



Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

EFFECT OF CHITOSAN NANO-ENCAPSULATED *VITEX AGNUS-CASTUS* PHENOLIC EXTRACT ON BIOLOGICAL ASPECTS OF *EPHESTIA CAUTELLA*

Amna Hamed Shaker, Soolaf Abud Kathiar

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

ARTICLE INFO

Article history

Received: 26th March, 2025

Revised: 9th June, 2025

Accepted: 17th June, 2025

Keywords

Chitosan nanoparticles

Vitex agnus-castus

Ephestia cautella

Phenolic extract

Insecticidal activity

ABSTRACT

Stored product pests such as *Ephestia cautella* threaten global food security and storage systems. Recently, plant-based nanotechnology has emerged as an eco-friendly, more effective alternative to conventional pesticides with lower environmental impact. The present study, therefore, investigated the physicochemical characterization and insecticidal efficacy of *Vitex agnus-castus* leaf extract nano-capsules against different life stages of *Ephestia cautella*. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed key functional groups, including hydroxyl groups ($\sim 3414\text{ cm}^{-1}$), aliphatic C-H stretches ($\sim 2923\text{ cm}^{-1}$), and aromatic or carbonyl-related bands ($1619\text{--}1401\text{ cm}^{-1}$), along with C-O stretching ($1300\text{--}1000\text{ cm}^{-1}$), and metal-oxygen bonds in the fingerprint region ($800\text{--}400\text{ cm}^{-1}$), indicating the formation of an organic-inorganic nanocomposite. Scanning Electron Microscopy (SEM) showed that the nano-capsules were semi-spherical with minimal aggregation, averaging 43.77 nm in diameter. Dynamic Light Scattering (DLS) analysis further confirmed a consistent average particle size of 227.1 nm . The bioassay results demonstrated significant toxicity of the nano-capsules against *E. cautella* eggs, larvae, and pupae. At 2000 ppm , egg mortality peaked at 60.96% , while lower concentrations (1000 , 500 , and 250 ppm) resulted in 50.52% , 37.04% , and 25.93% mortality, respectively. Larval mortality increased with both dose and exposure time, reaching 66% at 2000 ppm after 72 hours . Similarly, pupal mortality reached 57.69% at 2000 ppm , with lower but notable mortality at 1000 ppm (50%). Minimal effect was observed at 500 and 250 ppm . These findings suggest that *V. agnus-castus* nano-capsules possess promising insecticidal properties and could serve as an eco-friendly biopesticide for controlling *E. cautella* infestations.

Corresponding Author: Soolaf Abud Kathiar

Email: soolafak_bio@cs.w.uobaghdad.edu.iq

© 2025 EScience Press. All rights reserved.

INTRODUCTION

The date palm, belonging to the family Arecaceae and the genus *Phoenix*, includes about fourteen species, among which *Phoenix dactylifera* is the most economically important. Iraq has historically been a significant center for date palm cultivation, with over 30 million trees

planted by 1980 . At that time, Iraq was one of the world's leading producers of dates, with an annual production of approximately $980,000\text{ tons}$ (Jaradat, 2003).

Date palms are vulnerable to several insect pests that cause significant damage in both field and storage conditions. Among these, *Ephestia cautella* (the almond

moth) is a major pest, infesting not only dates, whether on the tree, fallen, or stored, but also a wide range of stored products such as dried figs, raisins, apricots, grains, and legumes (Ahmed et al., 1985). Other notable pests include *Oryctes elegans* (the rhinoceros beetle) and *Carpophilus hemipterus* (the dried fruit beetle), which further reduce fruit quality and yield.

Female *E. cautella* lay their eggs singly or in clusters on the outer surface of the dates, with an average of 138 eggs per female. After hatching, the larvae emerge and burrow between the date flesh and the outer skin, creating grooves. They feed on the date pulp for about a month, progressing through five larval stages. Upon reaching the final stage, they either pupate inside the date or exit to pupate elsewhere (Ress, 2007).

Recent research trends have focused on identifying effective natural plant compounds to manage insect pests, as many plants contain secondary metabolites with insecticidal or repellent properties. *Vitex agnus-castus*, native to the Mediterranean region, is one of the few temperate-zone species within the largely tropical and subtropical *Vitex* genus (David, 2008). A study by Mehlhorn and Schmahl (2005) demonstrated that seed extracts of *V. agnus-castus* were highly effective as repellents against ticks, fleas, mosquitoes, and biting flies. Nanotechnology has emerged as a promising field in pest management (Khan et al., 2021; Jabbar et al., 2022). Many conventional pesticides are organic compounds with poor water solubility, necessitating the development of nanoparticle-based formulations to enhance their solubility without altering their chemical structure (Fernandes et al., 2014; Shahbaz et al., 2022). Formulating pesticides as nanoparticles can significantly improve their water solubility and efficacy (Margulis-Goshen and Magdassi, 2013; Shahbaz et al., 2023). Moreover, biodegradable nanoparticles are considered more effective, cost-efficient, and environmentally friendly due to their non-toxic nature and degradability (Abdel-Gawad, 2018; Nazir et al., 2019).

Nanotechnology also helps preserve the stability and concentration of active plant compounds under various environmental conditions. These nano-formulations are characterized by their short action duration on target organisms, primarily due to their small size (100-1000 nm), and their environmental safety. Thus, nanoparticles offer a sustainable alternative to conventional chemical pesticides.

Although previous studies have primarily focused on the

fruits of *V. agnus-castus*, the present study investigates the bioactive components in the leaves of the plant. Specifically, it evaluates the efficacy of chitosan nano-encapsulated *V. agnus-castus* leaf phenolic extract against the egg, larval, and pupal stages of *E. cautella* under laboratory conditions.

MATERIALS AND METHODS

Rearing of *E. cautella*

A sample of *E. cautella* was obtained from the laboratories of the Scientific Research Commission in Iraq. The insects were reared on an artificial diet composed of 81% wheat grits, 12% glycerin, 6% date molasses, and 1% dry yeast, following the method described by Hamid (2002).

Extraction of *V. agnus-castus*

Leaves of *V. agnus-castus* were collected from the gardens of the University of Baghdad. The leaves were cleaned, washed to remove dust, and air-dried by turning them regularly to prevent fungal growth. Once completely dried, the leaves were ground using an electric grinder and stored in tightly sealed glass containers at room temperature until further use.

To prepare the alcoholic extract, 100 g of powdered leaves was placed in a Soxhlet apparatus with 300 ml of 99% absolute ethanol for 24 h. The extract was then cooled and filtered using filter paper, concentrated with a rotary evaporator, and transferred into Petri dishes. The final extract was collected and stored in opaque bottles until use.

Ion gelation-mediated loading of phenolic compounds onto chitosan nanoparticles

Nano-capsules were prepared from the alcoholic extract of *V. agnus-castus* leaves using the ion gelation method, a commonly used technique for encapsulating active compounds onto chitosan particles. This method utilizes non-toxic, suitable organic solvents and is based on ionic interactions between the positively charged amine groups of chitosan and a polyanion such as sodium tripolyphosphate (STPP), forming a stable network (Hosseini et al., 2013).

To prepare the chitosan nanoparticles, 40 g of chitosan was dissolved in 1000 ml of 2% acetic acid (prepared by diluting 20 ml of glacial acetic acid with distilled water) and stirred continuously for 24 h to obtain a clear 4% (w/v) solution. The plant extract was added to the chitosan solution, followed by the addition of a 2% STPP solution in a 2:1 (chitosan:STPP) ratio. Tween 80 was

added to enhance stability. The mixture was stirred for 5 h, sonicated for 15-20 min, and centrifuged at 10,000 rpm for 15 min to isolate the nanoparticles. The resulting pellet was washed three times with a 1:1 ethanol-water mixture, re-suspended in distilled water, and stored at 4°C. For powder formation, the nanoparticles were oven-dried at 45-50°C, ground into a fine powder, and stored in a dry, opaque container for further analysis (Kain and Kumar, 2020; Salama et al., 2022).

Characterization of chitosan nano-encapsulated *V. agnus-castus* phenolic extract

Fourier Transform Infrared (FTIR) spectroscopy

FTIR analysis was performed using an MIR8035 FTIR spectrometer (Thermo Fisher, Bremen, Germany) to identify the functional groups present in the nano-capsules (Amibo et al., 2022).

Scanning Electron Microscopy (SEM)

SEM was used to examine the shape and structure of the nano-capsules. Sample preparation followed the method described by Conti et al. (2022).

Dynamic Light Scattering (DLS)

The particle size distribution of the nano-capsules was measured using a Zetasizer Nano ZS (Malvern Instruments, UK) (Conner et al., 2022).

Bioassay of chitosan nano-encapsulated *V. agnus-castus* extract on eggs, larvae, and pupae of *E. cautella*

Four concentrations of the nano-encapsulated extract were prepared: 250, 500, 1000, and 2000 ppm. Their effects on different life stages of *E. cautella* were evaluated under laboratory conditions.

Egg stage

Fifty one-day-old eggs were used per replicate, with three replicates per concentration. Eggs were transferred to Petri dishes using a fine brush and sprayed with 1 ml of the extract using a hand sprayer. The dishes were incubated at 26 ± 1°C, 60-70% relative humidity, and a 16:8 (light:dark) photoperiod. Mortality was recorded after hatching.

Larval stage

Ten third-instar larvae per replicate (three replicates per concentration, including a control) were placed in 7 cm Petri dishes containing 1 g of the rearing diet. The larvae and diet were sprayed with 1 ml of the extract. The dishes were incubated under the same conditions, and larval mortality was recorded after 24, 48, and 72 h.

Pupal stage

The same procedure used for larvae was applied to pupae. Corrected mortality rates were calculated using Abbott's

formula to account for natural mortality in the control group (Abbott, 1925):

$$\text{Corrected mortality (\%)} = \frac{T - C}{100 - C} \times 100$$

T = Mortality in treatment and

C = Mortality in control

Statistical Analysis

The experiments were conducted using a completely randomized design (CRD) in a factorial arrangement. Treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level. Data analysis was performed using GenStat version 12.

RESULTS AND DISCUSSION

FTIR analysis

The FTIR analysis results (Figure 1) indicate the presence of hydroxyl (O-H) groups around ~3414 cm⁻¹, suggesting the presence of alcohols or phenols. Aliphatic C-H stretching is observed around ~2923 cm⁻¹. The bands between 1619-1401 cm⁻¹ suggest the presence of aromatic rings or carbonyl groups. In the region between 1300-1000 cm⁻¹, C-O stretching typical of ethers, esters, or acids was detected. Absorption in the fingerprint region (800-400 cm⁻¹) indicates metal-oxygen bonds, supporting the presence of an organic-inorganic nanocomposite.

SEM analysis of nano-capsules

SEM images of the nano-capsules derived from the extract revealed irregular semi-spherical shapes with minimal clumping or aggregation. The diameter of the capsules ranged from 34.11 to 48.65 nm, with an average diameter of 43.77 nm (Figure 2).

DLS particle size analysis

As shown in Figure 3, the particle size of the nano-capsules of the extract was also measured using a DLS device. The average particle size was found to be 227.1 nm, which aligns with the SEM results, confirming the consistency of the size analysis.

Toxic effect on eggs of *E. cautella*

Figure 4 illustrates the toxic effect of nano-capsules of *V. agnus-castus* extract on the eggs of *E. cautella*. The highest egg mortality rate, 60.96%, was recorded at the 2000 ppm concentration. This was significantly higher than the 1000 ppm concentration, which showed a 50.52% mortality rate, and the 500 ppm concentration with 37.04%. The lowest mortality rate was 25.93% at 250 ppm, while the control group exhibited only 1% mortality.

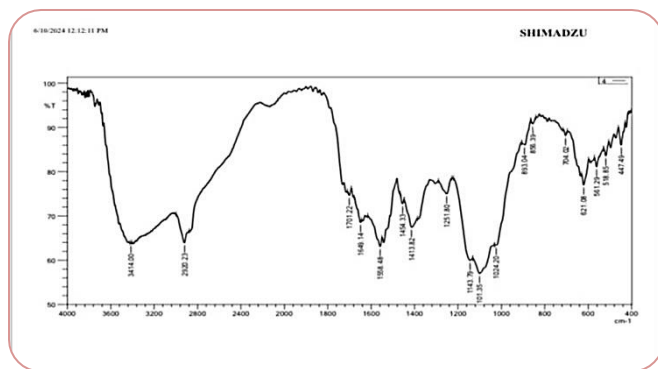


Figure 1. FTIR spectrum showing functional groups in the *V. agnus-castus* leaf extract.

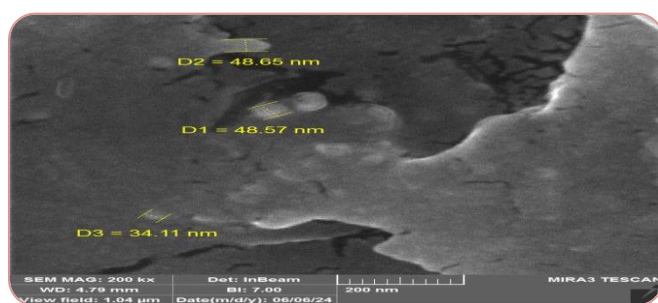


Figure 2. SEM analysis showing the diameter and morphology of nano-capsules containing *V. agnus-castus* leaf extract.

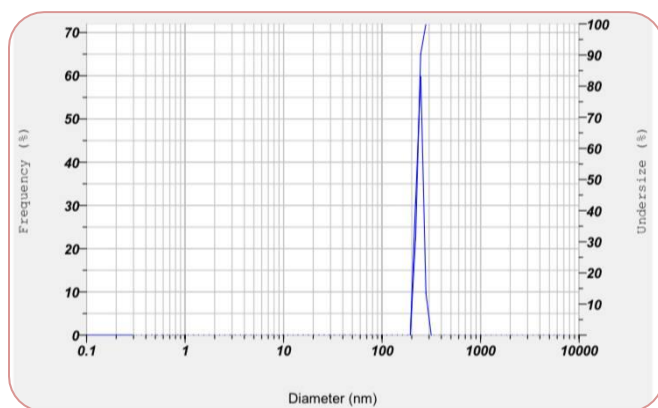


Figure 3. DLS analysis showing the size distribution of nano-capsules of *V. agnus-castus* leaf extract.

Toxic effect on larvae of *E. cauttella*

Table 1 shows the toxic effects of *V. agnus-castus* leaf nano-capsules on the mortality of *E. cauttella* larvae. Larval mortality increased with higher concentrations and longer exposure times. The most effective exposure time was 72 h, during which mortality rates reached 66% at 2000 ppm, 60% at 1000 ppm, 63.3% at 500 ppm, and 46.7% at 250 ppm.

Toxic effect on pupae of *E. cauttella*

Figure 5 shows the toxic effect of *V. agnus-castus* nano-capsules on the pupae of *E. cauttella*. The highest mortality rate was 57.69% at 2000 ppm, which was significantly higher than the 50% mortality observed at 1000 ppm. No significant difference was observed between the 500 ppm and 250 ppm treatments.

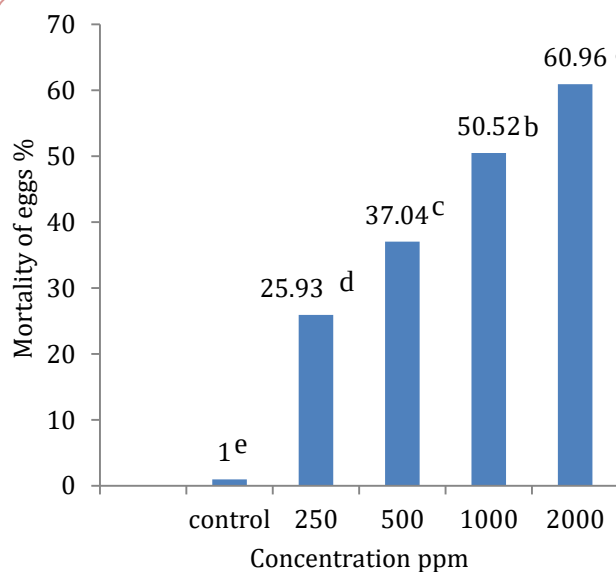


Figure 4. Effect of *V. agnus-castus* extract nano-capsules on egg mortality of *E. cauttella*.

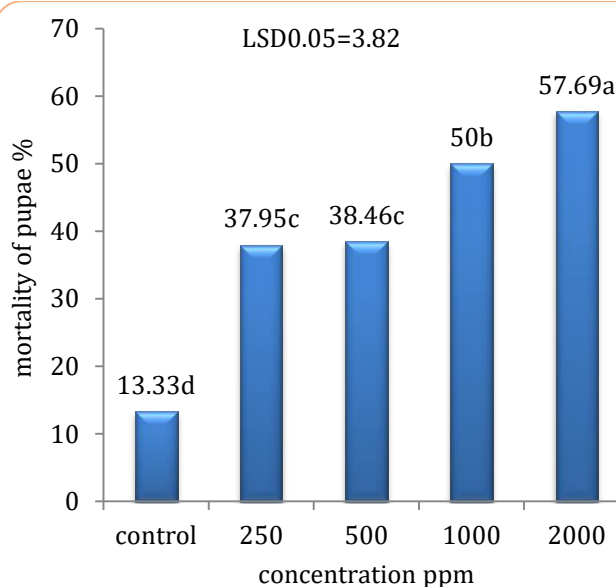


Figure 5. Effect of *V. agnus-castus* extract nano-capsules on pupal mortality of *E. cauttella*.

The methanol extract of *V. agnus-castus* leaves was found to contain significantly higher levels of phenolic and flavonoid compounds compared to both the methanol and petroleum ether extracts of *V. agnus-castus* seeds. These phytochemicals are known for their strong antioxidant and bioactive properties, which contribute to their insecticidal potential. In particular,

the essential oil extracted from *V. agnus-castus* leaves has demonstrated remarkable efficacy against the Mediterranean fruit fly (*Ceratitis capitata*), a major agricultural pest. Due to its potent insecticidal activity, the essential oil can serve as a promising eco-friendly alternative to conventional chemical insecticides for pest control (Hamdouch et al., 2022).

Table 1. Effect of *V. agnus-castus* nano-capsules on larval mortality of *E. cautella*.

Concentrations	Mortality rate per day					
	24 h	48 h	72 h	96 h	120 h	Average
Control	0	0	0	13.3	13.3	5.3
250	43.3	36.7	46.7	38.5	18.5	40.7 d
500	26.7	30.7	63.3	57.7	57.7	47.1 c
1000	30	40	60	53.8	53.8	47.5 b
2000	20	30	66	61.5	61.5	47.9 a
Average of time	24	45	45	27.3	47.3	65.47

In a study conducted by Sabbour and Abd El-Aziz (2019), the use of nano-extracts derived from plant sources was recommended for the control of *E. cautella* larvae. The researchers highlighted the high efficiency and environmentally friendly nature of these nano-formulations, which offer targeted toxicity and reduced ecological impact compared to synthetic pesticides.

Jiang et al. (2015) further elucidated the mechanism of insecticidal action of nanoparticles. According to their findings, nanoparticles exert toxicity by penetrating the insect cuticle and subsequently entering the internal cellular environment. Once inside, they interact with intracellular components such as proteins, sulfur, and phosphorus groups in DNA. This interaction disrupts normal cellular processes, leading to rapid denaturation of organelles and enzymes. Moreover, nanoparticles compromise membrane permeability and disturb the proton motive force, ultimately resulting in a loss of cellular function and cell death (Benelli, 2018).

In another relevant study, Al Hilfy et al. (2022) investigated the toxicity of a nanoemulsion of castor oil against the fourth instar larvae of *Culex quinquefasciatus*. Their results demonstrated that the nanoemulsion was significantly more effective than the bulk emulsion, achieving high larval mortality even at low concentrations. This enhanced efficacy was attributed to the small particle size, which facilitates better penetration and increased surface interaction with the target organism.

Moreover, a study by Hammed et al. (2024) reported the

insecticidal activity of *Metarhizium anisopliae*-polyethylene glycol 4000 nano-capsules loaded with a dichloromethane (DCM) crude extract of the fungus. These nano-capsules, rich in secondary metabolites and toxins, exhibited a strong inhibitory effect on acetylcholinesterase activity in *Musca domestica* larvae. Acetylcholinesterase is a key enzyme in the nervous system, and its inhibition leads to the accumulation of acetylcholine, causing paralysis and eventual death of the insect. This finding underscores the potential of fungal-based nano-formulations as effective biopesticides.

AUTHORS' CONTRIBUTIONS

SAK and AHS designed the study; AHS prepared the materials, collected and analyzed the data; SAK supervised the studies; SAK wrote the manuscript; All the authors proofread and approved the final manuscript.

RESEARCH FUNDING

This research did not receive any grant from funding agencies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

REFERENCES

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18, 265-267.
- Abdel-Gawad, R.M., 2018. Insecticidal activity of *Moringa oleifera* synthesized silver and zinc nanoparticles against the house fly, *Musca domestica* L. *Egyptian Academic Journal of Biological Sciences. A, Entomology* 11(4), 19-30.
- Ahmed, M.S.H., Al-Maliky, S.K., Al-Tawoel, A.A., Jabo, N.F., Al-Hakkak, Z.S., 1985. Effect of three temperature regimes on rearing and biological activities of *Bracon hebetor*. *Journal of Stored Products Research* 21(2), 65-68.
- Al Hilfy, A.A., Al Shammari, H.I., Kathiar, S.A., 2022. Toxicity of nanomulsion of castor oil on the fourth larval stage of *Culex quinquefasciatus* under laboratory conditions. *Baghdad Science Journal* 19(5), 999-1007.
- Amibo, T.A., Beyan, S.M., Damite, T.M., 2022. Production and optimization of bio-based silica nanoparticle from teff straw (*Eragrostis tef*) using RSM-based modeling, characterization aspects, and adsorption efficacy of methyl orange dye. *Journal of Chemistry* 2022(1), 9770520.
- Benelli, G., 2018. Mode of action of nanoparticles against insects. *Environmental Science and Pollution Research* 25, 12329-12341.
- Conner, C.G., McAndrew, J., Menegatti, S., Velez, O.D., 2022. An accelerated antibody aggregation test based on time sequenced dynamic light scattering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2022, 129833.
- Conti, S., Remuzzi, G., Benigni, A., Tomasoni, S., 2022. Imaging the kidney with an unconventional scanning electron microscopy technique: analysis of the subpodocyte space in diabetic mice. *International Journal of Molecular Sciences* 23(3), 1699.
- Fernandes, C.P., de Almeida, F.B., Silveira, A.N., Gonzalez, M.S., Mello, C.B., Feder, D., Apolinário, R., Santos, M.G., Carvalho, J.C.T., Tietbohl, L.A.C., Rocha, L., 2014. Development of an insecticidal nanoemulsion with *Manilkara subsericea* (Sapotaceae) extract. *Journal of Nanobiotechnology* 12(1), 1-9.
- Hamdouch, A., Asdadi, A., Bijla, L., Gharby, S., Bouharroud, R., Chebli, B., Hassani, L.M., 2022. Leaves and seeds extracts of *Vitex agnus-castus* L., an ecological and effective alternative to conventional insecticides against fruit flies (Diptera: Tephritidae). *Caspian Journal of Environmental Sciences* 20(5), 1117-1125.
- Hamid, A.A., 2002. Laboratory and field studies of the use of the fig moth parasite *Bracon hebetor* in the control of insect fig moth *Ephestia cautella* and spiny cotton nut worm *Earias insulana*. Master's thesis, Mechanism of Agriculture, University of Baghdad, p. 106.
- Hammed, A.A.A., Al Shammari, H.I., Kathiar, S.A., 2024. Effect of nanocapsules and extract of *Metarhizium anisopliae* in inhibiting acetylcholine esterase enzyme in *Musca domestica* larvae. *Baghdad Science Journal* 21(1), 41-52.
- Hosseini, S.F., Zandi, M., Rezaei, M., Farahmandghavi, F., 2013. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and *in vitro* release study. *Carbohydrate Polymers* 95(1), 50-56.
- Jabbar, A., Tariq, M., Gulzar, A., Mukhtar, T., Zainab, T., 2022. Lethal and sublethal effects of plant extracts and green silver nanoparticles against *Culex pipiens* mosquitoes. *Pakistan Journal of Zoology* 54(3), 1259-1267.
- Jaradat, A.A., 2023. Agriculture in Iraq: Resources potential, constraints and research needs and priorities. *Food Agriculture and Environment* 1(2), 160-166.
- Jiang, X., Miclăuş, T., Wang, L., Foldbjerg, R., Sutherland, D.S., Autrup, H., Chen, C., Beer, C., 2015. Fast intracellular dissolution and persistent cellular uptake of silver nanoparticles in CHO-K1 cells: implication for cytotoxicity. *Nanotoxicology* 9, 181-189.
- Kain, D., Kumar, S., 2020. Synthesis and characterization of chitosan nanoparticles of *Achillea millefolium* L. and their activities. *F1000Research* 9, 1297.
- Khan, H.S., Tariq, M., Mukhtar, T., Gulzar, A., 2021. Insecticidal toxicity of plant extracts and green silver nanoparticles against *Aedes albopictus*. *Pakistan Journal of Zoology* 53(6), 2123-2128.
- Margulis-Goshen, K., Magdassi, S., 2013. Nanotechnology: An advanced approach to the development of potent insecticides. In: Ishaaya, I., Palli, S., Horowitz, A. (Eds.), *Advanced Technologies for Managing Insect Pests*. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-4497-4_15.

- Mehlhorn, H., Schmahl, G., Schmidt, J., 2005. Extract of the seeds of the plant *Vitex agnus-castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. *Parasitology Research* 95(5), 363-365.
- Nazir, K., Mukhtar, T., Javed, H., 2019. In vitro effectiveness of silver nanoparticles against root-knot nematode (*Meloidogyne incognita*). *Pakistan Journal of Zoology* 51(6), 2077-2083.
- Ress, D., 2007. *Insects of Stored Grain: A Pocket Reference*. CSIRO Publishing, pp. 81.
- Sabbour, M.M., Abd El-Aziz, S.E., 2019. Impact of certain nano oils against *Ephestia kuehniella* and *Ephestia cautella* (Lepidoptera: Pyralidae) under laboratory and store conditions. *Bulletin of the National Research Centre* 43, 80.
- Salama, R., Osman, H., Ibrahim, H.M., 2022. Preparation of biocompatible chitosan nanoparticles loaded with *Aloe vera* extract for use as a novel drug delivery mechanism to improve the antibacterial characteristics of cellulose-based fabrics. *Egyptian Journal of Chemistry* 65(3), 589-604.
- Shahbaz, M., Akram, A., Raja, N.I., Mukhtar, T., Mashwani, Z.R., Mehak, A., Fatima, N., Sarwar, S., Haq, E.U., Yousaf, T., 2022. Green synthesis and characterization of selenium nanoparticles and its application in plant disease management: a review. *Pakistan Journal of Phytopathology* 34(1), 189-202.
- Shahbaz, M., Akram, A., Raja, N.I., Mukhtar, T., Mehak, A., Fatima, N., Ajmal, M., Ali, K., Mustafa, N., Abasi, F., 2023. Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease. *PLoS ONE* 18(2), e0274679. <https://doi.org/10.1371/journal.pone.0274679>