Original Article

Development and Optimization of a Nanoparticle-Based Imidacloprid Insecticide for Effective Control of *Blattella germanica*

Ismail Saba[1], Amir Amani[2], Mojgan Baniardalani[1], *Hamid Reza Basseri[1]

[1]Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran

***Corresponding author:** Dr Hamid Reza Basseri, E-mail: basserih@tums.ac.ir

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Abstract

Background: The German cockroach (*Blattella germanica*) is a pest with a global distribution that has adapted to live in human environments. *Blattella germanica* threatens human health by producing asthma-inducing allergens, carrying pathogenic/antibiotic-resistant microbes, and contributing to unhealthy indoor environments. Effective application of insecticides can play an important role in cockroach control programs. The purpose of this research was to develop and optimize a nanoparticle-based imidacloprid insecticide and evaluate its effectiveness against the German cockroach.

Methods: A bioassay was conducted to determine the LC_{50} and LC_{90} of imidacloprid technical against adult German cockroaches. The appropriate initial concentration of 3mg/m^2 was then utilized in the synthesis of nanoencapsulated imidacloprid via the ionic gelation method. The average particle size was determined using Dynamic Light Scattering (DLS) and the dried nanoparticles were analyzed using a Scanning Electron Microscope (SEM). The LC_{50} and LC_{90} values of Nano-imidacloprid were then compared with the technical grade of the insecticide.

Results: A comparison of the bioassay results for nanoencapsulated and imidacloprid technical revealed a superior insecticidal effect of the nanoencapsulated imidacloprid against the German cockroach. The LC_{50} value for the nanoencapsulated imidacloprid decreased from 4.656 to 3.081 mg/m^2 and the LC_{90} value decreased from 8.381 to 4.486 mg/m^2 when compared to imidacloprid technical.

Conclusion: The use of nanotechnology in insecticides can lead to increased efficacy and reduced consumption. This is because the smaller particle size of nanomaterials allows for better penetration and targeted delivery to pest organisms, reducing the overall amount needed for control.

Keywords: Nanoparticle; Imidacloprid; Insecticide; German cockroach

Introduction

German cockroaches are among the most prevalent pests globally, known for their small size, dietary habits, and specific behaviors. These insects are also known to act as vectors for a range of pathogens, including bacteria, fungi, protozoa, and viruses (1–13). Despite the use of various methods to control these pests in different parts of the world, taking into account the unique conditions and resources of each region, the frequent and widespread use of insecticides has resulted in increasing resistance among German cockroach populations (14–16). Studies have shown that these insects possess a high level of physiological resistance to various types of insecticides (17, 18).

The widespread resistance among cockroach populations presents significant challenges for pest control efforts. Concerns about the environmental effects of synthetic insecticides, including resistance and toxicity to humans and beneficial insects, have led to an increasing interest in low-risk alternatives. In recent years, insecticides based on micro and nanomaterials have emerged as a promising alternative to traditional chemical insecticides (19, 20).

Nanotechnology can help control insecticide resistance by developing novel insecticides with nanoparticles. Nanoparticles can be used as ingredients in insecticidal formulations or to deliver active components that are effective against targeted pests (21). At present, nano-scale insecticide formulations have been found to effectively protect against environmental factors such as temperature, rain, UV, and light (22). Studies on these formulations have shown that they are more stable than commercial formulations and retain their effectiveness for longer periods (23). The use of a nano-synthetic formulation for the insecticide eththiamethoxam has been found to improve delivery to pest tissues and enhance effectiveness (24). Various materials, including natural and synthetic polymers, lipids, surfactants, and dendrimers, have been utilized as carriers in drug delivery, insecticide optimization, and other industries such as health and cosmetics. Among these, polysaccharides have gained significant attention due to their exceptional physical and biological properties (25).

Chitosan nanoparticles have become a popular carrier for drugs due to their simple and mild preparation methods, as well as their ability to associate with macromolecules and facilitate their transport across mucosal barriers (26). Encapsulation is a common nanoformulation method for insecticides, in which the chemical, such as an insecticide, is physically or chemically coated and protected by specific substances. This protects the active ingredients from incompatible interactions, loss, or exposure to light (27). The primary goal of research on the transfer of insecticides using this technology is to increase toxicity by successfully delivering the active ingredients to the intended target. Microencapsulation of insecticides also increases the uptake of nano- or micro-compounds by the target organism compared to the pure compound (28).

Imidacloprid is a systemic insecticide that acts as a neurotoxin and belongs to a group of chemicals called neonicotinoids that target the central nervous system of insects. It interferes with the transmission of stimuli in the insect nervous system, specifically by blocking the nicotinergic nerve pathway (29). In the current study, we carried out the synthesis of a nanoformulation of imidacloprid using the modified ionic gelation technique, specifically for the formulation of imidacloprid-loaded Chitosan nanoparticles. The insecticidal activity of the imidacloprid-loaded chitosan nanoparticles was then studied in comparison to the technical formulation.

Materials and Methods

Materials

Imidacloprid, with a purity of 95%, was procured from Exirkeshavarz Yazd Company. Tripolyphosphate (TPP) and chitosan were sourced from Sigma Aldrich Co. Additionally, 5% acetic acid, acetone, and NaOH were acquired from Merk Co.

Breeding cockroaches

In this study, we utilized a sensitive strain of German cockroaches that had been maintained in the insectarium of the School of Public Health at Tehran University of Medical Sciences for nearly 20 years. The cockroaches were maintained in optimal breeding conditions, with a temperature of 25±2 degrees Celsius, the humidity of 60±10 percent, and a 12-12-hour light-dark cycle. They were fed a diet of dry bread, soy, and water, and were housed in rectangular plastic containers.

Bioassays

The experiments in this study were conducted following the guidelines determined using the WHO glass jar method as described by Ladonni (30). The initial dose of insecticide was calculated based on the manufacturer's guidelines, and the required solvent was provided for each jar with a surface area of 269.18 cm^2.

To determine the minimum and maximum insecticide concentrations associated with mortality rates ranging from 0% to 100% among German cockroaches, a series of serial concentrations of technical grade imidacloprid insecticide, ranging from 0.5 mg/m^2 to 12 mg/m^2, was prepared.

The solvent was infused into glass jars and rolled until it was fully vaporized, ensuring that the insecticide dose was deposited evenly over the inner surface of the jars. Bioassays were carried out as recommended by Ladonni (30). German cockroaches were anesthetized with CO_2, and 10 male cockroaches were introduced into each jar and exposed to the inner surface(31). The exposure time was 30 minutes, and the mortality rates were recorded 24 hours later. Each dose of insecticide was tested in 4 replications, and 10 cockroaches were used as a control group per concentration, with exposure to only the solvent. After several stages of preliminary testing, the minimum and maximum insecticide concentration values were determined, and from that the logarithmic concentration needed to perform the biometric test was calculated. The calculated concentrations for reaching from minimum to maximum mortality rates are 1.48, 2.019, 3.026, 4.08, 7.08, and 10 mg/m^2.

Preparing the nanoparticles

Nonencapsulated imidacloprid was synthesized using the ionic gelation method as previously described by Keawchaoon et al. (32). The ionic gelation method is based on the electrostatic interaction between the free amine groups of chitosan and the tripolyphosphate polyanion groups and causes the formation of a hydrogel of microparticles or nanoparticles that can be used to encapsulate various compounds. Based on the above-mentioned bioassay results, it was determined that 3 mg/m^2 represented the optimal quantity of imidacloprid to be utilized in the synthesis.

Chitosan was dissolved in a 0.05% aqueous solution of acetic acid and the pH was adjusted to 5. The solution was filtered and mixed with a 3 mg/m^2 solution of imidacloprid and tween 80, then stirred for 2 hours. Tripolyphosphate (TPP) was dissolved in deionized water and added to the chitosan solution in a 3:1 ratio, and the solution was stirred for 120 minutes to achieve crosslinking. The size and polydispersity index (PDI) was determined using dynamic light scattering DLS, and the amount of imidacloprid loaded was evaluated using an optical spectrophotometer based on the amount of imidacloprid loaded in nanoparticles, the required amount of nano encapsulated imidacloprid was calculated to reach the equivalent dose of insecticide in bioassay tests with technical imidacloprid and Bioassay tests were performed for the nano encapsulated imidacloprid on German cockroaches. SEM was used for morphological analysis. Unloaded nanoparticles were also prepared as a control.

Structure Characterization of nanoparticles

The mean particle size of nanoparticles was quantitatively determined using dynamic light scattering (DLS) techniques with a Vasco/Cordouan Technologies instrument, sourced from France. The samples were dispersed, and their intensity was measured at a temperature of 25 °C, with a refractive index of 1.335. The electrokinetic properties of the drug-loaded nanoparticles were evaluated using Zeta potential measurement with a Zeta sizer (sz-100/ Horiba Scientific) instrument, sourced from Japan. To investigate the morphological characteristics of the nanoparticles, scanning electron microscopy (SEM) was employed. The nanoparticles were deposited onto a thin sheet of metal and subsequently coated with a thin layer of gold to enhance their conductivity. The resulting images were obtained by detecting electrons emitted from the sample surface during SEM imaging.

Quantifying the quantity of encapsulated insecticide

To assess the nanoparticle formation efficiency, the unincorporated insecticide content was quantified, and its efficiency was determined by subtracting it from the initial insecticide amount. The insecticide content remaining after centrifugation was determined using an optical spectrometer. Centrifugation was conducted at room temperature at 15,000 rpm for 15 minutes, and the concentration of unbound insecticide in the supernatant was measured at 270 nm using an optical spectrometer. A standard calibration curve for imidacloprid loaded onto the nanoparticles is shown in Fig. 1. The loaded insecticide ranged from 0.005 to 0.3 mg per square meter, yielding a correlation coefficient of 0.98. Subsequently, the absorbance value of the supernatant solution, post-centrifugation, was fitted into the previously derived linear equation to determine the concentration of unbound insecticide. The loaded insecticide amount was then calculated using the following formula equation(33):

Loaded Insecticide (mg/m^2):

$$\frac{\text{Insecticide amonunt}\left(\frac{mg}{m^2}\right)}{\text{Unbonded insecticied amount}\left(\frac{mg}{m^2}\right)-\text{Initial insecticide amount}\left(\frac{mg}{m^2}\right)}$$

Release test of insecticide from nanoparticles

Release testing of imidacloprid from nanoparticles was conducted employing the dialysis method (34). This method is a venerable technique for eliminating low molecular weight compounds from a solution and substituting them with a buffer referred to as the dialysis bath or dialysate.

After preparing the phosphate buffer solution and the dialysis bag, a volume of 20 ml of the suspension, previously determined to contain the loaded insecticide nanoparticles, was subjected to centrifugation. The resultant nanoparticles were transferred into the dialysis bag, which was then immersed in the phosphate buffer solution. Subsequently, the vial containing the dialysis bag and buffer was positioned on a magnetic stirrer operating at a speed of 150 rpm. At specific time intervals (1, 2, 4, 6, 8, 10, 12, and 24 hours), one milli liter of the solution was withdrawn as a sample and substituted with fresh buffer. The collected samples were then analyzed using a spectrophotometer to quantify the released amount of insecticide.

Determining the nanoparticles' properties

The experimental results indicated that the production of chitosan nanoparticles is feasible within a specific range of chitosan and tripolyphosphate concentrations. The DLS analysis revealed that the mean particle size of the nanoparticles was in the range of 70–100 nm, as illustrated in (Fig. 2). The percentage of insecticide loaded in the nanoparticles was 54%. Furthermore, scanning electron microscopy (SEM) images of the dried particles revealed that they had a homogeneous structure, with an average size of 40–50 nm and an almost spherical shape, as depicted in (Fig. 3). Following a 24 hours evaluation of insecticide release from nanoparticles within a phosphate buffer solution, a release profile for imidacloprid was constructed, depicting the percentage of released insecticide as a function of time (Fig. 4). The maximum release observed after 24 hours amounted to 76%. The release profile exhibited a gradual, sustained release of the insecticide from the nanoparticles at each time interval.

Bioassay

The comparison of the results of probit analysis for insecticidal activity of imidacloprid technical and nanoencapsulated imidacloprid against *B. germanica* is represented in Table 1. As the results presented in Fig. 5. The insecticidal potency of imidacloprid exhibited enhancement, with the lowest test concentration (1.48 mg/m^2) resulting in a mortality rate of 3.36%, whereas nanoencapsulated imidacloprid yielded a slightly higher mortality rate of 3.59%. Conversely, at the highest test concentration (10 mg/m^2), the mortality rate increased from 95% with conventional imidacloprid to 100% with nanoencapsulated imidacloprid.

As evidenced by a decrease in the 50% lethal concentration (LC_{50}) from 4.656 mg/m^2 for the technical sample to 3.081 mg/m^2 for the nano-formulation of imidacloprid, and also a decrease in the 90% lethal concentration (LC_{90}) from 8.381 mg/m^2 for the technical sample to 4.486 mg/m^2 for the nano-formulation of the insecticide. In addition, statistical analyzing by T-test was performed to compare two series of data and there is a statistically significant difference ($P< 0.05$) between the means of the two variables.

However, these findings highlight the enhanced potency of the nanoencapsulated form of imidacloprid in controlling German cockroaches. The reduction in LC_{50} and LC_{90} values indicates improved efficacy.

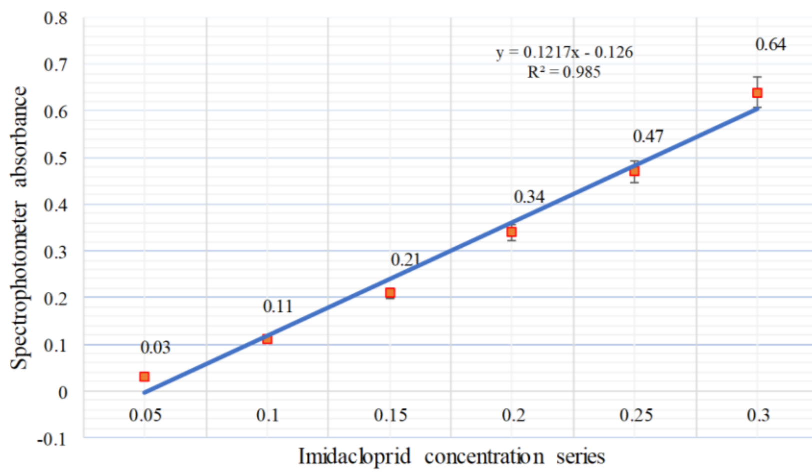

Fig. 1. A standard calibration curve was constructed for imidacloprid loaded onto the nanoparticles. The loaded insecticide exhibited a concentration range of 0.005 to 0.3 mg per square meter, resulting in a high correlation coefficient of 0.98

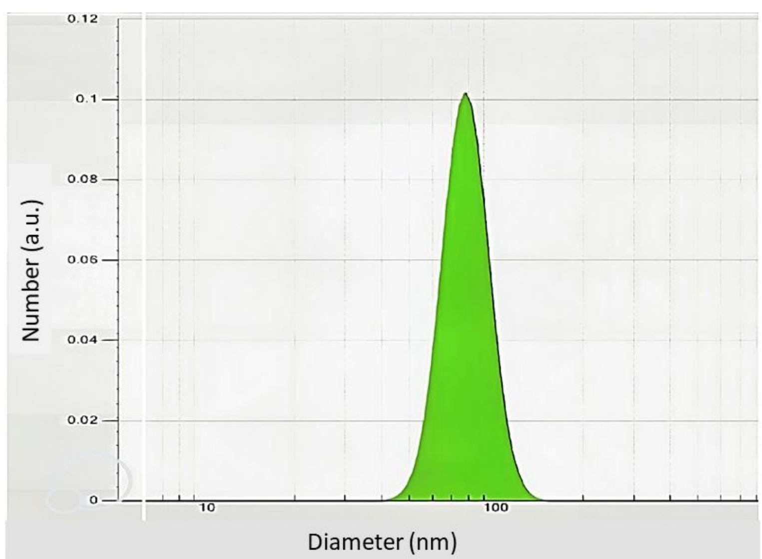

Fig. 2. Result of Dynamic Light Scattering (DLS) employed to characterize the nanoencapsulated imidacloprid. The mean particle size of the nanoparticles fell within the range of 70–100 nm

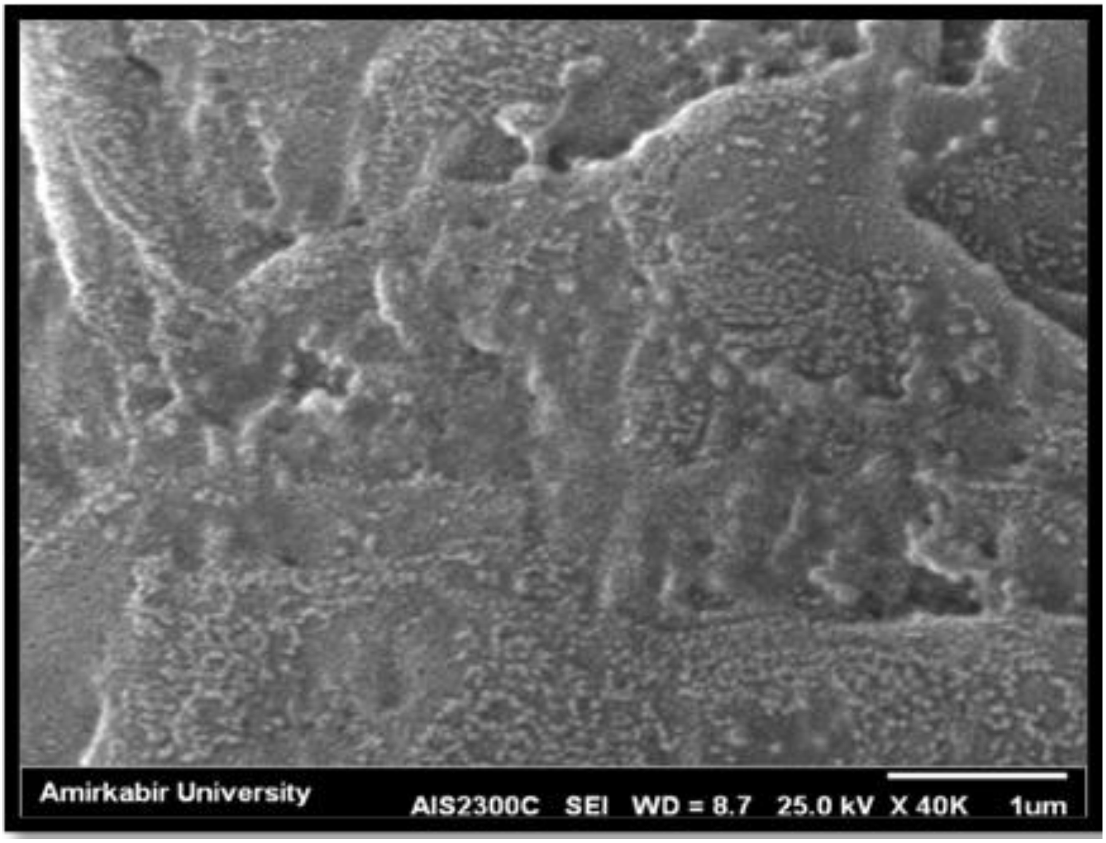

Fig. 3. Scanning electron microscopy (SEM) images of the dried particles of nanoencapsulated imidacloprid reveal a homogeneous structure, with an average size of 40–50 nm and an almost spherical shape

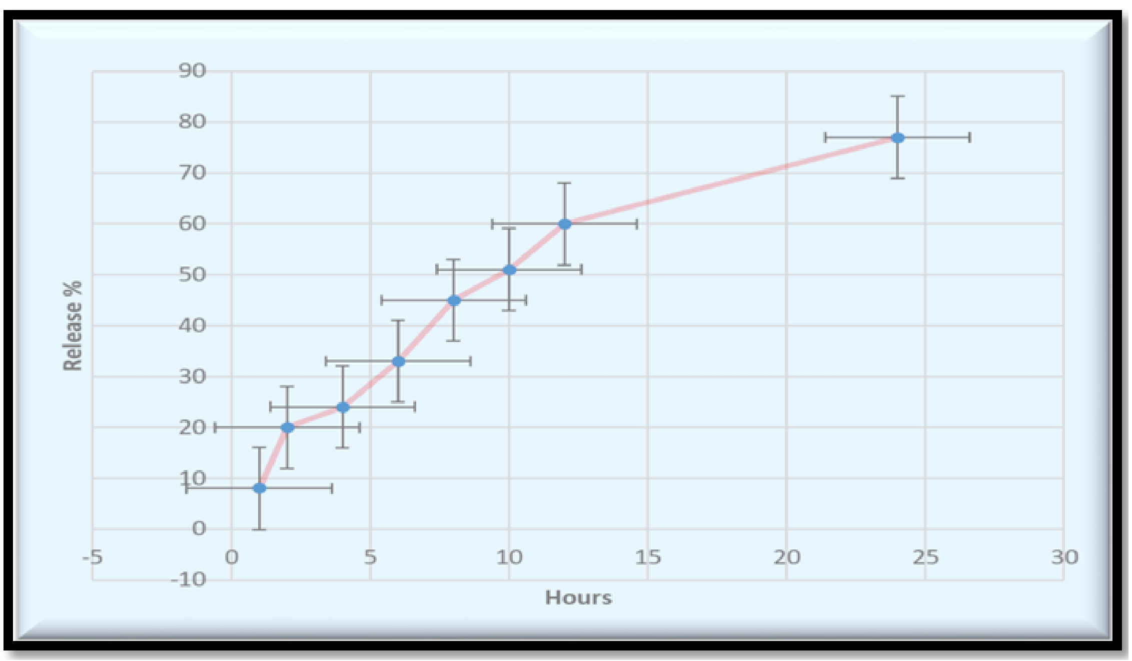

Fig. 4. The release pattern of imidacloprid from nanoparticles over time (Hour). Imidacloprid is gradual and sustained released from the nanoparticles at any given point in time. The error bars represent the standard error of the mean (SE)

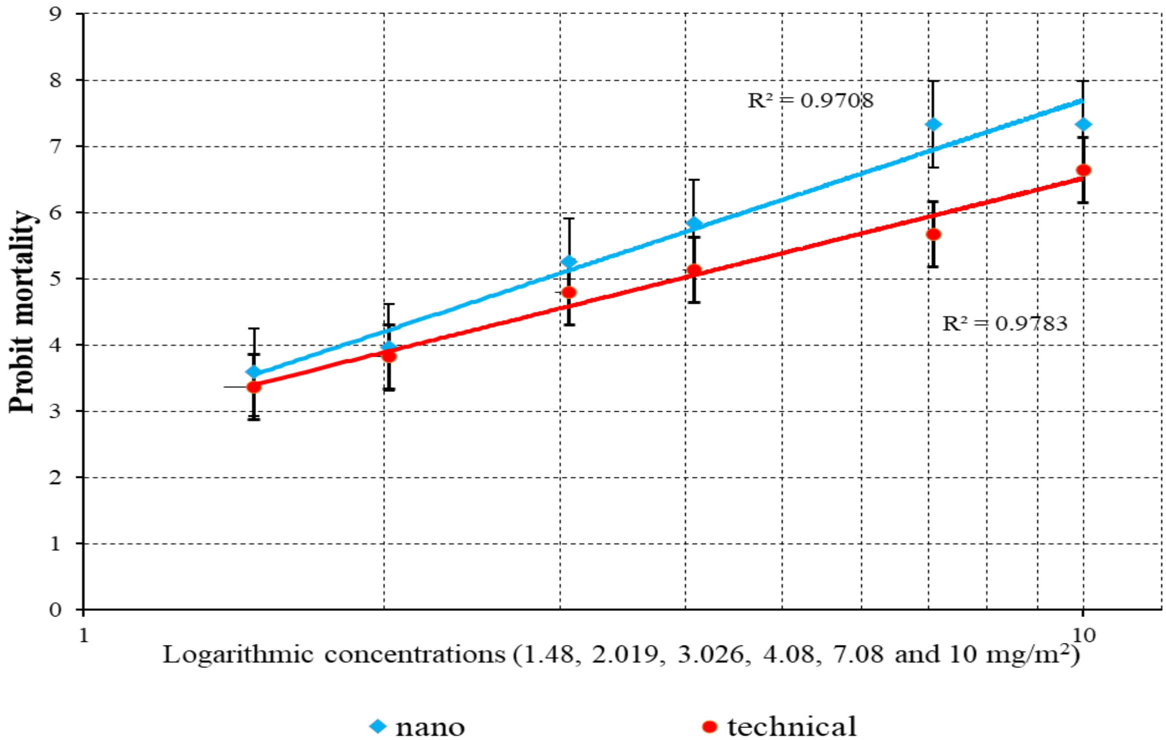

Fig. 5. Probit linear regression line equation of the insecticidal activity of imidacloprid technical vs nanoencapsulated imidacloprid against a susceptible laboratory strain (SPH-TUMS) of *Blattella germanica* in laboratory condition. The LC_{50} value for the nanoencapsulated imidacloprid decreased from 4.656 to 3.081 mg/m^2 and the LC_{90} value decreased from 8.381 to 4.486 mg/m^2. The error bars represent the standard error of the mean (SE)

Discussion

Nanotechnology can reduce the application of high amounts of insecticides as nanomaterials have a high surface area with enhanced reactivity, thus lowering the cost with increasing yields (19). Thus, the less frequent application is good for costs and human and environmental safety. Nanotechnology can offer advantages to insecticides, like improving the shelf-life and increasing the solubility of poorly water-soluble insecticides, all of which could have positive environmental impacts (35). In a study on Cu nanoparticles, results indicate that ingestion of copper nanoparticles could affect the growth of the German cockroach through an unclear mechanism that does not involve a reduction in the overall bacterial microbiota load (36). In another study the lethal, sublethal, and ecotoxic effects of peppermint and palmarosa essential oils (EOs) and their polymeric nanoparticles (PNs) were evaluated on the adults of rice weevil (*Sitophilus oryzae* L.), cigarette beetle (*Lasioderma serricorne* F.), and the larvae of *Culex pipiens pipiens* Say. On *S. oryzae* and *L. serricorne*, PNs increased EOs' lethal activity, extended repellent effects for 84 h, and also modified behavioral variables during 24 h. Moreover, EOs and PNs generated toxic effects against *Cx. pipiens pipiens*. These results show that nanoformulation can have a positive effect on the toxicity of insecticides (37). In a study essential oils (EO) from peppermint and palmarosa were nano-formulated and tested against the German cockroach, *B. germanica*. Peppermint and palmarosa nanoparticles enhanced the lethal and sublethal effects of the essential oils on *B. germanica*. These results suggest that the newly developed nano insecticides could be successfully used to control German cockroaches (38). Another study on the effects of orally administered gold nanoparticles (AuNPs) on the reproduction and growth of the insect *B. germanica* showed that ingestion of AuNPs caused lethal effects in *B. germanica* that compromised critical traits involved in population dynamics (39).

The main objective of encapsulation is to increase the efficacy of the insecticide by delivering the active ingredients to the intended target in a controlled manner. The study of encapsulation as a method of transferring insecticides is an area of ongoing research, aimed at developing more efficient and effective pest management strategies (40).

In recent years, researchers have explored diverse techniques and materials for the nanoformulation of imidacloprid. In one such study, nano-imidacloprid was synthesized through direct encapsulation with ABA triblock linear dendritic copolymers. Bioassay outcomes for this nanoformulation, evaluated on *Glyphodes pyloalis* (Lepidoptera, Crambidae) demonstrated a substantial reduction in the required insecticide dosage and environmental risks when employing the nanoform of imidacloprid. These results underscore the promising performance and efficacy of this formulation (41). In a separate investigation, amphiphilic copolymers were employed to create controlled-release formulations of imidacloprid. Analysis of the imidacloprid release kinetics in water from various formulations revealed that the controlled-release formulation exhibited a more rapid release profile in comparison to commercial formulations. These newly developed controlled-release formulations offer promising potential for effective pest management in a variety of crop applications (42).

We utilized a straightforward ion gelation method, which involved chitosan and Tripolyphosphate, to nanoencapsulate imidacloprid. Subsequently, we assessed its effectiveness against German cockroaches, representing the first instance of such an approach. Research has examined various applications of chitosan in the nanoencapsulation of insecticides, aiming to enhance efficiency and regulate the controlled release of these agents. The findings of these studies have demonstrated the effectiveness of this approach in a variety of cases. For example, in a study by Guan et al (43). imidacloprid microcrystals were directly encapsulated with natural polysaccharides chitosan and sodium alginate. The toxicity of the insecticide was evaluated against the adult stage of *Martianus dermestoides* (Coleoptera: Tenebrionidae)). The results showed that the polysaccharide capsules prolonged the release time of the encapsulated imidacloprid crystals. The toxicity of the novel 50% nanoformulation imidacloprid was higher in the adult stage compared to the 95% imidacloprid as indicated by the lower LC_{50} value. In another study, researchers explored the use of chitosan nanoparticles to formulate the metabolite of *Nomureae rileyi*, which possesses insecticidal properties. They compared the insecticidal efficacy of this nanoformulation with that of the main metabolite on *Spodoptera litura*. The results revealed a significant increase in effectiveness after nanoencapsulation with chitosan nanoparticles (44). Additionally, a separate study investigated poneem nano formulated with chitosan, and the synergistic effect of chitosan was demonstrated in enhancing the efficacy of the insecticide (45). Furthermore, research has shown that the combination of chitosan and insecticide can lead to a decrease in the activity of the *Phytophthora infestans* fungus. This synergistic effect highlights the potential of chitosan-based formulations for pest management strategies (46). In this study, using chitosan nanoparticles, control tests were performed on German cockroaches, and no significant mortality was observed in bioassay tests.

These findings, along with others, demonstrate the potential of using chitosan nanoparticles as a means of encapsulating insecticides to improve their effectiveness and stability.

The limitations and ineffectiveness of conventional pest management methods have necessitated the exploration of modern approaches to pest control, which can be a time-consuming process. The application of nanotechnology in pest management has the potential to offer efficient and effective solutions to this problem (23). At the nanometer scale, the properties of materials, both physicochemical and biological, exhibit variations from those of single atoms, molecules, and bulkier materials, as demonstrated in various studies (47). The use of micronutrients at the nanoscale could thus provide promising opportunities for the development of innovative pest management strategies.

The integration of nanotechnology into insecticides holds promise for enhancing efficacy while minimizing consumption. The reduced particle size of nanomaterials facilitates improved penetration and targeted delivery to pest organisms, ultimately decreasing the overall required dosage for effective control. However, it remains crucial to acknowledge that the long-term environmental and human health implications of nanotechnology are still under investigation. Further research is necessary to comprehensively assess its potential impact.

Conclusion

Utilizing nanotechnology insecticides would provide efficient alternatives for pests and diseases. The current research highlights the enhanced potency of the nanoencapsulated form of imidacloprid in controlling German cockroaches. The reduction in LC_{50} and LC_{90} values indicates improved efficacy.

Acknowledegments

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Ethical consideration

This research was approved by the ethics committee of the Tehran University of Medical Sciences (ir.tums.sph.rec.1399.095).

Conflict of interest statement

The authors declare there is no conflict of interest.

References

1. Fardisi M, Gondhalekar AD, Ashbrook AR, Scharf ME (2019) Rapid evolutionary responses to insecticide resistance management interventions by the German cockroach (*Blattella germanica L.*). Sci Rep. 9(1): 1–10.
2. Crawford JA, Rosenbaum PF, Anagnost SE, Hunt A, Abraham JL (2015) Indicators of airborne fungal concentrations in urban homes: understanding the conditions that affect indoor fungal exposures. Sci Total Environ. 517: 113–124.
3. Elgderi R, Ghenghesh K, Berbash N (2006) Carriage by the German cockroach (*Blattella germanica*) of multiple-antibiotic-resistant bacteria that are potentially pathogenic to humans, in hospitals and households in Tripoli, Libya. Ann Trop Med Parasitol. 100(1): 55–62.
4. Fakoorziba M, Eghbal F, Hassanzadeh J, Moemenbellah-Fard M (2010) Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential vectors of the pathogenic bacteria found in nosocomial infections. Ann Trop Med Parasitol. 104(6): 521–528.
5. Fathpour H, Emtiazi G, Ghasemi E (2003) Cockroaches as reservoirs and vectors

of drug resistant *Salmonella spp*. Iran Biomed J. 7(1): 35–38.

6. Menasria T, Moussa F, El-Hamza S, Tine S, Megri R, Chenchouni H (2014) Bacterial load of German cockroach (*Blattella germanica*) found in hospital environment. Pathog Glob Health. 108(3): 141–147.
7. Menasria T, Samir TI, Mahcene D, Benammar L, Megri R, Boukoucha M, Debabza M (2015) External bacterial flora and antimicrobial susceptibility patterns of *Staphylococcus spp*. and *Pseudomonas spp*. isolated from two household cockroaches, *Blattella germanica* and *Blatta orientalis*. Biomed Environ Sci. 28(4): 316–320.
8. Pai H-H, Ko Y, Chen E (2003) Cockroaches (*Periplaneta americana* and *Blattella germanica*) as potential mechanical disseminators of *Entamoeba histolytica*. Acta Trop. 87(3): 355–359.
9. Pomés A, Mueller GA, Randall TA, Chapman MD, Arruda LK (2017) New insights into cockroach allergens. Curr Allergy Asthma Rep. 17: 1–16.
10. Smith DD, Frenkel J (1978) Cockroaches as vectors of *Sarcocystis muris* and of other coccidia in the laboratory. J Parasitol. 64(2): 315–319.
11. Zurek L, Schal C (2004) Evaluation of the German cockroach (*Blattella germanica*) as a vector for verotoxigenic *Escherichia coli* F18 in confined swine production. Vet Microbiol. 101(4): 263–267.
12. Zarchi AAK, Vatani H (2009) A survey on species and prevalence rate of bacterial agents isolated from cockroaches in three hospitals. Vector Borne Zoonotic Dis. 9 (2): 197–200.
13. Akbari S, Oshaghi MA, Hashemi-Aghdam SS, Hajikhani S, Oshaghi G, Shirazi MH (2015) Aerobic bacterial community of American cockroach *Periplaneta americana*, a step toward finding suitable paratransgenesis candidates. J Arthropod Borne Dis. 9(1): 35–48.
14. Wang C, Lee CY, Rust MK (2021) Biology and management of the German cockroach: CABI publisher. New York, p. 304.
15. Chai RY, Lee CY (2010) Insecticide resistance profiles and synergism in field populations of the German cockroach (Dictyoptera: Blattellidae) from Singapore. J Econ Entomol. 103(2): 460–471.
16. Hu I-H, Chen SM, Lee CY, Neoh KB (2020) Insecticide resistance, and its effects on bait performance in field-collected German cockroaches (Blattodea: Ectobiidae) from Taiwan. J Econ Entomol. 113(3): 1389–1398.
17. Scharf ME, Neal JJ, Marcus CB, Bennett GW (1998) Cytochrome P450 purification and immunological detection in an insecticide resistant strain of German cockroach (*Blattella germanica*, L.). Insect Biochem Mol Biol. 28(1): 1–9.
18. Ghaderi A, Baniardalani M, Basseri HR (2021) Level of pyrethroid-resistance associated with cytochrome P450 expression in German cockroach, *Blattella germanica* (Blattodea: Ectobiidae) in the field collected strains. J Arthropod Borne Dis. 15(2): 152–161.
19. Madhuban G, Rajesh K, Arunava G (2012) Nano-pesticides-A recent approach for pest control. J Plant Prot Sci. 4(2): 1–7.
20. An C, Sun C, Li N, Huang B, Jiang J, Shen Y, Wang C, Zhao X, Cui B, Wang C, Li X (2022) Nanomaterials and nanotechnology for the delivery of agrochemicals: strategies towards sustainable agriculture. J Nanobiotechnology. 20 (1): 1–19.
21. Bhattacharyya A, Bhaumik A, Rani PU, Mandal S, Epidi TT (2010) Nanoparticles-A recent approach to insect pest control. Afr J Biotechnol. 9(24): 3489–3493.

22. Kumar S, Nehra M, Dilbaghi N, Marrazza G, Hassan AA, Kim KH (2019) Nano-based smart pesticide formulations: Emerging opportunities for agriculture. J Control Release. 294: 131–153.
23. Dangi K, Verma AK (2021) Efficient and eco-friendly smart nano-pesticides: Emerging prospects for agriculture. Mater Today: Proceedings. 45: 3819–3824.
24. Liu X, He B, Xu Z, Yin M, Yang W, Zhang H, Cao J, Shen J (2015) A functionalized fluorescent dendrimer as a pesticide nanocarrier: application in pest control. Nanoscale. 7(2): 445–449.
25. Plucinski A, Lyu Z, Schmidt BV (2021) Polysaccharide nanoparticles: From fabrication to applications. J Mater Chem C Mater. 9(35): 7030–7062.
26. Nagpal K, Singh SK, Mishra DN (2010) Chitosan nanoparticles: a promising system in novel drug delivery. Chem Pharm Bull. 58(11): 1423–1430.
27. Nuruzzaman M, Rahman MM, Liu Y, Naidu R (2016) Nanoencapsulation, nano-guard for pesticides: a new window for safe application. J Agric Food Chem. 64 (7): 1447–1483.
28. Fojtová D, Vašíčková J, Grillo R, Bílková Z, Šimek Z, Neuwirthová N, Kah M, Hofman J (2019) Nanoformulations can significantly affect pesticide degradation and uptake by earthworms and plants. Environ Chem. 16(6): 470–481.
29. Elbert A, Becker B, Hartwig J, Erdelen C (1999) Imidacloprid-a new systemic insecticide. Pflanzenschutz Nachr Bayer 44: 113–136.
30. Ladonni H (2001) Evaluation of three methods for detecting permethrin resistance in adult and nymphal *Blattella germanica* (Dictyoptera: Blattellidae). J Econ Entomol. 94(3): 694–697.
31. Choo LEW, Tang CS, Pang FY, Ho SH (2000) Comparison of two bioassay methods for determining deltamethrin resistance in German cockroaches (Blattodea: Blattellidae). J Econ Entomol. 93 (3): 905–910.
32. Keawchaoon L, Yoksan R (2011) Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids and surfaces B: Biointerfaces. 84(1): 163–171.
33. Tao Z, Deng J, Wang Y, Chen H, Ding Y, Hua X, Wang M (2019) Competitive immunoassay for simultaneous detection of imidacloprid and thiacloprid by upconversion nanoparticles and magnetic nanoparticles. Environ Sci Pollut Res Int. 26: 23471–23479.
34. Farajzadeh R, Pilehvar-Soltanahmadi Y, Dadashpour M, Javidfar S, Lotfi-Attari J, Sadeghzadeh H, Shafiei-Irannejad V, Zarghami N (2018) Nano-encapsulated metformin-curcumin in PLGA/PEG inhibits synergistically growth and hTERT gene expression in human breast cancer cells. Artif Cells Nanomed Biotechnol. 46(5): 917–925.
35. Huang B, Chen F, Shen Y, Qian K, Wang Y, Sun C, Zhao X, Cui B, Gao F, Zeng Z, Cui H (2018) Advances in targeted pesticides with environmentally responsive controlled release by nanotechnology. Nanomater. 8(2): 102.
36. Zha C, Turner M, Ray R, Liang D, Pietri JE (2023) Effects of copper and zinc oxide nanoparticles on German cockroach development, indoxacarb resistance, and bacterial load. Pest Manag Sci. 79(8): 2944–2950.
37. Yeguerman CA, Urrutia RI, Jesser EN, Massiris M, Delrieux CA, Murray AP, González JO (2022) Essential oils loaded on polymeric nanoparticles: bioefficacy against economic and medical insect pests and risk evaluation on terrestrial and aquatic non-target organisms. Environ Sci Pollut Res Int. 29(47): 71412–71426.
38. Yeguerman C, Jesser E, Massiris M, Delrieux C, Murray AP, González JW (2020)

Insecticidal application of essential oils loaded polymeric nanoparticles to control German cockroach: Design, characterization and lethal/sublethal effects. Ecotoxicol Environ Saf. 189: 110047.
39. Small T, Ochoa-Zapater MA, Gallello G, Ribera A, Romero FM, Torreblanca A Garcerá MD (2016) Gold-nanoparticles ingestion disrupts reproduction and development in the German cockroach. Sci Total Environ. 565: 882–888.
40. Madhavi V, Reddy A, Madhavi G, Reddy NB (2020) Nanoencapsulation of pesticides: Sustainable perspective in agriculture. AIP Conf Proc. 2280: 040029.
41. Memarizadeh N, Ghadamyari M, Adeli M, Talebi K (2014) Preparation, characterization and efficiency of nanoencapsulated imidacloprid under laboratory conditions. Ecotoxicol Environ Saf. 107: 77–83.
42. Adak T, Kumar J, Shakil N, Walia S (2012) Development of controlled release formulations of imidacloprid employing novel nano-ranged amphiphilic polymers. J Environ Sci Health, Part B. 47(3): 217–225.
43. Guan H, Chi D, Yu J, Li X (2008) A novel photodegradable insecticide: Preparation, characterization and properties evaluation of nano-Imidacloprid. Pestic Biochem Physiol. 92(2): 83–91.
44. Chandra JH, Raj LA, Namasivayam SKR, Bharani RA (2013) Improved pesticidal activity of fungal metabolite from *Nomureae rileyi* with chitosan nanoparticles. International Conference on Advanced Nanomaterials and Emerging Engineering Technologies; IEEE.
45. Paulraj MG, Ignacimuthu S, Gandhi MR, Shajahan A, Ganesan P, Packiam SM, Al-Dhabi NA (2017) Comparative studies of tripolyphosphate and glutaraldehyde cross-linked chitosan-botanical pesticide nanoparticles and their agricultural applications. Int J Biol Macromol. 104: 1813–1819.
46. Huang X, You Z, Luo Y, Yang C, Ren J, Liu Y, Wei G, Dong P, Ren M (2021) Antifungal activity of chitosan against Phytophthora infestans, the pathogen of potato late blight. Int J Biol Macromol. 166: 1365–1376.
47. Hodes G (2007) When small is different: some recent advances in concepts and applications of nanoscale phenomena. Adv Mater. 19(5): 639–655.