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Biological effect of Silver Nanoparticles synthesis by Leaves of *Cymbopogon citratus on eggs* and larvae of *Cadra cautella* (Walk.) (Lepidoptera: Pyralidae)

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***Abstract**. During storage and before consumption, grains often experience a decline in quality, nutritional content, and cleanliness due to pest attacks. Botanical insecticides are considered an alternative method to reduce dependence on harmful chemical pesticides. This study aimed to evaluate the effectiveness of three concentrations of silver nanoparticles (AgNPs) synthesized from the leaves of Cymbopogon citratus against Cadra cautella (Walk.) (Lepidoptera: Pyralidae) and their potential as biological control agents. The selection of C. cautella as the test organism was based on the limited number of studies examining this pest. The AgNPs used were synthesized through an eco-friendly method, and their optical and physical properties were analyzed using UV-Vis spectroscopy. The results demonstrated that AgNPs synthesized from C. citratus extract showed high effectiveness in controlling the eggs and second instar larvae of C. cautella. Egg mortality reached 80.3% at a concentration of 40 µg/ml after 24 hours of exposure, followed by 72.9% at 30 µg/ml, and 65.8% at 20 µg/ml. In contrast, the control group showed no egg mortality. Similarly, larval mortality rates were 74.9%, 65.8%, and 60.2% for concentrations of 40 µg/ml, 30 µg/ml, and 20 µg/ml, respectively. These findings indicate that higher concentrations of AgNPs lead to greater mortality in both eggs and larvae. This study highlights the potential of green-synthesized AgNPs from C. citratus leaves as an effective and eco-friendly biopesticide. Their significant impact on egg and larval mortality suggests that they can be developed as an alternative pest control strategy in stored grains, thereby reducing reliance on conventional chemical pesticides that are detrimental to human health and the environment. Further research is recommended to explore their long-term efficacy, safety, and integration into sustainable grain storage management.*

***Keywords:** Biological control, Cadra cautella, Cymbopogon citratus, Insecticides, Lemongrass*

1. INTRODUCTION

Cadra cautella (Walk.) (Lepidoptera: Pyralidae) is a significant pest causing storage losses worldwide. [1] It adversely affects cereals, grain-derived products, pulses, dried fruits, and nuts [2].The urgency of addressing this bug's detrimental consequences cannot be overstated. The larvae contaminate food when they feed on dates and dried fruits with their droppings, the remains of deceased insects, and the threads they produce [3]. This contamination not only affects the food material, creating holes and grooves that allow fungi to enter, but also greatly increases crop contamination with aflatoxins. [4]. The highest levels of aflatoxin contamination were found in dates compared to other stored food items. Additionally, aflatoxin levels are significantly higher in food affected by insects than in healthy foodstuffs. with the insect than in healthy foodstuffs [5].Diverse ways have been utilized to prevent pests in stored food. The utilization of chemical pesticides and fumigation continues to be the primary control approach. It has occupied a significant position in this domain, causing environmental and health detriments to humans and the ecosystem, as well as

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contributing to ozone layer depletion. This situation prompted international concern and led to the establishment of measures to cease the production and usage of chemical pesticides globally [6].So the researchers have developed alternative pest control methods, such as heat treatment, physical control, and biological control, which are being increasingly used [7].Recent studies have increasingly focused on replacing chemicals with natural compounds derived from essential plant sources. *C. citratus,* originally native to Asia, now grows worldwide. [8]. Both aqueous and alcoholic extracts from dried leaves have been used as antimicrobials. [9]. Several studies have shown that the biological aactivity of lemongrass arises from its high levels of active compounds and essential oils [10].The green synthesis of nanoparticles using plant extracts offers various advantages. because plants are environmentally sustainable and can be managed with minimal effort [11]. The main goal of this study is to assess the effectiveness of thebiological effect of silver nanoparticles of the leaves of *C. citratus* extract for controlling the eggs and larval stages of *C. cautella.*

2. MATERIAL AND METHOD

Population of *C. cautella* insects

C. cautella was meticulously obtained from a sample of infected grains in storage in Baghdad. The sample for the study was carefully created by placing wheat seeds in plastic containers measuring 15 cm x 30 cm. 5 g of dry bread yeast were added with precision. The culture was enveloped in a fine cloth, and the cover was secured with a rubber band to prevent any disturbance. The culture was maintained at 30 ± 2°C and 60 ± 5% relative humidity in a carefully controlled setting and kept until the experiment, ensuring the validity of the results.

Lemongrass *C. citratus* collection

The lemongrass leaves were collected in Baghdad. They were meticulously rinsed under running tap water while being swirled and put in a large bowl to remove the majority of dirt and unnecessary weeds. The items were subsequently dried using a large stainless steel colander, arranged on stainless steel trays, and allowed to air dry in the room. The leaves were ground in the laboratory electric grinder to a fine powder. To preserve the lemongrass, it was stored in airtight glass containers in the refrigerator until needed [12].

Preparation of Leaf Extracts

We prepared the plant extract following the method [13]. About 20 grams of *C. citratus* leaves were weighed and put into a 500-mL conical flask with 100 mL of D.W. We thoroughly mixed the mixture and heated it to a boil at 60-70°C for 40 minutes. The extracts underwent filtering using Whatman No. 1 , packaged in a dark glass container, and were stored in the freezer. The plant extract played a crucial role in the silver nanoparticle synthesis. We used the *C. citratus* extraction was performed using a pre-prepared silver nitrate solution. A flask containing 80 ml of $AgNO_3$ (1 mM) was combined with 20 ml of the essential plant extract, the pH of the solution was recorded, and the mixture was subsequently heated at 60 °C for 25 minutes. The observed colour shift in the reaction mixture, from light yellow to red-brown, demonstrates the efficacy of the biosynthesis process [14].

Characterization by (UV-vis)

UV-visible (UV-vis) spectrophotometry was performed with an Agilent UV-Cary 60 device. The measurements were conducted on materials suspended in glycerine and water solutions.

Optimization studies for *C. citratus* -AgNPs synthesis

Various criteria were examined to determine the best conditions for the synthesis of *C. citratus* AgNPs. The criteria encompassed the volume of $AgNO_3$ solution, the volume of *C. citratus* extract, pH, temperature, and duration. The resultant solution was employed to verify the production of nanoparticles, which were examined by UV-visible absorption spectroscopy [15].

Enhancement of the extract volume ratio and $AgNO_3$

To achieve the optimal peak of the resultant silver particles, the ratio of silver (Ag) to extract concentration was varied to maximize the production of (AgNPs). The reaction process was monitored utilizing various ratios of Ag to extract (1:1, 2:1, 3:1, 4:1, 1:2, 1:3, and 1:4), and the absorption value of the final solution was assessed spectrophotometrically. The reaction temperature was maintained at 100°C for approximately 80 minutes [16].

Optimization of temperature

To synthesize *C. citratus* AgNPs, the reaction was optimized by maintaining the temperature between 20 and 100°C for approximately three hours.

Enhancement of time to reaction

The UV–vis absorbency for *C. citratus* -AgNPs was derived from the medium of reaction utilizing an appropriate volume of $AgNO_3$ solution and *C. citratus* extract. The result solution was observed with varying contact durations at 100°C, spanning from 0 to 80 minutes.This study elucidates the impact of reaction time.

Stability analysis

The stability of the resulting solution was assessed at room temperature during intervals of 1 day, 1 week, and 1 month.

Impact of *C. citratus* Extracts on 24-Hour-Old Eggs of *C. cautella*

Twenty eggs with three replicates were systematically chosen for the experiment. They were placed in Petri dishes, with the treatment involving the systematic spraying of the extract at three concentrations (40, 30, and 20 µg/ml), in addition to a control test. The eggs were then systematically transferred The sample was transferred to a Petri dish and placed in the laboratory incubator, maintaining a systematic temperature of 30 ± 2°C and a systematic relative humidity of 60 ± 5%. The mortality ratio and hatching period were systematically recorded, ensuring the reliability of the results.

Impact of Extracts *C. citratus* on second larvae of *C. cautella*

By isolating the eggs and tracking their development through the instars until they reached the second instar larvae, the second instar larvae were collected. Using 20 larvae with three replicates, they were placed in Petri dishes and the treatment involved spraying an extract at three different concentrations. (40, 30, and 20 µg/ml), along with a control test. After treatment, the larvae were moved to an incubator set at 30 ± 2°C and 60 ± 5% relative humidity, where the mortality ratio and larval stage period were recorded.

Statistical analysis

All experiments were performed in triplicate, and results were represented as mean ± standard deviation. Statistical significance was assessed at a level of $p < 0.05$.

3. RESULTS AND DISCUSSIONS

Green synthesizes of Ag-NPs

The process involved synthesizing silver nanoparticles by using *C. citratus* extract in combination with a pre-prepared solution of silver nitrate. A conical flask holding 80 ml of 1 mM AgNO3 was combined with 20 ml of leaf and heated at 60ºC for 25 mins [17]. The successful acquisition of the biosynthetic process has been confirmed by observing the colour shift of the reaction mixture, depicted in Figure (1), from green-yellow to red-brown.

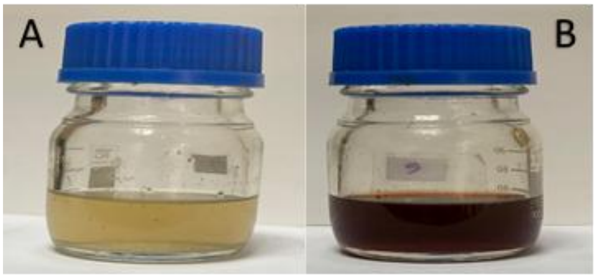

Fig 1 A: *C. citratus* extract, B: subsequent addition of AgNO3

UV-Visible Spectroscopy

After witnessing the colour change from green-yellow to dark brown, the silver nanoparticles created using the *C. citratus* extract were detected by UV-visible analysis at 1100 to 189 nm. At 420 nm, as shown in Figure 2, these results reveal the formation of silver nanoparticles. The findings from this study align with previous research by [18]

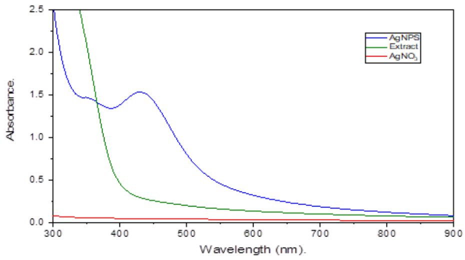

Fig. 2. UV– Visible Spectroscopy of C. citratusis AgNPs.

Impact of ratio of extract to AgNPs

C. citratus -AgNPs reaction was conducted in the ratio between silver solution and extract volume, the study showed that the best value for silver to extract is 7 to 0.5, respectively. It is clear that when the silver concentration is twice the extract solution, the beak is at its highest point, but when the amount increases, the stability decreases. This decline may be due to the amount of extract no longer being enough to reduce all the silver. After confirmation of the silver ratio, the extract ratio was changed. The stability decreased with the increase in the amount of extract. This may be because aggregations and agglomerations happened due to the large amount of *C. citratus* extract. The optimum conditions determined for AgNPs synthesis were 1mM of AgNO3 with Hylocereus undatus; the ratio was 0.5 ml of extract: 7ml of (1mM) AgNO3 mixed for 50 min at 100°C and reacted in the extract medium. Once all conditions were established, Fig. 3 emerged. A comparable study examines the volumetric impact of AgNPs in UV–Vis spectroscopy, aligning with the findings reported [19].

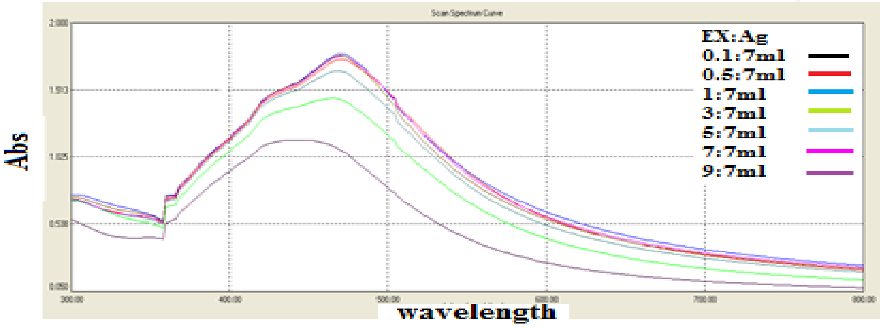

Fig 3. Effect of different ratio of extract and AgNPs

Impact of Reaction Time

The influence of contact time was investigated through a series of reactions conducted at ambient temperature for up to 80 minutes, utilizing 0.5 ml of *C. citratus* extract combined with 7 ml of 1 mM AgNO3 for the production of –AgNPs. UV–vis spectra were employed to monitor the reactions of *C. citratus* with AgNPs at one-hour intervals. The distinct SPR peaks, centered at 80 minutes and 430 nm, were evident in the UV–vis spectroscopy. The intensity of peak to absorbance augmented with prolonged reaction time, becoming more pronounced and sharper at 80 minutes (Figure 4). The absorption plot against reaction time indicates that the SPR peak markedly rises until 80 minutes (Figure 4). This discovery indicates that *C. citratus* -AgNPs interference may elicit a rapid response within 50 minutes. The rise in absorbency with time signifies a reduction in metal ions and an accumulation of nanoparticle. This resulted from the tiny nuclei that diminish the size of particles, as seen by a rise in SPR intensity at the peak [20]. The SPR and colloidal solution maintain stability for 50 minutes.

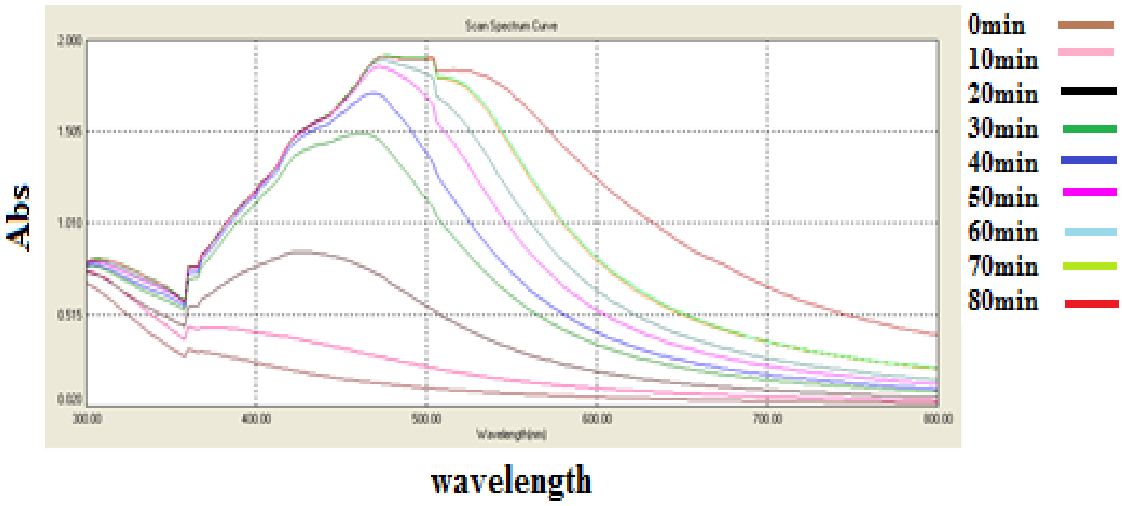

Fig 4. Effect of reaction time (min)

Impact of temperatures

This study evaluated the influence of temperatures on the formation of *C. citratus*-AgNPs by altering the reaction temperatures from 20 to 100 ◦C for roughly 50 minutes, while adhering to the previously optimized parameters to produce *C. citratus* - AgNPs. The influence of temperature has significance in the production of AgNPs. The production of AgNPs from *C. citratus* resulted in a more consistent variation in size and an increased rate of formation of particles as the reaction mixture temperature was elevated. Elevated temperatures accelerate the reduction of precursor silver and the formation of nanoparticles. Consequently, the UV spectrum of the reaction mixture exhibited a distinct SPR peak at 430 nm with increasing temperatures, with the optimal peak occurring at 100°C (Figure 5). Consequently, the rate of reaction escalates at the optimal temperature of 100°C, which promotes and amplifies the kinetic energy of the reaction[21].

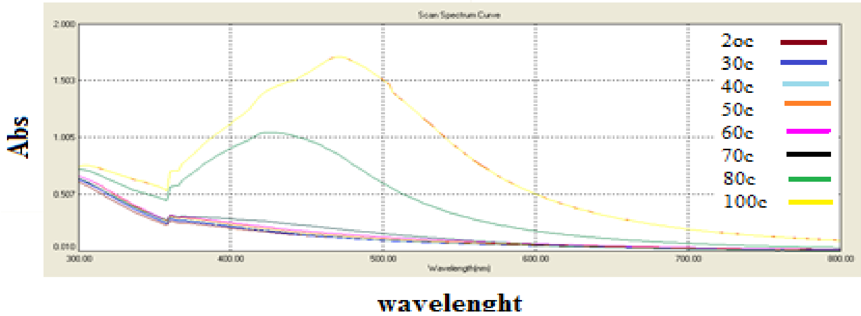

Fig 5. Effect of temperture (°C)

The consistency of interaction time in synthesis of AgNPs through the reaction between silver ions and lowering agent in *C. citratus* extract was monitored over a period of one month. Figure 6 illustrates the UV-VIS spectrum of produced AgNPs over time following the introduction of *C. citratus* extract. Demonstrate that an increase in reaction time leads to a progressive enhancement in the spectrum of absorbance with surface plasmon resonance at λmax among 430-450 nm. This indicates that the produced AgNPs remained stable for almost one month [17]. This work demonstrated no fluctuation in the peak at 430 nm even after one month of storage, showing the robust stability of biosynthesized AgNPs.

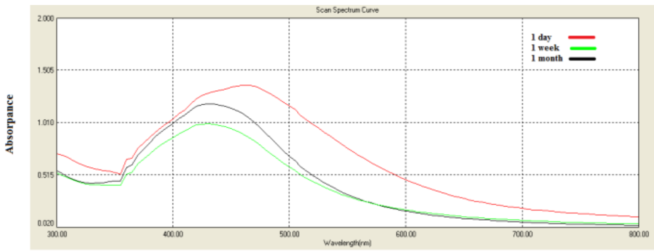

Fig 6. Stability of AgNPs

Effect of Silver Nanoparticles Leaves of *C. citratus* on Eggs and Second Larvae of *C. cautella*

The study's results demonstrated the effectiveness of silver nanoparticles from the leaves of *C. citratus* on Eggs (24 old). Second Larvae of *C. cautella* Tables 1 and 2 show that the most incredible egg mortality rate after 24 hours of treatment was 80.3% at a concentration of 40 µg/ml, followed by 72.9% at 30 µg/ml, and it decreased to 65.8% at 20 µg/ml; in contrast, the control treatment recorded no egg mortality. Furthermore, larval mortality rates were 74.9%, 65.8%, and 60.2% at 40 µg/ml, 30 µg/ml, and 20 µg/ml, respectively. Studies have shown the presence of α-citral (geranial) and β-citral in the chemical composition of *C. citratus* oil. These are acyclic monoterpene aldehydes, commonly referred to as citral [22]. The insecticidal activity of C. citratus may be due to citral [23]. Plant compounds delay the development of insects because of their toxic effects on them [24]. As a result of the study, the exposure of *C. cautella* to *C. citratus* extract significantly prolonged the larval durations. Furthermore, the active ingredients in *C. citratus* may cause a wide range of physiological disturbances, leading to a reduction in the development of treated insects. Such changes may lead to malnutrition, disrupt metabolic processes, hinder the absorption of specific nutrients, and impair the insects' ability to digest food [25]. In another study [26], it was mentioned that lemongrass leaf extract significantly inhibits alpha-amylase activity, significantly inhibits lipase activity, and selectively blocks the digestive system's ability to break down starch and fats. The synthesis of nanoparticles increases the efficiency of the extract and increases the mortality rate in insects. This increased activity of nanoparticles compared over their bulk counterparts arise from their increased surface-to-volume ratio. [27]. Additionally, [28] indicated that nanoparticles of the substance accumulated on the spiracle, resulting in obstruction, which impaired the insect's respiratory function and ultimately led to its demise.The findings of this study align with [29] when studying the effect of *C. citratus* oil nano on the Larvae of Galleria mellonella .The results are also consistent with the findings presented in study [30], which discusses the toxic effects of the essential plant, lemongrass oil, on adult *Sitophilus oryzae.* This proved the efficiency of the extract in controlling the insect.

Table 1.Effect of Silver Nanoparticles Leaves of *C. citratus* on Eggs *C. cautella*

Insect	concentrerions	death rates %	Incubation Period (Days)
C. cautella	40µg/ml	80.3 ± 4.38 a	4.2 b
	30µg/ml	72.9 ± 3.01 b	5.4ab
	20µg/ml	65.8 ± 3.22 b	5.6a
	Control	0 ± 0 c	4.6b

LSD value	6.79 *	1.045*
* (P<0.05).		

Table 2. Effect of Silver Nanoparticles Leaves of C. citratus on Second Larvae C. cautella

Insect	Concentrations	death rates %	Duration of the Larval Stage (in Days)
C. cautella	40µg/ml	74.9 ± 3.56 a	4.4 b
	30µg/ml	60.2 ± 2.64 a	5.6 b
	20µg/ml	55.6 ± 2.98 b	5.9 a
	Control	0 ± 0 c	4.2 b
LSD value		6.92 *	1.198 *
* (P<0.05).			

4. CONCLUSION

The results of this current study conclude that Silver nanoparticles derived from the leaves of *C. citratus* are effective for controlling insects, serving as a safe substitute for managing 24-day-old eggs and second-stage larvae of *C. cautella*. Encouraging future research on research on other insect pests is also encouraged.

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