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Insecticidal Potential of Garlic Nanoparticles Versus Raw Extract against *Callosobruchus maculatus* (F.) and Their Non-target Effects on the Parasitoid *Anisopteromalus calandrae* (Howard)

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ABSTRACT

Background: Stored grains are still being protected and preserved with synthetic chemical pesticides, which has major environmental and health concerns and needs to be met with more sustainable and biocompatible means. In this study, we examined the insecticidal activity of biosynthesized silver nanoparticles (Ag NPs) derived from Iraqi garlic extract and their synergistic effects against the larvae of southern cowpea beetle *Callosobruchus maculatus* (F.), which is a major stored-products pest of this plant.

Methods: The garlic aqueous extract (*Allium sativum* L.) was used to synthesize green silver nanoparticles (Ag NPs) which were characterized by XRD, FESEM, FTIR and EDS techniques. To calculate adult mortality of *Callosobruchus maculatus*, three concentrations (20, 30 and 50 ppm) of both the Nano and crude extracts of garlic were tested and compared for seven days. Mortality of third- and fourth-instar larvae of *C. maculatus* within the seeds was also calculated using the same concentrations of the Nano and crude extracts. Additionally, larval mortality due to the ectoparasitoid *Anisopteromalus calandrae* was calculated. Finally, larval mortality due to the synergistic effect between the Nano and crude extracts at the same concentrations mentioned above and the ectoparasitoid *A. calandrae*, was studied and the number of resulting ectoparasitoid offspring was calculated after 25 days.

Result: Biosynthetic silver nanoparticles (Ag NPs) exhibited the best toxicity against the adults *C. maculatus* with a high mortality of 73% at 50 ppm ($p<0.01$). A noteworthy synergistic effect was also observed for killing *C. maculatus* larvae. Ag NPs had 75% mortality at 50 ppm, while Ag NPs combined with *A. calandrae* had 100% mortality at 30 ppm of *C. maculatus* larvae. The bioavailable silver nanoparticles and the parasitoid synergized extremely well, which was confirmed through statistical analysis. Also, a high rate of parasitoid emergence was obtained, indicating that there was excellent selection and biocompatibility between the bioavailable nanoparticles and natural enemy.

Key words: *Anisopteromalus calandrae, Callosobruchus maculatus*, Garlic nanoparticles.

INTRODUCTION

Strategic food commodity stocks, especially cereal and pulses stocks, are imperative for the world. This is because countries are dedicated to having adequate stocks on hand to reduce the impact of natural disasters and bad harvests each year. Yet, stored agricultural commodities are very vulnerable to many biological deterioration agents including insects and rodents resulting in deterioration of quality and loss of nutritional values (Mahdi and Hammadi, 1987).

Pulsaceous crops are especially susceptible to many insect pests of which many are members of the order Coleoptera and have the ability to live and reproduce on dry seeds (Al-Bakr, 1999; Ismail, 2005). Of these, *Callosobruchus maculatus* (F.) (southern cowpea beetle) is one of the most destructive which infests over 35 species of pulses, reducing seed weight by up to 62% (Al-Azzawi *et al*., 1990). Larval development causes loss of weight in stored legumes because larval development and pupation occur within a single seed and the adult beetle does not feed (Kang *et al*., 2013). The average number of eggs produced by an adult female is 74.8±1.8 and the total average lifespan is 33.3±2.4 days in the laboratory conditions (Sharma *et al*., 2018). In these "stored product ecosystems," some natural enemies are very important to help control pest populations. The ectoparasitoid, *Anisopteromalus calandrae* (Howard) is one of the most effective biological control agents reducing the density of major stored cereal pests such as *Sitophilus oryzae* and

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C. maculatus. This parasitoid is more successful in locating the host larvae in the grains to paralyze them by inserting its ovipositor and also deposits eggs on or near the host (Al-Azzawi and Mahdi, 2024).

The use of sophisticated techniques has enabled the creation of nanopesticides, which are either new pesticides based on nanoparticles or biomolecules delivered by nanoparticles. Garlic, which contains a variety of sulfur based bioactive compounds like allyl disulfide (Lee *et al*., 2007) is a significant plant source for green synthesis in this context. Moreover, silver nanoparticles (Ag NPs) have emerged as a popular and effective insecticide against several insect pests (Owolade *et al*., 2008). The bio-derived nanoparticles products prepared using plant extracts are likely to be the front runners in next few years for sustainable pest management strategies due to their improved efficiency and environmental friendly behavior. In this regard, the purpose of this study was to evaluate the insecticidal activity of garlic mediated Ag NPs and to find the compatibility among them with the ectoparasitoid *A. calandrae* for integrated pest management.

MATERIALS AND METHODS

Insect rearing

Adult Southern Cowpea Beetles (*Callosobruchus maculatus*) were collected in December 2025 from infested chickpeas in local markets in Karbala Governorate. To establish laboratory cultures, 1.250 kg of uninfested chickpeas were frozen at -18°C for 72 hours to eliminate any potential prior infestation (eggs or hidden larvae). Subsequently, the chickpeas were removed and allowed to equilibrate at room temperature for 24 hours to dissipate residual cold, ensuring optimal conditions for insect activity or mating. The beetles were reared in plastic containers in a relatively dark location at a temperature and relative humidity of 25±5°C and 50±10% respectively, using a thermohygrometer to monitor environmental conditions. Newly emerged adults (at least 48 hours post-pupal stage) were used for subsequent experiments.

Preparation of garlic powder

The local garlic cloves (*Allium sativum*) were purchased from the local markets in Karbala region. Dust and surface contaminants from the cloves were washed off with distilled water. After that, they were chopped to facilitate drying. The garlic slices were placed in a convection oven and dried for 15-20 minutes at 80-90°C until they were fully dry and rigid. The dried pieces were ground with an electric mixer until a fine homogeneous powder was obtained. The last powder was kept in airtight opaque glass containers in a dark and cool place until used for green synthesis experiments to preserve the quality and prevent its degradation by light or oxygen.

Synthesis and preparation of silver nanoparticles (Ag NPs)

Preparation of AgNO3 solution

For the green synthesis process, a highly purified aqueous solution of silver nitrate (AgNO3) was prepared at a precise concentration of 0.35 mM. The solution was prepared by dissolving 0.0642 g of AgNO3 crystals in 1000 mL of deionized water. The mixture was continuously stirred using a magnetic stirrer to ensure complete homogeneity and full dissolution of the salt. The resulting solution was stored in an amber glass bottle to prevent the photolysis of the silver ions.

Green synthesis protocol

A total of 10 grams of dried garlic was weighed and then mixed with 100 mL of distilled water. This mixture was then heated at 50-60°C on a magnetic stirrer for 20 minutes to get extraction of the active compounds. The solution was then allowed to cool and filtered using What man No. 1 filter paper to obtain a clear aqueous extract free of plant impurities. 20 mL of the previously prepared garlic extract was mixed with 80 mL of silver nitrate solution (in a 1:4 mixing ratio) in an opaque glass flask. The mixture was then allowed to stand at room temperature in complete darkness for 24 hours, to avoid oxidation and photo degradation of the silver. The successful synthesis was evidenced by the color change in the solution from clear to dark brown/black. To overcome the speed limitation, the nanoparticle precipitate was collected by the help of a centrifuge at 2500 rpm for one hour. The precipitate was washed with distilled water to wash out unreacted residues. Later, it was placed in a heat-resistant dish and dried in the oven at 40-50°C to get the Nano powder.

Insecticidal bioassays

Preparation of concentrations

A series of concentrations (20, 30 and 50 ppm) were prepared for both the nanoparticle formulation and the crude extract using deionized water. The dilutions were calculated based on the standard dilution equation:

$$C_1V_1 = C_2V_2$$

Here,

C_1 and V_1= The initial concentration and volume.

C_2 and V_2= The target concentration and final volume, respectively.

The exact volumes were measured and transferred using a calibrated micropipette and the final volumes were adjusted to 100 mL in volumetric flasks.

Experimental setup and treatment application

Effect of different concentrations on adult insect of *C. maculatus* mortality

To evaluate the effect of various concentrations of garlic plant extract (both nano-formulation and crude extract) on mortality rates of adult insects *C. maculatus*, adult southern cowpea weevils (48 hours old) were isolated and placed

in plastic containers containing 10 g of chickpeas. Three replicates were prepared for each concentration, in addition to a control treatment sprayed with deionized water. The chickpeas were treated by direct spraying with 1.5 mL of each treatment (Nano and crude extracts) and allowed to air-dry for 30 minutes prior to introducing the insects . Five adults (males and females, without parasitoids) were added to each replicate. Adult mortality was recorded after 24, 48 and 72 hours, with a final mortality reading taken after one week.

Toxicity bioassay of garlic extracts (Ag NPs and Crude) and their synergistic effect with ectoparasitoid *A. calandrae* on larvae of *C. maculatus*

To evaluate the effects of various concentrations of Garlic Extracts (Ag NPs and Crude) on the mortality of third and fourth instar larvae of *C. maculatus*, an experiment was conducted using three concentration levels (20, 30 and 50 ppm) for both the Ag NPs and the crude extract, along with two control groups. For each treatment, 10 g of infested grains containing the target larvae were prepared per replicate, with two replicates per concentration. The application was performed by spraying 1.5 mL of the respective extract solution onto each replicate, after which the treated grains were air-dried at room temperature. The control treatments were designated as: negative Control (Control-), sprayed with 1.5 mL of deionized water alone without the addition of extracts or ectoparasitoids; and positive Control (Control+), sprayed with 1.5 mL of deionized water and subsequently exposed to four adult individuals of the ectoparasitoid *A. calandrae*. The experiment was designed to assess the direct contact toxicity of the extracts alone, as well as their combined effect when paired with the parasitoid. Larval mortality rates and the overall efficiency of the ectoparasitoid were recorded 25 days post-treatment.

RESULTS AND DISCUSSION

Characterization of the synthesized nanoparticles

Visual observation

The first confirmation of the successful reduction of silver ions ($AgNO_3$) to the nanoparticles is when the color changes are visually observed. A brown coloration of the solution of silver nanoparticles is scientifically proven to be caused by the excitation of surface plasmon resonance in the metallic particles. In the experiment, Iraqi garlic was extracted with water and mixed with a clear solution of silver nitrate, the color of the silver nitrate solution was gradually changed from clear to dark brown as shown in (Fig 1). This process is biochemical reaction with the active compounds in garlic (allicin, phenols) can be confirmed, as it inevitably leads to a decrease in the amount of silver ions and the formation of nanoparticles. The experimental results obtained are found to be similar to that of Singh *et al.* (2010), thus confirming the accuracy and reliability of the biosynthetic process adopted in the present study.

FTIR analysis and bio-efficacy

The FTIR analysis of garlic nanoparticles is shown in (Fig 2), showing that several bands of absorption of the chemical functional groups present in the plant extract that were

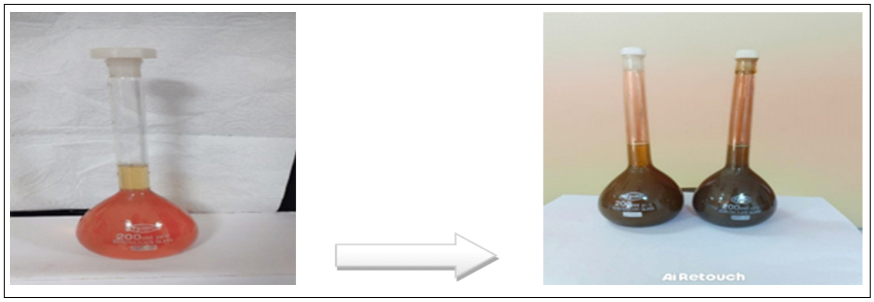


Fig 1: Visual observation of the green synthesis of silver nanoparticles (Ag NPs); (Left) pure aqueous extract of *Allium sativum*, (Right) the brown color formation indicating the reduction of Ag NPS ions after 24 hours.

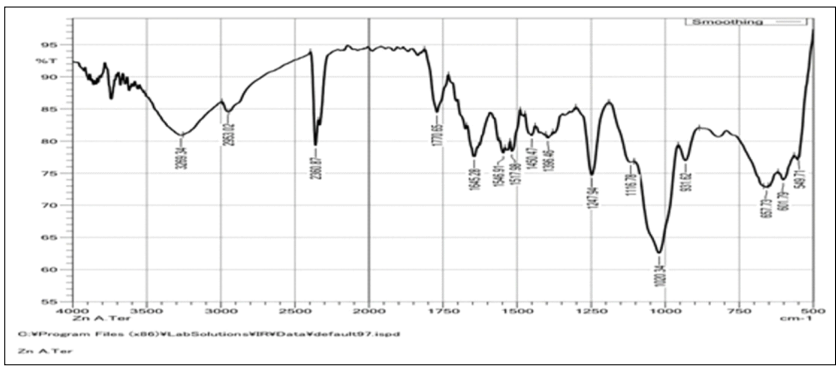


Fig 2: FTIR spectra of vacuum-dried powder of synthesized Ag NPs using aqueous garlic (*Allium sativum*) extract.

involved in the synthesis and stabilization of the garlic nanoparticles were found. A wide and strong band was observed at 3269 cm^{-1}, which was attributed to tensile vibration of hydroxyl (O-H) group, suggesting the presence of phenolic compounds that are good reducing agents. Aliphatic carbon-hydrogen (C-H) bonds were also found at 2953 cm^{-1}. The sharp peaks in the spectrum also appeared at 1770 cm^{-1} and 1645 cm^{-1}, corresponding to carbonyl (C=O) and amide (Amide I) groups of protein bonds. Presence of these clusters shows that the proteins present in garlic have coated the nanoparticles and made them highly stable in solution without agglomeration. The results of this are in accordance with (Singh *et al*., 2010) who reported that secondary metabolites of garlic offer exceptional long-term stability to metallic nanoparticles.

The peaks in the 1020 cm^{-1} and 1247 cm^{-1} ranges, however, are linked to the C-S and S=O disulfide bonds of allicin and the volatile sulfur compounds of garlic. The continuity of these bonds after the preparations accounts for the fact that the Nano-extract was more effective in disrupting the vital tissue of southern cowpea beetle than the crude extract after passing through the beetle's cuticle. This proves that in addition to their role in reduction and encapsulation, bioactive plant secondary metabolites are directly responsible for the strong insecticidal activity against stored pests, which is in complete agreement with the results obtained by Shunmugadevi and Anbu Radhika (2020).

XRD patterns and crystallinity

The XRD analysis indicated that the synthesized green AgNPs were crystalline (Fig 3). The diffraction pattern showed distinct reflection peaks at 2θ values of 29.11°, 38.00°, 64.30° and 77.85°. The Bragg reflection is very sharp with a full width at half maximum (FWHM) of 0.129, which shows a high crystallinity. The calculated crystal sizes fell in a wide range (4.2 to 77.8 nm) with help of Debye-Scherrer equation. Such variation in size is mainly attributed to a calculated micro-strain of 2.84% in the crystal lattice. The lack of strong peaks showed the purity of the phase and the integrity of the structure of the composite nanomaterial (Abdul-Zahra *et al*., 2025).

EDS and elemental composition

The energy-dispersive X-ray spectroscopy (EDS) further validated the elemental characteristics of the prepared matrix (Fig 4). As listed in Table 1, the analysis suggested a majority composition of carbon (46.0%) and oxygen (51.9%), as well as a few quantities of phosphorous (0.4%), sulfur (0.5%) and potassium (1.1%). Such elemental constitution clearly indicates the validity of the "organic shell," implying that the bioactive substances obtained from garlic (such as allicin and associated proteins) successfully coated the metal core. Elemental mapping revealed the existence of elemental silver within the organic matrix. Most importantly, the absence of any heavy metal contamination makes these nanoparticles environmentally friendly and safe for biological usage (Abdul-Zahra *et al*., 2025) (Singh *et al*., 2010).

FESEM morphology

From (Fig 5), one can observe that the synthesized nanoparticles have the spherical and spherical shape with very uniform size distribution, which is the most common form of silver particles formed biologically using plant extracts. This type of particle formation is suitable for biological use because of the high surface area available to the particles with low volume. At higher magnification, such as 100 nm, one observes that the particles have individual structures with simple cluster-type aggregation of particles. This is as a result of the effect of the biomolecules present in the garlic extract as the capping agents. Such organic layer acts as the stabilizer and inhibits fusion and uncontrolled growth of particles according to the explanation of the scientists regarding the stabilizing nature of proteins and phenolic substances (Abdul-Zahra *et al*., 2025; Singh *et al*., 2010). There are both very tiny Nano-sized particles (lesser than 20 nm) and somewhat larger particles in the provided images, which is consistent with the results calculated by using XRD analysis (the smallest crystal size is 4.2 nm). This indicates that the presence of somewhat larger particles in the images obtained through FESEM technique is due to either surface aggregation or measurement of the apparent size.

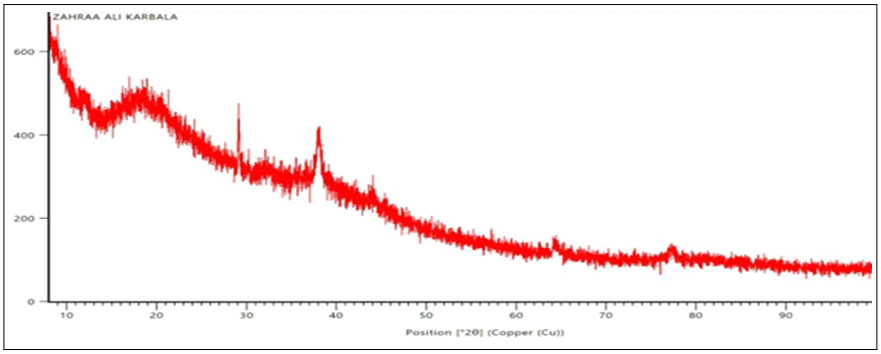


Fig 3: XRD pattern of the green synthesized silver nanoparticles (Ag NPs) using garlic extract.

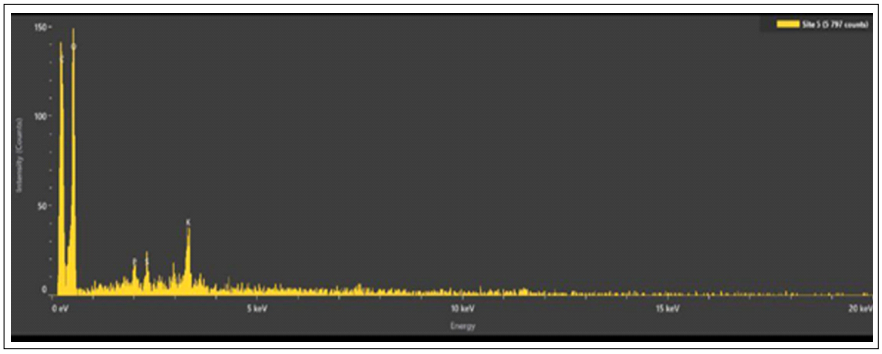


Fig 4: EDS spectrum and elemental mapping of the synthesized Ag NPs confirming the presence of elemental silver.

Table 1: Elemental composition of the synthesized Ag NPs as determined by energy dispersive X-ray spectroscopy (EDS).

Weight % Error	Weight %	Atomic % Error	Atomic %	Element
1.4	37.9	1.7	46.0	C
2.2	57.0	2.0	51.9	O
0.1	0.9	0.1	0.4	P
0.1	1.2	0.1	0.5	S
0.2	3.1	0.1	1.1	K

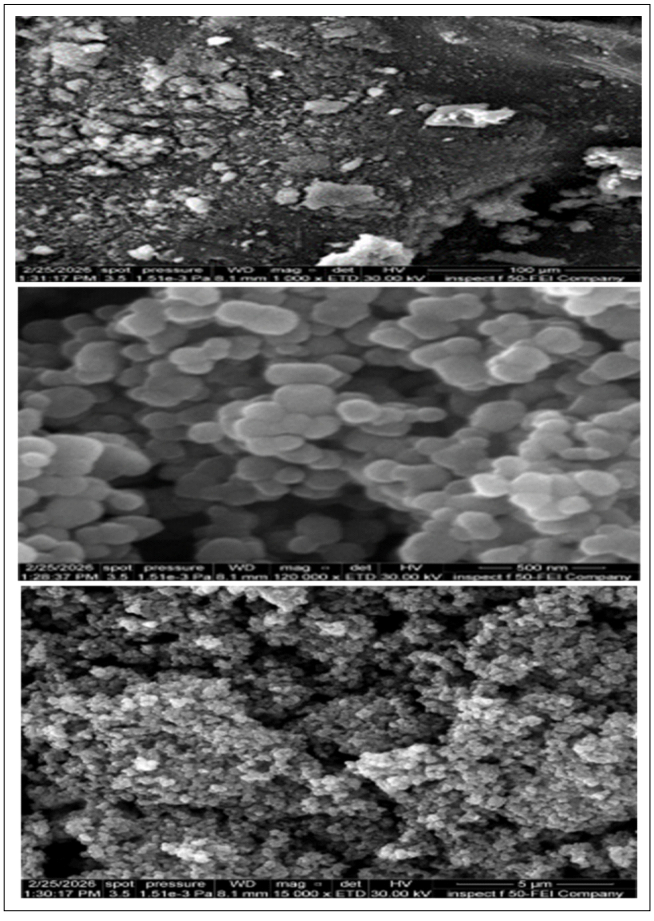


Fig 5: FESEM micrographs of the synthesized silver nanoparticles at different magnifications, showing the spherical morphology and the distribution of the particles.

Table 2: Morality rates of *Callosobruchus maculatus* adults treated with normal garlic extract versus biogenic silver nanoparticles (Ag NPs) after 7 days of exposure.

X^2	P-Value	Nanoscale	Crude extract	Concentration (ppm)
0.792645	0.373302	60%	47%	20
6.492137	**0.010835	60%	53%	30
0.128630	0.719856	73%	67%	50
0.000	1.000	0.00	0.00	Control
		**0.00000	**0.0000	P-Value
		55.852870	47.880747	X^2

Insecticidal bioassays against *Callosobruchus maculatus* adults

From Table 2, it is evident that there exists significant effectiveness of silver nanoparticles (Ag NPs) in managing the southern cowpea beetle (*C. maculatus*). The biological effectiveness did not only encompass the ability to kill but also include the ability to paralyze completely and motor inactivity of the specimens. This fast and strong effect of the Nano- treatment during the early days of the experiments can be attributed to the distinctive properties of silver nanoparticles, especially their small size and greater specific surface area which enabled their easy penetration into the insect's body wall (cuticle). It is only through its comparison with other similar local researches that the economic feasibility of this work has become evident. This is exemplified by the recent study by (Kashmir and Ali 2024), where crude oil extracts from black pepper fruits (*Piper nigrum*) and eucalyptus leaves (*Eucalyptus camaldulensis*) proved to be very effective only at higher doses (3 ml/L) in eliminating (100%) the insect within 7 days of the treatment. Plants have fast and powerful insecticidal effects on stored product insects. For instance, plant extracts like black pepper have proven to cause 100% mortality of cowpea beetles within 24 hours of treatment (Govindan *et al*., 2023). However, although some plant oils, such as neem oil, can provide relatively high mortality rates (84-100%) of adult *Callosobruchus maculatus*, they have to be kept in storage for more than 135 days to ensure their maximum effect (Sharma *et al*., 2018). On the other hand, the Nano-formulation exhibited efficient toxicity and eradication effect even at very low concentration levels, which are measured in parts per million (ppm), illustrating the ability of nanotechnology to minimize the quantity of plants used and multiply their efficacy several times. The high mortality rates observed in this experiment agree with the results from Carbone *et al*. (2020), where 52% mortality rate was observed on cowpea beetle adults using Nano-concentration prepared with aqueous tomato peels extract. This indicates that this insect is highly sensitive to silver nanoparticles physiologically. The extreme quiescence and immobility of the insect in the laboratory could be attributed to penetrating silver nanoparticles causing high oxidative stress in insect's cells due to ROS overproduction. This directly causes the breakdown of cell membranes and the inhibition of energy enzymes, thus affecting the stability of the nervous system and leading to a total lack of motor control until the death of the insect. These findings validate some previous research works, such as Goswami *et al*. (2010); Zahir *et al*. (2012) and Pathipati *et al*. (2021) where the superiority of the efficacy of silver nanoparticles in reducing the viability of storage pests was confirmed. The findings revealed that there is a gradual increase in the number of deaths within the seven days of exposure. This trend is fully in agreement with Isman (2020) where the effectiveness of nanoparticles in offering protection for plant molecules from environmental breakdown was confirmed. The stability provided by the Nano-biopesticide means that it has an increased longevity and stability of the residue in the storage environment than the regular fast-evaporating aqueous extracts. Additionally, the dual action of repellency and lethality of the Nano-biopesticide in this study is supported by the results of (Gulzar *et al*., 2020), which indicated the high mortality rates and repellence properties of silver nanoparticles used to target other stored-product insects. The impressive ability of the artificially synthesized silver nanoparticles to kill insects is shown when it is compared to the crude plant extract using solvents. According to (Saranya *et al*., 2019), the mortality of 100% was achieved in *C. maculatus* only after 5 days of treatment with *Acorus calamus* hexane extract at a concentration of 0.1%. In the current study, the artificial nanoparticles synthesized from garlic showed impressive mortality rates within a shorter period at lower concentrations (in parts per million) . This is consistent with the findings of Shneen *et al*. (2024), where the silver nanoparticle compound prepared using moringa leaves at a concentration of 2% achieved a mortality rate of 80%, compared to both the moringa extract alone and the control group, which achieved mortality rates of 43% and 0%, respectively, after 7 days of treatment.

Synergistic effect of Ag NPs and *Anisopteromalus calandrae* on larval mortality

The results obtained from Table 3 show very clearly that biosynthesized garlic silver nanoparticle (Ag NPs) were markedly more effective at killing *C. maculatus* larvae than the crude extract. In addition, applying Ag NPs alongside the ectoparasitoid *A. calandrae* produced a

Table 3: Synergetic effect of garlic-mediated silver nanoparticles (Ag NPs) and the parasitoid *Anisopteromalus calandrae* on the mortality rates of *Callosobruchus maculatus* larvae after 25 days.

X^2	P-Value	Crude extract with ectoparasitoid	Crude extract without ectoparasitoid	Nanoscale with ectoparasitoid	Nanoscale without ectoparasitoid	Concentration (ppm)
2.791461	0.424907	50%	30%	65%	35%	20
48.236906	**0.0000	60%	50%	100%	65%	30
5.999513	0.111634	70%	60%	100%	75%	50
7.508707	**0.057335	25%	0	25%	0	Control + (Adding parasitoid only)
0.000	1.000	0	0	0	0	Control - (Without adding extract or ectoparasitoid)
		**0.002808	**0.004368	***0.000	*0.034393	P-Value
		14.072591	13.128129	72.923889	8.645614	X^2

noticeable synergistic effect, yielding higher mortality rates than either treatment applied individually. Analysis of the control groups revealed zero mortality in the untreated negative control (Control-, only deionized water), whereas the positive control (Control +, parasitoid alone) recorded a 25% mortality rate, confirming the baseline effectiveness of the parasitoid . At a low concentration of 20 ppm, Ag NPs alone caused 35% larval death, which rose substantially to 65% when the parasitoid was introduced. The concentrations of 30 ppm and 50 ppm recorded the maximum biological activity. At these levels, the combined regimen (Ag NPs and parasitoid) achieved complete larval elimination (100% mortality). In comparison, Ag NPs alone reached 65% and 75% mortality, while the crude extract paired with the parasitoid reached 60% and 70% at the same respective concentrations. These variations across treatments were statistically significant ($P < 0.001$). This enhanced efficacy likely stems from a dual action mechanism: the garlic nanoparticles weaken the larval cuticle and impair their physiological defenses, thereby making the larvae far more susceptible to being attacked, paralyzed and utilized for egg-laying by the female parasitoid wasps.

CONCLUSION

The process of Ag NPs synthesis via the use of garlic extract (*Allium sativum*) offers an extremely efficient, sustainable and ecological way of handling the insects of stored products, specifically *Callosobruchus maculatus*. This research proves that garlic-based AgNPs show much higher capability of killing adults than usual crude extracts from plants. It is mostly due to improved body wall penetration and cell disruption. Importantly, the combination of such biologically active particles with ectoparasitoid *Anisopteromalus calandrae* results in synergy and leads to 100% death of larvae within 30 and 50 ppm after 25 days of storage. In addition, the high level of parasitoid survival after the treatment highlights the exceptional biocompatibility of the formulation with the natural enemies. Selective toxicity, which makes these nanoparticles fatal to the targeted pest and harmless to the natural enemy of the pest, further proves that these nanoparticles should be included as an essential part of IPM strategies. The entire process, in essence, is an effective and safe alternative to hazardous chemicals and guarantees that the strategic stocks of grains and pulses will always remain intact.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

This study did not involve vertebrate animals. All experiments were conducted using insect models (*Callosobruchus maculatus* and *Anisopteromalus calandrae*) under standard laboratory conditions, in accordance with the institution's guidelines for ethical research and environmental safety.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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