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BIO-EFFICACY OF SYNTHESIZED NANOPARTICLES AND METHANOLIC LEAF EXTRACT OF *MELIA AZEDARACH* ON *MEGASELIA HALTERATA*

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ABSTRACT

Megaselia halterata (Diptera: Phoridae), commonly known as the scuttle fly is cosmopolitan insect pest. *M. halterata* poses a significant threat to agriculture as it can infest various crops including mushrooms, causing 30-70% yield losses. The current research explores the potential use of silver nanoparticles synthesized from *Melia azedarach* (Chinaberry) (MA-AgONPs) in combating the mushroom pest *Megaselia halterata* on *Agaricus bisporus*. The biosynthesis of AgONPs was confirmed through various characterization techniques, i.e., UV-visible spectroscopy, x-ray diffraction, fourier-transform infrared spectroscopy, scanning electron microscopy and transmission electron microscopy. *In vivo* experiments demonstrated the efficacy of MA-AgONPs in reducing the emergence of adult *M. halterata* and minimizing larval damage to *A. bisporus* mushrooms. The highest reduction in adult emergence (94%) and the least sporophore damage (5.12–5.34%) were achieved at a concentration of 50 ppm. Additionally, this treatment yielded the highest mushroom production (4.74 kg/bag), surpassing both the methanolic extract application (4.60 kg/bag) and control treatments. Furthermore, *in vitro* experiments revealed a concentration and time dependent increase in the mortality of *M. halterata* larvae. The highest mortality rate (91.67%) was observed at a concentration of 50 ppm after 48 hours. The green-synthesized MA-AgONPs shows greater effectiveness as compared to methanolic leaf extract as evident by lower LC_{50} and LC_{90} values. These findings suggest that *Melia azedarach*-derived AgONPs present a promising biocontrol solution for sustainable mushroom cultivation.

Keywords: *Megaselia halterata, Melia azedarach, Agaricus bisporus,* green synthesis, Silver oxide nanoparticles, bio-efficacy

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1.INTRODUCTION

Fungal pathogens and insect pests represent significant challenges in agricultural production, especially in the cultivation of economically valuable crops such as *Agaricus bisporus* (button mushroom). Among these pests, *Megaselia halterata* (commonly known as the scuttle fly) is a notorious dipteran species that inflicts severe damage on mushroom crops during all growth stages. This pest not only affects the yield but also compromises the quality of the production, leading to substantial economic losses (Shamshad, 2010). Conventional chemical pesticides, although effective, raise environmental and health concerns due to their persistence, toxicity, and potential for developing pesticide resistance (Rijal et al., 2021). Hence, there is a pressing need for sustainable and eco-friendly pest management strategies.

In recent years, nanotechnology has emerged as a promising field for developing innovative pest control methods. Silver oxide nanoparticles (AgONPs) have received a lot of attention due to their powerful antibacterial and insecticidal characteristics (Kumar et al., 2020). Unique physicochemical properties of silver-based nanoparticles such as their small size, high surface area-to-volume ratio, and bioactivity, make them beneficial options for targeted agricultural applications. (Shafey, 2020). Recent studies have demonstrated that biosynthesized nanoparticles derived using plant extracts offer a green and sustainable alternative to chemically synthesized nanoparticles (Gomes et al., 2015). Plant-mediated synthesis not only eliminates the use of hazardous chemical based insecticides but also enhances the biocompatibility and efficacy of nanoparticles (Iravani, 2011).

Melia azedarach, a member of the Meliaceae family, is a fast-growing deciduous tree widely known for its bioactive secondary metabolites, such as limonoids, alkaloids, and flavonoids. These compounds exhibit strong insecticidal, antimicrobial, and antifungal properties, making the plant an excellent candidate for green nanoparticle synthesis (Ali et al., 2024; D. Sharma and Paul, 2013). Previous studies have reported the successful synthesis of silver-based nanoparticles using *M. azedarach* leaf extract, demonstrating their effectiveness against a range of pathogenic microorganisms and pests (Haq et al., 2023).

The current study focuses on the green synthesis of silver oxide nanoparticles using *Melia azedarach* leaf extract and evaluates their efficacy against *M. halterata* in the context of *A. bisporus*. The *in vitro* and *in vivo* experiments were designed to assess the nanoparticles insecticidal activity and their potential to mitigate pest infestations without adversely affecting mushroom production. By integrating nanotechnology with natural plant-derived compounds, this work aims to propose a sustainable and effective alternative for insect pest control in mushroom farming.

2.MATERIALS AND METHODS

2.1. Chemicals and reagents

Silver nitrate ($AgNO_3$, purity ≥ 99.9 %, Sigma-Aldrich) served as the precursor for synthesizing silver oxide nanoparticles. Throughout the study, double-distilled and deionized water (MilliQ) was consistently utilized. Sodium hydroxide pellets (NaOH, purity ≥ 97 %, Sisco Research Laboratories Pvt. Ltd.) were used to ensure the appropriate reduction of the nanoparticles.

2.2. Collection and extraction of *Melia azedarach* leaves

Leaves of *Melia azedarach* were collected from the vicinity at latitude 32.315054ºN and longitude 76.08718ºE in the Chamba District of Himachal Pradesh, India. The plant leaves were isolated and rinsed with Milli-Q water. After washing, the leaves were dried in the shade at room temperature for few days. Using the Soxhelt apparatus, 20 g of crushed leaves and

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200 mL of Milli-Q water were extracted (Ramya et al., 2009; Sharma et al., 2021). The resultant suspension was saved for further use after being filtered via Whatman filter paper No. 1.

2.3. Green synthesis of silver oxide nanoparticles

Silver nanoparticles were synthesized by reducing an $AgNO_3$ using an extract derived from *M. azedarach* leaves. The synthesis was based on a modified method previously described by Shafey et al., 2020 . To prepare MA-AgONPs, 25 ml of leaf extract was gradually mixed with a 0.5 M $AgNO_3$ solution using a magnetic stirrer at room temperature for 3 hours. Afterward, 50 ml of NaOH was added dropwise to the mixture. The solution was then centrifuged at 8000 rpm for 20 minutes. The resulting pellet (MA-AgONPs) was washed six times with distilled water. Finally, the nanoparticles were dried in an oven at 40°C for 24 hours and were subsequently analyzed for their larvicidal properties**.**

2.4. Characterization of *Melia azedarach* derived silver oxide nanoparticles

The UV-Vis spectrum of MA-AgONPs was measured between 300 to 550 nm using a UV-Vis spectrophotometer. The phase and crystal structure of the nanoparticles was determined by XRD analysis, conducted with a PANalytical Netherlands X'Pert Pro Cu Kα instrument over a 2-theta range of 20° to 80°. FTIR analysis was carried out using a Perkin Elmer device in ATR mode, covering the range of 4000 cm^{-1} to 400 cm^{-1} to identify various functional groups. The morphology and surface characteristics were observed with a Zeiss EVO40 SEM, while TEM images were obtained using a TALOS instrument.

2.5. Maintenance of test insect culture

The compost samples collected from different locations containing eggs, larval, and pupal stages were maintained in 200cc spawned mushroom compost contained in a beaker (500 ml cap.) and then placed in the plastic cages. The population of different species of mushroom flies collected during the survey was maintained by keeping the cages in dark places and was used for conducting various laboratory experiments.

2.6. *In vitro* bioassay

The bio-efficacy of different concentrations (10, 20, 30, 40, 50 ppm) of methanolic and AgONPs of *M. azedarach* leaf extract were evaluated *in vitro* through poisoned food technique (Nene and Thapliyal, 1993) and a control was also set up in Petri Plates. Third instar larvae (n=20/replicates) of mushroom flies were selected and added into fully grown Petri Plates of *A. bisporus*. Per cent larval mortality of each concentration along with uninoculated and untreated control was observed at exposure periods of 12, 24, 36 and 48 hrs. The data thus obtained were further subjected to Abbott's correction as per the formula given below:

$$\%\ corrected\ mortality = \frac{\%\ mortality\ of\ treatment - \%\ mortality\ in\ control}{100 - \%\ mortality\ in\ control} \times 100\%$$

The log concentration-mortality regression was estimated by probit analysis as per Finney, (1971). Based on this analysis, LC_{50} and LC_{90} values of the respective extracts were calculated.

2.7. *In vivo* bioassay

2.7.1 Experimental site

The study was carried out at the farmer field in two constitutive growing periods (2023-2024). The growth cell was designed similarly to commercially used systems but on a smaller scale. Each growth cell was treated as an individual replicates with three replicates assigned to each treatment.

2.8. Statistical analysis

In vitro and *in vivo* data are represented as mean values ± standard error (SE). The *in vivo* experimental data were analyzed using analysis of variance (ANOVA) and regression coefficient

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analyses with SPSS software (IBM SPSS 17.0). Treatment means were compared using Duncan's multiple range test (DMRT). All statistical analyses were performed at the significance level of $p \leq 0.05$. FTIR, XRD, and UV spectra were plotted using Origin software, while SEM and TEM images were analyzed using Image J software version 1.54i.

The percentage reduction in adult emergence for each treatment in each growing periods was calculated by using the formula (Erler et al., 2011; Muhammad Hussnain Babar, 2012):

$$\% \text{ Reduction} = \frac{\text{Y-X}}{\text{Y}} \times 100$$

Where Y= Total number of emerging adults in the negative control and X= Total number of emerging adults in each of the treatments.

Whereas the percentage damage of mushroom was determined by using the formula (F Erler et al., 2011):

$$\% \, Damage \; incidence = \frac{Number \; of \; sporophores \; damaged}{Total \; number \; of \; sporophores} \times 100$$

The mushroom yield was expressed as kg/bag.

3. RESULTS

3.1. Characterization of green synthesized nanoparticles

M. azedarach aqueous extract was added to the $AgNO_3$ solution, the reaction mixture exhibited a color changes from yellow to brown. This shift in color is attributed to the bioactive compounds present in the *M. azedarach* extract, which reduce silver ions, leading to the formation of stable silver nanoparticles (AgONPs). It is well-established that AgONPs demonstrate strong surface plasmon resonance (SPR) behavior, resulting from the collective oscillation of free electrons on the surface of metallic particles (Ider and Eddahbi, 2016). These electron oscillations are influenced by particle size, which subsequently determined these absorption wavelengths range within the visible spectrum. The UV-Visible spectroscopy analysis confirmed the synthesis of AgONPs by displaying a distinct SPR peak at around 395.67 nm (Fig.1A). The presence of a single SPR peak indicates that the AgNPs were predominantly spherical in shape. XRD analysis further verified the crystalline nature of the MA-AgONPs revealing diffraction peaks at 2θ values of 38.54°, 44.08°, 64.20° and 77.90°, corresponding to the (111), (200), (220) and (311) crystal planes, respectively, (Fig. 1B). FTIR spectroscopy identified the functional groups associated with MA-AgONPs, showing seven prominent peaks at 595, 832, 3120, 1138, 1695, 2156 and 3433 cm−1 (Fig. 1C). Scanning Electron Microscope imaging showed a spongy, porous morphology with a loosely arranged texture (Fig. 1D). The SEM analysis also indicate that the nanoparticles were spherical and tended to aggregate into clusters, with a uniform average size of 13.07 nm as depicted in the histogram (Fig.1D). TEM analysis revealed the internal structure of the nanoparticles, including visible lattice fringes, which could be attributed to the deposition of Ag metal onto the *M. azedarach* extract (Fig. 1E).

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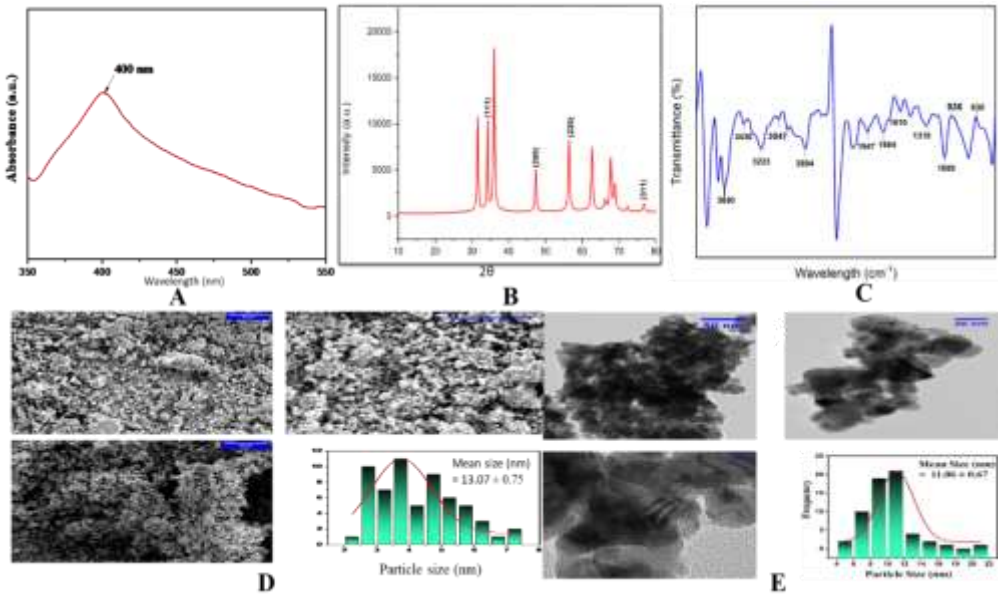

Fig. 1. (A) UV-Vis absorption spectra of MA-AgONPs (B) XRD analysis represented by four peaks corresponding to crystal planes (C) FTIR spectrum (D) SEM images at different magnifications and their corresponding size distribution histogram (E) TEM images at different magnification showing lattice fringes, and size distribution histogram.

3.2. *In vitro* bioassay

All the concentrations of MA-AgONPs showed a significant effect on larval mortality of *M. scalaris*. With the increase in concentrations and exposure periods, the larval mortality also increases. The highest mortality was observed at 50 ppm concentration of MA-AgONPs at 48 h of treatment (91.67 %) and the lowest mortality was observed at 10 ppm (38.33%) of methanolic leaf extract of *M. azedarach* (Table 1). At 36 h of treatment of MA-AgONPs, the highest mortality was observed at 50 ppm (86.67 %) and the lowest mortality was observed at 10 ppm (28.33) of methanolic leaf extract of *M. azedarach*. There were 78.33% and 28.33% mortality at 50 ppm and 10 ppm respectively after 24 h exposure period of MA-AgONPs. Furthermore, at 24 h exposure period of methanolic leaf extract the percent mortality was 53.33% and 20.00% at 50 ppm and 10 ppm respectively.

Table 1. Bio-efficacy of methanolic and synthesized silver nanoparticles at 48 hours exposure period using *Melia azedarach* leaf extract against third instar larvae of *Megaselia halterata*

Per cent mortality (Mean ± SE) at different exposure periods					
Extracts	**Concentrations (ppm)**	**12 h**	**24 h**	**36 h**	**48 h**
Methanolic extract	10	11.67^{b} ±1.66	20.00^{b}±2.88	28.33^{b}±4.40	38.33^{b} ±1.66
	20	20.00^{c}±0.00	28.33^{c}±1.67	38.33^{c}±1.66	53.33^{c} ±1.67
	30	26.67^{d}±1.66	36.67^{d}±1.67	51.67^{d}±1.66	63.33^{d} ±1.66
	40	38.33^{e}±1.66	48.33^{e}±1.67	61.65^{e}±1.66	71.67^{e} ±1.66

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	50	46.67^{f}±1.67	53.33^{e}±1.67	68.33^{e}±1.66	76.67^{e} ±1.66
Synthesized AgONPs	10	16.67^{b}±1.66	28.33^{b}±1.66	38.33^{b}±1.66	48.33^{b} ±1.66
	20	26.67^{c}±1.66	36.67^{c}±1.66	48.33^{c}±1.66	58.33^{c} ±1.67
	30	36.67^{d}±1.66	51.67^{d}±3.33	62.33^{d}±1.66	70.00^{d} ±0.00
	40	46.67^{e}±1.66	61.67^{e}±1.66	80.00^{e}±2.88	81.67^{e} ±1.66
	50	58.33^{f}±1.66	78.33^{f}±1.66	86.67^{e}±3.33	91.67^{f} ±3.33

The values are mean ± SE followed by common letter (s) are not significantly different at $p \leq 0.05$.

The *in vitro* larvicidal activity data of the green synthesized nano particles were presented as LC_{50} and LC_{90} values along with their respective 95% confidence interval (Table 2). An increase in larval mortality was noted as the nanoparticle concentration increases from 10 to 50 ppm. The MA-AgONPs exhibited strong toxicity against larvae, with LC_{50} values of 43.12, 24.03, 16.83 and 12.50 ppm and LC_{90} values of 253.74, 110.29, 74.80 and 64.67 ppm after 12, 24, 36 and 48 h exposure, respectively. Similarly, the methanolic leaf extract also showed significant toxicity, with LC_{50} values of 62.39, 46.62, 26.57 and 17.37 ppm and LC_{90} values of 390.58, 400.67, 210.78 and 121.85 ppm across the same exposure times. These findings indicated that MA-AgONPs were more effective against third instar larvae after 48 h exposure followed by 36 hours.

Table 2. Bio-efficacy activity of methanolic and synthesized silver nanoparticles using *Melia azedarach* leaf extract against third instar larvae of *Megaselia scalaris*

Periods of bioassay (h)	LC values in ppm (95% CL)			
	MA-AgONPs		MA-Meth	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
12	43.12	253.74	62.39	390.58
24	24.03	110.29	46.62	400.67
36	16.83	74.8	26.57	210.78
48	12.5	64.67	17.37	121.85
LC_{50}, LC_{90}: Lethal concentrations caused 50% and 90% mortality after 12, 24, 36, 48 h at 95% confidence limits CL: Confidence limit				

3.3. *In vivo* bioassay

3.3.1 Effect of methanolic leaf extract and green silver nanoparticles of *M. azedarach* in field test on emergence of adult *M. scalaris*

The effect of biosynthesized silver oxide nanoparticles (AgONPs) on the adult emergence of *M. halterata* was evaluated by determining the percentage reduction relative to two control groups: uninoculated control (UUC) and inoculated control (UIC). All treatments significantly suppressed adult fly emergence compared to the inoculated control (DMRT, $P \leq 0.05$). The average number of *M. halterata* adults emerging from the treated groups consistently remained significantly lower than that of the

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inoculated control across two consecutive periods (DMRT, P ≤ 0.05).

During the first growing period, significant differences were observed among treatments

(Table 3; F = 2620.32, P ≤ 0.05), with the 50 ppm AgONPs treatment leading to the highest reduction in adult emergence (93.66%). This reduction was statistically comparable to both the uninoculated and inoculated controls. A similar pattern was observed during the second growing period, where 50 ppm AgONPs again achieved a 94.20% reduction in adult emergence (F = 3994.17, P ≤ 0.05). Furthermore, all tested concentrations significantly contributing to *M. halterata* mortality. Across both growing periods, the uninoculated and inoculated controls remained the least effective treatments.

Table 3. Percentage reduction in adult emergence and sporophore damaged rate of *Megaselia halterata* and their larvae at different concentration of synthesized silver nanoparticles of *Melia azedarach* in each of the two growing periods.

Reduction in adult emergence (RAE) and sporophore damage rate (SDR)				
Concentrations (ppm)	Period I		Period II	
	RAE (%)	SDR (%)	RAE (%)	SDR (%)
10	76.96^{c}	12.07^{b}	78.00^{c}	13.09^{b}
20	86.30^{d}	10.74^{b}	86.43^{d}	11.20^{b}
30	87.10^{de}	9.09^{ab}	87.86^{de}	9.69^{ab}
40	88.96^{e}	8.11^{ab}	89.43^{e}	8.83^{ab}
50	93.66^{f}	5.12^{a}	94.20^{f}	5.34^{a}
UIC	0.00^{a}	33.13^{d}	0.00^{a}	34.52^{d}
UUC	56.33^{b}	25.26^{c}	57.33^{b}	26.45^{c}

[a] Values of two growing periods
[b] Means within the columns followed by the same lower case letter are not significantly different (DMRT≤ 0.05).

Similarly, the effect of the methanolic leaf extract of *M. azedarach* (Table 4) showed significant differences between treatments (F = 3477.27, P ≤ 0.05). At concentration of 50 ppm the extract resulted in the highest reduction in adult emergence (71.26%) during the first growing period. This reduction was statistically comparable to both the uninoculated and inoculated controls. In the second growing period, similar results were observed, with a 72.23% decrease in adult emergence at 50 ppm (F = 9927.35 P ≤ 0.05). All tested concentrations significantly contributed to *M. halterata* mortality, while the uninoculated and inoculated controls remained the least effective across both periods.

Table 4. Percentage reduction in adult emergence and sporophore damaged rate of *Megaselia halterata* and their larvae at different concentration of methanolic leaf extract of *Melia azedarach* in each of the two growing period.

Reduction in adult emergence (RAE) and sporophore damage rate (SDR)				
Concentrations (ppm)	Period I		Period II	
	RAE (%)	SDR (%)	RAE (%)	SDR (%)
10	55.43^{b}	18.65^{ab}	57.23^{b}	19.90^{bc}
20	59.63^{c}	16.79^{ab}	61.33^{c}	16.93^{abc}

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30	62.06[d]	14.44[a]	62.60[d]	15.37[ab]
40	65.60[e]	13.03[a]	67.13[e]	13.77[ab]
50	71.26[f]	8.58[a]	72.23[f]	8.62[a]
UIC	0.00[a]	33.13[d]	0.00[a]	34.52[d]
UUC	56.33[b]	25.26[c]	57.33[b]	26.45[c]

[a] Values of two growing periods
[b] Means within the columns followed by the same lower case letter are not significantly different (DMRT≤ 0.05).

3.3.2. Effect on larval damage

All the given treatments of methanolic leaf extract and synthesized AgONPs of *M. azedarach* reduced the incidence of mushroom damage by the larvae of *M. halterata* and had significantly less sporophore damage rates compared with the uninoculated control ($P \leq 0.05$, DMRT) (Table 4). In terms of percentage of sporophores damaged by the larvae, the lowest damage rates in both growing periods were 5.12, 5.34 % respectively at 50 ppm in the MA-AgONPs treatment. The other concentrations of MA-AgONPs, were 8.11, 8.83 at 40 ppm in both growing periods.

During two growing periods, significantly lower damage rates occurred in methanolic leaf extract compared with uninoculated control ($P \leq 0.05$, DMRT) (Table 3) were 8.58 and 8.62 % respectively. Of the ten treatments tested, the extract of MA-AgONPs had significantly lower damage rates in all growing periods than the methanolic leaf extract at 50 ppm concentration ($P \leq 0.05$, DMRT).

3.3.3. Effect on yield of button mushroom

The data recorded during each flush presented in Table 5 with respect to the effect on yield of mushroom with respect to usage of different concentration of MA-AgONPs revealed that maximum yield (2.35 kg/ bag in first flush and 2.44 kg/bag in second flush) at concentration 50 ppm was obtained in MA-AgONPs of *M. azedarach* (table 5). However, the lowest yield during first flush and second flush (1.30 kg/ bag and 1.12 kg/ bag) respectively was recorded in inoculated control.

Table 5. Effect of AgONPs of *Melia azedarach* on *Agaricus bisporus* yield obtained during two growing periods.

Concentration (ppm)	Yield obtained from the treatments tested in overall two growing periods [mean yield ± (SE)][a]		
	First flush yield (kg/bag)	Second Flush yield (kg/bag)	Total Yield (kg/bag)
10	2.11[b] ± 0.00	2.11[b] ± 0.00	4.23[b] ± 0.00
20	2.12[b] ± 0.02	2.12[b] ± 0.00	4.24[b] ± 0.02
30	2.14[bc] ± 0.01	2.16[bc] ± 0.01	4.31[b] ±0.03
40	2.16[bc] ± 0.02	2.19[b] ± 0.02	4.34[bc] ±0.04
50	2.35[c] ± 0.16	2.44[c] ± 0.16	4.74[c] ± 0.32
UIC	2.06[b] ± 0.02	2.08[b] ± 0.02	4.18[b] ± 0.04
UUC	1.30[a] ± 0.02	1.12[a] ± 0.02	2.38[a] ± 0.04

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[a] Values of two growing periods
[b] Means within the columns followed by the same lower case letter are not significantly different (DMRT≤ 0.05).

All treatments, including the inoculated control, produced significantly higher yields for each flush and total yield (across two flushes) compared to the uninoculated treatment (P≤0.05). However, methanolic leaf extract at a concentration of 50 ppm resulted in the highest yield, with 2.30 and 2.29 kg per bag. Yield in all treatments during both growing periods were significantly greater than those of the uninoculated control (DMRT, P≤0.05). Notably, the highest mushroom yield in both flushes (4.74 kg per bag, respectively) was obtained.

Table 6. Effect of methanolic leaf extract of *Melia azedarach* on *Agaricus bisporus* yield obtained during two growing periods.

Concentration (ppm)	Yield obtained from the treatments tested in overall two growing periods [mean yield ± (SE)]		
	First flush yield (kg/bag)	Second Flush yield (kg/bag)	Total Yield (kg/bag)
10	[b] 2.08 ± 0.01	[bc] 2.14 ± 0.01	4.24± 0.02
20	[b] 2.10 ± 0.01	[bc] 2.17 ± 0.02	4.30± 0.03
30	[b] 2.15 ± 0.02	[bc] 2.18 ± 0.04	4.33± 0.06
40	[b] 2.17 ± 0.04	[cd] 2.22 ± 0.02	4.36± 0.06
50	[c] 2.30 ± 0.08	[d] 2.29 ± 0.06	4.60± 0.14
UIC	[b] 2.06 ± 0.01	[b] 2.08 ± 0.01	4.18± 0.02
UUC	[a] 1.30 ± 0.05	[a] 1.12 ± 0.03	2.27± 0.08

[a] Values of two growing periods
[b] Means within the columns followed by the same lower case letter are not significantly different (DMRT≤ 0.05).

4. DISCUSSION

The global accessibility of crop protection agents specifically designed for mushroom cultivation remains significantly limited. Concurrently, stringent regulatory frameworks and advocacy by consumer pressure groups shows reluctances for their usage, considering substantial concerns over public health risks and environmental sustainability. Consequently, future crop protection strategies in mushroom farming must prioritize the implementation of pest prevention measures. In the event of persistent insect infestations, alternative solutions to chemical crop protection are become necessary. However, these alternatives are expected to be produced on a limited scale, emphasizing the urgent need for sustainable pest management approaches.

The present study demonstrates the efficacy of green-synthesized silver oxide nanoparticles (AgONPs) using *Melia azedarach* (MA) leaf extract, particularly in the context of their larvicidal properties against *Megaselia halterata* and their potential to enhance mushroom

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(*Agaricus bisporus*) productivity. Numerous recent investigations have emphasized the green synthesis and application of biogenic nanoparticles due to their non-toxic and environmentally friendly nature (Chinnasamy et al., 2019; Ghareeb et al., 2020). Earlier research has reported the successful green synthesis of silver nanoparticles exhibiting notable antimicrobial and antibacterial properties (Chinnasamy et al., 2019; Khan and Shahid, 2020). In this process, the phytochemical constituents present in plant extracts serve as reducing agents, facilitating the conversion of metal precursors into stable metallic nanoparticles.

The successful synthesis of MA-AgONPs was initially confirmed by the color change of the reaction mixture from yellow to brown, a visual indicator of nanoparticle formation due to surface plasmon resonance (SPR). UV-Vis spectroscopy further validated this by showing a strong SPR absorption peak at ~395.67 nm, indicating spherical nanoparticles (Jebril et al., 2020). These findings align with prior studies (Ider and Eddahbi, 2016) supporting the idea that the shape and optical properties of AgONPs are heavily influenced by the reducing phytochemicals in the extract. XRD analysis confirmed the crystalline nature of the nanoparticles, while FTIR spectroscopy revealed the presence of bio-functional groups involved in nanoparticle capping and stabilization (Jebril et al., 2020) . SEM and TEM further characterized the morphology, showing spherical, porous, and spongy particles with uniform distribution and observable silver lattice fringes (Khan and Shahid, 2020; Zangeneh, 2019).

In vitro bioassays studies of MA-AgONPs exhibited significantly higher larvicidal activity than the methanolic extract alone across all tested concentrations and exposure durations. The highest mortality (91.67%) at 50 ppm after 48 hours suggests potent toxic action, likely due to the enhanced surface area and reactivity of the nanoparticles. LC_{50} and LC_{90} values support this, with lower values for AgONPs (LC_{50} = 12.50 ppm at 48h) compared to the methanolic extract (LC_{50} = 17.37 ppm at 48h), indicating increased toxicity. These findings are consistent with the growing body of literature suggesting that biogenic AgNPs are more effective insecticidal agents than crude plant extracts due to their ability to penetrate biological membranes and disrupt physiological processes at the cellular level (Subarani and Sabhanayakam, 2013). AgNPs can be used as a medical supplement owing to their non- cytotoxic, antioxidant, antibacterial and cutaneous wound-healing properties(Hajialyani, 2018). Silver oxide nanoparticles prevented the growth of bacteria (Zangeneh and Joshani, 2019)

In vivo bioassays study further supported these results. The application of MA-AgONPs significantly suppressed adult emergence of *M. halterata* and reduced larval damage to mushroom sporophores, especially at higher concentrations (Erler et al., 2011). The 50 ppm treatment consistently resulted in over 93% reduction in adult emergence and minimal damage to sporophores (5.12–5.34%), outperforming both the methanolic extract and the control treatments. These findings highlight the nanoparticles' ability to maintain efficacy under more complex environmental conditions, offering practical benefits for integrated pest management in mushroom cultivation systems. However, the reduction in adult emergence and sporophore damage rate increased with the increase in concentration of different treatments as compared with inoculated control and the observed results were supported by earlier studies (Erler et al., 2011; Fedai Erler et al., 2009a, 2009b; Fedai Erler and Polat, 2008, 2015; Muhammad, 2012).

The yield data of *A. bisporus* confirmed that treatment with MA-AgONPs did not adversely affect mushroom growth. On the contrary, the 50 ppm concentration improved yield significantly, with a total yield of 4.74 kg/bag compared to 2.38 kg/bag in the uninoculated control.

Interestingly, the enhancement of yield could be attributed not only to the reduction of pest-induced damage but also to the possible antimicrobial and growth-promoting effects of silver nanoparticles. Similar positive impacts of AgNPs on plant and fungal health have been documented, suggesting their dual role as both protective and growth-stimulatory agents (Erler et al., 2011; Fedai Erler et al., 2009a; Muhammad, 2012)

5. CONCLUSION

The study proved that silver oxide nanoparticles derived from *M. azedarach* possess significant larvicidal activity against *M. halterata*. Mortality rates of third instar larvae of *M. halterata* increased in the direct proportion to the higher concentrations of MA-AgONPs across all tested levels. In an *in vivo* experiment with *A. bisporus*, MA-AgONPs could potentially control mushroom fly population by reducing adult emergence offering a possible alternative to conventional chemicals. The results also indicate that plant extracts helped to reduce phorid fly emergence and larval damage to mushrooms. While considering the yield outcomes from test treatments, the uninoculated control showed the lowest values. This results suggest that green-synthesized AgONPs from *M. azedarach* offer a sustainable, eco-friendly, and effective alternative to conventional chemical insecticides. Their superior larvicidal activity, protective role in mushroom cultivation, and non-toxic impact on crop yield suggest wide applicability in agricultural and entomological pest management practices. However, further studies on environmental safety, potential bioaccumulation, and long-term impacts are necessary before widespread application.

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Conceptualization and Methodology: Sunil Kumar, Sachin Upmanyu; Writing – original draft: Kumari Ruchika, Visualization: Kanika Choudhary, Alka Rana; Validation: Dixit Sharma, Pankaj Thakur; Formal analysis: Dixit Sharma, Alka Rana Writing – review & editing: Sunil Kumar, Pankaj Thakur; Supervision: Sunil Kumar, Sachin Upmanyu.

Declaration of Competing Interest

The authors have nothing to declare.

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