of linear regression, it was found that the mathematical model that best fit the release data was the Korsmeyer-Peppas model, with a determination coefficient (R^2) of 0.9767, as illustrated in Figure 3-B.

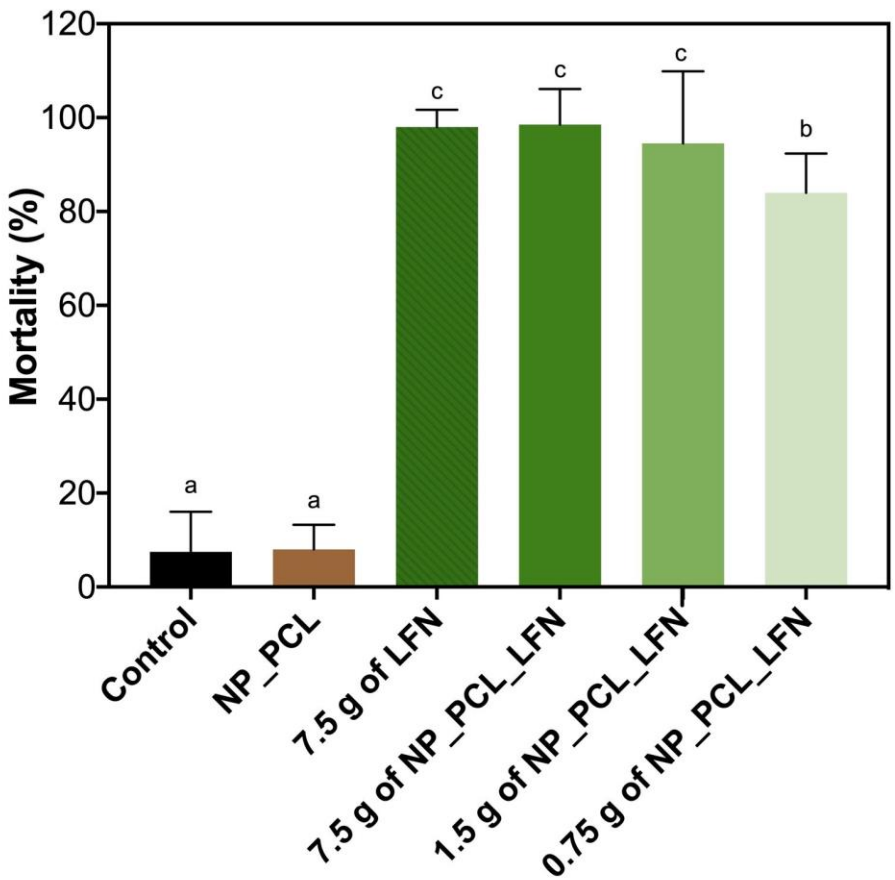
The Korsmeyer-Peppas equation used in the analysis provided a value of n greater than 1, indicating that the release of the active compound follows a non-Fickian mechanism, characterized as super case II. This type of release is primarily dominated by polymer chain erosion, suggesting that the active ingredient is released in a controlled manner and is dependent on the degradation of the polymeric material. This controlled erosion can be influenced by factors such as polymer composition, formulation structure, and release medium conditions.

Additionally, the observed release behavior may be related to a combination of mechanisms, including the diffusion of the active ingredient through the polymer matrix and its concomitant degradation. The predominance of erosion as the controlling mechanism may be advantageous in applications where sustained and controlled release of the active ingredient is desired, especially in contexts where delivery efficiency and protection of the active compound are crucial for the formulation's performance.[30]

Evaluation of biological activity in artificial diet

The evaluation of biological activity against *R. nu* provides insights into the effectiveness of the tested formulations. As demonstrated in Figure 4, the nanoparticle formulations containing lufenuron (NP_PCL_LFN) demonstrated even higher mortality rates compared to the commercial formulation, reaching 98.5% mortality. Even when diluted five times, this formulation still exhibited a significant activity with 95% mortality. Furthermore, a dilution of 10 times resulted in a mortality rate of 84%. This highlights the potent effect of the LFN-loaded nanoparticle formulations. These findings indicate that the nanoparticle formulations effectively control *Rachiplusia nu,* even at lower concentrations.

Figure 4: Biological activity evaluation against *Rachiplusia nu* in artificial diet. Comparison of mortality (%) of nanoparticle formulations containing lufenuron (NP_PCL_LFN), commercial formulation (LFN) and nanoparticles only (NP_PCL).



Bars with the same letter are not significantly different (Skott Knott test at $P \leq 0.05$).

Similar results were obtained by Boff et al. (2022), where it was found that nanoencapsulated bifenthrin and lambda-cyhalothrin with synergists in diet-overlay bioassays increased the control of soybean looper caterpillar (*Chrysodeixis includens*) for both chemicals.[31] In the same way, equivalent control results were observed with the development of two microemulsion-based nanoformulations, using novaluron, in the control of *Spodoptera littoralis*, in addition, the authors emphasize that it is possible to nanoencapsulate insecticides with methods that require low energy and are environmentally preferable.[32]

The control and NP_PCL did not exhibit significant effect on the tested organisms. On the other hand, the commercial formulation (LFN) displayed mortality of 98% at the recommended dose. This indicates the efficacy of the commercial product in controlling these pests.

Before transitioning to the analysis of effects under simulated field conditions, it is imperative to contextualize the findings from the artificial diet assays. These tests are pivotal in the initial assessment of control agent efficacy, facilitating the evaluation of dosage and the product's potential against target organisms. Building upon the promising outcomes observed with the formulated product, subsequent evaluation was conducted under conditions that closely mimic real-world scenarios, using semi-controlled environments.

This phase aims not only to validate the observed efficacy but also to offer additional insights into the practical applicability of the product. The upcoming semi field trial will validate the findings from the artificial diet experiments and provide a comprehensive assessment of this product's potential as an effective pest control solution.

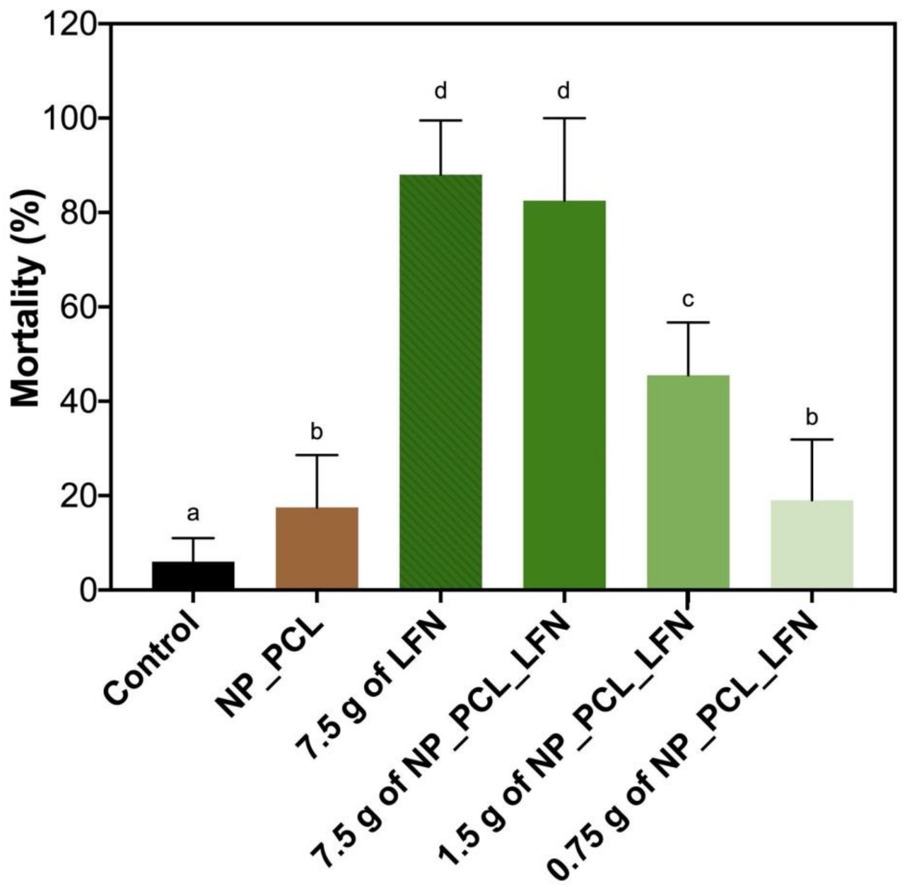
Evaluation of biological activity in semi-field

A second experiment was conducted to evaluate the biological activity of the formulation on soybean leaflets. Figure 5 shows the effects of this experiment, showing that the commercial formulation and the nanoformulation at equivalent doses achieved statistically equal; both treatments demonstrated satisfactory control greater than 80%, demonstrating that nanoencapsulation is a tool with potential use in control.

Whatever with a five-times dilution, control decreased to 46%, and with a ten-times dilution, control further dropped to 19%. Despite the significant reduction in the amount of active ingredient applied, considerable results were observed in the control of *R. nu*.

A study using teflubenzuron, from the same chemical group as lufenuron (Chitin Synthesis Inhibitors), tested the control efficacy of this insecticide in an artificial diet and semi-field trials of *C. includens* combined with different adjuvants.[33] The results were similar regarding their efficacy in diet and field conditions. It was observed that when contrasting the two results, there is a natural loss of control efficiency, probably due to factors such as environmental interactions with the plant-insect-insecticide relationship.

Figure 5: Biological activity evaluation against *Rachiplusia nu* in semi field. Comparison of mortality (%) of nanoparticle formulations containing lufenuron (NP_PCL_LFN), commercial formulation (LFN) and nanoparticles only (NP_PCL).



Bars with the same letter are not significantly different (Skott Knott test at $P \leq 0.05$).

Nanopesticides can maintain or enhance pest control efficacy while reducing the required dosage, improving environmental safety and sustainability.[34] Nanopesticides with reduced doses of active ingredients can achieve efficient pest control, as demonstrated by studies showing significant efficacy against agricultural pests. This approach highlights the potential of nanoencapsulation to enhance the environmental safety and sustainability of pesticide applications.[35]

CONCLUSION

In conclusion, the nanoparticles containing lufenuron (NP_PCL_LFN) demonstrated favorable physicochemical properties and stability over time, indicating their potential to deliver lufenuron to target pests effectively. Biological activity assays showed that encapsulated lufenuron retained its effectiveness even at lower concentrations, underscoring the potential of these systems for pest management. It is important to note the distinction between in diet and semi field tests and emphasize the significance of evaluating nanopesticides in various environments. At recommended doses, the formulation has shown consistent efficacy in diet studies, highlighting its practical potential. However, further investigations into the effects of nanopesticides across different environments are crucial. Looking ahead, optimizing the formulation to enhance the concentration of active ingredients in nanoparticles could prove advantageous in developing competitive solutions for pest control. This approach holds promise for advancing sustainable and environmentally friendly strategies in agriculture.

FUNDING SOURCES

The authors are grateful for the financial support provided by the São Paulo State Research Support Foundation (FAPESP, grants: CBioClima - #2021/10639-5; #2017/21004-5), Brazilian National Council for Scientific and Technological Development (CNPq-MCTI-INCT NanoAgro #405924/2022-4, #308439/2021-0-LFF), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES-MEC INCTNanoAgro #88887.953443/2024-00).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

The authors would like to thank the Grupo de Pesquisa em Resistência de Insetos (GPRI - UFSM) for providing the insects used in the work and the group Manejo Inteligente em Tecnologia de Aplicação (MITA – UFSM) for providing the structure to conduce the biologicals trials. The authors are grateful for the financial support provided by the São Paulo State Research Support Foundation (FAPESP, grants: CBioClima - #2021/10639-5; #2017/21004-5), Brazilian National Council for Scientific and Technological Development (CNPq-MCTI-INCT NanoAgro #405924/2022-4, #308439/2021-0-LFF), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES-MEC INCTNanoAgro #88887.953443/2024-00).

ABBREVIATIONS AND NOMENCLATURE

LFN, Commercial Lufenuron; NP_PCL, Polycaprolactone nanoparticles; NP_PCL_LFN, Polycaprolactone nanoparticles with lufenuron; AFM, Atomic Force Microscopy; IPM, Integrated Pest Management; PDI, Polydispersity Index; DLS, Dynamic Light Scattering; NTA, Nanoparticle Tracking Analysis; EE, Encapsulation Efficiency.

REFERENCES

(1) Bruinsma, J. *World Agriculture: Towards 2015/2030: An FAO Study*; Routledge, 2017. https://doi.org/10.4324/9781315083858.

(2) Weller, S., Culbreath, A., Gianessi, L., Godfrey, L., Jachetta, J., Norsworthy, J., Madsen, J. (2014). The contributions of pesticides to pest management in meeting the global need for food production by 2050.

(3) Nicolopoulou-Stamati, P.; Maipas, S.; Kotampasi, C.; Stamatis, P.; Hens, L. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Front. Public Health* **2016**, *4*. https://doi.org/10.3389/fpubh.2016.00148.

(4) Shiva, V. *The Violence of the Green Revolution: Third World Agriculture, Ecology, and Politics*; University Press of Kentucky, 2016.

(5) Fernandes, C. L. F.; Volcão, L. M.; Ramires, P. F.; Moura, R. R. D.; Da Silva Júnior, F. M. R. Distribution of Pesticides in Agricultural and Urban Soils of Brazil: A Critical Review. *Environ. Sci. Process. Impacts* **2020**, *22* (2), 256–270. https://doi.org/10.1039/C9EM00433E.

(6) Boisduval, J. A. (1874). *Natural History of Insects: General Species of Lepidoptera. Heterocerans. Sphingids, Sesiids, Castnids* (Vol. 1). Encyclopedic Library of Roret.

(7) Sosa-Gómez, D. R., Corrêa-Ferreira, B. S., Hoffmann-Campo, C. B., Corso, I. C., Oliveira, L. J., Moscardi, F., ... & Roggia, S. (2014). *Manual for the Identification of Insects and Other Invertebrates in Soybean Cultivation*.

(8) Mascarenhas, R.N.; Boethel, D. J. Development of diagnostic concentrations for insecticide resistance monitoring in soybean looper (Lepidoptera:Noctuidae) larvae using an artificial diet overlay bioassay. Journal of Economic Entomology, v. 93, p. 897-904, 2000. https://doi.org/10.1093/jee/93.3.897

(9) EFSA (2008). *Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance Lufenuron*. European Food Safety Authority, p. 130, 2008. Available at: [http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/189r.pdf. Accessed on: Aug. 2024.

(10) FAO (2008). *Lufenuron. FAO Specifications and Evaluations for Agricultural Pesticides*. Food and Agriculture Organization of the United Nations, p. 22, 2008. Available at:

[http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Lufenuron08.pdf. Accessed on: Aug. 2024.

(11) Hamoutene, D., Ryall, E., Porter, E., Page, F.H., Wickens, K., Wong, D., Martell, L., Burridge, L., Villeneuve, J., Miller, C. 2023. Discussion of Environmental Quality Standards (EQS) and their development for the monitoring of impacts from the use of pesticides and drugs at marine aquaculture sites. DFO Can. Sci. Advis. Sec. Res. Doc. 2022/066. vii + 117 p.

(12) Wells, C., & Collins, C. T. (2022). A rapid evidence assessment of the potential risk to the environment presented by active ingredients in the UK's most commonly sold companion animal parasiticides. Environmental science and pollution research, 29(30), 45070-45088. https://doi.org/10.1007/s11356-022-20204-2

(13) Flint, M. L. *IPM in Practice, 2nd Edition: Principles and Methods of Integrated Pest Management*; University of California Agriculture and Natural Resources, 2012.

(14) Fraceto, L. F., Grillo, R., de Medeiros, G. A., Scognamiglio, V., Rea, G., & Bartolucci, C. (2016). Nanotechnology in agriculture: which innovation potential does it have?. *Frontiers in Environmental Science*, *4*, 186737. https://doi.org/10.3389/fenvs.2016.00020

(15) Liu, C., Zhou, H., & Zhou, J. (2021). The applications of nanotechnology in crop production. *Molecules*, *26*(23), 7070. https://doi.org/10.3390/molecules26237070

(16) Chaud, M., Souto, E. B., Zielinska, A., Severino, P., Batain, F., Oliveira-Junior, J., & Alves, T. (2021). Nanopesticides in agriculture: Benefits and challenge in agricultural productivity, toxicological risks to human health and environment. *Toxics*, *9*(6), 131. https://doi.org/10.3390/toxics9060131

(17) Agostini, A., Mondragón, L., Coll, C., Aznar, E., Marcos, M. D., Martinez-Manez, R., Amorós, P. (2012). Dual enzyme-triggered controlled release on capped nanometric silica mesoporous supports. *ChemistryOpen*, *1*(1), 17. https://doi.org/10.1002%2Fopen.201200003

(18) Mascarin et al., 2019 G. M. Mascarin, R. B. Lopes, Í. Delalibera, É.K.K. Fernandes, C. Luz, M. Fari Current status and perspectives of fungal entomopathogens used for microbial control of arthropod pests in Brazil. J. Invertebr. Pathol., 165 (2019), pp. 46-53. https://doi.org/10.1016/j.jip.2018.01.001

(19) Meirelles, L. N.; Mesquita, E., Corrêa, T. A., Bitencourt, R. D. O. B., Oliveira, J. L., Fraceto, L. F., Bittencourt, V. R. E. P. Encapsulation of entomopathogenic fungal conidia: evaluation of stability and control potential of Rhipicephalus mcroplus. **Ticks and Tick-borne Diseases**, v. 14, n. 4, p. 102184, 2023. https://doi.org/10.1016/j.ttbdis.2023.102184

(20) Sabbour, M. M. A. (2018). Pathogenicity of imidacloprid and its nano against Rhyzopertha dominica (Coleoptera: Bostrichidae) under laboratory and store conditions.

(21) Sabry, A. K. H., Mohamady, A. H., Sleem, R. A., Abolmaaty, S. M., & Helmy, R. (2023). Role of etofenprox nanoformulation in suppression of the silver whitefly, Bemisia tabaci and its residue in eggplant fruits. *Journal of Plant Protection Research*, 30-38. https://doi.org/10.24425/jppr.2023.144501

(22) Greene, G. L., Leppla, N. C., & Dickerson, W. A. (1976). Velvetbean caterpillar: a rearing procedure and artificial medium. *Journal of economic entomology*, *69*(4), 487-488. https://doi.org/10.1093/jee/69.4.487

(23) R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Avaliable in: <https://www.R-project.org> (Accessed on March 10, 2024).

(24) Pascoli, M.; Lopes-Oliveira, P. J.; Fraceto, L. F.; Seabra, A. B.; Oliveira, H. C. State of the Art of Polymeric Nanoparticles as Carrier Systems with Agricultural Applications: A Minireview. *Energy Ecol. Environ.* **2018**, *3* (3), 137–148. https://doi.org/10.1007/s40974-018-0090-2.

(25) Ramazani, A.; Keramati, M.; Malvandi, H.; Danafar, H.; Kheiri Manjili, H. Preparation and in Vivo Evaluation of Anti-Plasmodial Properties of Artemisinin-Loaded PCL–PEG–PCL Nanoparticles. *Pharm. Dev. Technol.* **2018**, *23* (9), 911–920. https://doi.org/10.1080/10837450.2017.1372781.

(26) Diyanat, M.; Saeidian, H.; Baziar, S.; Mirjafary, Z. Preparation and Characterization of Polycaprolactone Nanocapsules Containing Pretilachlor as a Herbicide Nanocarrier. *Environ. Sci. Pollut. Res.* **2019**, *26* (21), 21579–21588. https://doi.org/10.1007/s11356-019-05257-0.

(27) Wang, J., Zhang, J., Li, S., Liu, D., Bhambhani, A., & Zeng, H. (2023). Characterization of structures and molecular interactions of RNA and lipid carriers using atomic force

microscopy. *Advances in Colloid and Interface Science*, *313*, 102855. https://doi.org/10.1016/j.cis.2023.102855

(28) Abrantes, D. C., Rogerio, C. B., de Oliveira, J. L., Campos, E. V., de Araújo, D. R., Pampana, L. C., ... & Fraceto, L. F. (2021). Development of a mosquito repellent formulation based on nanostructured lipid carriers. *Frontiers in Pharmacology*, *12*, 760682. https://doi.org/10.3389/fphar.2021.760682

(29) Campos, E. V. R.; Oliveira, J. L. de; da Silva, C. M. G.; Pascoli, M.; Pasquoto, T.; Lima, R.; Abhilash, P. C.; Fernandes Fraceto, L. Polymeric and Solid Lipid Nanoparticles for Sustained Release of Carbendazim and Tebuconazole in Agricultural Applications. *Sci. Rep.* **2015**, *5* (1), 13809. https://doi.org/10.1038/srep13809.

(30) Trucillo, P. (2022). Drug carriers: A review on the most used mathematical models for drug release. *Processes*, *10*(6), 1094. https://doi.org/10.3390/pr10061094

(31) Boff, J. S., Bernardi, O., Lopes, C. S., Gonçalves, R. S., & Silva, F. A. Development and biological evaluation of nanoencapsulated-based pyrethroids with synergists for resistance management of two soybean pests: insights for new insecticide formulations. Pest Management Science, 79(3), 1204-1212, 2023. https://doi.org/10.1002/ps.7295

(32) Elek, N., Hoffman, R., Raviv, U., Resh, R., Ishaaya, I., & Magdassi, S. (2010). Novaluron nanoparticles: Formation and potential use in controlling agricultural insect pests. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *372*(1-3), 66-72. https://doi.org/10.1016/j.colsurfa.2010.09.034

(33) Hettwer, B. L., Godoy, D. N., Hanich, M. R., Caye, M., Moreira, R. P., Luchese, E. F., Melo, A. A. (2022). Influence of adjuvants added to teflubenzuron spray on resistant and susceptible strains of the soybean looper Chrysodeixis includens (Lepidoptera: Noctuidae). *Ciência Rural*, *53*, e20210710. https://doi.org/10.1590/0103-8478cr20210710

(34) Abdollahdokht, D., Bayat, S., Javan-Nikkhah, M., Abouie Mehrizi, A., Alizdeh, H., & Rezapour, S. Conventional agrochemicals towards nano-biopesticides: An overview on recent advances. Chemical and Biological Technologies in Agricuture, vol. 9, no. 1, p. 13, 2022. https://doi.org/10.1186/s40538-021-00281-0

(35) Shekhar, S., Sharma, S., Kumar, A., Taneja, A., & Sharma, B. (2021). The framework of nanopesticides: a paradigm in biodiversity. *Materials Advances, 2*(20), 6569-6588. https://doi.org/10.1039/D1MA00329A

3 DISCUSSÃO

A nanoencapsulação tem se destacado como uma tecnologia inovadora no desenvolvimento de formulações agrícolas, oferecendo vantagens significativas sobre os pesticidas convencionais. Nanoformulações podem ser caracterizadas pela incorporação de ingredientes ativos em nanocápsulas, que possuem propriedades distintas como menor tamanho de partícula e maior área de superfície, essas características proporcionam várias vantagens, incluindo maior solubilidade de compostos com baixa solubilidade em água, proteção contra condições ambientais adversas e maior biodisponibilidade devido à liberação controlada (MADHAVI *et al*., 2020).

Estudos, como o de Jiang *et al*. (2024) demonstraram que a nanoencapsulação pode melhorar a eficácia de pesticidas ao aumentar sua solubilidade e estabilidade, permitindo uma aplicação mais eficiente em campo como observado no presente trabalho. Além deste, um estudo demonstrou que a adição de compostos sinérgicos às formulações de piretróides por meio da nanotecnologia teve um impacto significativo na estabilidade dos inseticidas, também evidenciou uma melhora na preservação da atividade inseticida ao longo do tempo, com uma redução na taxa de perda de eficácia (BOFF *et al.*, 2022). A estabilidade da formulação aumentada pode levar a uma maior eficiência dos inseticidas, reduzindo a necessidade de reaplicações e, consequentemente, os impactos ambientais e custos associados ao controle de pragas.

Dentro do campo das nanoformulações, os inseticidas nanoencapsulados emergem como uma ferramenta promissora para o manejo de pragas. Um exemplo é o uso de nanocápsulas carregadas com piridalil para o controle da lagarta-do-algodão (*Helicoverpa armigera*) (SAINI *et al*., 2014). Além disso, nanocápsulas de azidobenzaldeído contendo metomil, têm se mostrado eficazes no combate à lagarta-do-cartucho (*Spodoptera frugiperda*) (SUN *et al*., 2014). Outra aplicação notável é o uso de nanocarreadores de quitosana para veicular o *Metarhizium rileyi*, um agente de controle biológico contra a lagarta-desfolhadora (*Spodoptera litura*), oferecendo uma alternativa sustentável e direcionada no combate a essa praga (CHANDRA *et al*., 2013).

Além destes, um estudo que focou na nanoencapsulação do acetamiprido, usando alginato de sódio e polietilenoglicol, demonstrou que a nanoencapsulação do acetamiprido melhorou significativamente sua eficiência inseticida em comparação com a formulação tradicional (EBADOLLAHI *et al*., 2022). Por fim, a nanoencapsulação se apresenta como uma abordagem promissora e eficiente no desenvolvimento de novas formulações agrícolas, destacando-se por sua capacidade de melhorar a solubilidade, estabilidade e eficácia dos pesticidas. A incorporação de ingredientes ativos em nanocápsulas não só aprimora as propriedades físicas e químicas dos compostos, como também promove uma aplicação mais direcionada e sustentável, reduzindo a necessidade de reaplicações e minimizando os impactos ambientais.

Os estudos apresentados ao longo dessa discussão reforçam o potencial das nanoformulações como uma ferramenta inovadora no manejo de pragas, com resultados significativos em termos de aumento da eficácia inseticida e durabilidade das formulações. Assim, a nanotecnologia desponta como uma aliada indispensável na busca por soluções agrícolas mais eficazes e sustentáveis, com potencial de transformar práticas tradicionais de controle de pragas, contribuindo para a segurança alimentar e a proteção do meio ambiente.

CONCLUSÕES

O desenvolvimento de uma nanoformulação de lufenurom é possível a partir de nanopartículas de policaprolactona na concentração de 1,0 mg/mL.

A nanoformulação a base de lufenurom apresentou propriedades físico-químicas favoráveis e estabilidade ao longo do tempo, indicando seu potencial para fornecer o ingrediente ativo para controle da praga-alvo de forma eficaz.

O ensaio de atividade biológica em dieta determinou que lufenurom encapsulado manteve sua eficácia mesmo em concentrações mais baixas.

Nas doses recomendadas de bula, a nanoformulação desempenhou eficácia consistente em estudos de semi-campo.

REFERÊNCIAS BIBLIOGRÁFICAS

BAMISAYE, Abayomi et al. Recent advances in nanoemulsion for sustainable development of farm-to-fork systems. **Journal of Cleaner Production**, p. 139226, 2023.

BERNARDI, Oderlei et al. Manejo da resistência de insetos a plantas Bt. **Edição. PROMIP– Manejo Integrado de Pragas, Engenheiro Coelho, SP, Brasil**, 2016.

BOFF, Jéssica S. et al. The effect of synergistic compounds on the susceptibility of *Euschistus heros* (Hemiptera: Pentatomidae) and *Chrysodeixis includens* (Lepidoptera: Noctuidae) to pyrethroids. **Environmental Entomology**, v. 51, n. 2, p. 421-429, 2022.

BUENO, Adeney de Freitas; SOSA-GÓMEZ, Daniel Ricardo. **Nota Técnica - Embrapa Soja**: ocorrência de rachiplusia nu e crocidosema aporema em soja-bt na safra 20/21 e principais orientações de manejo aos produtores para a safra 21/22. Londrina: Embrapa, 2022.

CHANDRA, J. Helan et al. Improved pesticidal activity of fungal metabolite from *Nomureae rileyi* with chitosan nanoparticles. In: **International Conference on Advanced Nanomaterials & Emerging Engineering Technologies**. IEEE, 2013. p. 387-390.

EBADOLLAHI, Asgar et al. Nanoencapsulation of acetamiprid by sodium alginate and polyethylene glycol enhanced its insecticidal efficiency. **Nanomaterials**, v. 12, n. 17, p. 2971, 2022.

GAZZONI, Décio Luiz; DALL'AGNOL, Amélio. Paralelo entre a soja no mundo e no Brasil. 2018.

JIANG, Xin et al. Chitosan nanoparticles loaded with *Eucommia ulmoides* seed essential oil: Preparation, characterization, antioxidant and antibacterial properties. **International Journal of Biological Macromolecules**, v. 257, p. 128820, 2024.

LI, Mingshan et al. Recent advances in nanoparticle-mediated co-delivery system: A promising strategy in medical and Agricultural Field. **International Journal of Molecular Sciences**, v. 24, n. 6, p. 5121, 2023.

LIMA, Jonathan Dias de. **Desenvolvimento de nanoformulações biopesticidas aplicadas na agricultura**. 2020.

MADHAVI, V. et al. Nanoencapsulation of pesticides: Sustainable perspective in agriculture. In: **AIP conference proceedings**. AIP Publishing, 2020.
MAPA, Ministério da Agricultura Pecuária e Desenvolvimento. **Sistema de Agrotóxicos Fitossanitários** (AGROFIT).. 2023. Disponível em: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Acesso em: 14 jan. 2023.

MOSCARDI, Flavio et al. Artrópodes que atacam as folhas da soja. **Soja: manejo integrado de insetos e outros artrópodes-praga**, v. 4, p. 859, 2012.

RUSSO, María Leticia et al. Entomopathogenic Fungi as Dual Control Agents against Two Phytopathogens and the Lepidopteran Pest Rachiplusia nu in Soybean (Glycine max (L.) Merr). **Journal of Fungi**, v. 10, n. 2, p. 93, 2024.

SAINI, Priya et al. Development of pyridalyl nanocapsule suspension for efficient management of tomato fruit and shoot borer (*Helicoverpa armigera*). **Journal of Environmental Science and Health, Part B**, v. 49, n. 5, p. 344-351, 2014.

SEDIYAMA, Tuneo; TEIXEIRA, R. C da; BARROS, H. B. Origem, evolução e importância econômica. **Tecnologias de produção e usos da soja**, v. 1, p. 1-5, 2009.

SOSA-GÓMEZ, Daniel Ricardo et al. Manual de identificação de insetos e outros invertebrados da cultura da soja. 2014.

SPECHT, Alexandre et al. Biotic potential and life tables of *Chrysodeixis includens* (Lepidoptera: Noctuidae), *Rachiplusia nu*, and *Trichoplusia ni* on soybean and forage turnip. **Journal of Insect Science**, v. 19, n. 4, p. 8, 2019.

SUN, Dequan et al. Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. **Plant cell reports**, v. 33, p. 1389-1402, 2014.