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RESEARCH ARTICLE

Enhancing the insecticidal efficacy of *Allium sativum* extracts through microencapsulation via complex coacervation

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Abstract

Garlic (*Allium sativum* L.) has been widely studied for its insecticidal properties. The primary bioactive molecule in garlic extracts include allicin, alliin, S-allylcysteine, diallyl disulfide, diallyl trisulfide, diallyl sulfide and ajoene. However, these compounds degrade under environmental conditions once extracted. This study aimed to enhance the effectiveness of garlic extracts in controlling *Tenebrio molitor* by optimizing microencapsulation techniques. The garlic extracts were encapsulated using the complex coacervation method, with independent variables including pH levels (3, 6 and 9), whey protein isolate (WPI) (4 %, 6 % and 8 % w/v) and pectin (0.50 %, 0.75 % and 1.00 % w/v). A Taguchi L9 (3^3) orthogonal array was employed to design 9 treatments and *T. molitor* mortality was assessed 72 h after a 10 sec immersion of the insects in the treatments. Statistical analysis revealed that WPI had the most significant influence (24.52 %), followed by pH (18.82 %) and pectin (7.79 %). The interaction between pH and pectin had the greatest effect on the encapsulation process, accounting for 38.65 % of the influence. The optimal microencapsulation conditions were predicted by software to be pH 3, a pectin concentration of 0.75 % w/v and a WPI concentration of 4.00 % w/v, resulting in a signal-to-noise (S/N) ratio of 42.30. Experimental validation of these conditions produced an S/N ratio of 18.54, corresponding to a *T. molitor* mortality rate of 92 ± 4.47 %. The resulting microcapsules had diameters ranging from 1-5 µm. Complex coacervation is a highly promising method for microencapsulating garlic extracts and preserving their insecticidal properties.

Keywords

Maceration extract; Taguchi methodology; *Tenebrio molitor*; bioactive compounds; biocontrol

Introduction

In recent decades, the pursuit of sustainable solutions in agriculture has become increasingly important as the global population continues to grow, placing unprecedented pressure on food production systems (1, 2). Central to

this effort is the development and adoption of environmentally friendly practices that minimize the ecological impact of agriculture while ensuring food security for current and future generations (3). In this context, the use of natural products for pest management has emerged as a promising approach, offering a viable alternative to conventional synthetic pesticides, which often pose risks to human health and the environment (4, 5).

Among the many natural substances investigated for their potential in pest control, garlic (*Allium sativum* L.) stands out as a multifunctional powerhouse. Garlic, a member of the genus *Allium* and the family Liliaceae, consists of 6 to 35 bulblets, known as cloves, which are encased in a white skin (6). When garlic cloves are crushed or the tissue is physically disrupted, the enzyme alliinase (EC 4.4.1.14) is released, converting alliin into allicin (7). Allicin is the primary bioactive compound in garlic, accounting for 70 -80 % of its sulfur compounds and responsible for the characteristic garlic odor (8). Additionally, a diverse array of sulfur-based active compounds has been identified in garlic, including allicin, alliin, S-allylcysteine, diallyl disulfide, diallyl trisulfide, diallyl sulfide and ajoene (9).

The bioactivities of garlic extracts encompass antimicrobial, anticancer, antioxidant and anti-diabetic effects, along with the ability to reduce cardiovascular diseases and improve immune functions (10). Renowned for its culinary and medicinal properties across diverse cultures for centuries, garlic has also demonstrated remarkable efficacy as an insecticide and repellent against a wide range of agricultural pests (11-13). The bioactive compounds responsible for garlic's insecticidal properties exert their effects through various mechanisms, including interference with insect respiratory systems, disruption of cellular processes and deterrence of feeding and oviposition (14, 15). Additionally, garlic extract and garlic essential oil is known to target several specific proteins in insect metabolism (11). Common targets for insecticides include neurochemical/neurohormonal proteins such as the acetylcholinesterase enzyme (AChE), γ-aminobutyric acid receptor (GABAa_aaR) and octopamine receptor (OctpR). Other targets involve insect growth regulators (IGRs) that affect molting timing, notably the Methoprene-tolerant receptor (MET) and the ecdysone hormone receptor (EcR). The juvenile hormone pathway is also indirectly targeted through enzymes and transporters like the juvenile hormone binding protein (JHBP), which is crucial for the adult reproductive cycle (16).

The appeal of garlic as a natural insecticide lies in its effectiveness and its relative safety for non-target organisms and the environment. Unlike many synthetic pesticides that can harm both pests and beneficial organisms, garlic-based formulations typically exhibit lower toxicity to non-target species. This reduces the risk of ecological disruption and minimizes collateral damage to beneficial insects, pollinators and soil microorganisms (13, 16). Additionally, garlic's high biodegradability ensures minimal persistence in the environment, alleviating concerns about chemical residues and long-term ecological impacts.

Despite the promising attributes of garlic as a biopesticide, its practical application in pest management faces challenges related to formulation and delivery. Garlic extracts, though potent in their raw form are susceptible to degradation from factors such as light, heat, oxygen and microbial activity, which compromise their efficacy and shelf life (17).

Encapsulation technology offers a versatile and effective solution to address formulation challenges. Encapsulation enhances the stability, solubility and controlled release of extracts by entrapping them within a protective matrix. This process extends the bioavailability and effectiveness of active compounds while minimizing environmental exposure and off-target effects (18). Various encapsulation methods have been employed to entrap garlic extracts, including molecular inclusion, spray drying, complex coacervation and lipid-based nanoencapsulation techniques such as nanoemulsions, nanoliposomes and nanophytosomes. The choice of the most suitable encapsulation technique depends on the processing conditions involved in the production of nano (micro) particles and their final applications (6). Among the techniques, complex coacervation has gained attention for its ability to encapsulate both hydrophilic and hydrophobic compounds in a single formulation, offering versatility and efficiency in formulation design (19).

Complex coacervation involves the phase separation of oppositely charged polyelectrolytes in aqueous solutions, leading to the formation of a polymer-rich coacervate phase that encapsulates the active ingredients. This process creates 2 distinct, incompatible phases: a polymer-rich phase (coacervate) and a diluted solvent phase, which are maintained in dynamic equilibrium through hydrogen bonding and electrostatic interactions between the oppositely charged colloidal species (6, 20, 21). The coacervate shell acts as a protective barrier, shielding the encapsulated compounds from external factors such as pH fluctuations, temperature variations and enzymatic degradation (19). Additionally, complex coacervation allows for the incorporation of multiple active ingredients and additives, enabling the development of multifunctional formulations with synergistic effects and tailored properties (20).

The Taguchi method, a design of experiments (DOE) approach, provides a systematic framework for optimizing processes by adjusting parameters to achieve desired product attributes (22-25). Through the Taguchi method, researchers can confidently identify the optimal conditions for maximizing encapsulation efficiency while minimizing process variability and resource consumption (26). This method involves systematically varying factors such as polymer concentration, pH, temperature and agitation speed (27). The benefits are clear: improved efficiency, reduced variability and better resource management (25). The objective of this study was to optimize the encapsulation process of garlic extracts via complex coacervation using the principles of the Taguchi design of experiments to enhance the insecticidal efficacy of garlic-based formulations. Our research provides sustainable

alternatives to conventional pesticides by leveraging the insecticidal properties of garlic in a formulation that balances efficacy, safety and environmental sustainability. We conducted a comprehensive investigation of encapsulation parameters and insecticidal activity evaluations to contribute to the development of innovative solutions for sustainable pest management in agriculture. Our efforts aim to bridge the gap between traditional knowledge of garlic's insecticidal properties and cutting-edge encapsulation methods, offering powerful tools for pest control while upholding principles of environmental and human health protection.

Materials and Methods

Plant material and insect culture

The *A. sativum* bulbs were sourced from the local market at Ciudad Valles, San Luis Potosí, México. The cloves were manually separated from the bulbs and stored at 4 °C until use. *Tenebrio molitor* larvae and wheat bran were purchased from the supplier tenebrios.com (Mexico City, Mexico). The larvae were housed in a plastic box measuring 53.34 cm x 36.06 cm x 13.97 cm, which contained a 2.5 cm layer of wheat bran as food. The insects were maintained in a controlled environment at 28 ± 2 °C. For the assays, larvae in the 10^{th} to 20^{th} instar were selected (28). To maintain the reproduction cycle, adults and pupae were separated from the larvae weekly and placed under the same controlled conditions.

Garlic extracts

Ground cloves of *A. sativum* were added to a flask containing 50 % ethanol at a solid-to-liquid ratio of 1:12, following the method described (19). The extraction was conducted using static maceration for 24 h at room temperature (~25 °C) in the dark. The extract was then filtered through Whatman No. 1 filter paper (GE Healthcare Life Sciences) and subsequently microfiltered through a 0.45 µm PTFE membrane (Whatman, Cytiva). The ethanol was removed from the resulting filtrate using a Büchi R-200 rotavapor (Büchi, Switzerland). The remaining aqueous solution was immediately used for encapsulation (Fig. 1).

Microencapsulation process

Whey protein isolate (WPI, Isolate 80 % protein, Isopure®, USA) and pectin (JR foods, low methoxy, esterification degree ≤ 50) were each suspended in 50 mL of distilled water at the required concentration according to the experimental design. The WPI solution was stirred for 15 min at 50 rpm on a magnetic stirrer at room temperature. The pectin solution was heated to 50 °C, stirred at 150 rpm for 10 min and then cooled to room temperature. To ensure complete solubilization, the polymer solutions were stored overnight at 4 °C. Next, Tween 80, constituting 10 % of the total solids and added to the solution and mixed until fully dissolved. Subsequently, 100 mL of the extract was added and the pH was adjusted to 3, 6 or 9 using 0.1 N or 1 N HCl and NaOH respectively. Finally, the solution was homogenized for 5 min using an ultrasonic bath (40 kHz, Branson 3800).

Taguchi methodology

The coacervation method was used to encapsulate the garlic extracts, following the optimization process (19, 29). The Taguchi methodology was employed, testing 3 main factors, each at 3 levels (Table 1).

An orthogonal array L9 (3^3) was generated, allowing for 9 experimental runs that combined the 3 factors at their respective levels. The experimental procedure was carried out according to the specifications detailed in Table 2. After obtaining the microcapsules, each treatment was applied to a total of 50 larvae. All treatments were performed in triplicate and *T. molitor* mortality was assessed after 72 h. The experimental data were analyzed using Statistica 7 software (Statsoft, OK, USA) through the "higher the better" function. The signal-to-noise ratio (S/N) was used as an indicator of treatment reproducibility and quality, calculated with the following equation:

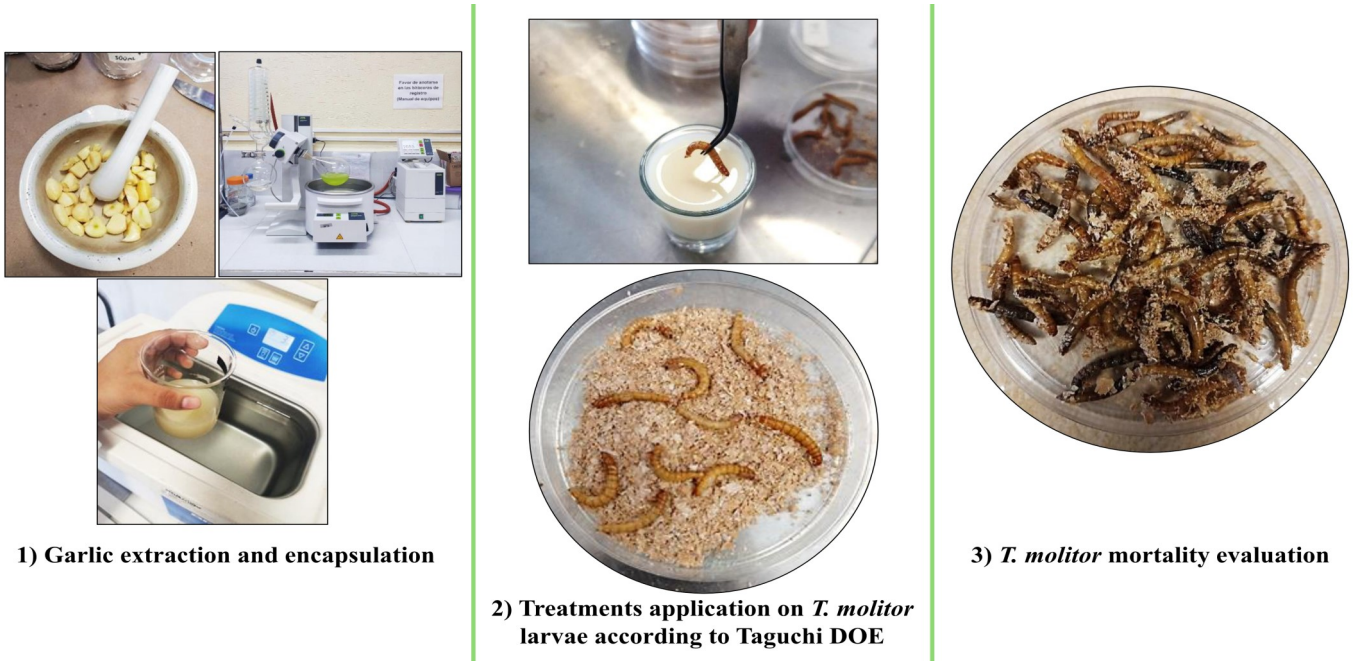

Fig. 1. General process for the obtention and application of garlic extracts coacervates.

Table 1. Factors and assigned level values considered for the microencapsulation process.

No.	Factor	Level 1	Level 2	Level 3
1	pH	3	6	9
2	Pectin (g)*	0.50	0.75	1.00
3	WPI (g)*	4.00	6.00	8.00

*g/ 100 mL of garlic extract.

$$S/N = -10\,Log10\,(\frac{1}{N} * \sum(\frac{1}{y^2}))$$

In this equation, the factor of -10 ensures the ratio reflects the inverse of "bad quality," where y represents the experimental value obtained in each trial and N is the number of samples. The analysis of variance (ANOVA) was used to determine the contribution percentage of each

$$P = \frac{SSi}{SST} * 100\,\% = \frac{SSi - MSi * dfi}{SST} * 100\,\%$$

factor, calculated as follows:

Where *P* is the contribution percentage, *SSi* is the individual sum of squares, *SST* is the total sum of squares, *MSi* is the individual mean square and *dfi* represents the individual degrees of freedom.

The optimal encapsulation conditions were validated by evaluating insect mortality 72 h after treatment. The treatment was applied to a total of 50 insects, as described in the following section and all treatments were conducted in triplicate.

Insecticidal activity assay

The mortality of *T. molitor* was evaluated as a response factor in this study, following the assay method with some modifications (30). Each larva was individually immersed for 10 seconds in 30 mL of microencapsulates solution placed in a 100 mL beaker. After drying for 5 min at room temperature, the larvae were transferred to Petri dishes containing 3 g of wheat bran as food. The Petri dishes were then stored at 28 °C. Larval mortality was monitored every 24 h for 72 h after treatment application and the results were recorded as the percentage of larvae mortality. The control treatments consisted of water and a mixture of polymers.

Morphological characterization of the microcapsules

The optimal treatment was analyzed using scanning electron microscopy (SEM). Before SEM analysis, the treatment underwent spray drying and pulverization using a spray dryer (Buchi, Mini spray dryer B-290, Switzerland). The spray drying process was conducted with a feed flow rate of 6 mL/min, an inlet temperature of 160 °C and a drying airflow rate of 40 m^3/h. The resulting powder was then collected and stored in hermetically sealed bags at room temperature until the SEM analysis was conducted.

Scanning Electron Microscopy (SEM) was used to examine the morphological characteristics of the microcapsules containing *A. sativum* extracts. The analysis was performed on a JEOL JSM-6610LV (JEOL Inc., USA) microscope, operated at an acceleration voltage of 10 kV. Prior to imaging, each sample was sputter-coated with gold using a JEOL JFC-1100 sputter coater for 3 min. All samples were processed and visualized at 20 °C. Digital images were captured using the Quartz PCI imaging software, version 8 (Quartz Imaging Corp., Vancouver, British Columbia, Canada).

Results

Taguchi methodology and insect mortality

The Taguchi methodology was instrumental in conducting experiments with 3 levels. The experiments were facilitated by the orthogonal array L9 (3^3) design (Table 2). A total of 9 treatments were tested, with 50 larvae subjected to each treatment. The conditions tested in treatment 1 resulted in high insect mortality (88 ± 8.37 %). Although treatments 2 and 8 were statistically similar, their S/N ratio values differed due to variance (33.32 and 29.15 respectively). The lower insect mortality (8.4 ± 7.89 %) and S/N value (3.93) were observed in treatment 7.

Factors contribution to the encapsulation process

The S/N values from Table 2 were used to analyze the effects of various factors on the encapsulation process, specifically in relation to the mortality of *T. molitor*. The result of the analysis of variance (ANOVA) as presented in Table 3. The ANOVA table highlights the relative contributions of each factor to the process. Individually, the most influential factor was WPI, followed by the pH.

Table 2. Experimental matrix for the orthogonal array L9 (3^3) and experimental results.

Run	pH	Pectin	WPI	S/N	Insect mortality (%)
1	1	1	1	38.80	88 ± 8.37^{a}
2	1	2	2	33.32	48 ± 8.37^{b}
3	1	3	3	27.78	26 ± 5.48^{bc}
4	2	1	2	23.06	20 ± 10.00^{bc}
5	2	2	3	27.68	30 ± 14.14^{bc}
6	2	3	1	25.84	34 ± 16.73^{bc}
7	3	1	3	3.93	8.4 ± 7.89^{c}
8	3	2	1	29.15	40 ± 18.71^{b}
9	3	3	2	29.78	36 ± 11.40^{bc}

WPI = whey protein isolate; S/N = signal-to-noise ratio; pH values = 3, 6 and 9; pectin values (g/100 mL) = 0.50, 0.75 and 1.00; WPI values (g/100 mL) = 4.00, 6.00 and 8.00. The same letters show no significant differences (Tukey test $p \leq 0.01$).

Table 3. Analysis of variance for the encapsulation process.

Factors	SS	df	MS	F	*p*	Contribution (%)
[1] pH	563.48	2	281.74	31.26	0.000000	18.82
[2] Pectin	233.21	2	116.61	12.94	0.000066	7.79
[3] WPI	734.28	2	367.14	40.74	0.000000	24.52
1 by 2	1157.44	4	289.36	32.11	0.000000	38.65
Error	306.41	34	9.01			10.23
Total	2994.82	44				100.00

Pectin had the lowest individual contribution. However, the ANOVA table also reveals that the interaction between pH and pectin had the most significant effect on the encapsulation process, contributing 38.65 % to the overall outcome. The error factor accounted for 10.23 % of the variation.

The influence of each factor at different levels is illustrated in Fig. 2. A high S/N ratio was achieved at the lower pH value (pH 3). As the pH increased, the response factor decreased. A similar trend was observed for WPI. In contrast, pectin exhibited a quadratic effect, with the highest S/N ratio at level 2 (0.75 g/100 mL). The S/N ratio decreased when either level 1 or level 3 was used.

Optimal encapsulation conditions

After analyzing the experimental data and the performance of the factors in the encapsulation process, the optimal encapsulation conditions were predicted. Table 4 presents the conditions predicted by the software, which suggested a S/N ratio of 40.27 with pH 3, 0.75 g/100 mL of pectin and 4.00 g/100 mL of WPI. This mathematical prediction was then tested experimentally, yielding a S/N ratio of 42.30. This result was achieved because the optimal encapsulation conditions led to a *T. molitor* mortality of 92 ± 4.47 %.

Morphology of microcapsules

SEM images of the microcapsules, shown in Fig. 3, reveal irregularly shaped spheres with smooth surface, each having a diameter of approximately 5 µm or smaller. The shrunk morphology of the pectin-WPI complexes is attributed to dehydration during the spray-drying process. It is important to note that the type of wall material used can influence the microcapsule's morphology. However, the primary objective of this study was not compromised by the size or shape of the microcapsules. The microcapsules effectively absorbed the garlic extract, ensuring that its components were retained within the coating materials.

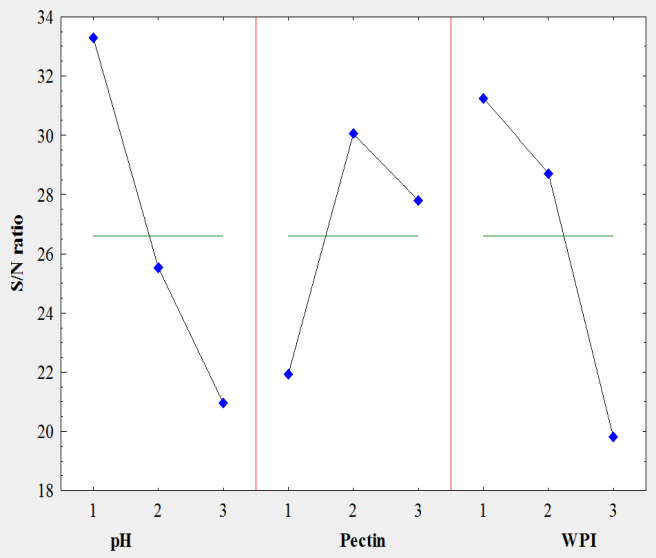

Fig. 2. Individual factors performance at different levels.

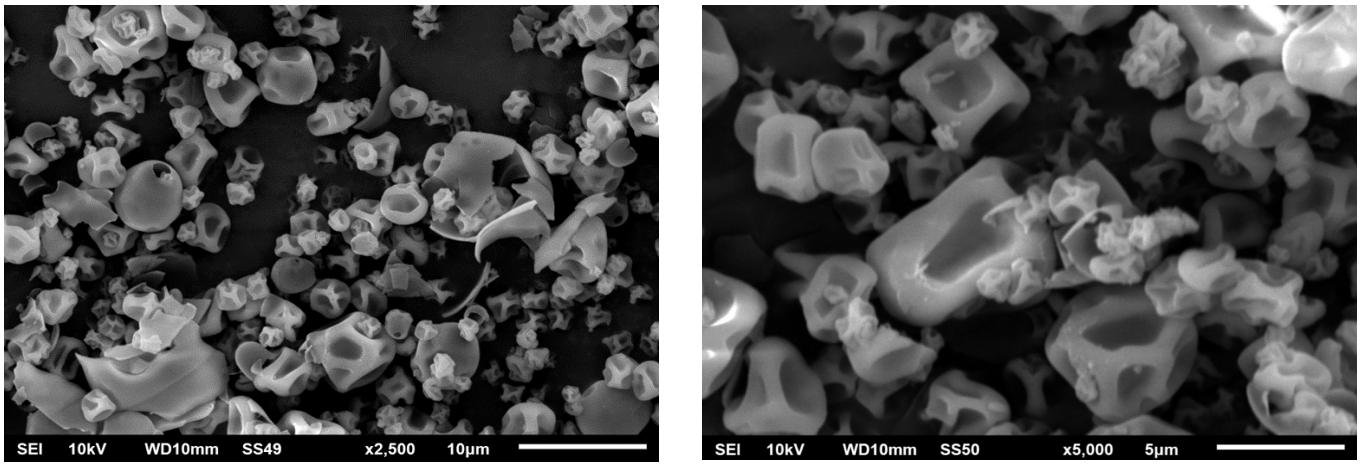

Fig. 3. Scanning electron microscope image of the microcapsules.

Table 4. Optimal conditions for the microencapsulation process of *A. sativum* extracts.

Factors	Level	Value	Effect Size	Standard Error
[1] pH	1	3.00	4.75	3.58
[2] Pectin	2	0.75 g/100 mL	2.39	3.58
[3] WPI	1	4.00 g/100 mL	4.37	3.58
Expected S/N		40.27		
Experimental validation S/N		42.30		
T. molitor mortality (%)		92 ± 4.47		

Discussion

Effect of microencapsulated garlic extracts on T. molitor mortality

Garlic (*Allium sativum* L.) is well-known for its insecticidal properties, attributed to a range of bioactive compounds such as allicin, diallyl disulfide, diallyl trisulfide and ajoene (15). These lipophilic compounds are effectively extracted using organic solvents (6). In this study, garlic extracts were obtained through maceration with 50 % aqueous ethanol over a 24 h period. Following extraction, the ethanol was removed via rotary evaporation, resulting in a fully aqueous extract for experimental use. The encapsulation of these bioactive compounds was performed using a modified complex coacervation method, based on the technique optimized (29) for encapsulating lipid-soluble d-limonene. This encapsulation process is critical as it ensures the entrapment and controlled release of the insecticidal compounds, thereby enhancing their efficacy against *T. molitor*.

The differential mortality rates of *T. molitor* observed in this study are directly linked to the varied encapsulation conditions applied across nine distinct treatments. While most treatments resulted in mortality rates below 50 %, treatment 1 proved particularly effective, achieving a mortality rate of 88 ± 8.37 %. In contrast, treatment 7 demonstrated the lowest efficacy, with a mortality rate of only 8.4 ± 7.89 %. These findings underscore the importance of optimizing encapsulation conditions to maximize the insecticidal potential of garlic extracts. The results are consistent with previous research on neem extracts encapsulated through complex coacervation, which also reported variable insect mortality rates depending on the encapsulation techniques used (19). The high mortality rate observed in treatment 1 can likely be attributed to optimal entrapment and release dynamics of the bioactive compounds within the microcapsules. This treatment likely provided a more sustained release of allicin, diallyl disulfide, diallyl trisulfide and ajoene, ensuring prolonged exposure of *T. molitor* to the insecticidal agents. Conversely, the lower efficacy observed in treatment 7 suggests suboptimal encapsulation conditions, potentially leading to a less effective release profile of a less effective release profile of the bioactive compounds. These variations highlight the critical role of encapsulation technology in enhancing the bioavailability and potency of plant-derived insecticides.

By applying the S/N ratio using the "higher-the-better" function, we were able to identify treatments with high reproducibility. Achieving a high S/N ratio requires both a high response variable and low variance. Therefore, treatments with high S/N values demonstrated both high insect mortality and low standard deviation. Notably, treatment 1 achieved the highest S/N value (38.80), while treatment 7 had the lowest value (3.93). It is important to emphasize that the data presented in Table 2 was crucial for conducting the statistical analysis and for mathematically predicting the optimal conditions.

Contribution of factors to the encapsulation process

The performance of particles produced through complex coacervation can be influenced by various factors, including total polymer concentration, protein-polysaccharide ratio, core-to-wall material ratio, pH and others. The Taguchi methodology allows for the evaluation of these factor's performance, with analysis of variance (ANOVA) used to assess the individual contributions of each factors, expressed as percentage.

In this study, the most influential factor in the microencapsulation process was Whey Protein Isolate (WPI), which accounted for 24.52 % of the effect, followed by pH, contributing 18.82 %. WPI primarily contains β-lactoglobulin, a protein capable of interacting with other compounds due to its β-barrel structure, which effectively dock hydrophobic compounds (31). This makes WPI a crucial component in forming the core of the microcapsules. As shown in Fig. 2, using a lower WPI concentration (4 g/100 mL) resulted in a high S/N ratio, correlating with increased insect mortality. Conversely, increasing WPI concentration led to a decrease in the S/N ratio.

pH is another critical factor in the complex coacervation process, as it influences the ionization state of functional groups on biomolecules such as proteins and polysaccharides (29). By adjusting the pH, one can control the charge density on these molecules, which affects the strength of electrostatic interactions between oppositely charged molecules-key to the formation and stability of coacervates. As depicted in Fig. 2, increasing pH values resulted in decreased *T. molitor* mortality. Higher pH levels alter the electrostatic charges of WPI and pectin, causing WPI to lose its isoelectric point and shift from positively charged hydrogen groups to negatively charged hydroxyl groups (19). This change reduces the entrapment of insecticidal compounds within the WPI and induces repulsive forces with pectin, hindering effective electrostatic interaction between the polymers (32). In contrast, lower pH levels significantly enhanced insect mortality. At pH 3, the amino groups of WPI are protonated, bringing WPI closer to its isoelectric point and promoting electrostatic attraction with negatively charged pectin (33). This optimal interaction at lower pH levels facilitates the effective entrapment of insecticidal compounds, thereby increasing *T. molitor* mortality.

Individually, pectin had the lowest influence on the encapsulation process, contributing just 7.79 %. It is generally recommended that factors with less than 10 % influence should be either pooled or set at a fixed level (34). However, the Taguchi DOE allows for the evaluation of factor interactions, which revealed that the interaction between pH and pectin had the most significant effect on the complex coacervation process, contributing 38.65 %. This finding underscores the importance of including pectin in the optimization process. Pectin plays a crucial role in complex coacervation by forming complexes with oppositely charged WPI, leading to the formation of a

coacervation phase. Within this phase, pectin helps create the encapsulation matrix. The interaction between pectin and WPI results in a network that effectively entraps the encapsulated extract (35). The pH of the solution is particularly important in this interaction because it alters the charges on the carboxyl groups of pectin (33). The pH influences the formation and stability of complexes between pectin and oppositely charged molecules like WPI. At pH levels where pectin is highly charged, it readily interacts with positively charged molecules through electrostatic forces, leading to stable complex formation. However, at extreme pH values, these interactions may weaken, adversely affecting the formation and stability of the coacervate phase (36).

The statistical analysis identified the optimal encapsulation conditions as pH 3, pectin at 0.75 g/100 mL, and WPI at 4 g/100 mL, with a predicted S/N ratio of 40.27. Experimental validation of these conditions resulted in an S/N ratio of 42.30, achieving the highest insect mortality rate of 92 ± 4.47 %. In comparison, our previous research using *Azadirachta indica* leaf extracts microencapsulated through complex coacervation yielded an insect mortality rate of 85 ± 10.49 %, with a significantly lower S/N ratio of 18.54 (19), indicating higher experimental variability.

The consistency of the results in this study underscores the effectiveness of the Taguchi methodology in optimizing encapsulation processes to produce highly reproducible outcomes. The S/N ratio, a key feature of the Taguchi method, facilitates the evaluation of response consistency across diverse experimental conditions. Depending on research objectives, the S/N ratio can be applied in 3 forms: larger-the-better, smaller-the-better and nominal-is-best. In this study, the larger-the-better condition was employed to maximize insect mortality (37). The S/N ratio, derived from experimental data, provides a quantitative measure of how parameter changes impact the formulation process (38). This approach highlights the superior performance and reliability of the optimized encapsulation conditions, offering a robust framework for enhancing the efficacy of insecticidal compounds through precise and consistent microencapsulation techniques.

Size and morphology of microcapsules

The size and morphology of the microencapsules were thoroughly analyzed using Scanning Electron Microscopy (SEM). Coacervates obtained under optimal encapsulation conditions were used and the microcapsules were subjected to spray-drying before SEM analysis. The results, illustrated in Fig. 3, revealed particles with diameters below 5 µm. Although another report achieving nanoencapsulation of d-limonene using a similar complex coacervation methodology (29), our study did not reach particle sizes below 1 µm.

The SEM images displayed microparticles with irregular shapes, surface roughness and varied sizes, consistent with findings (19). It was expected that the shape, morphology and size of the microencapsulates would be affected by the spray-drying treatment. The rapid dehydration caused by the spray-dryer led to irregular and shrunken morphologies in the microcapsules. This irregular morphology and rough surface texture are likely result due to structural collapse and surface deformation during the quick dehydration process. Despite these morphological changes, the biological efficacy of the encapsulated insecticidal compounds was not compromised. The achieved *T. molitor* mortality rate of 92 ± 4.47 % indicates that these changes did not adversely impact insect mortality. Future research could investigate alternative drying methods or process modifications to achieve more uniform particle sizes and shapes, potentially broadening the application of these encapsulated formulations.

The successful use of SEM in characterizing these microcapsules underscores its importance in assessing the quality and consistency of encapsulated products. SEM analysis confirmed the presence of microcapsules with sub-5 µm diameters and varied morphologies, reaffirming the potential of microencapsulation in developing effective, natural insecticidal agents. SEM remains a crucial tool for quality assessment and optimization of the encapsulation process.

Conclusion

The Taguchi methodology proved to be an invaluable tool for optimizing the microencapsulation conditions of *Allium sativum* L. extracts through complex coacervation, representing a significant advancement in this field. By using *Tenebrio molitor* as a model insect, we identified the optimal encapsulation parameters as pH 3, pectin at 0.75 g/100 mL and WPI at 4.00 g/100 mL. These conditions resulted in an impressive *T. molitor* mortality rate of 92 ± 4.47 %. Our analysis revealed that the most influential factors in the encapsulation process were pH, WPI and the interaction between pH and pectin. The particles obtained under optimal conditions exhibited irregular shapes, surface roughness and sizes ranging from 1 to 5 µm. Notably, the use of *A. sativum* extracts free from organic solvents aligns with environmentally sustainable practices and reduces potential ecological impacts. This research not only reinforces the insecticidal efficacy of garlic but also underscores the critical role of encapsulation methodologies in achieving effective pest control. By further refining these techniques, we can fully exploit the insecticidal potential of garlic extracts, offering a viable natural alternative to synthetic pesticides. Future research should focus on optimizing encapsulation parameters to ensure consistent and high mortality rates across a broader range of insect pests. This approach will significantly contribute to the development of eco-friendly pest management strategies that leverage the potent, natural insecticidal properties of plant extracts. Ultimately, this study provides a comprehensive foundation for the sustainable development of bioinsecticides and advanced pest management strategies, emphasizing the importance of innovative encapsulation techniques and environmentally friendly practices.

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Authors' contributions

MRM: Methodology, statistical analysis, wrote the draft manuscript; MAZ: SEM analysis, wrote the draft manuscript; DPR: wrote the manuscript; GCGMA: revised the manuscript; RGG: revised the manuscript; JCTA: revised the manuscript; RR: lab resources, revised the manuscript; PAZ: funding, lab resources, revised the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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